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Ear metabolism and genotype-by-environment interaction in field-grown durum wheat: identification of novel traits for crop improvement.

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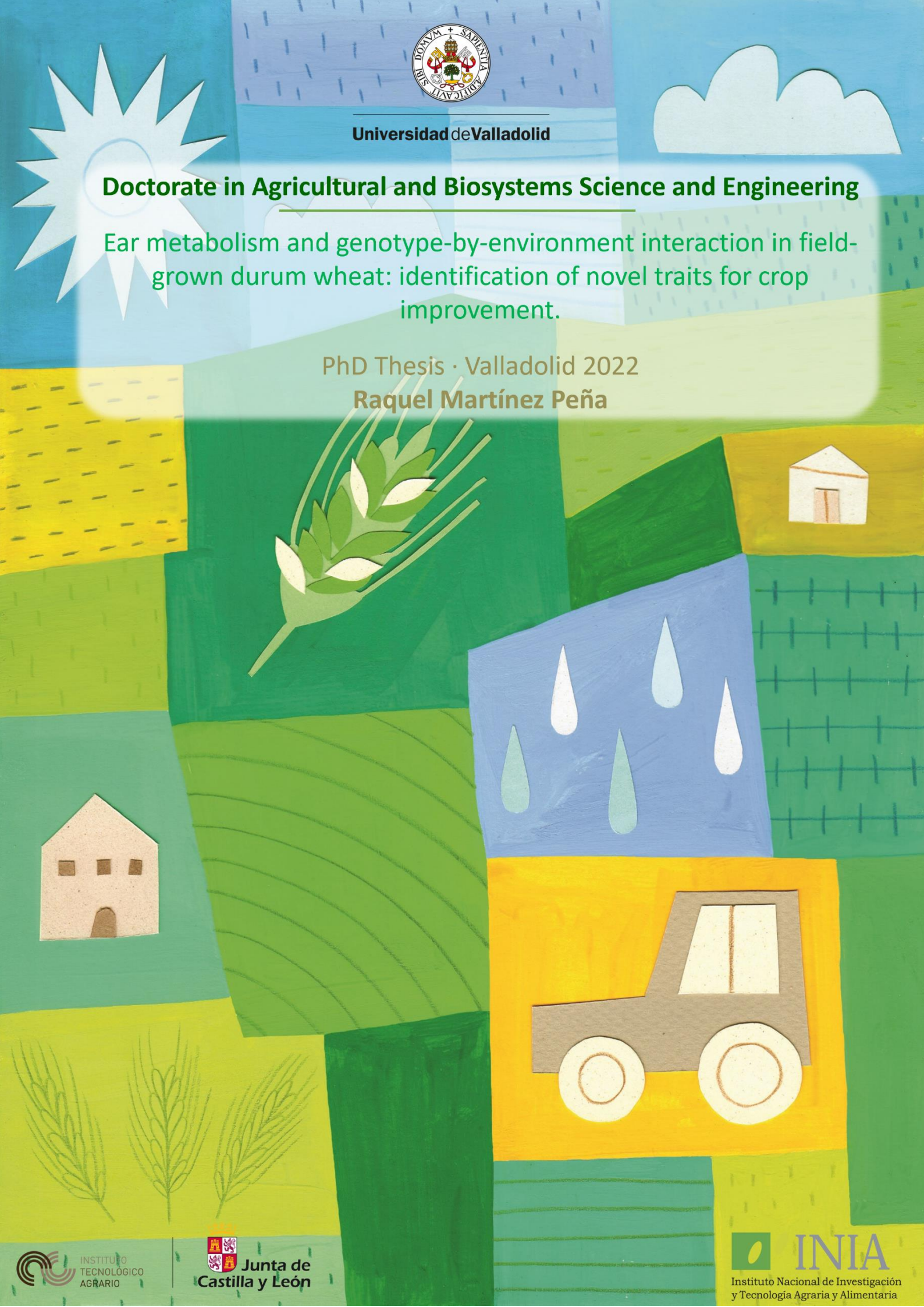

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PROGRAMA DE DOCTORADO EN CIENCIA E INGENIERÍA AGROALIMENTARIA Y DE BIOSISTEMAS

TESIS DOCTORAL:

Ear metabolism and genotype-by-environment interaction in field-grown durum wheat: identification of novel traits for crop improvement.

Presentada por Raquel Martínez Peña para optar al grado de
Doctora por la Universidad de Valladolid

Dirigida por la **Dra. Nieves Aparicio Gutiérrez** y
el **Dr. Rubén Vicente Pérez**
Tutora: **Dra. Mercedes Sánchez Bascones**

Valladolid, mayo 2022

Cuentan de un sabio que un día
tan pobre y mísero estaba,
que sólo se sustentaba
de unas hierbas que cogía.
¿Habrá otro, entre sí decía,
más pobre y triste que yo?;
y cuando el rostro volvió
halló la respuesta, viendo
que otro sabio iba cogiendo
las hierbas que él arrojó.

Quejoso de mi fortuna
yo en este mundo vivía,
y cuando entre mí decía:
¿habrá otra persona alguna
de suerte más importuna?
Piadoso me has respondido.
Pues, volviendo a mi sentido,
hallo que las penas mías,
para hacerlas tú alegrías,
las hubieras recogido.

Fragmento de "**La vida es sueño**", *Calderón de la Barca*

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"La verdadera ciencia enseña, por encima de todo, a dudar y a ser ignorante."

Miguel de Unamuno

Cover and interior illustrations composed by 

Sketch of the ears and view of the field in oil painted by *Eutiquio Martín*

PREFACE

Today's world faces many challenges, such as population growth, economic instability, resource scarcity and impending climate change. This raises the need to make more efficient use of our resources and develop our resilience, primarily to ensure our food security, as one of the main challenges facing agricultural research in this century.

Climate change projections predict an increase in average land surface temperature, more frequent and severe heavy rainfall events, and periods of drought leading to water scarcity and soil degradation, which will affect plant growth and development and impact agricultural productivity and yields in many regions of the world. Durum wheat, is one of the most important crops for human consumption, grown mainly in semi-arid climates with limited availability of nutrients and water resources. Its cultivation represents a valuable source of nutrients for the human diet, such as proteins, carbohydrates, essential vitamins and minerals. As part of human history, durum wheat was a key crop in the Neolithic Revolution that supported the dawn of civilisation. This crop has spread worldwide from the Middle East, and currently, the central cultivated regions are concentrated in few suitable areas, being the Mediterranean Basin the most representative. In addition, a wide variety of products can be obtained from its semolina, some with strong cultural background, such as pasta, burghul a, couscous and unleavened bread. All indications show that durum wheat will remain a staple food crop in the future.

The environmental conditions of the current climate scenario will aggravate these constraints, especially in the semi-arid Mediterranean regions typical of our geographical environment. To maintain crop yields, short-term solutions are usually taken, such as increasing the application of nitrogen fertilisers, which in the long-term will undoubtedly be economically costly and harmful to the environment, generating water and soil pollution problems. To cope with this scenario, it is estimated that the demand for cereals, both for food and feed, must increase by 70% by 2050, which will require new strategies and innovative approaches to achieve a Golden Revolution in agriculture.

The stagnation or even reduction of land suitable for cultivation, the problems of contamination, and the lack of genetic gains present in some areas lead us to be more creative in our research and seek new avenues of study and objectives. In this sense, one of the biggest challenges for physiologists, molecular biologists, agronomists, and breeders will be identifying traits or attributes to select cereal varieties that maximise their production under climate change conditions in specific areas, as there will be different climatic variations depending on the area in which we are located. To achieve this goal, it will first be necessary to perform holistic studies to understand the response of key physiological, biochemical and molecular processes occurring in the different organs of the plant to individual and combined environmental factors associated with climate change.

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SUPPLEMENTARY MATERIAL

I. ABBREVIATIONS

%C	Percentage of carbon
%N	Percentage of nitrogen
aa	Amino acids
ADP	Adenosine diphosphate
AMMI	Additive Main effect and Multiplicative Interaction
ANOVA	Analysis of variance
Anth	Anthocyanins
ATP	Adenosine triphosphate
b*	Yellow pigment index
BSA	Bovine serum albumin
C	Carbon
C/N	Carbon-nitrogen ratio
CAN	Calcium ammonium nitrate
Chl	Chlorophyll
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo.
CO_2	Carbon dioxide
CSI	Crop senescence index
CV	Coefficient of variation
DAS	Days after sowing
DF	Dietary fibre
df	Degrees of freedom
DH	Days from emergence to heading
DW	Dry weight
E	Environment
FAO	Food and Agriculture Organization
Fd	Ferredoxin
Fd-GOGAT	Ferredoxin-dependent glutamate synthase
Flav	Flavonoids
Fru	Fructose
FW	Fresh weight
G	Genotype
G6PDH	Glucose-6-phosphate dehydrogenase

GA	Green area
GCV	Genotypic coefficient of variation
GCY	Grain carbon yield
GDDH	Growing degree days at heading
GEI	Genotype-environment interaction
GGA	Greener area
GI	Gluten index
Glc	Glucose
Glc6P	Glucose-6-phosphate
Gln	Glutamine
Glu	Glutamate
GluDH	Glutamate dehydrogenase
GNY	Grain nitrogen yield
GQI	General quality index
GS	Glutamine synthetase
GY	Grain yield
h^2	Heritability
HI	Harvest index
HK	Hexokinase
HTPP	High-Throughput Plant Phenotyping
IPCC	Intergovernmental Panel on Climate Change
LAI	Leaf area index
Mal	Malate
MAPA	Ministerio de Agricultura y Pesca Alimentación
Max	Maximum
MGE	Mega-environments
MGF	Mid-grain filling
Min	Minimum
MTT	Methylthiazolyldiphenyl-tetrazolium bromide
N	Nitrogen
NAD-GDH	NAD-specific glutamate dehydrogenase
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NBI	Nitrogen balance index

NDVI	Normalized difference vegetation index
NH_4^+	Ammonium
NIR	Near-infrared
NO_2^-	Nitrite
NO_3^-	Nitrate
NPK	Nitrogen-phosphorus-potassium
NR	Nitrate reductase
NSK	Number of kernels per spike
NSP	Number of spikes per m^2
NUE	Nitrogen-use efficiency
O_2	Oxygen
OAA	Oxaloacetate
PCA	Principal component analysis
PCV	Phenotypic coefficient of variation
PEP	Phosphoenolpyruvate
PEPCase	Phosphoenolpyruvate carboxylase
PES	Phenazine ethosulfate
PGI	Phosphoglucose isomerase
Pi	Inorganic phosphate
PL	Peduncle length
PROT	Protein content
RGB	Red-green-blue
rpm	Revolutions per minute
Rubisco	Ribulose-1,5-bisphosphate carboxylase-oxygenase
RuBP	Ribukise-1.5-bisphophate
RUE	Radiation use efficiency
RWC	Relative water content
SAN	Ammonium nitrosulfate
SDSS	Sodium dodecyl sulphate sedimentation
SL	Spike length
SS	Sum of squares
Suc	Sucrose
TCA	Tricarboxylic acid cycle
TKW	Thousand kernel weight

TW	Test weight
UAS	Small unmanned aerial vehicles
VIs	Vegetation indices
VPDB	Vienna Pee Dee Belemnite
VTR	Vitreousness
WC	Water content
WG	Wet gluten
WUE	Water use efficiency
$\delta^{13}C$	Carbon stable isotope
$\delta^{15}N$	Nitrogen stable isotope

II. ABSTRACT

Title Thesis: "Ear metabolism and genotype-by-environment interaction in field-grown durum wheat: identification of novel traits for crop improvement"

Durum wheat (*Triticum turgidum* L. ssp. *durum*) represents one of the world's most essential and widely grown crops. However, to cope with the increasing demand of food for the future world's population in a context of climate change, more efforts are needed to understand the responses of durum wheat plants to existing and upcoming abiotic stresses. The knowledge of the environmental influences on grain yield and quality, as well as phenotype and metabolism, is essential to successfully select varieties better adapted to specific local agro-environments in terms of final products, through the identification of those parameters with interest to increase the efficiency of breeding programmes. This is especially relevant in the Mediterranean basin due to the high inter-annual variability of climatic conditions. In addition, preliminary studies have shown that a significant part of the carbon and nitrogen remobilised to the grains during the filling phase comes from the ears, especially under stress conditions, such as water scarcity or nutrient availability in the soil.

Therefore, in this PhD thesis we have evaluated the effects of genotype by environment interaction on the phenotype at different levels (plant canopy, whole plant and photosynthetic organs) by conducting field trials on a panel of commercial durum wheat varieties registered after the Green Revolution and cultivated in Spain in the last 40 years. To identify varieties with a consistently specific and broader adaptation to defined agro-environments, the present and future growing conditions associated with climate change that could take place in the Spanish region of Castile and León region were simulated, including optimal and stress conditions, such as water deficit, irrigation, high temperatures (late sowing), and low nutrient availability (different levels of fertilisation). A wide range of techniques and methodologies were used, from the evaluation of agronomic components and the industrial and nutritional quality of the grain to phenotyping and biochemical analyses. More specifically, in-depth physiological and biochemical analyses of the source and sink organs were carried out to understand the role of each organ, especially the ear, in the assimilation and translocation of carbon and nitrogen during grain filling in response to different abiotic stresses. All this under a holistic approach to identify new traits for the improvement of yield and grain quality in C_3 cereals in Mediterranean climate conditions and to

elucidate the role played by the different laminar and non-lamilar organs of the plant, mainly at the later stages of growth.

Different statistical analyses were used to elucidate the effect of the genotype, the environment and their interaction. They showed that the environment represents the main factor that strongly influences the canopy and plant growth, and the metabolism of photosynthetic organs, ultimately affecting the yield and the nutritional and industrial quality of the grain obtained at harvest. Nevertheless, the genetic influences were also notable for most of the evaluated agronomic and grain quality parameters. We identified the durum wheat varieties presented in our panel with high stability for the region of Castile and León under optimal and limiting growing conditions associated with climate change, also considering high yield and quality according to industrial standards. A retrospective study of the evolution of nutritional quality in the last forty years revealed that, despite the slight tendency of increased grain yield, the mineral concentration in the mature grains remain stagnant. Furthermore, we determined the varieties with the highest grain nutrient composition among our panel. Moreover, the correlations obtained between specific parameters such as grain yield and protein content with the concentrations of Ca, K, S and Fe could be of interest for crop improvement.

Next, source-sink dynamics were studied in durum wheat in response to contrasting nitrogen fertilisation levels to identify phenotypic and metabolic parameters at the whole plant level related to yield and grain quality in response to nitrogen. Low nutrient availability led to an imbalance in the carbon and nitrogen metabolism coordination at the whole plant level, associated with reduced grain yield and nutrient composition. The activities of key enzymes in carbon and nitrogen metabolism, as well as the levels of photoassimilates, showed not only the flag leaf but that each organ plays an essential role during grain filling, some with a higher photosynthetic capacity, others for the storage of nutrients that will be remobilised at later stages of grain filling, or that will play an essential role in the assimilation and recycling of nitrogen. Interestingly, the enzymatic activities of relevant enzymes such as Rubisco and sucrose content of the ear organs were positively associated with grain yield and quality, unlike leaves, suggesting, together with the regression models obtained with organ-specific isotopic signatures, the potential contribution of non-photosynthetic organs during grain filling.

Finally, concerning the previous study, the effect of water stress on the content of carbon and nitrogen metabolites with a role in grain filling was studied to elucidate the tolerance of photosynthetic organs under water deprivation and to identify new breeding strategies involving the development of resilient varieties adapted to limiting conditions at the whole plant level. Water

stress led to a significant decrease in yield, biomass, carbon and nitrogen assimilation, promoted water use efficiency and differentially modified grain quality traits in a subset of five durum wheat varieties. The results showed that the response to water stress is different according to the photosynthetic organ, with blades and peduncles being the most susceptible to water stress. In contrast, ear organs, mainly glumes and lemmas, showed tolerance at the most vulnerable stages of the plant, such as anthesis and grain filling. Quantitative calculations of metabolite content per organ showed surprisingly that the peduncle is the organ with the highest potential to provide nutrients to grain filling as a reservoir of carbon- and nitrogen-rich compounds, although it was susceptible to stress while the ears showed higher stability regardless of the water regime.

All these results highlighted the importance of combining plant agronomy, physiology and biochemistry to understand the mechanisms at the whole plant level controlling complex traits, such as grain yield and quality, especially in response to abiotic stresses to develop resilient crops adapted to future climate scenario.

Keywords: Agronomic components, Durum wheat, Ear, Grain quality, Grain yield, GxE interaction, Nutrient composition, Phenotyping, Primary metabolism.

III. RESUMEN

Título tesis: "Metabolismo de la espiga e interacción genotipo-ambiente en el trigo duro cultivado en campo: identificación de nuevos rasgos para la mejora del cultivo"

El trigo duro (*Triticum turgidum* L. ssp. *durum*), es considerado uno de los principales cultivos y ampliamente extendidos en el mundo. Es por ello, que para hacer frente a la creciente demanda de alimentos, debido al incremento esperado de la población mundial, en un contexto de cambio climático, se necesita dedicar más esfuerzos para comprender las respuestas de las plantas de trigo duro cultivadas a los estreses abióticos existentes y previstos.

El conocimiento del grado en que el medio ambiente influye en la calidad del grano y el rendimiento de los cultivos, así como en su fenotipo y metabolismo, es esencial para identificar con éxito las variedades mejor adaptadas a los agroambientes locales específicos y así poder identificar aquellos parámetros de interés que permitan aumentar la eficiencia en los programas de mejora. Esto es especialmente relevante en la Cuenca Mediterránea debido a la gran variabilidad interanual existente de las condiciones climáticas. A su vez, en estudios preliminares se ha demostrado que una parte significativa del carbono y del nitrógeno removilizados a los granos durante la fase de llenado, provienen de las espigas, especialmente en condiciones de estrés, como es el caso de escasez de agua o una disponibilidad limitada de nutrientes en el suelo.

Por todo ello, en la presente tesis doctoral hemos evaluado los efectos del genotipo, del ambiente y de la interacción genotipo por ambiente sobre el fenotipo a distintos niveles (dosel vegetal, planta entera y órganos fotosintéticos), mediante la realización de ensayos de campo con un panel de variedades comerciales de trigo duro registradas tras la Revolución Verde y ampliamente cultivadas en España en los últimos 40 años. Con objeto de identificar aquellas variedades con una adaptación específica o más amplia a determinados agroambientes, simulándose las condiciones de desarrollo presentes y futuras asociadas al cambio climático que pueden tener lugar en la región española de Castilla y León, incluyendo tanto condiciones óptimas como de estrés, como son el déficit hídrico, el riego, las altas temperaturas (siembra tardía), y la baja disponibilidad de nutrientes (diferentes niveles de fertilización). Se utilizó para ello una amplia gama de técnicas y metodologías, desde la evaluación de los parámetros agronómicos y la calidad industrial y nutricional del grano hasta el fenotipado y los análisis bioquímicos. Más concretamente, se llevó a cabo un profundo análisis fisiológico y bioquímico de los órganos fuente y sumidero para entender el papel de cada órgano, especialmente la espiga, en la asimilación y translocación de carbono y nitrógeno durante el llenado del grano en respuesta a diferentes estreses abióticos. Todo ello bajo un enfoque holístico con objeto de identificar nuevos rasgos para la mejora del rendimiento y la calidad del grano en cereales C_3

en condiciones climáticas mediterráneas y para dilucidar el papel que juegan los diferentes órganos laminares y no laminares de la planta, principalmente en las últimas etapas de crecimiento.

Se utilizaron diferentes análisis estadísticos para explicar el efecto del genotipo, el ambiente y su interacción. Se ha confirmado que el ambiente representa el principal factor que influye en el crecimiento de las plantas y en el metabolismo de los órganos fotosintéticos, afectando en última instancia al rendimiento y a la calidad nutricional e industrial del grano obtenido en la cosecha. Sin embargo, el efecto del genotipo también fue significativo para la mayoría de los parámetros agronómicos y de calidad del grano evaluados. A lo lardo de su estudio, se han identificado variedades de trigo duro, dentro de nuestro panel con alta estabilidad para la región de Castilla y León en condiciones de cultivo óptimas y limitantes asociadas al cambio climático, considerando además un alto rendimiento y calidad según los estándares industriales. Un estudio retrospectivo de la evolución de la calidad nutricional en los últimos cuarenta años reveló que, a pesar de la ligera tendencia al aumento del rendimiento del grano, la concentración de minerales en los granos maduros permanece estancada. Además, se identificaron las variedades con una mayor composición nutricional del grano en nuestro panel. Las correlaciones obtenidas entre parámetros específicos como el rendimiento de grano y el contenido de proteína con las concentraciones de Ca, K, S y Fe podrían ser de interés para la mejora del trigo duro.

A continuación, se estudió la dinámica fuente-sumidero en respuesta a distintos niveles contrastantes de fertilización nitrogenada para identificar parámetros fenotípicos y metabólicos a nivel de toda la planta relacionados con el rendimiento y la calidad del grano en respuesta al nitrógeno. La baja disponibilidad de nutrientes condujo a un desequilibrio en la coordinación del metabolismo del carbono y del nitrógeno a nivel de toda la planta, asociado a una reducción del rendimiento del grano y de la composición de nutrientes. Las actividades de las enzimas clave en el metabolismo del carbono y del nitrógeno, así como los niveles de fotoasimilados, mostraron no sólo que la hoja bandera, sino que cada órgano juega un papel esencial durante el llenado del grano, algunos con una mayor capacidad fotosintética, otros para el almacenamiento de nutrientes que serán removilizados en etapas posteriores del llenado del grano, o que jugarán un papel esencial en la asimilación y reciclaje del nitrógeno. Curiosamente, las actividades de enzimas relevantes como la Rubisco y el contenido de sacarosa de los órganos de la espiga se asociaron positivamente con el rendimiento y la calidad del grano, a diferencia de las hojas, sugiriendo, junto con los modelos de regresión obtenidos con firmas isotópicas específicas de los órganos, la potencial contribución de los órganos no fotosintéticos durante el llenado del grano.

Por último, en relación con el estudio anterior, se evaluó el efecto del estrés hídrico sobre el contenido de metabolitos de carbono y nitrógeno y su papel en el llenado del grano para estudiar la tolerancia de los órganos fotosintéticos al estrés hídrico y para identificar nuevas estrategias de mejora que impliquen el desarrollo de variedades resistentes y adaptadas a las condiciones limitantes a nivel de toda la planta. El estrés hídrico provocó una disminución significativa del rendimiento, la biomasa, la asimilación de carbono y nitrógeno, promovió la eficiencia en el uso del agua y modificó diferencialmente los rasgos de calidad

del grano en un subconjunto de cinco variedades de trigo duro. Los resultados mostraron que la respuesta al estrés hídrico es diferente según el órgano fotosintético, siendo las hojas y los pedúnculos los más susceptibles al estrés hídrico. Por el contrario, los órganos de la espiga, principalmente glumas y lemas, mostraron tolerancia en las etapas más vulnerables de la planta, como la antesis y el llenado del grano. Los cálculos cuantitativos del contenido de metabolitos por órgano mostraron sorprendentemente que el pedúnculo es el órgano con mayor potencial para aportar nutrientes durante el llenado del grano como reserva de compuestos ricos en carbono y nitrógeno, aunque fueron susceptibles al estrés mientras que las espigas mostraron mayor estabilidad independientemente del régimen hídrico.

Todos estos resultados ponen de manifiesto la importancia de combinar la agronomía, la fisiología y la bioquímica de las plantas para comprender los mecanismos que controlan caracteres complejos, como el rendimiento y la calidad del grano, especialmente en respuesta al estrés abiótico, con el fin de desarrollar cultivos resistentes y adaptados al futuro escenario climático.

Palabras claves: Calidad del grano, Componentes Agronómicos, Composición de nutrientes, Espiga, Fenotipado, Interacción GxE, Metabolismo Primario, Rendimiento, Trigo duro.

Chapter 1

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Introduction

1. CHAPTER 1: INTRODUCTION

1.1. Durum wheat

1.1.1. History

Wheat is a crop belonging to the family Poaceae, subfamily Pooideae, tribe Triticeae, and genus *Triticum*. The genus *Triticum* L. comprises the plants generally known as grasses. This genus is complex, with a rich number of wild and cultivated species. The word wheat comes from the Latin (*Triticum*), which means "broken", "crushed", or "threshed" due to the manipulation necessary to separate the wheat grain from the sheaths covering it (León & Rosell, 2007). Wheat exhibits allopolyploidy, as do other important crops such as cotton, rapeseed, and oats (Huang & Brûlé-Babel, 2010). Allopolyploid species contain two or more similar chromosomes in the same nucleus by interspecific hybridisation followed by spontaneous chromosome duplication or gamete reduction (Huang & Brûlé-Babel, 2010). Phylogenetically, wheat species constitute a classical polyploid series based on a seven-chromosome endowment (Akhunov *et al.*, 2005). The species of this genus are classified according to their chromosome number as diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42), with the primary chromosome number (x) equal to seven and their genomes AA, AABB and AABBDD, respectively.

In 1753, Carl von Linnaeus proposed the first classification of wheat based on physiological and morphological differences. Later, Sakamura (1918) proposed a new classification based on the number of chromosomes present in each previously recognised morphological type. Following this fundamental discovery, many botanists and geneticists have proposed its classification. The most widely accepted is the classification of MacKey (1988). It is one of the most respectful of the rules of botanical nomenclature and the only one that gives equal importance to morphological, physiological, cytological, genetic, biochemical and evolutionary criteria. The most recently published evolutionary scenario of the modern wheats cultivated nowadays is represented in Figure 1.1 (Pont *et al*., 2019).

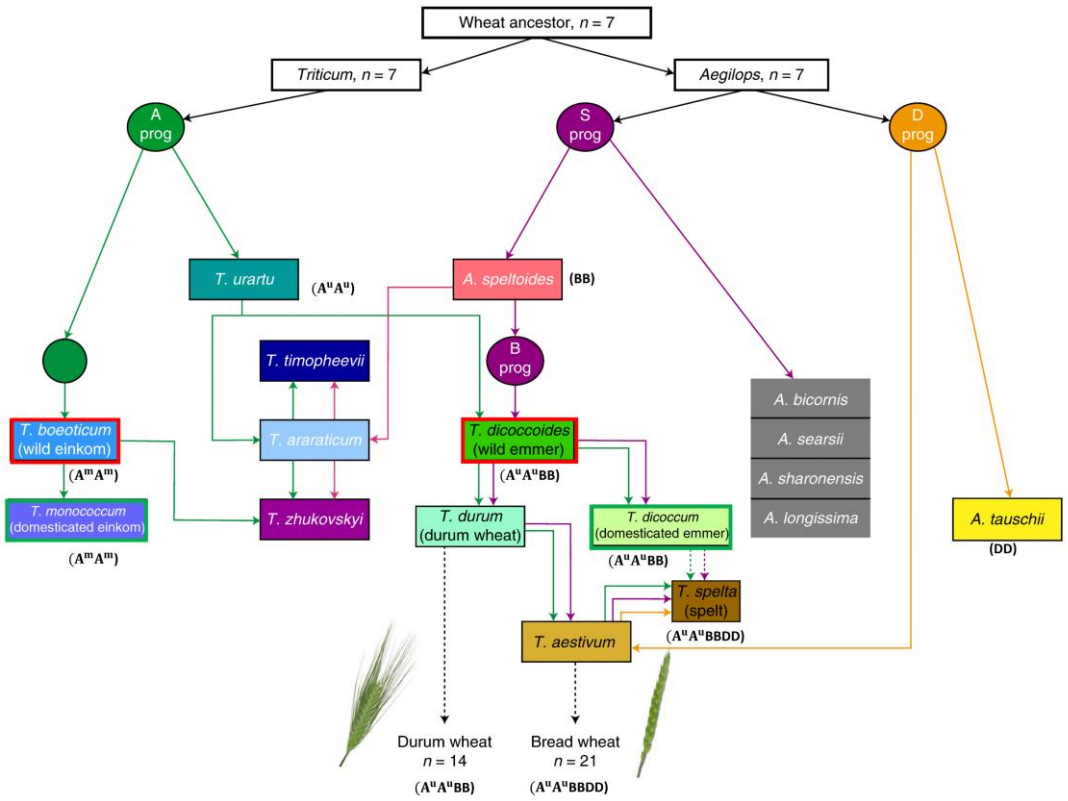


Figure 1.1. Schematic illustration of the evolutionary scenario of the hexaploid bread and tetraploid durum wheat based on the more substantial edges of the subgenomes phylogenetic networks with green, purple and brown lines denoting the paths from the A, B and D subgenomes. Arrow colours illustrate the phylogenetic relatedness between subgenomes (plain arrows indicate the primary, vertical signal; dashed arrows show alternative paths well supported by the inferred topologies and indicative of introgression or gene flow). Circles show putative extinct ancestor intermediates. In red wild cultivars, in green domesticated cultivars. The schematic is based on current data, analysis and prior assumptions from the literature (modified from Pont *et al*., 2019).

Throughout the history of humanity, there have been several domestication phenomena in which plants have been involved. These occurred over a long period and even simultaneously in the great ancient civilisations. The aim was the induced selection for a higher yield of edible or usable fruits to satisfy man's survival needs. This allowed them to move from nomadic to sedentary in the Neolithic period (Peng *et al.*, 2011). Historically, cereals have been one of the most important sources of nutrients for humanity and have been associated with the origin of civilisations and cultures. Traditionally, a functional classification of grasses has been used based on the plant part of the interest and the organism for which they would be used as food, distinguishing cereals and forage grasses. While cereals (including wheat, barley, rice, oats, maize, etc.) have been selected as human and livestock feed because of the high starch content of their grains, the vegetative biomass of forage grasses (fescue, wheatgrass or goatgrass) is often used for ruminant animal feed (Tetlow & Emes, 2017).

The so-called "Fertile Crescent" region of Southwest Asia bounded by the Tigris and Euphrates rivers (mountainous areas of Southwest Asia and in Iran, Turkey, Syria, Lebanon, Israel, Palestine and Jordan; Figure 1.2) witnessed several domestications processes around 10000-15000 BC, which included three cereal species: diploid wheat (einkorn; *Triticum monococcum* ssp. *monococcum*), tetraploid wheat (emmer; *Triticum turgidum* ssp. *dicoccum*) and barley (*Hordeum vulgare*) (Brown *et al.*, 2009). From this area, cereals spread westwards across the Mediterranean basin around 3000 years ago to finally reach the Iberian Peninsula (MacKey, 2005). During the westward migration of wheat from its centre of origin, the dynamic environmental conditions facilitated its spread and induced evolutionary changes (Harlan, 1992; Zeven, 1998).

Maritime transport also allowed the spread of the einkorn species along the Mediterranean coast and its subsequent cultivation in Italy, Spain (7000 years ago) and south of Gibraltar (MacKey, 2005). North Africa was also used as a dispersal route, allowing wheat to enter the Iberian Peninsula during the Middle Ages (Moragues *et al.*, 2006). Einkorn coexisted with emmer and barley crops throughout this dispersal process, the latter with higher yields and better adaptation to domestication (MacKey, 2005), so the dispersal process of emmer could probably have been in line with that of einkorn, as suggested by Özkan *et al.*, (2007). However, it only reached Egypt (MacKey, 2005), from where it spread southwards (Feldman, 2001). Today, einkorn cultivation is a relict, growing only on a small scale in parts of western Turkey, Balkan countries, Germany, Switzerland, Spain and the Caucasus. Similarly, emmer is only found in Jordan, Syria and Israel, central south-eastern Turkey, and eastern Iraq and western Iran (Özkan *et al*., 2007).

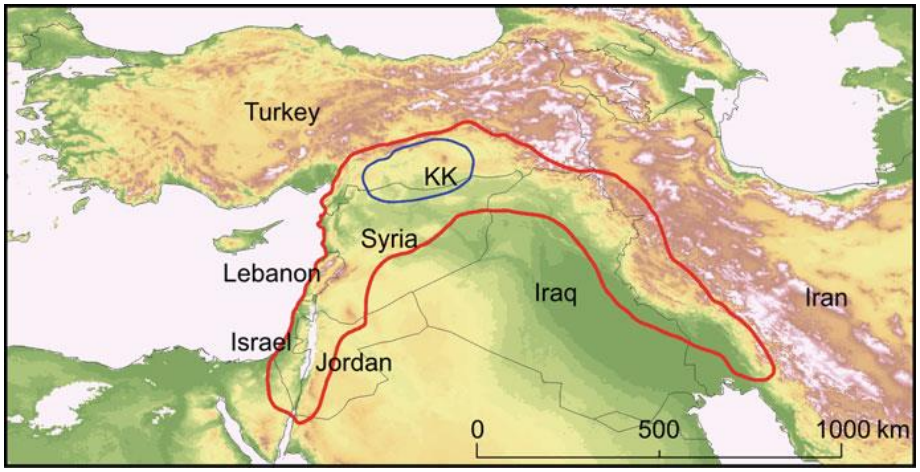


Figure 1.2. Fertile Crescent and core area of plant domestication. The Fertile Crescent is indicated with a red line, and the core area is shown with a blue line. KK, Karacadag mountain range in south-eastern Turkey (Glaubrecht, 2010).

From these species, landraces have been established through a combination of natural selection of traits that increased their adaptability in the various agro-ecological zones existing in the Mediterranean basin (Cleveland & Soleri, 2007) and farmer-mediated selection of traits considered to be of interest. The so-called "landraces" represented the totality of commercial crops until the first half of the 20th century before they were progressively abandoned in favour of other more productive types, favouring selected varieties with more desirable aspects. But from the early 1970s onwards, they were displaced from farmers' fields by the semi-dwarf improved crops of the Green Revolution (Royo *et al.*, 2009), which led to a global production increase of almost threefold in the case of wheat (FAOSTAT, 2022). The development of these varieties took place at the International Rice Research Institute (IRRI) in the Philippines and the International Maize and Wheat Improvement Center (CIMMYT) in Mexico (Khush, 2001). Using and disseminating the semi-dwarf gene *Rht-B1b*, transferred initially from the Japanese bread wheat variety "Norin10" (Autrique *et al.*, 1996). This gene increased earliness, reduced plant height without substantially decreasing total plant dry weight and drastically improved harvest index (McCaig & Clarke, 1995; De Vita *et al.*, 2007; Royo *et al.*, 2007), transforming the wheat plant to produce more grain per unit biomass. This change in plant architecture made the wheat plant much more competitive in modern agricultural production systems. By making the plant shorter, less susceptible to lodging and more sensitive to inputs (water and fertiliser), dwarfing genes also provided a unique opportunity for crop intensification, especially in irrigated and high rainfall environments. However, it has been suggested that the genetic diversity remaining after modern cultivars' drastic displacement of landraces may have been significantly reduced and their quality traits, partly due to a reduced number of ancestors in current breeding programmes.

1.1.2. Production

Cereals have attracted interest because of their productivity, dietary importance and ease of transport and storage (Feuillet *et al.*, 2008). As a result, wheat is one of the world's most widely grown crops (Fischer *et al.*, 2014). It is cultivated from Japan in the east to the plains of the USA in the west; from Scandinavia and Canada in the north to Patagonia and New Zealand in the south; from sea level in many countries to over 1700 meters above sea level in Nepal. It has reached a global area of approximately 220 Mha in recent years (FAOSTAT, 2022). Wheat constitutes about 20% of the energy and protein in the human diet required worldwide (Braun *et al.*, 2010) and is, therefore, a key pillar for ensuring food security (Reynolds *et al.*, 2012).

Durum wheat [*Triticum durum* L. ssp. *durum* (Desf.)] is a self-pollinated annual plant, a crop of great economic and cultural importance, widely grown in the Mediterranean basin. It is one of

the most essential cereals globally since it accounts for between 5% and 10% of the total cultivated wheat production (Beres *et al.*, 2020). By 2020, world durum wheat production reached 33.6 million tonnes through the cultivation of 17 million hectares. Its production is concentrated in the variable and often rainless regions of the Mediterranean basin and the northern plains of Canada, the United States and Chile, among other countries (Figure 1.3). Specifically, the so-called Mediterranean basin covers countries between 27º and 47ºN and between 10ºW and 37ºE, extending over three continents and a coastline of 46000 km (Royo *et al*., 2017). It is a significant contributor, as about half of the world's durum wheat crop is produced in this region. It is generally sown between November and December and harvested between May and July. Much of it is imported to other countries despite being one of the largest consumers of the grain, mainly for the production of pasta and couscous and for the production of a range of other semolina products such as frike bourghul and unleavened bread. These are considered the basis of a good food pyramid (Grant *et al.*, 2012; Royo *et al.*, 2017).

During the 19th century in Spain, the area under wheat increased dramatically to 5.1 million hectares in 1830 (Casanova, 1857). In 1934 wheat already occupied 4.2 million hectares, with an average yield of 1.3 t ha^{-1} (Nagore, 1934). However, the area subsequently decreased to 2.7 million hectares in 1975 and 2.1 million hectares in 2006 (Figure 1.4). In the 1990s, part of the area under common wheat was planted with durum wheat due to European Union subsidies for traditional durum wheat growing areas (Royo, 2005). Nowadays, bread wheat is mainly grown in Castile and León and Castile La Mancha regions, located in the country's northwest and southeast. Other important areas are Aragon, Catalonia, Andalusia, Extremadura and Navarre (Royo & Briceño-Félix, 2011). Moreover, durum wheat is concentrated in the areas where it has traditionally been grown Andalusia (71.6%) and Aragon (20.4%) (Figure 1.4) (MAPA, 2022). Winter wheat and facultative bread types are common in the north, while spring types prevail in the south. Durum wheat is of spring habit throughout the country. As more than 91% of wheat areas are located in rainfed Mediterranean environments, under low and erratic rainfall conditions, yields and production vary significantly from year to year (Royo & Briceño-Félix, 2011).

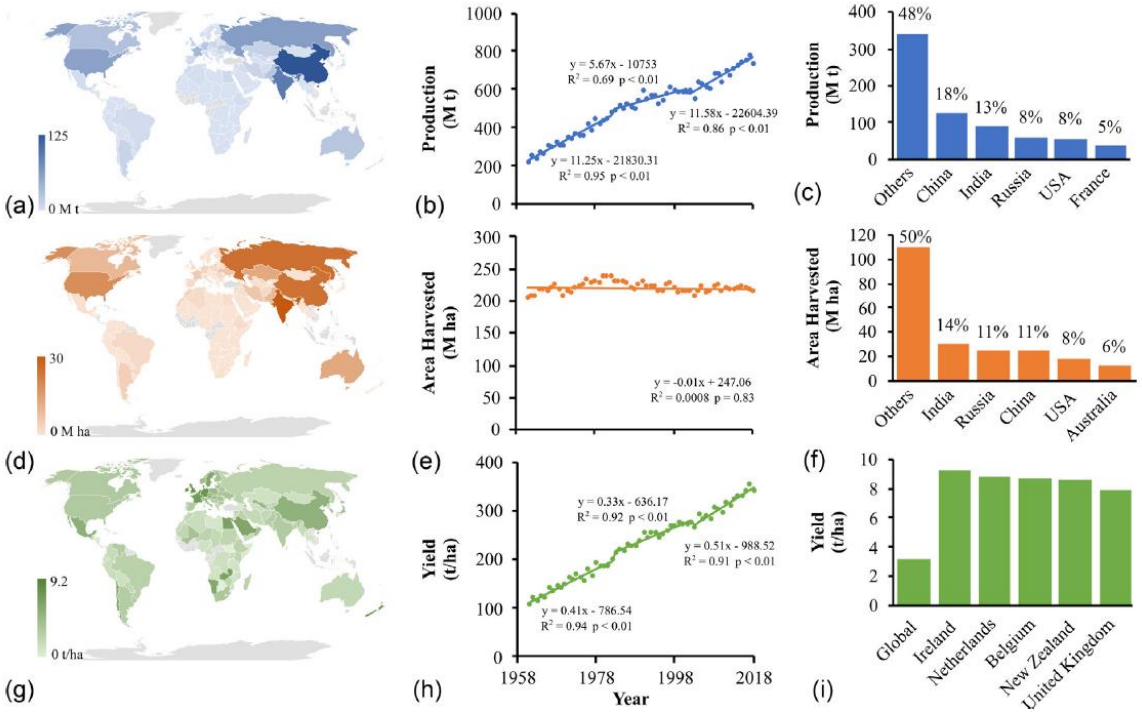


Figure 1.3. World scheme generated from FAOSTAT (2020), (a) wheat production, (d) harvested area, and (g) grain yield recorded between 2009 and 2018; (b, e, h) trends observed from 1961 to 2018; (c, f, i) average of the production, area harvested and yield, from 2009 to 2018, of the top five producers' countries. (Slafer *et al.*, 2021).

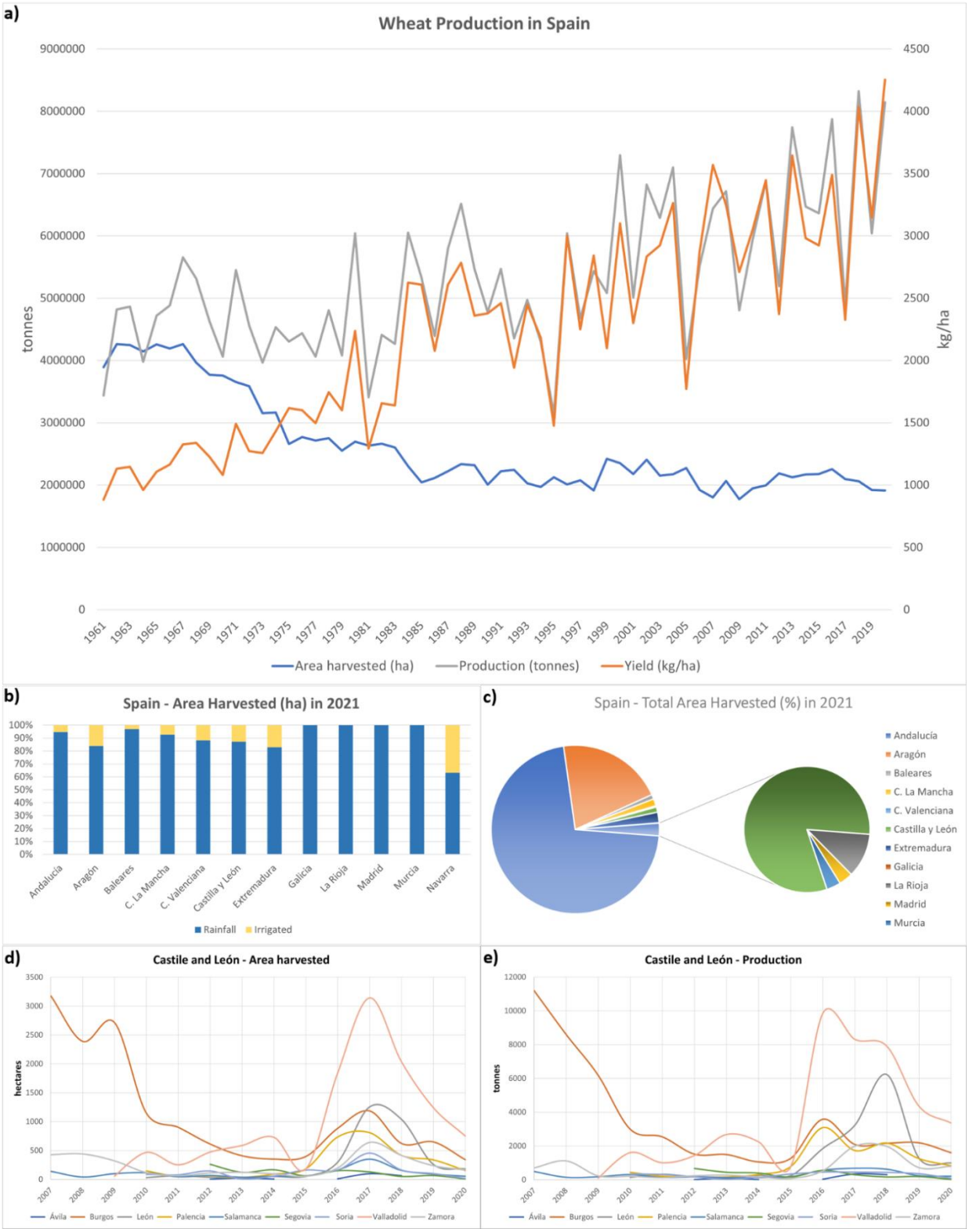


Figure 1.4. Description of wheat production in Spain, (a) Historical records of area harvested, production and yield of durum and bread wheat (FAOSTAT, 2022); (b, c) Percentage of area harvested in Spain in the last campaign per the different autonomous communities (MAPA, 2022); (d, e) Area harvested and production obtained in Castile and León over the previous years (MAPA, 2022).

1.1.3. Crop development

The development of a plant can be defined as the sequence of phenological events, those that are controlled by genetic and environmental factors, which determine the morphological and functional changes of the plant leading to the accumulation of biomass and the formation of yield components (Koch *et al*., 2009). The life cycle of wheat is a complex process, and several stages of development can coexist simultaneously in different parts of the same plant. However, considering external morphology, wheat crop development can generally be divided into four main phases, the first three comprising plant development up to anthesis. These phases are (i) **crop establishment,** from sowing to the beginning of tillering when seedlings have approximately three expanded leaves, and (ii) **tillering**, from the appearance of the first tillers to the beginning of stem elongation, which usually coincides with the cessation of tillering, (iii) **stem elongation**, from the first detectable node above the ground until anthesis, covering relevant stages such as flag leaf emergence, heading and anthesis, and (iv) **grain filling**, which describes the progress concerning the water content of the grain, from watery to hard, milky and pasty (Figure 1.5).

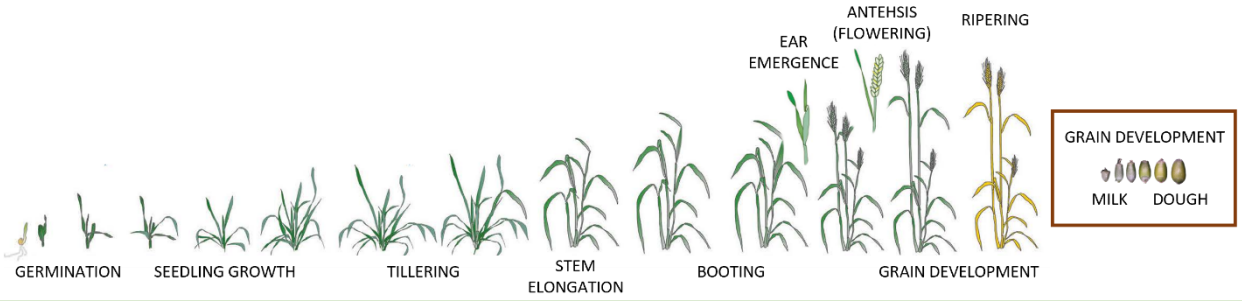
Several developmental scales (or codes) can be used to characterise the development of wheat, which describe the different growth stages visually without the need to dissect the plant. These scales include:

- The **Feekes scale** (Feekes, 1941). Popularised by Large (1954), it is less detailed, with only one digit for each stage, from pre-sprouting and tillering, stages 1-5; through stem extension and spiking, stages 6-10 to maturity, stage 11.

- The **Haun scale** (Haun, 1973). It is widely used to define the different stages of vegetative development. It focuses on the appearance of leaves, i.e. the Haun stage is a number describing the number of leaves (and the fraction of leaves) that have appeared on the main shoot.

- The **Zadoks scale** (Zadoks *et al*., 1974). It provides a good description of wheat's vegetative and reproductive stages. It classifies the phenology of cereals into ten different levels and one hundred subcategories (Z00-Z100) according to observable characteristics (Figure 1.5).

Zadoks scale is the most detailed and user-friendly developmental scale, frequently used for agronomic research and agricultural decision-making (e.g., spraying agrochemicals and fertilisation). This scale considers two digits in the "decimal code". The first digit, with values from 0 (germination) to 9 (maturity), refers to the main growth stage and the second digit, also with values from 0 to 9, reveals more details of each of the primary growth stages, quantifies the progress of that stage or number of organs, with position five corresponding to the average value (Figure 1.5). The leaf numbers, for example, have decimal codes from 11 to 19 and the offspring of the primary tillers from 21 to 29. The main stages from 0 to 3 refer to the vegetative organs (0: germination, 1: leaves, 2: tillers and 3: internodes), from 4 to 6 refer to the ear stage (4: heading, 5: earling and 6: anthesis), and from 7 to 9 to the stages of grain growth and development (Hyles *et al.*, 2020; Slafer *et al.*, 2021) (Figure 1.5).



Germination	*Seedling growth*	*Tillering*	*Stem Elongation*	*Booting*	*Anthesis*	*Milk Development*	*Dought Development*	*Ripering*
Z00 Dry seed	**Z10** First leaf through coleoptile	**Z20** Main shoot only	**Z30** Stem starts to elongate, "head at 1 cm"	**Z41** Flag leaf sheath extending	**Z50** First spikelet of Ear just visible above flag leaf ligule	**Z71** Seed watery ripe	**Z83** Early dough	**Z91** Seed hard (difficult to divide by thumbnail)
Z01 Start of imbibition	**Z11** First leaf Unfolded (ligule visible)	**Z21** Main shoot and 1 tiller	**Z31** First node detectable	**Z43** Flag leaf sheath just visibly swollen	**Z53** 1/4 of ear emerged	**Z73** Early milk	**Z85** Soft dough	**Z92** Seed hard (can no longer be dented by thumbnail)
Z03 Imbibition complete	**Z12** 2 leaves unfolded	**Z22** Main shoot and 2 tillers	**Z32** Second node detectable	**Z45** Flag leaf sheath swollen	**Z55** 1/2 of ear emerged	**Z75** Medium milk	**Z87** Hard dough (thumbnail impression held)	**Z93** Seed loosening in daytime
Z05 Radicle emerged from Seed	**Z14** 4 leaves unfolded	**Z24** Main shoot and 4 tillers	**Z34** Fourth node detectable	**Z47** Flag leaf sheath opening	**Z57** 3/4 of ear emerged	**Z77** Late milk		**Z94** Overripe, straw dead and collapsing
Z07 Coleoptile emerged	**Z16** 6 leaves unfolded	**Z26** Main shoot and 6 tillers	**Z36** Sixth node detectable	**Z49** First awns visible	**Z59** Emergence of ear complete			**Z95** Seed dormant
Z09 Leaf just at coleoptile tip	**Z18** 8 leaves unfolded	**Z28** Main shoot and 8 tillers	**Z37** Flag leaf just visible					**Z96** Viable seed giving 50 % germination
			Z39 Flag leaf blade all visible					**Z97** Seed not dormant
								Z98 Seed dormancy induced

Figure 1.5. Development stages in wheat describe following the Zadoks Decimal Scale (Zadoks *et al.*, 1974), with a score of 0–100 (modified from Hyles *et al.*, 2020).

These stages are accelerated by increasing temperature and photoperiod (Slafer & Rawson, 1994). However, in winter wheat, from sowing to double-cresting, vernalisation is the main factor controlling development (Kirby *et al*., 1999). Each phase has its range of optimal temperatures, in which the organs developing in that phase will be enhanced (Porter & Gawith, 1999). One of the most critical phases is flowering, which is crucial for establishing the final number of grains. This phase is also one of the most sensitive to temperature extremes, when cold temperatures below 9°C, high temperatures above 31°C, and water stress should be avoided (Porter & Gawith, 1999; Russell & Wilson, 1994). The growth conditions during the grain-filling period are crucial for higher grain weight (Royo *et al*., 2006) (Figure 1.6).

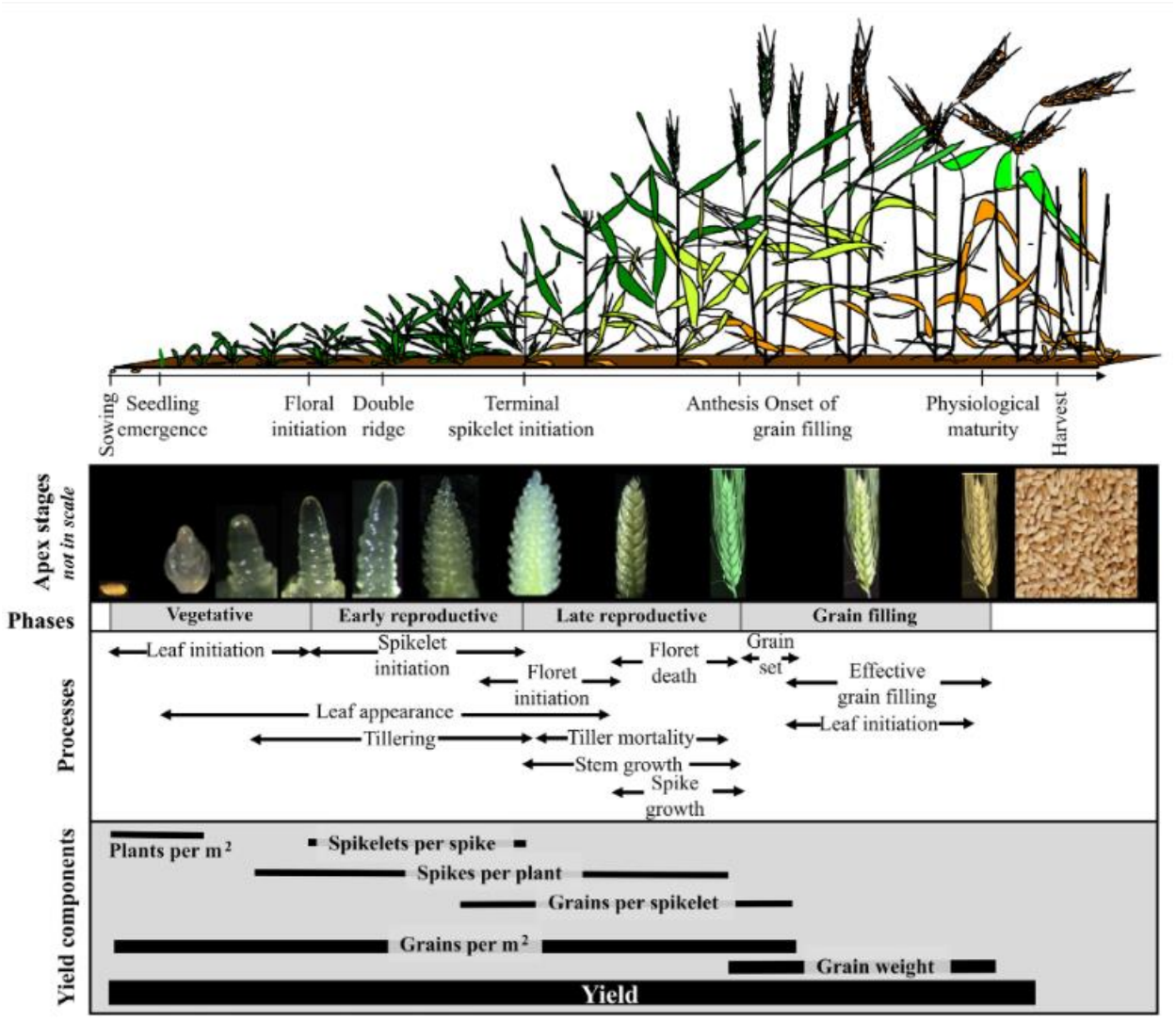


Figure 1.6. Key development stages of wheat from sowing to harvest. The boxes underneath illustrate the appearance of the apex/spike, the four major component phases, the timing of differentiation or growth of organs, and the timing of formation and definition of yield components (Slafer *et al*., 2021).

1.1.3.1. The main structures of the plant

The main structures of wheat plants are the coleoptile, the stem, the daughter stems (also called tillers or side shoots), the leaves, the spikes and the roots (White, 2008). The coleoptile is a specialised structure that allows seedlings to emerge. The stem comprises two structures: the nodes, organs where the rest of the plant parts converge, and the internodes, which are the parts of the stem that elongate during growth (Figure 1.7). Stems are also called primary stems when they originate from the leaves of the main stem, while secondary stems are produced from the primary stems. The primary stem is erect and hollow inside, except at the node, and serves as a temporary assimilated reservoir, supplying nutrients to developing structures, mainly the grains. Secondary or daughter stems arise from the base of the main stem and build their structures.

The leaves are long and narrow, arranged alternately along the stem and consisting of two distinct parts: the basal sheath, which is inserted at the node and envelops the stem, giving it firmness, and the lamina or blade, which separates from the stem, the primary photosynthetic tissue of the plant. There are also lobed structures called the ligule and auricle at the point on the stem where the blade and sheath meet. The last leaf on the stem is the flag leaf (Figure 1.7). Wheat plants have primary or seminal roots, which appear during germination to support the plant in the early stages, and secondary or adventitious roots, which arise during tillering from the crown nodes (Benlloch-González *et al*., 2014).

The ear (also called spike) is the inflorescence of wheat composed of a central rachis (a stem-like structure) and two rows of spikelets. A typical wheat ear has 15 to 20 spikelets, each with ten individual flowers (called florets) contained in two spongy bracts (lower and upper glumes). Each of the florets is enclosed in two other bracts (lemma and palea) and includes the reproductive organs (carpel and stamens). The awns are extensions of the tip of the lemma (Brinton & Uauy, 2019). The fertilised ovule forms the reproductive unit, the grain, composed of the embryo, the endosperm (which provides energy and nutrients to the embryo during germination) and the bran (protective outer covering). After germination, the embryo will generate a new wheat plant (Figure 1.7).

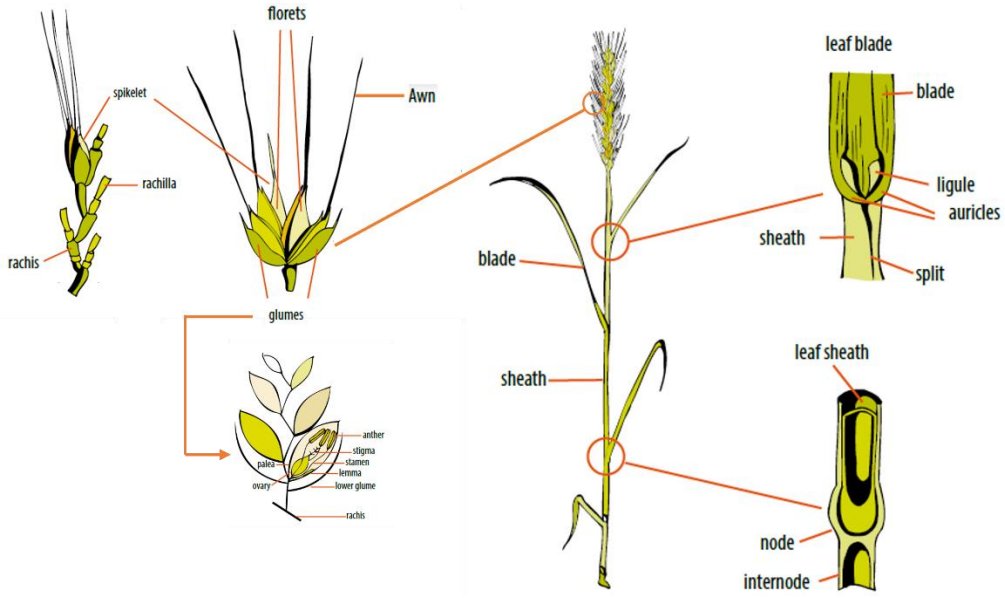


Figure 1.7. Structure of the different wheat organs at the end of the reproductive stage (modified from White, 2008).

1.1.3.2. Wheat components and their formation

Crop yield is a highly complex quantitative trait controlled by many plant traits. It results from complex interactions throughout the growth development between the environment and the genetic background, affecting most genes' direct or indirect expression (Slafer *et al.*, 2005). Therefore, any attempt to increase yield would be based on a thorough understanding of its components. One of the most common approaches for yield improvement, according to Donald and Hamblin (1976), is to consider it as the product of plant biomass and harvest index (the proportion of aboveground biomass allocated to grain):

$$YIELD = Aboveground\ biomass\ at\ maturity\ x\ Harvest\ Index$$

In Mediterranean climate regions, all the yield components are influenced by the environmental conditions of each growing season, especially during the wheat development stage, where some stress may occur (Slafer *et al.*, 2021). Grain yield represents the end product of the interaction of these conditions with the genotype, the individual effects of which are difficult to specify. It can be broken down into agronomic and physiological components. From an agronomic point of view yield can be expressed in its main agronomic components: the number of ears per unit area harvested, the number of grains per ear and the final grain weight (Maçãs *et al.*, 2000) (Figure 1.8).

The seed sown density and germination index determine the number of plants per area. This component is the first to establish between sowing and part of the vegetative phase. After germination, seedling development begins, and at some point, in the vegetative stage, the number

of sprouts is determined. Some of these shoots will generate an ear, but sometimes the shoot may be aborted, or the ear never fully develops, ultimately determining the number of ears. The number of grains per spike depends on the number of florets initiated on each spike, of which only the fertile and pollinated ones will be able to generate the grain and may be aborted during the grain set process depending on environmental conditions (Miralles *et al.*, 2000; Russell & Wilson, 1994) (Figure 1.6).

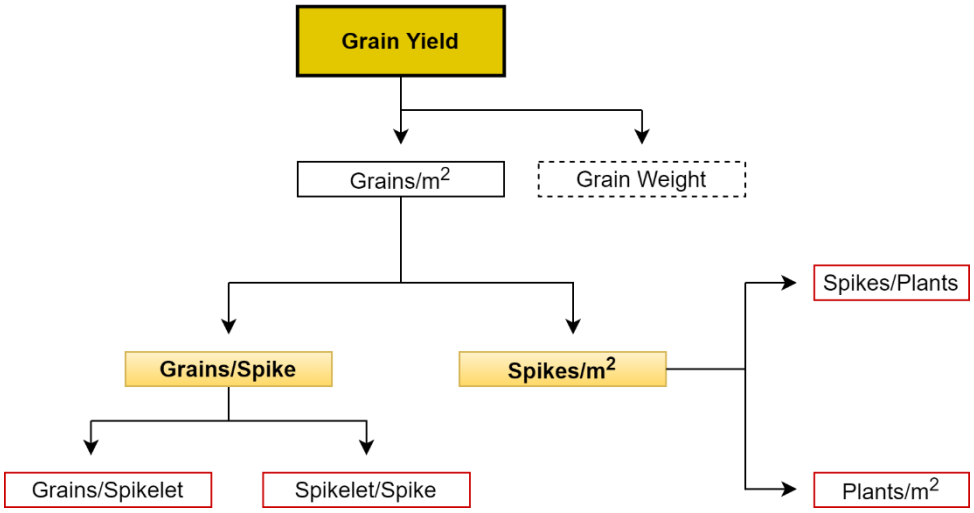


Figure 1.8. The formation sequence of the agronomic components directly determines the final grain yield (modified from Slafer *et al.*, 2021).

From a physiological point of view, yield results from an interaction between the number of resources taken up by the plant and the efficiency in using these resources. By resources, we mean any input that can determine and limit productivity, such as radiation, water or any essential nutrient. The productivity of crops depends on the amount of resources the crop captures and the efficiency with which they are used. Efficiency is understood in terms of biomass produced per unit or resource, e.g. radiation use efficiency (RUE), water use efficiency (WUE) and nutrient use efficiency (Araus *et al.*, 2021).

1.2. Primary metabolism: the coordination of carbon and nitrogen pathways

Carbon (C) and nitrogen (N) metabolism constitutes the plants' primary metabolism. Although they occur independently in the first phase, they interact closely and have many feedback

regulators (Stitt & Krapp, 1999; Vicente *et al*., 2018a). Photosynthesis is primarily driven by the photon output of light-harvesting complexes in the chloroplast. Several carrier proteins or enzyme complexes are involved in the photophosphorylation reactions, ultimately leading to energy production (i.e. ATP and NADPH) (Lawlor, 1993). The subsequent reactions belong to the Calvin-Benson cycle. They are strongly dependent on the first step of CO_2 fixation, the catalytic activity of Rubisco, the primary source of N during senescence, and the energy produced in the previous steps. Afterwards, both the synthesis and cleavage of complex carbohydrates require the participation of several enzymes and the energy (e.g. ATP and NADH) generated into the mitochondria (Urry *et al.*, 2020). These energy-carrying molecules and C skeletons are necessary to synthesise N-rich metabolites from nitrate (NO_3^-) and ammonium (NH_4^+), such as amino acids, nucleotides, proteins, nucleic acids and cofactors. Thus, regulating both metabolic pathways is critical for proper plant growth and development (Kaur *et al.*, 2017).

Plants undergo several processes of vital importance, such as photosynthesis, respiration, and nutrient absorption and transformation. Nevertheless, the photosynthetic fixation of CO_2 is considered the essential chemical process that takes place on the earth's surface and that allows the development not only of the plant but also of other forms of life on Earth since, through this autotrophic process, they incorporate matter and energy into the biosphere (Medrano & Flexas, 2000; Xiong & Bauer, 2002).

Photosynthesis occurs in a subcellular organelle called chloroplast (Staehelin, 2003). These give plants their characteristic greenness (chlorophylls) and are found in greater or lesser density depending on the organ and its internal structure (Urry *et al.*, 2020). The design of a chloroplast includes a double membrane envelope that separates the chloroplast from the cytosol and modulates the translocation of metabolites and proteins between the organelle and the cell. In addition, it contains a third membrane system with an inner lumen, called the thylakoids, and is suspended in a dense, enzyme-rich fluid known as the stroma. This thylakoid membrane system can form individual or stacked sacs, the latter called grana and contains the components necessary for photosynthetic light harvesting, electron transport and adenosine triphosphate (ATP) synthesis (Staehelin, 2003) (Figure 1.9).

In photosynthetic processes, which are essential for their growth and development, plants use light energy from the sun and hydrogen (H) from the water absorbed by the roots (together with

minerals) to reduce the assimilated CO_2 present in the atmosphere, to transform it into chemical energy that can be used for the maintenance of different metabolic pathways, e.g. in complex C-rich compounds such as starch, sucrose or fructans (Nelson & Yocum, 2006). Some amino acids, fatty acids and isoprenoids are also synthesised in the chloroplast from the C fixed during this process (Geigenberger *et al*., 2005). In summary, photosynthesis comprises two phases: (i) the generation of chemical energy in the form of ATP and reducing power (NADPH), and (ii) the utilisation of this energy for the fixation and assimilation of CO_2 into C-intermediates for different biosynthetic pathways inside and outside the chloroplast via the photosynthetic carbon reduction cycle (PCRC) or Calvin-Benson cycle (Geiger & Servaites, 1994).

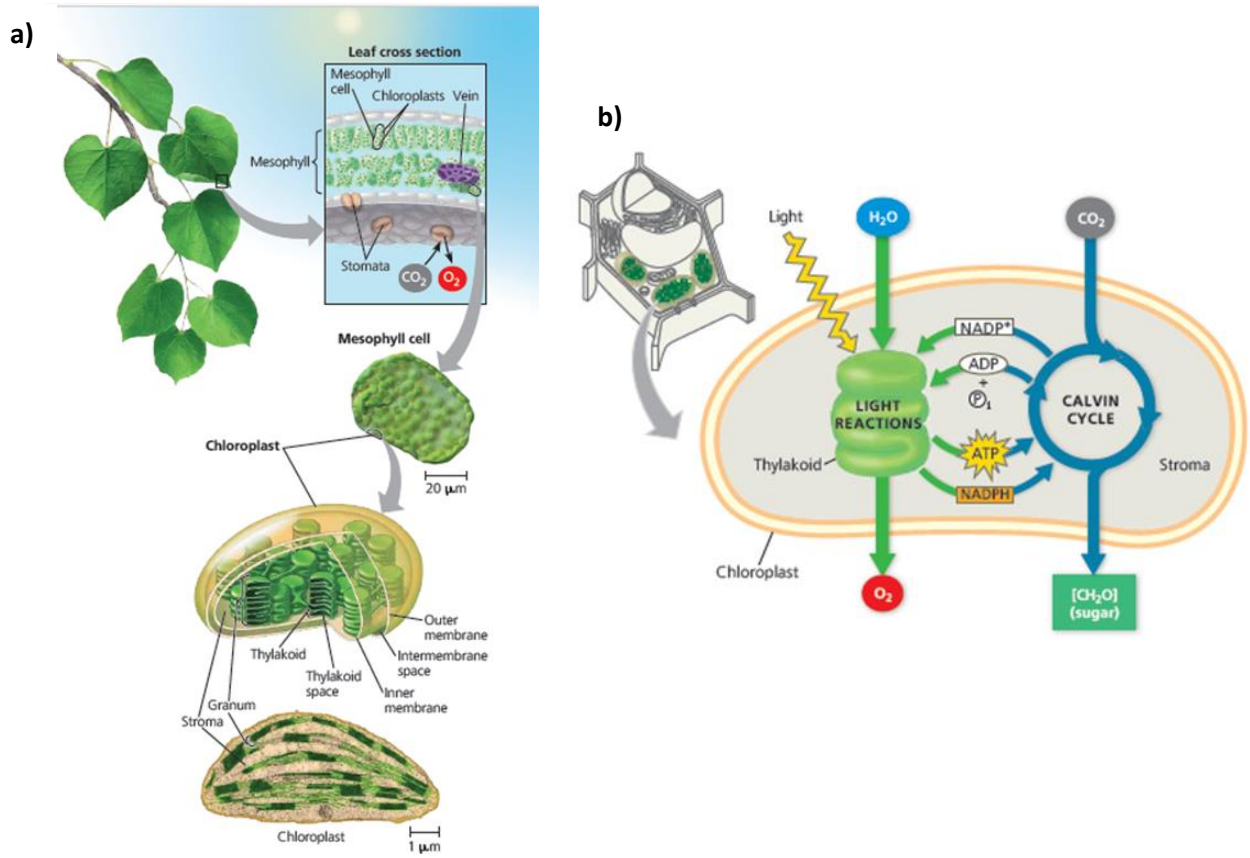


Figure 1.9. (a) Zooming in on the location of photosynthesis in a plant, from the leaf to the chloroplast, the organelle where photosynthesis occurs. (b) An overview of photosynthesis: cooperation of the light reactions and the Calvin cycle. In the chloroplast, the thylakoid membranes are the sites of the light reactions, whereas the Calvin cycle occurs in the stroma. The light reactions use solar energy to produce ATP and NADPH, which supply chemical energy and reducing power to the Calvin cycle. The Calvin cycle incorporates CO_2 into organic molecules, converted to sugar (Urry *et al*., 2020).

However, more than 70% of the total C, N, and H fixed during photosynthesis is returned to the atmosphere through cellular respiration. During aerobic respiration, carbohydrates are oxidised, and their products are used to generate usable energy and C-intermediates needed to synthesise various precursors (Atkin *et al*., 2000). It is the reverse reaction of photosynthesis and describes the complete oxidation of sucrose to CO_2 and reduction of O_2 to water (Taiz *et al*., 2015).

Cellular respiration coordinates several biochemical reactions in the plastids, cytosol and mitochondria. It involves three main metabolic pathways: glycolysis, the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (or mitochondrial electron transport chain).

On the other hand, N is an essential nutrient for plant growth and productivity of crops (Good *et al*., 2004; Barbanti *et al*., 2007) and cereals such as wheat (Hirel *et al.*, 2007). It is a primary component of nucleotides, amino acids, proteins, cofactors, and secondary metabolites (Martin & Marschner, 1988; Scheible *et al*., 2004). Quantitatively speaking, it is the nutrient required in the most significant quantities by the plant. Therefore, it often represents a limiting element, affecting their deficit from metabolism to resource allocation and growth and development, including changes in root architecture, senescence, flowering, etc. (Stitt, 1999; Nunes-Nesi *et al*., 2010).

Most N (98 %) is found in soils as organic matter, not available to plants. The fixation of molecular nitrogen (N_2) into ammonia (NH_3) and the transformation of organic N into inorganic matter requires the participation of soil microorganisms (Taiz *et al*., 2015; Castro-Rodríguez *et al*., 2017). Higher, non-N-fixing plants acquire inorganic N from the soil in the form of NO_3^- or NH_4^+ (Bloom, 2015). The most predominant form of inorganic N in agricultural soils is NO_3^- (Nacry *et al*., 2013; Bhattacharya, 2019). Due to the low affinity of NO_3^- to form surface complexes with soil minerals and the fact that it can be easily leached out, its availability is spatially variable and dependent on several factors, including microbial activity or soil type (Dechorgnat *et al*., 2010).

Ammonium is the reduced form of N taken up by plants from the soil for assimilation and subsequent use in amino acid and protein biosynthesis via the GS/GOGAT cycle, a pathway comprising the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT; glutamine:2-oxoglutarate aminotransferase). NO_3^- must be reduced to NH_4^+ by the sequential action of the cytosolic and chloroplastic enzymes, nitrate reductase (NR) and nitrite reductase (NiR), respectively (Lea & Ireland, 1999; Hodges, 2002), thus allowing its subsequent incorporation into organic matter (Martin & Marschner, 1988). The GS/GOGAT pathway plays an essential role in N assimilation, as the glutamine and glutamate produced can be used as precursors for the biosynthesis of other nitrogenous compounds, including amino acids, nucleotides, chlorophylls, and polyamines (Lea & Ireland, 1999), which are necessary for proper plant growth and development.

1.2.1. Source-sink dynamics

Plants are made up of several organs with different purposes. Broadly speaking, fully developed vascular plants consist of two main parts: the roots and the aerial organs. Plant organs function in an integrative way so that there is an exchange of resources between the organs that produce them (source organs) and the organs that demand them (sink organs). Source-sink communication is regulated by a complex signalling network involving sugars, hormones, environmental factors, and unknown mechanisms yet to be described (Yu *et al*., 2015).

Roots are the main source organs that capture water for the plant, from which, in turn, mineral nutrients are taken up. Nevertheless, they do not fix C and therefore are a sink mainly for C-rich compounds and energy-carrying molecules to support their metabolism. During most of the plant's life, leaves are the main water sink organs. Still, at the same time, they are the organs that produce most of the photosynthetically assimilated sugars and assimilate a high percentage of the nutrients taken up in the roots, e.g. N, which is then distributed to the plant for growth. In cereals, the last fully developed leaves, commonly referred to as the "flag leaf", are considered the main sucrose-contributing organ for grain filling (Yu *et al*., 2015) (Figure 1.10). Nutrients are transported through the plant from one organ to another in multiple directions, depending on the demand, sometimes even changing throughout the day (McCormick *et al*., 2008). Source-sink regulation impacts plant growth efficiency and, subsequently, crop yield, as yield correlates with the ability of the plants to efficiently assimilate, store and remobilise nutrients before and during the grain filling process.

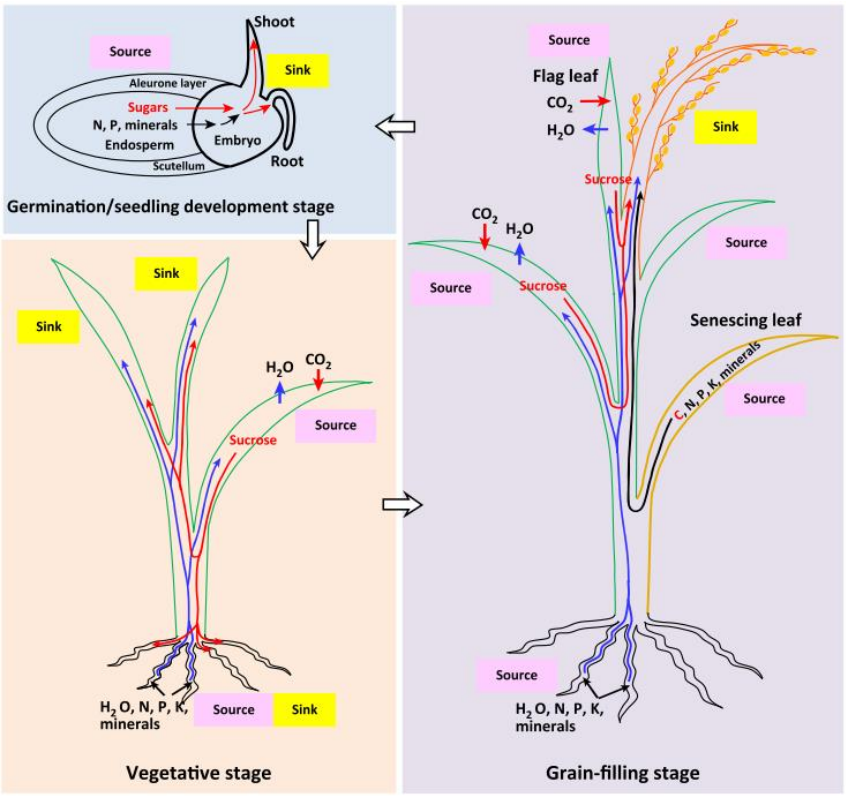


Figure 1.10. Representation of the translocation of Sucrose and other nutrients from the source to the sink organs during the development of cereals (Yu *et al*., 2015).

The contribution of each of these organs depends on the plant species, genotype and environment. In the case of cereals, under ideal growth conditions, grain filling occurs by the acquisition of assimilates from (i) photosynthesis of the flag leaf (laminae and sheath), (ii) translocation of nutrients stored mainly in the internodes, and (iii) photosynthesis of the ear (Tambussi *et al*., 2007). However, grain filling is limited by source capacity under water stress due to inhibiting C fixation and N assimilation, mainly reported in flag leaves (Medina *et al*., 2016; Vicente *et al*., 2018b). While leaves and shoots (aboveground biomass) are limited under water stress, root metabolism is induced to enhance nutrient and water uptake (Gargallo-Garriga *et al*., 2014). The peduncle and the sheath constitute photosynthetically active organs that even senesce more slowly than the flag leaf and participate in the transport and storage of photoassimilates (Kong *et al*., 2010).

For the latter stages of plant development, the source-sink balance becomes relevant because not all seed components vary to the same degree when the availability of assimilates per seed is altered, so seed composition and quality may change. However, previous research exploring the impacts of source-sink manipulations during grain filling on grain weight potential under multiple

conditions has found little or no significant variation (Fischer, 2011). This indicates that sink limitation may dominate over source availability of assimilates during grain filling, affecting grain development capacity. Overall, these results suggest an unlikely competition between grains for assimilation after the aqueous phase of grain filling (Slafer, 2007).

Understanding the mechanisms and their regulation under different growth conditions during grain filling is crucial for grain development, notably when growth conditions can fluctuate instantaneously as in the field. An example could be defoliation caused by a leaf-eating insect that attacks the crop in the middle of grain filling, which alters the source-sink balance of the plant. In addition, knowledge in this area can provide strategies to improve crop productivity and help meet the future food demands of a growing world population under a climate change scenario (Rodrigues *et al*., 2019; Smith & Myers, 2018).

1.2.2. Carbohydrates

Carbohydrates are the main products of photosynthesis and can be involved in several metabolic and signalling pathways. Sucrose and starch are the primary direct products of photosynthetic C assimilation in the leaves of most plants (Zeeman *et al*., 2007). The C fixed during the day produced during the Calvin-Benson cycle can be exported from the chloroplast to the cytosol as triose phosphates via the action of a specific translocator (Flügge *et al*., 1989), making sucrose synthesis possible, while the excess of C can be stored in the form of starch or other storage carbohydrates, depending on the plant species. Sucrose is a disaccharide consisting of a fructose molecule and a glucose molecule linked at carbons 1 and 2 (α1→β2), respectively. This carbohydrate is subsequently loaded into the phloem of the source tissues for distribution to heterotrophic plant organs (roots, seeds, developing leaves, fruits or tubers) and to be utilized in growth processes, in the biosynthesis of cellulose and storage carbohydrates (e.g. starch and fructans) (Fallahi *et al*., 2008).

On the other hand, starch is synthesized simultaneously as sucrose, mainly in leaves, through a gluconeogenic pathway in the chloroplast. It makes up almost 70% of the dry weight of wheat grain (Huang & Brûlé-Babel, 2010). It is an insoluble polysaccharide composed of amylose, a linear polymer of glucose residues linked by α(1-4) bonds, and amylopectin, a highly branched polymer in which α(1-4) bonds are interspersed with α(1-6) bonds (Grennan, 2006; Leterrier *et al*.,

2008; Orzechowski, 2008). Amylopectin usually constitutes 75% of starch and amylose, the remaining 25% (Huang & Brûlé-Babel, 2010). Starch is deposited in semicrystalline granules in the chloroplasts (Grennan, 2006; Geigenberger, 2011) and represents the primary transient reserve carbohydrate in most plants, mobilized during the night to support leaf metabolism and continue the export and maintenance of sucrose synthesis (Niittylä *et al*., 2004). Carbohydrates not only function as metabolic sources and structural components of cells but also act as regulatory molecules that control gene expression and metabolic processes (Jang *et al*., 1997; Sheen *et al*., 1999; Paul *et al*., 2008), thus contributing to plant homeostasis (Kleczkowski *et al*., 2004).

About 15% of angiosperms store fructans as reserve carbohydrates, as is the case of durum wheat (Hendry, 1993; Kawakami *et al*., 2005). Fructans constitute linear or branched soluble polymers of fructose, formed by a sucrose molecule with fructoses linked by β(2-1) or β(2-6) bonds and are synthesized and stored in the vacuole from sucrose by the action of fructosyl transferases (FT) (Xue *et al*., 2011). They can be stored both in the grain and in other vegetative organs (leaves, stems, roots), depending on the stage of development and growth conditions (Morcuende *et al*., 2005; Kawakami *et al*., 2005; Vicente *et al*., 2016).

Respiration is the catabolic pathway in which carbohydrates are partially oxidized, leading to the formation of ATP, NADH, pyruvate and malate and the production of substrates for anabolism (Taiz *et al*., 2015). In plants, the first step of respiration is the glycolysis that takes place in the cytosol or within plastids. Both sucrose and starch act as primary substrates of the pathway, but the immediate products of their degradation (pentoses or trioses) can also be used (Plaxton, 1996). Depending on the organelle, different functions have been attributed to glycolysis. For example, non-green plastids and chloroplasts, in the absence of light, use glycolysis to participate in starch degradation and in the production of C-skeletons, ATP or reducing the power used in other plastid-specific biosynthetic pathways (fatty acids or Shikimate pathway). This is followed by TCA cycle, which uses the products of glycolysis to produce energy and reducing power, together with the mitochondrial electron transport chain, to generate C skeletons that are used for the synthesis of nitrogenous compounds, being the main link between C and N metabolism (Fernie *et al*., 2004) (Figure 1.11).

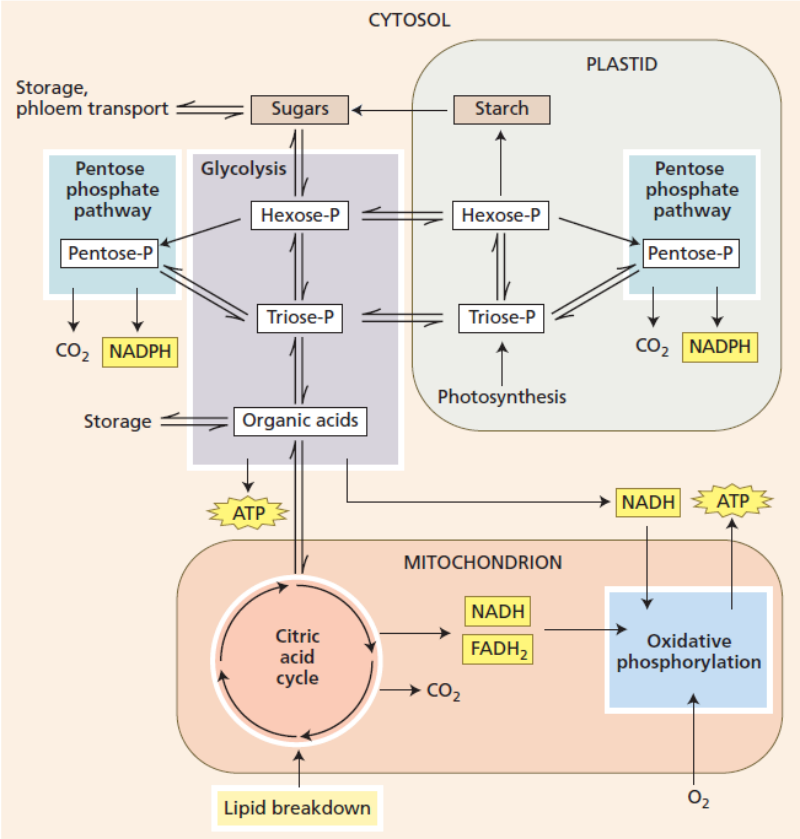


Figure 1.11. Overview of the respiration process (Taiz *et al.*, 2015).

1.2.3. Amino acids and Proteins

As described above, most plant species absorb mineral N from the soil through the root system, generally inorganic forms (NO_3^- and NH_4^+) and organic forms (Crawford, 1995; Dechorgnat *et al.*, 2010). Absorbed NO_3^-, besides being an essential macronutrient, represents a signal molecule that modulates many aspects of plant metabolism, morphology, growth and differentiation (Scheible *et al.*, 1997; Kaiser & Huber, 2001). High concentrations of NO_3^- promote aerial part development and inhibit lateral root development, while its deficiency increases root growth (Campbell, 1999; Castaings *et al.*, 2010). The assimilation of NO_3^- into nitrogenous organic compounds requires the prior reduction of NO_3^- taken up by the root to NH_4^+ (Nunes-Nesi *et al.*, 2010; Hawkesford *et al.*, 2012), which can either take place directly in the root or the leaves after NO_3^- is transported to the aerial part.

In plants, fungi and bacteria, the process is initiated by the reduction of NO_3^- to nitrite (NO_2^-) in the cytosol by the enzyme NR (Masclaux-Daubresse *et al.*, 2010; Dechorgnat *et al.*, 2010). NR

is considered the key limiting enzyme for N assimilation and is subject to different levels of regulation: enzyme synthesis, degradation, reversible inactivation, effector regulation and substrate concentration (Campbell, 1999; Hawkesford *et al.*, 2012). This high degree of regulation allows amino acid biosynthesis to match the supply of C skeletons provided by photosynthesis and prevents the accumulation of NO_2^- during the night, which is toxic to the cell (Kaiser & Huber, 2001; Hawkesford *et al.*, 2012).

The NH_4^+ absorbed by the root or generated by NO_3^- reduction may also be assimilated in the root or leaves themselves or accumulate in the vacuoles of these organs (Hawkesford *et al.*, 2012). The reduced to assimilated N ratio is unknown and depends on plant species, developmental stage, exogenous NO_3^- concentration, and other factors (Nunes-Nesi *et al.*, 2010; Hawkesford *et al.*, 2012). Root-absorbed NH_4^+ is usually not transported long distances and is rapidly assimilated in the root itself to avoid toxicity (Smart *et al.*, 1998), whereas NH_4^+ from NO_3^- reduction is mainly incorporated aerially. $NH_4^+$is also generated in photorespiration, lignin biosynthesis, senescence-induced N remobilisation and N_2 fixation in legumes (Temple *et al.*, 1998; Hawkesford *et al.*, 2012). The key enzymes in NH_4^+ assimilation, irrespective of their origin and the tissue where they occur, are GS and GOGAT.

Amino acids are organic compounds containing at least one amino group and one carboxyl group. They represent the initial products of N assimilation. They are involved in the biosynthesis of proteins and other nitrogenous compounds and the response of plants to different stresses (Galili *et al.*, 2008). Plants can synthesise all the amino acids they need, although only 20 constitute the structural units that form proteins (Hawkesford *et al.*, 2012). From the union of several amino acids by associations of peptide bonds, so-called peptides are generated. They are a type of nitrogenous molecule linked by bonds established between the carboxyl group of one amino acid and the amino group of another (Pallardy, 2008).

1.2.4. Enzymes

1.2.4.1. RUBISCO

Rubisco, ribulose-1.5-bisphosphate carboxylase-oxygenase (Rubisco), represents the most abundant protein on Earth. It is estimated to contain 15-35% of all leaf N in C_3 plants, thus

constituting a vital N reservoir (Evans, 1983; Suzuki *et al.*, 2009). This enzyme is located in the stroma of the thylakoids and is directly involved in C fixation and consists of two types of subunits, a large subunit encoded in the chloroplast (eight large subunits LSU, 50-55 kDa) and a small subunit encoded in the nucleus (eight small subunits (SSU, 12-18 kDa). The latter are synthesised in the cytosol and then transported to the chloroplast, where the enzyme is assembled to participate in photosynthesis (Taiz *et al.*, 2015).

The enzyme catalyses the carboxylation of ribulose-1.5-bisphosphate, fixing CO_2 and releasing two molecules of 3-phosphoglycerate. However, Rubisco can also catalyse the oxygenation of ribulose-1.5-bisphosphate, which leads to the beginning of the photorespiration pathway (Suzuki & Makino, 2013). The enzyme has a low affinity for CO_2, which makes photorespiration an inherent process of carboxylation in C_3 plants, leading to the loss of a significant part of the C fixed. This also explains why large amounts of the enzyme are needed in the plant to compensate for its low affinity for CO_2. The catalytic activity of Rubisco is regulated by a mechanism involving reversible carbamylation of an ε-amino group of a particular lysine residue located in the active centre of the protein and subsequent stabilisation of the carbamate by magnesium (Mg^{2+}) binding (Gutteridge & Gatenby, 1995; Andersson, 2008). The activation state of the enzyme responds to light-induced changes in the phosphorylation potential determined by the ATP:ADP ratio in the stroma and requires the action of the enzyme Rubisco activase (Portis *et al*., 2007; Pérez *et al*., 2011) (Figure 1.12).

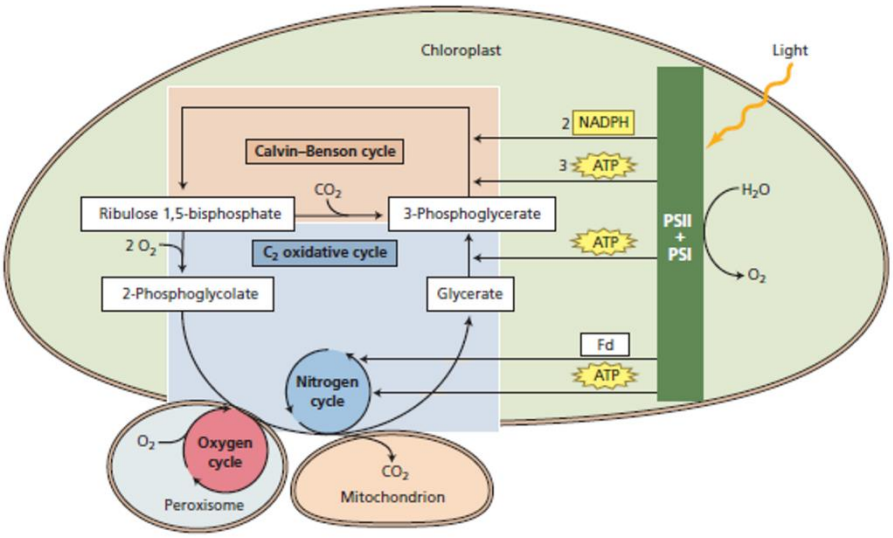


Figure 1.12. Dependence of the CO_2 oxidative photosynthetic carbon cycle on chloroplast metabolism. The supply of ATP and reducing equivalents from light reactions in thylakoid membranes are needed to function the C_2 oxidative photosynthetic process in three compartments: chloroplasts, peroxisomes and mitochondria (Taiz *et al*., 2015).

1.2.4.2. PEPCase

The enzyme phosphoenolpyruvate carboxylase (PEPCase) catalyses the carboxylation of phosphoenolpyruvate (PEP) using HCO_3^- to produce oxaloacetate (OAA) and inorganic phosphate (Pi) (González *et al*., 2002). This allows the replenishment of intermediates to the TCA cycle, either as OAA or as malate, after the conversion catalysed by malate dehydrogenase (MDH), a key enzyme in the respiration pathway that provides C skeletons for the assimilation of N compounds (Taiz *et al.*, 2015). However, it is more widely represented in C_4 plant metabolism, in which, instead of Rubisco, it catalyses the initial carboxylation in mesophyll cells near the outer atmosphere (Taiz *et al.*, 2015).

1.2.4.3. GS/GOGAT

Plant cells avoid NH_4^+ toxicity generated by NO_3^- assimilation or photorespiration by converting it rapidly, irrespective of its origin and the tissue produced (Nussaume *et al.*, 1995). The main pathway for the conversion of NH_4^+ involves the sequential actions of GS and GOGAT, forming a GS/GOGAT cycle (Ward & Keys 1978) that produces both glutamine and glutamate (Figure 1.13), the precursors of all nitrogenous compounds in plants, as they act as N donors for the biosynthesis of amino acids, nucleotides, chlorophylls, etc. (Lea & Ireland, 1999).

GS combines NH_4^+ with a glutamate molecule to form glutamine, consuming ATP (Forde & Lea, 2007). Plants contain two classes of isoforms of the enzyme, one in the cytosol (GS1), found in vascular tissues of the root, mature leaves and seeds, and one in the plastids (GS2), located in photosynthetically active young leaves and, to a lesser extent, in roots and other tissues. The cytosolic forms are expressed in germinating seeds or the vascular bundles of roots and shoots and produce glutamine for intercellular N transport. GS2 in root plastids mainly generates amide N for local consumption; GS2 in shoot chloroplasts resimilates photorespiratory NH_4^+ (Lancien *et al*., 2006; Galili *et al*., 2008).

On the other hand, the enzyme GOGAT transfers the amino group of glutamine to 2-oxoglutarate, producing two glutamate molecules, one that can be exported for anabolic processes and the other to be reused in the cycle (Hawkesford *et al.*, 2012). Plants contain two types of GOGAT; one accepts electrons from NADH from respiration, and the other accepts electrons from ferredoxin (Fd), produced in the chloroplasts during the day. The NADH-dependent enzyme

(NADH-GOGAT) is located in the plastids of non-photosynthetic tissues, such as roots or vascular bundles of developing leaves. In roots, NADH-GOGAT is involved in the assimilation of NH_4^+ absorbed from the rhizosphere (the soil close to the root surface); in vascular bundles of developing leaves, NADH-GOGAT assimilates glutamine translocated from roots or senescing leaves (Taiz *et al.*, 2015). The ferredoxin-dependent GS (Fd-GOGAT) is found in chloroplasts and promotes photorespiratory N metabolism. Fd-GOGAT is predominantly localised in the chloroplasts of leaf midrib cells and appears to be involved in the supply of glutamate for amino acid biosynthesis during photosynthesis. Both the amount of protein and its activity increase with light levels. Roots, especially those under NO_3^- nutrition, have Fd-GOGAT in the plastids. Fd-GOGAT in roots presumably functions to incorporate glutamine generated during nitrate assimilation. Electrons to reduce Fd in roots are caused by the pentose phosphate oxidative pathway (Taiz *et al.*, 2015).

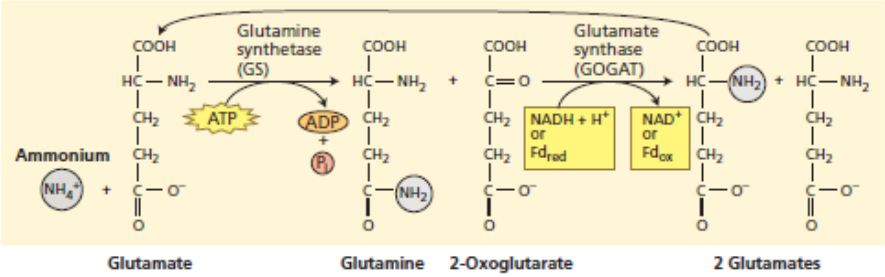


Figure 1.13. The GS-GOGAT pathway forms glutamine and glutamate. A reduced cofactor is required for the reaction: ferredoxin (Fd) in green leaves and NADH in non-photosynthetic tissues (Taiz *et al*., 2015).

1.2.4.4. GDH

Glutamate dehydrogenase, or GDH, is an alternative pathway for ammonium assimilation. This enzyme catalyses a reversible reaction that synthesises or deaminates glutamate (Figure 1.14). Again, two enzyme forms are present; the first one is NADH-dependent and is found in the mitochondria of phloem companion cells, but can also be located in the cytoplasm when the NH_4^+ concentrations are very high (Fontaine *et al*., 2012). The other isoform is NADPH-dependent and is found in the chloroplasts of photosynthetic organs. Although both forms are relatively abundant, they cannot replace the GS-GOGAT pathway for NH_4^+ assimilation, and their primary function is to deaminate glutamate during N reallocation (Taiz *et al.*, 2015). Thus, this enzyme is mainly involved in NH_4^+ release, specifically during senescence, by catalysing the oxidative deamination of glutamate and supplying C skeletons such as 2-oxoglutarate for respiration and oxidative phosphorylation (Fontaine *et al*., 2012; Hawkesford *et al*., 2012).

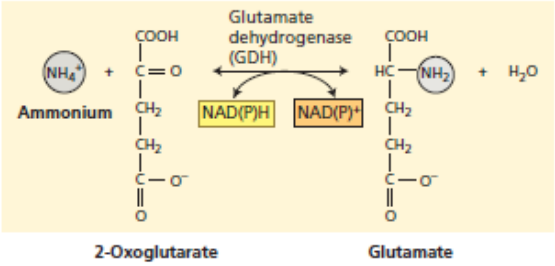


Figure 1.14. The GDH pathway forms glutamate using NADH or NADPH as a reductant (Taiz *et al*., 2015).

1.3. Grain quality parameters

Bread and durum wheat are used for various types of food: bread, noodles, biscuits, cakes, etc. In addition, wheat is used in non-food applications such as starch, dry vital gluten, biodegradable plastics, and ethanol (Day *et al*., 2006; Uthayakumaran & Wrigley, 2010). Wheat quality has different meanings depending on the end-use and the step in the value chain from breeding, field production, marketing, manufacturing of the final product, and the consumer (Rondanini *et al*., 2019).

Quality is a very complex concept, and the determination of its components is somewhat subjective and can be approached from various points of view. Furthermore, factors affecting quality, such as protein content, gluten strength, vitreousness and colour, have different priorities in durum wheat markets. The intermediaries in the grain industry define their own quality concepts. Thus, throughout the durum wheat production process, from the time the grain is sown until the final product reaches the consumer, the idea of quality varies according to seed companies, farmers, seed and pasta industries and market demand (Troccoli *et al*., 2000) (Table 1.1). Therefore, improvement cannot be directed at each of them but has to act globally given the number of characters involved, without forgetting that the final product must satisfy the consumer, who demands pasta with an excellent visual appearance and good culinary quality. Therefore, the aim must be to improve the quality of the final product, bearing in mind that the varieties obtained must be productive for the farmer; otherwise, he will not buy the seed, they must have a good yield in semolina, which satisfies the seed industries, and they must give a final product that is well accepted in the market.

According to the point of view of the intermediaries mentioned above, the ideal durum wheat seed attributes belong to three different orders. **Agronomic quality** defines the variables that lead to high yields per unit area with adaptation to environmental and cultivation conditions and

guaranteed production stability over the years. **Nutritional quality** refers to the nutritional value of the final product, especially for the industry in those countries where durum wheat is a fundamental part of their diet, whether in the form of pasta, couscous, bulgur or other related products. And **technological quality**, which in the semolina industry refers to the possibility of extracting as much high-purity semolina as possible from the grain, while the pasta industry wants semolina that can be transformed into pasta whose appearance and culinary quality satisfy consumers' desires.

Table 1.1. Shows the main aspects related to the quality of durum wheat according to the different intermediaries involved in the commercial and industrial chain up to the final consumer (modified from Troccoli *et al*., 2000).

Seed company	Grain merchant	Farmer	Milling Industry	Pasta Industry	End Consumer
Varietal purity	Cleaning	Grain yield	Semolina yield	Protein content	Cooking quality
Germination	Safety	Grain quality	Ash content	Gluten quality	Visual appearance
	Protein Content	Stability and production	Grain uniformity	Semolina size	Quality/Price ratio
	Test weight		Impurities	Yellow index	
	Grain moisture		Grain moisture		

Durum wheat produces an amber and vitreous grain from the milling of which a yellowish flour with a particle size of 150-500 μm is obtained, called semolina (Mellado, 2007), an essential product from which traditional foods of vital importance for the subsistence of populations are made (Sissons, 2008). Pasta is the main product made from durum wheat semolina and is preferred for its superior quality. After cooking, it retains its shape, firmness, and bright yellow colour to consumers' taste. Therefore, to obtain good quality pasta, the durum wheat variety must be of good and uniform quality (Igrejas *et al.,* 2020). Couscous is another prominent food made from durum wheat and widely consumed in North African countries. In addition, bread made from durum wheat flour and bulgur is also a staple food in Middle Eastern, Balkan and North African countries (Igrejas *et al.*, 2020).

For marketing, the most common grain quality traits are test weight, moisture content, protein content, and limits for particular grain defects (sprout, fungal or insect damage) or limits for weed

seeds and other contaminants (Wrigley, 1994). These attributes are used for the segregation or grading of wheat by grain buyers and traders to separate healthy wheat, suitable for human consumption, from weather-damaged grain affected by disease or drought, among other lower value factors. Some countries have more complex grading systems than others, but many common attributes and values among the different standards (Delwiche, 2010).

1.3.1. The grain

The widespread use of wheat as a staple food is a consequence of the unique rheological properties of wheat flour after milling. Its use as a staple food provides almost 20% of daily dietary protein and calories (Arzani & Ashraf, 2017). In addition, it offers other valuable nutrients such as vitamins, minerals or bioactive phytochemicals distributed throughout the different layers that make up the wheat grain bran (Wieser *et al*., 2020).

Although influenced by growing conditions, species and varieties, the chemical composition of the mature grain remains reasonably stable. Carbohydrates account for about 70% of the total nutrient content found in the grain, but most of this proportion (58%) belongs to the starch content. The remaining carbohydrates (13%) are mainly non-starch polysaccharides. To a lesser extent, proteins account for almost 11% of the nutrients, followed by lipids and minerals (2% each) and, to a lesser amount, vitamins and phytochemicals (minus than 0.1%) (Wieser *et al.*, 2020).

Three main components are found in the grain (listed from the outside to the inside, Figure 1.15):

(i) the bran or pericarp, which constitutes 10-15% of the weight of the grain and covers it, is characterised by a high fibre and ash content and is devoid of starch (Hoseney, 1991);

(ii) the endosperm, which makes up the bulk of the grain volume, constitutes approximately 80-85% of the grain weight and is found within the bran layer or bran cover. It consists of an aleurone layer and a starchy endosperm. It contains starch and proteins, most of which are gluten, the reserve proteins (Onipe *et al.*, 2015);

(iii) the embryo (germ or young plant; 3%), which is located at the base of the grain, divided into the scutellum (involved in the absorption of soluble sugars), the plumule (containing the leaf primordia and shoot apex) and the radicle (a primary root system) (Anderson & Garlinge, 2000; White, 2008). It is characterised by a lack of starch and a high content of oil, protein, soluble sugars and ash (Hoseney, 1991).

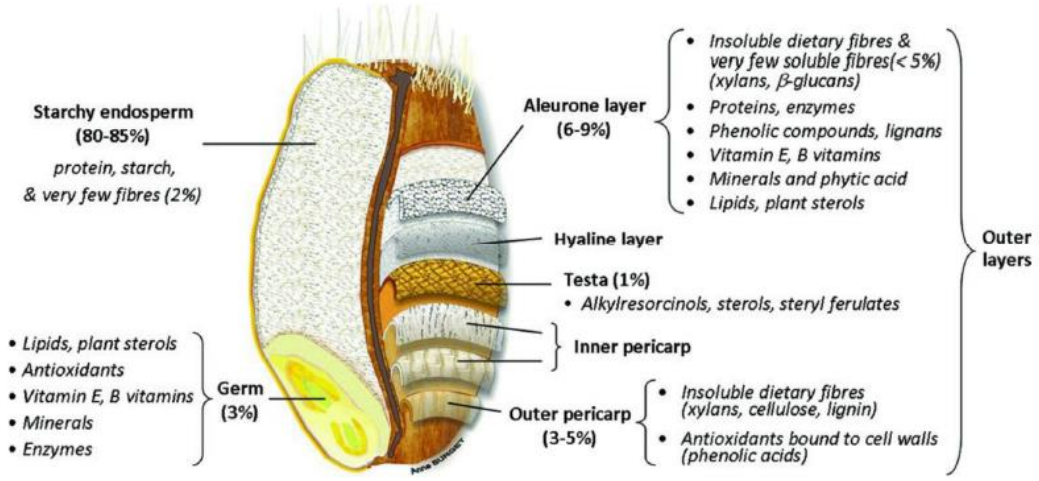


Figure 1.15. Layers and distribution of compounds in durum wheat grain (Onipe *et al*., 2015).

1.3.2. Industrial quality

There are numerous methods for estimating the industrial quality of wheat. The use of one or the other depends on whether the industry uses commercial tests to determine the quality of durum wheat or quality tests for selecting varieties in breeding programmes. Quality analyses, understood in the broadest sense, with some modifications for durum wheat concerning flour wheat, can be grouped into the following groups: (i) physical analyses of the grain (test weight, vitreousness, 1000-grain weight and moisture); and (ii) analyses of the chemical and biochemical characteristics of both grain and semolina (protein, ash, gluten index, semolina yield and carotene content –yellow pigments–) (Igrejas *et al.*, 2020).

The industrial quality of wheat can be simplified into three key traits: grain hardness, grain protein concentration and dough or protein quality. The relationships between these three traits have been graphically summarised by Moss (1973). For producing a given quality end product, there is a relatively narrow range of grain protein concentrations for each type of endosperm hardness (Figure 1.16).

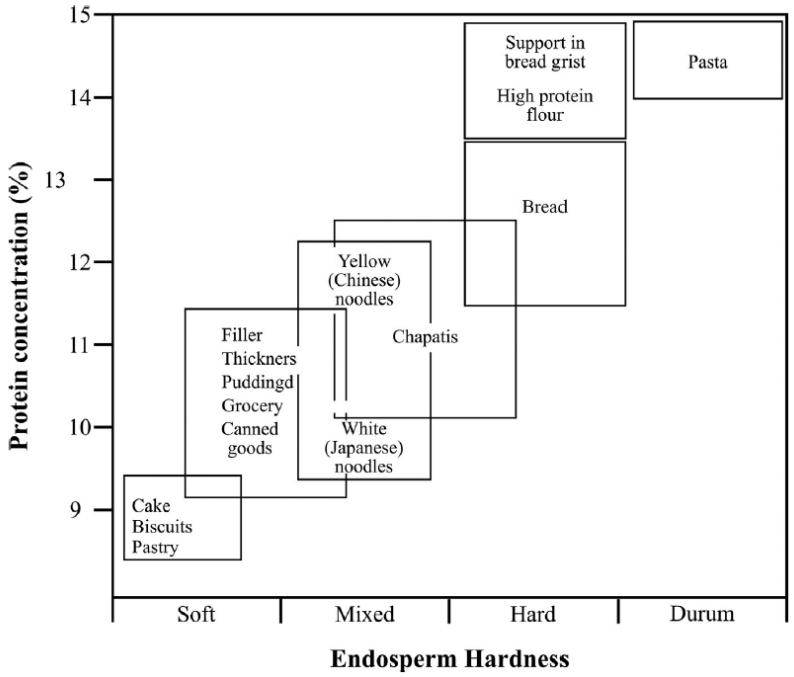


Figure 1.16. Relationship between the grain protein content, the endosperm type, and the end uses of wheat flour in the industry (Moss, 1973).

In Spain, the *Royal Decree 190/2013 from 15 march*, which modified the *Royal Decree 1615/2010 from 7 December*, defines some of the parameters indicated in the physical and chemical analyses mentioned above, used to determine the general quality index (GQI) (Table 1.2). This index is used to compare each grain variety with the parameters of the reference varieties, called control varieties. Then the variety can be included in the list if its GQI exceeds the value of 98, resulting from the following equation:

$$GQI = (40\% \times PC) + (30\% \times GI) + (20\% \times b^*) + (10\% \times TW)$$

where PC (protein content) is the protein of the variety/average protein of control, GI (gluten index) is the SDS sedimentation of a variety/average SDSS of control, b* (yellow pigment) is the β-carotene content of the variety/average content of control, and TW (test weight) is the test weight of the variety/average test weight of control.

Table 1.2. Classification of durum wheat into groups (a) and grades (b) established by Article 7 of the Spanish quality standard, Royal Decree 190/213.

(a)

		Protein content (%)	*Test weight (kg/hl)*	*Vitreousness (%)*
GROUP	1	≥ 13	≥ 80	> 80
	2	≥ 12	≥ 78	> 75
	3	≥ 11	≥ 77	> 60
	4		The rest	

(b)

		Humidity (%)	*Ash (%)*	*Fall index (seconds)*	*Impurities (%)*	*Other cereals (%)*	*Shrivelled (<1.9 mm) or broken (%)*
GRADE	I	≤ 12	< 1.75	> 300	< 3	< 2	< 4
	II	≤ 12.5	< 1.85	> 300	< 4	< 3	< 6
	III	≤ 13	< 2.00	> 250	< 6	< 3	< 10
	IV	> 13	> 2.00	< 250	> 6	> 3	> 10

In addition, in groups 1 and 2, a high gluten quality (≥ 75) and high yellow pigment content (b* ≥ 19 or β-carotene ≥ 8 ppm) can be considered to be different to the rest of the high-quality varieties.

1.3.2.1. Milling quality

Semolina yield is an important quality criterion for durum wheat. The quantity and homogeneity of the grain size (between 160 - 500 μm) of the semolina obtained will determine the quality of the dough.

There is a positive association between test weight and semolina yield, and it has long been used as an indicator of the industrial potential of durum wheat (Clarke *et al.*, 2008). Test weight is expressed per unit volume in kg hl^{-1} and measures the space occupied by the grain. Nowadays, the industry uses even more acceptable kernel sizes, which have increased yield advantages. They improve the hygroscopic properties of the particles, increasing the efficiency of the technological process of pulp production (Clarke *et al.*, 2008). Grain size is the most crucial factor in assessing semolina yield, as durum wheat milling yield is proportional to grain thickness and shrunken and damaged grains, reducing yield (D'Egidio *et al.*, 1990). In addition, the relative ratio of endosperm

to kernel hulls is a function of kernel size and kernel hull thickness. The proportion of husks is lower for round kernels than for elongated seeds and is more down the more significant the average kernel size is (López-Bellido, 1991).

The thousand kernel weight is closely related to the size (Godon, 1991) and density of each grain (Halverson & Zeleny, 1988). For example, let us suppose that we have two samples of grains of a similar size, but it turns out that their thousand kernel weights are different. This is because, depending on the proportion between the endosperm (higher density) and the hulls (lower density), the weight of the grain will be higher or lower (Posner and Hibbs, 2004). Grain size and density depend mainly on the variety and environmental conditions, especially during grain filling and ripening (Shewry *et al.,* 2010).

Another critical factor in the quality of durum wheat grains is the degree of vitreousness. Vitreousness is an important parameter in the case of durum wheat, mainly because the higher the presence of non-vitreous (floury) grains, the lower the amount of semolina produced in the industrial process and the higher the amount of flour, which for durum wheat is a by-product (Dexter *et al.,* 1988; Sissons, 2008). Although some can be incorporated into the semolina itself, excessive flour production forces it to be diverted to other less profitable uses. In turn, it negatively influences other quality criteria such as protein content, baking quality and dough colour, so the industry demands durum wheat with a low percentage of non-vitreous grains. Varietal sensitivity and unavoidable circumstances that do not favour the deposition of protein in the grain or a lower proportion of protein, such as the presence of high humidity during the final stage of grain maturation, either due to rainfall or irrigation in the weeks before harvest, are decisive for the loss of the vitreous conformation, transforming them in this case into floury grains (Sandhu *et al.*, 2009). The degree of endosperm hardness is another factor influencing milling quality, affecting the particle size after milling, water absorption by the flour and milling yield. Although it is not only a consequence of the texture of the grain (vitreous or floury) but also of the binding strength between proteins and starch, it can be estimated, to a certain extent, by the vitreousness percentage so that the greater the hardness or vitreousness of the endosperm, the less tendency it will have during milling to be reduced to flour, which in terms of semolina is a by-product. In contrast, a grain that is not very vitreous will tend to break down into excellent products to the detriment of the semolina yield.

1.3.2.2. Seed quality

Excluding those factors which are not influenced by the variety of durum wheat used (humidity, rate of impurities, thickness of broken kernels, etc.), the characteristics which determine the value of durum wheat seed are: (i) the fragility of the grain envelopes and the ease of separation between the endosperm and the rest of the envelopes; (ii) the protein content; and (iii) the carotenoid content (colour).

The fragility of the grain envelopes and the ease of separation between the endosperm and the envelopes are implicated in the difficulty encountered by the seed industry in cleaning the bran properly. Conversely, too tight a bond between the albumen and the peripheral envelopes of the grain will lead to a decrease in the semolina yield.

The protein content is the most important variable determining the quality of durum wheat seed (Dexter & Matsuo, 1977). Grain protein content varies as a function of the environmental conditions and the crop management practices (Peña *et al.*, 2002; Shewry, 2007). These variations are much more significant than the genotype (Aguirrezábal *et al.*, 2015). The protein concentration in the grain is mainly due to variations in the amount of C compounds, i.e. starch (Jenner *et al*., 1991), while the amount of N compounds, i.e. protein per grain, is relatively stable. The relationship between C and N compounds leading to a final grain protein concentration can be explained more simply by the effects of environmental factors during the grain filling period on the rate and duration of starch, oil and protein accumulation (Aguirrezábal *et al*., 2015). It has been shown that grain N concentration increases with increasing temperature and soil moisture deficit (Schipper, 1991).

The grain endosperm must be dark yellow to give the dough the desirable dark colour due to end-consumer consumption preferences. This character is related to the carotene content of the endosperm, depends on the genotype-environment interaction, and dramatically affects the final price of the end products (Subira *et al.*, 2014)

1.3.2.3. Pasta quality

Culinary quality in pasta production, considering the characteristics desired by the end consumer, is a comprehensive concept that includes water absorption or the degree of swelling. For this, we need semolina that absorbs just the right amount of water. For example, the absorption of a large amount of water during pasteurisation would result in pasta with less chewy and desirable consistency. This concept also encompasses texture, which depends on the viscoelasticity conferred by the gluten, and surface integrity, which means that cooked pasta must be consistent, firm and elastic when compressed with the teeth (commonly referred to as 'al dente'). It must also have a pleasant aroma, the characteristic taste of pasta, free of any other nuances and a uniform amber-yellow colour (which depends on the amount of carotenoid pigments in the grain and on the lipoxygenase enzymes capable of causing the oxidation of these pigments during the production process (Johnson & Quick, 1983).

The cooking quality of the dough is influenced by protein content, gluten quality and bright yellow colour (D'Egidio *et al.*, 1990). Again, the vitreous texture of durum wheat endosperm is essential in this processing procedure and, as was remarked earlier, for an excellent semolina yield (Ficco *et al.*, 2009). On the other hand, floury grains give rise to whitish spots, which are unpleasant for the appearance of the dough, conferring a lower hardness than vitreous grains and a worse performance during the milling process (Marchylo *et al.*, 2004).

The role played by the protein component in maintaining the integrity of cooked pasta is a function of the quantity and quality of gluten present in the grain. In terms of gluten quality, varieties with gluten strength produce dough with greater firmness in cooking, giving a greater or lesser density to the lattice together with the starch granules, increasing tolerance to overcooking and also reducing breakage losses during manufacture and transport, also affecting organoleptic properties (Josephides *et al.*, 1987). Therefore, the rheological quality of gluten (related to the viscoelasticity or strength of the dough) is the main factor determining the baking quality of the dough and one of the most critical parameters in evaluating durum wheat quality (Kovacs, 1997).

1.3.3. Nutritional quality

The nutritional value of the final product, made from durum wheat, depends primarily on the total amount of protein and its amino acid composition. However, starch, vitamins and mineral

substances also play a considerable role. Given the general scarcity of protein accessibility and intake globally, increasing the total amount of protein in durum wheat is a possible solution. Therefore, its consumption is promoted not only for its health benefits but also for its lower production cost and lower environmental impact than animal protein procurement. However, the protein-energy range of the total energy and its biological value (represented by the among of N absorbed) of durum wheat is usually low compared to animal proteins. This low nutritional value is because plant-based proteins are generally deficient in essential amino acids (e.g., branched-chain amino acids, lysine, methionine and tryptophan) and are less digestible in their natural form than animal-based proteins (Davies & Jakeman, 2020).

Minor nutritional components of grain weight (approximately 2%) include non-polar and polar lipids, such as triacylglycerols or free fatty acids, group E and B vitamins, minerals, such as potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), manganese (Mn) iron (Fe) and zinc (Zn), and phytochemicals. The latter are active non-nutritive biomolecules with beneficial effects on human health, promoting well-being and preventing diseases (Wieser *et al*., 2020). Nevertheless, these components differ in their distribution within the grain. For example, the starchy endosperm contains low contents of cell wall components, minerals and phytochemicals. In contrast, the pure bran, i.e. the aleurone layer, the outer layers of the grain and the embryo lack starch and are enriched in these minor components (Shewry *et al*., 2013) (Table 1.3). Therefore, from a nutritional point of view, it is essential to modify the milling process to recover most of the aleurone layer in flour or make better use of the bran in human food.

Micronutrient deficiency is a major global concern of public health importance. The root cause of this problem is the lack of availability of a balanced diet for resource-poor communities. Therefore, recently much attention has been given to improving the mineral nutrient content in crops, called Biofortification, which is essential in staple crops to restrict malnutrition and diseases and promote the welfare of target populations who base a large part of their diet on these crops with low consumption of animal products. Biofortification is a strategy that uses plant breeding techniques to produce staple food crops with higher levels of micronutrients, reducing the levels of antinutrients and increasing the levels of substances that promote their absorption (Bouis, 2003, Pfeiffer & McClafferty, 2007). As an example of an antinutrient, most of the Pi present in the aleurone layer of mature cereal seeds (40-80%) is stored as phytate or phytic acid, an antinutritional factor that forms insoluble complexes with minerals such as Ca, Mg, Fe and Zn reducing their bioavailability and intestinal absorption (Ficco *et al*., 2009).

Table 1.3. The micronutrient content of wheat and wheat fractions (mg kg^{-1}) and RDA requirements (mg) (modified from Igrejas *et al.*, 2020).

Wheat sample	Fe	Zn	Cu	Mn
Wheat	18-31	21-63	1.8-6.2	24-37
Bran	74-103	56-141	8.4-16.2	72-144
Germ	41-58	<100-144	7.2-11.8	101-129
Flour	3.5-9.1	3.4-10.5	0.62-0.63	2.1-3.5
Maximum adult RDA	15	15	1.5-3.0	2-5

RDA, recommended dietary allowances.

1.4. Challenges of agriculture

1.4.1. Impact of the climate change

It is true that throughout history, there have been other changes of considerable magnitude since the formation of the Earth, either due to external natural causes or endogenous natural causes (Foster *et al.*, 2018). However, they have not occurred in such a short period as the current global warming of the planet, which cannot be explained by natural variability alone. Consideration of natural causes together with simulations of anthropogenic causes produces a perfect coupling with imminent global warming (IPCC, 2013a). Thus, the United Nations Framework Convention on Climate Change (UNFCCC), in article 1, defines climate change as "a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods" (IPCC, 2013b).

The different climatic types existing in the world are defined by the combined annual variation of temperature and precipitation (UNESCO-FAO, 1963). Figure 1.17 shows the main area describing the so-called Mediterranean climate: all of southern/southwestern Europe and North Africa. Mediterranean climate can also be found in the United States of America (California), South America (Chile), South Africa and southern Australia. The climatic classification defined by W. Köppen in 1931 indicates that the entire southwest of Portugal and most parts of Spain are included in the temperate climates, in the Mediterranean or dry subtropical subdivision: mild, humid weather with hot, dry summer (Csa) in the south, or humid temperate climate with hot, dry summer (Csb) in the north (Köttek *et al.*, 2006). This climate is characterised by mild, rainy winters

and hot, dry summers, with variable autumns and springs in temperature and precipitation. It is named after the Mediterranean Sea, the area where this climate is typical and has the most considerable geographical extension (Royo & Briceño-Félix, 2011). However, it is projected to be more limiting at the end of this century (2071-2100), where a climatic BSk (arid, steppe, cold) according to the Köppen-Geiger classification is likely to occur in many regions of the Iberian Peninsula (Beck *et al*., 2018) (Figure 1.17).

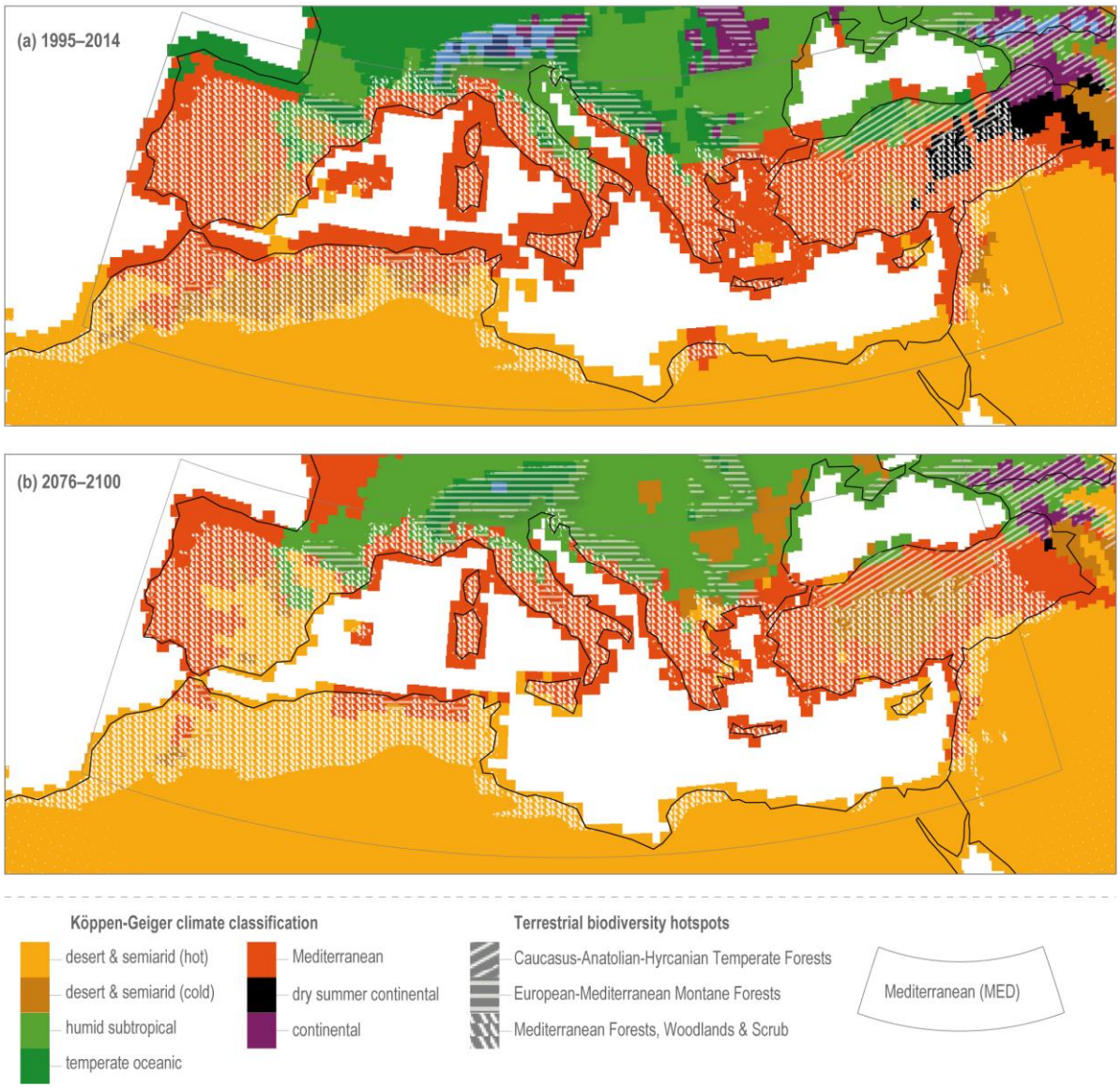


Figure 1.17. Köppen-Geiger climate classification over terrestrial biodiversity hotspots in the Mediterranean (IPCC, 2021).

Rapid growth in agricultural productivity since the 1960s has underpinned the development of the current global food system, which is both a significant driver of climate change and increasingly vulnerable to it (through production, transport and market activities). Several studies have shown that global crop production must double by 2050 to meet the demands of population

growth, nutritional needs and increasing biofuel consumption (FAO, 2018). This would significantly increase greenhouse gas emissions and other environmental impacts, including biodiversity loss. Therefore, we need to be prepared for climate change predictions, including higher temperatures and more extended periods of drought. By trying to increase crop yields to meet these increasing demands, rather than expanding the arable area, we will protect our ecosystems as much as possible (Cassman, 1999).

Under rising temperatures (2°C), rainfed wheat yields in most locations could be reduced by an estimated 2-59%, depending on farming practices (Brouziyne *et al*., 2018; Kheir *et al*., 2019), mainly due to the shortening of the crop growth cycle (up to about 30 days) due to rising temperatures (Bouregaa, 2019).

Accelerating crop improvement is an increasingly urgent issue to meet the growing global demand for food. To this end, the success of wheat breeding depends on the development of high-yielding varieties better adapted to changing climatic conditions. How crop yields are affected by stress conditions caused by climate change has received significant attention, as abiotic stress can lead to deficiencies in growth, crop yield, or permanent damage such as water and nutrient shortages (Lamers *et al*., 2020).

1.4.1.1. Water scarcity

One of the main abiotic stress factors affecting crop growth is water stress. Water stress occurs mainly when the water supply to the roots becomes limiting or transpiration rates excessive due to increased temperatures (Osakabe *et al*., 2014). During a period of drought, osmotic and metabolic imbalance of the plant occurs, leading to loss of turgor and impending stomatal closure. This leads to massive transcriptional reprogramming (Liu *et al*., 2015; Vicente *et al*., 2018b). As a result, genes involved in several primary and secondary metabolism pathways are altered. For example, those related to the synthesis of osmoprotectants (sugars, some amino acids, etc.) and protective proteins are generally induced, while those for photosynthesis and amino acid metabolism are repressed (Budak *et al*., 2013; Habash *et al*., 2014; Rybka & Nita, 2015).

Furthermore, depending on the degree of water deficit experienced and the time at which this stress originates are two crucial points at certain stages of development that are particularly sensitive for cereals. For example, during the early stages of development, there is a reduction in cell growth and leaf area, with a consequent decrease in photosynthetic area. If this drought event

occurs at more advanced stages, such as heading and flowering, there will be a significant reduction in the final yield obtained (Snape *et al.*, 2001) since this is when the number of grains is determined. Finally, at the end of the crop cycle, water stress will cause a shortening of the grain filling period and result in early senescence and then a smaller grain size (Christopher *et al.*, 2014).

Typically, crops are subjected to different stresses under field conditions, and it is widespread to find together drought and heat stress in semi-arid areas. High temperatures, e.g. heat waves, lead to accelerated plant development, dysfunctional photosynthesis, reduced fertility and fruit formation problems, and downstream effects on crop yield (Asseng *et al.*, 2014). Like water deficit, these adverse conditions affect wheat differently depending on the phenological stage in which they occur, showing that the most significant yield reductions occur when stress occurs in the late growing vegetative phase and during the grain filling period when it is combined with a decrease in precipitation and therefore crop evapotranspiration exceeds that of available water in the soil (Simane *et al.*, 1993).

1.4.1.2. Nutrient deficit

Deficiencies of certain limiting nutrients such as N and phosphorus are common in agroecosystems, leading to problems in average plant growth and development (Evans, 1983; Carstensen *et al.*, 2018). For example, N is the main limiting factor for crop yield and biomass after water deficiency. In addition, N is an essential element in chlorophyll production and other cellular components (Zhu *et al.*, 2008; Muñoz-Huerta *et al.*, 2013) and drives C assimilation in the canopy (Li *et al.*, 2014).

N management has significant economic and environmental implications (Bonfil *et al.*, 2004). An adequate N supply is crucial for the proper maintenance of the biochemical quality of the plant, which forms a significant part of photosynthetic proteins (Nobel, 2009) and for improving grain yield and quality. Insufficient N application would reduce photosynthetic capacity leading to symptoms of chlorosis, necrosis, reduced growth and tillering and consequently reduced grain yield (Corp *et al.*, 2003). On the other hand, over-fertilisation with N would lead to low N use efficiency and the generated negative environmental impacts affecting air and water quality and then biodiversity and human health (Lu & Zhang, 2000). In particular, this fertiliser abuse causes significant effects on the environment, such as NO_3^- leaching, which dramatically influences

eutrophication and groundwater pollution (Inoue *et al*., 2012), as well as nitrous oxide (N_2O) emissions, which contribute to global warming in greenhouse gas emissions (Muñoz-Huerta *et al*., 2013; Chen, 2015). All this highlights the need for proper management of this element.

1.4.2. Growing population food demand

The food system encompasses all activities and actors involved in the production, transport, manufacture, retail, consumption and waste of food and their impact on nutrition, health and welfare, and the environment. Achieving good food security is one of the fundamental concerns of a country. An increasing world population and global climate change are among the most significant challenges facing society today. According to FAO, food security is "a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (FAO, 2018).

The world's population has been increasing slowly and steadily throughout history, reaching 1 billion in 1800 for the first time and 2.5 billion in 1950 (Bongaarts, 2009). Since then, the population has increased rapidly, reaching 6.7 billion in 2007 (United Nations, 2019). Today, the world population has already exceeded 7.9 billion (United Nations, 2021; Worldometers, 2021), and although the global growth rate has decreased from 2.09 % in 1968 to 1.05 % in 2020, it is expected to continue to increase to about 10 billion people in 2050 and between 9.4 and 12.7 billion in 2100 (United Nations, 2019). This increased population growth is usually accompanied by a societal transformation, such as the shift from agriculture to industry (Bongaarts, 2009). As a result, the demand for food is subject to population growth. It is estimated that by 2050, food production must increase by at least 50 % (FAO, 2014) (Figure 1.18).

Crop productivity increased significantly worldwide during the Green Revolution due to increased cultivated land and improved agronomic practices, including nitrogenous agrochemical fertilisers, herbicides and pesticides (Khush, 2001). However, at the same time, as demand for food is increasing, production is gradually being constrained by the loss of arable land area due to urban expansion, land degradation, non-food uses of crops and climate change (Parry & Hawkesford, 2010). Reported annual increases in global productivity of major staple crops are close to 1 % over the last decades, while wheat yield increases were over 3 % from 1965 to 1974

(FAO, 2014). Therefore, to meet the growing demand for food, we need to increase the crop efficiency annually since it is highly unlikely that the cultivation area can be improved. In turn, increasing N fertiliser inputs does not seem to be the most appropriate option for the sustainability of agriculture (Ray *et al*., 2013).

The best and more sustainable approach to ensure food security in the coming decades is to develop high-yielding varieties with better tolerance to biotic and abiotic stresses to meet the growing demand for food. This implies that genetic improvement will have to maximise crop yields.

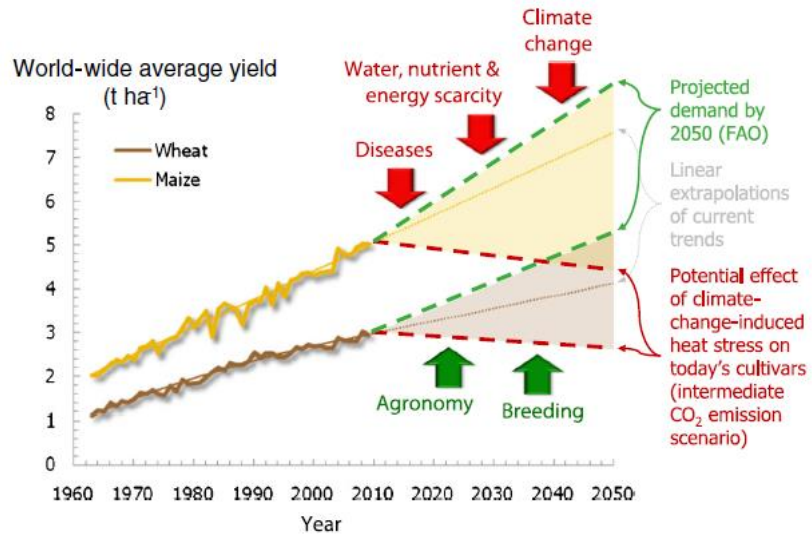


Figure 1.18. Average of maize and wheat yield and expected worldwide demand by 2050. Represented in red colour the liming factors and in green colour the factors that could increase it (Thierfelder *et al*., 2018).

1.4.3. Bottleneck in breeding

Crop improvement has become a permanent goal in breeding programmes, reinforced in this century by the challenge posed by the already described increase in world population and the consequent demand for food (Ray *et al*., 2013).

Improving yield potential has been achieved through wheat breeding in most countries throughout the 20th century (Slafer *et al*., 1994; Calderini *et al*., 1999; Foulkes & Reynolds, 2015). Figure 1.19 shows the relative genetic gains in yield from data obtained in several countries and

from different studies in the same region. In all of them, the average yield gain was 0.74% y^{-1}. Only in the case of five countries (Brazil, Chile, China, England and Mexico) did they show genetic gains ≥ 1% y^{-1}. However, Slafer *et al*. (2021) pointed out that it is essential to remember that the shorter the period evaluated, the higher the estimated genetic gain. Furthermore, the older the landraces or varieties with elder records studied, the more significant the genetic improvement. Genetic gain increased slowly in the first half of the 20th century and to a greater extent during the second half of the 20th century (Calderini & Slafer, 1999). This period corresponds to the so-called Green Revolution, led by the Nobel Prize winner Dr. Norman Borlaug, which marked a turning point in the actual yield obtained, which had been increasing, albeit slowly in the previous years, thus consolidating yield improvement worldwide (Austin *et al*., 1980; Slafer & Andrade, 1989; Siddique *et al*., 1989). With few exceptions, such as in low-performance environments in parts of Australia (Hyles *et al*., 2020). The main changes due to introgression of Rht alleles associated with yield improvement were an increase in harvest index with lower biomass and an increase in the number of grains, sometimes coupled with a slight decrease in the weight of grain obtained (Slafer *et al*., 1994; Calderini *et al*., 1999; Foulkes & Reynolds, 2015).

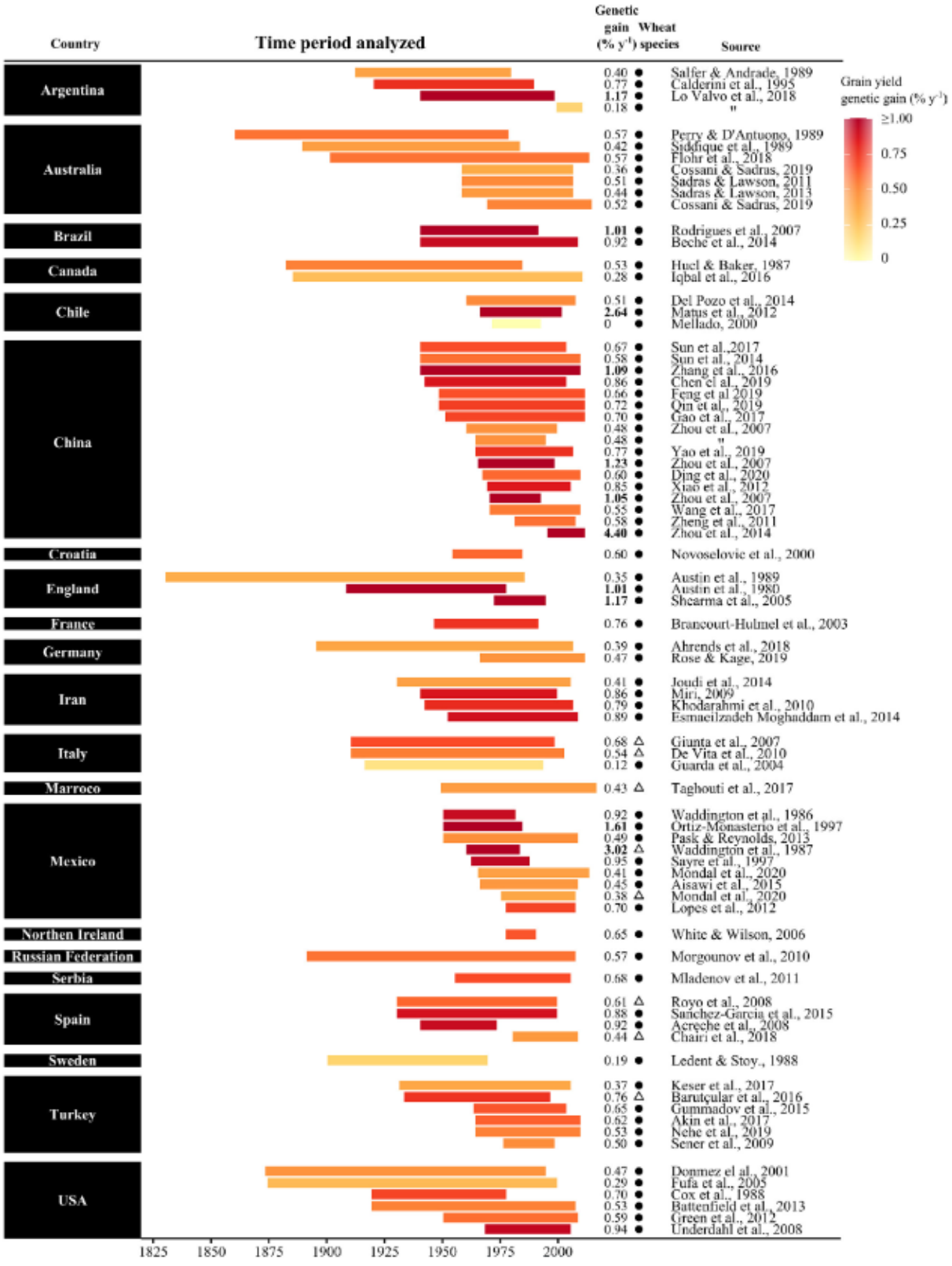


Figure 1.19. Relative genetic gain in yield was reported for different countries and periods of bread (closed circles) and durum (open triangles) wheat. This figure only included studies in which genetic gains were evaluated through growing side-by-side cultivars released at different times in the same experiment. Genetic gains were calculated as the ratio between the absolute genetic improvement and the average yield as a percentage. The bars' colour shows them and their intensity representing the period analysed in each study. Exact values are explicit at the right of the bars (Slafer *et al.*, 2021).

In the first half of the 20th century, the area under wheat cultivation increased in America, Australia and Africa, contributing to increased world production (Calderini & Slafer, 1998), stabilising since the 1960s between 200 and 240 Mha. Today, the largest wheat cultivated areas are in India, Russia, China, the USA and Australia, accounting for half of the world's wheat cultivated area. In a detailed study by Arata *et al*. (2020), they assessed yield trends and variability of 168 crops in 224 countries between 1961 and 2014 from data collected by FAO. They concluded that in the case of wheat, yields increased in 75% of the areas considered in the study, other areas showed no trend (17%), and in 8% of cases, the area even decreased. As for the stability of crop yields, only 27.6% showed an increase in variability. This paper argues that geographical area is the crucial factor affecting yield variability. Eastern Europe, Central Asia, the Middle East and North Africa had the highest variability recorded.

In contrast, Western Europe and North America had the lowest values. This is not surprising, as agricultural technologies that mitigate yield variability, such as modern irrigation techniques, are much less common in these areas. Over time, climate change and agricultural management practices are the leading cause of this increased variability (Arata *et al*., 2020). In the most productive areas (e.g. China and India), which account for 75% of global wheat production, climate variability accounted for 36% of the year-to-year yield variation (Ray *et al*., 2015). For example, in China and India, the leading wheat producers, 32% of their yield variation was associated with variability in both temperature and rainfall, respectively (Ray *et al*., 2015).

The main approaches by which the productivity of staple crops can be boosted include the continued exploitation of natural genetic variability and improved management practices (Araus *et al*., 2018). Strategies to cope with future environmental scenarios include selecting crop varieties by modifying plant traits to increase their resilience and thus reduce the risks of yield losses (Araus & Kefauver, 2018). Contemporary breeding programmes require the analysis of hundreds of lines. Alternatively, the other pathway involves adjusting agronomic management practices to mitigate crop exposure to environmental stress, such as implementing better irrigation systems and more efficient fertilisation, adjusting cropping, in terms of planting and harvesting times, to accommodate the entire crop cycle, or even applying the principles of conservation agriculture, through minimal disturbance and permanent soil cover and crop rotations. In this context, a so-called Golden Revolution in agriculture is needed to meet the goals of sustainably feeding the future population while meeting growing consumer expectations (Evans & Lawson, 2020).

1.4.3.1. Case of Spain

Spain produces approximately 21 million tonnes of cereals per year and imports 11 million tonnes to meet current demand. On average, about 250000 ha of durum wheat are sown, and during the last few years, it is estimated that the production obtained ranged from 826855 t to 1350420 t (Cooperativas Agro-alimentarias, 2020). However, durum wheat production in Spain has decreased significantly due to reducing hectares cultivated and tonnes produced. Nevertheless, its consumption has continued to grow, making Spain highly dependent on imports (Royo & Briceño-Félix, 2011) and contributing to national food insecurity in a scenario of climate change and political instability.

Faced with the impossibility of increasing the area devoted to agriculture, both globally and nationally, the only possible response to meet a growing population's current and future demand is to maintain or increase the productivity and stability of crops, either by agronomic means or through genetic improvement. However, in genetic modification, an evident lack of yield improvement has been observed after the Green Revolution, in line with previous studies (Xynias *et al.*, 2020). The limited genetic variability may explain, in part, the low rate of genetic progress in the case of durum wheat in Spain. The strategy of "crossing the best with the best" has increased overall genetic gains. However, in some countries where yields have stagnated (generally high-yielding countries, such as those in Western Europe), no genetic increases have been observed in the last decade. In our country, the study of the genetic variability in grain yield and many other traits was significant, suggesting the absence of a clear genetic improvement (Chairi *et al*. 2018). This highlights the need to create an innovative national strategy to maintain or increase the productivity of durum wheat grown in Spain.

1.5. Novel approaches for crop improvement

Genetic improvement through breeding is the best way to increase crop productivity (Mir *et al*., 2019). With the rapid advancement of functional genomics, an increasing number of crop genomes have been sequenced, and dozens of genes that play a key role in agronomic traits have been identified; among them, the wheat genome has been sequenced (Yao *et al*., 2018; Shi *et al*., 2019; Pont *et al*., 2019). However, the information obtained cannot be adequately exploited to understand the complex traits of multiple genes due to the lack of phenotypic data on crops (Jin *et al*., 2021). Due to the rapid development of genetic analysis techniques and the increasing size of crop populations, phenotyping represents the main bottleneck that restricts crop improvement

(Figure 1.20). The phenotypic performance involves a complex interaction between genotype and environment (climate, soil, abiotic/biotic stresses and crop management methods) (Xu, 2016). Traditional phenotyping procedures measured observable characteristics of crops during their growth cycle, i.e. by the date of crop establishment or flowering, and post-harvest yield and agronomic traits such as harvest index. These measurements are not only laborious but also time-consuming and subject to the subjectivity of the person performing them, which can lead to discrepancies.

To overcome this, it is necessary to resort to phenomics research, which is defined as the accurate acquisition and analysis of high yielding phenotypes during the crop growth cycle, under abiotic and biotic stress conditions, in a multidimensional manner during crop growth at various scales, at the level of the whole organism, from cells to organs, individual plants, plots and fields (Song *et al*., 2021).

The development of new technologies (sensors, imaging technology, analysis methods) and the use of more efficient platforms, which can even act automatically and are increasingly accurate, can help us capture phenotypic data to link them to available genetic resources (Rebetzke *et al*., 2019; Zhao *et al.*, 2019; Yang *et al*., 2020; Zhang *et al*., 2020). We can monitor the crops at all stages of their growth and even define ideotype attributes for the posterior selection of varieties or the evaluation of new study traits (Sanchez-Bragado *et al*., 2020c). This process is known as high-throughput phenotyping programmes (HTPP), which involve using different non-invasive remote sensing approaches (Atzberger, 2003; Reynolds & Langridge, 2016), allowing the faster screening of larger populations than conventional phenotyping procedures. However, information on the plant's metabolism is also relevant to understanding the metabolic changes associated with a phenotype and, ultimately, its grain yield and quality. Although these analyses are costly and time-consuming, they are an excellent contribution to phenotyping to help identify new traits at the canopy, whole plant or organ level that can contribute to crop improvement programmes (Figure 1.20).

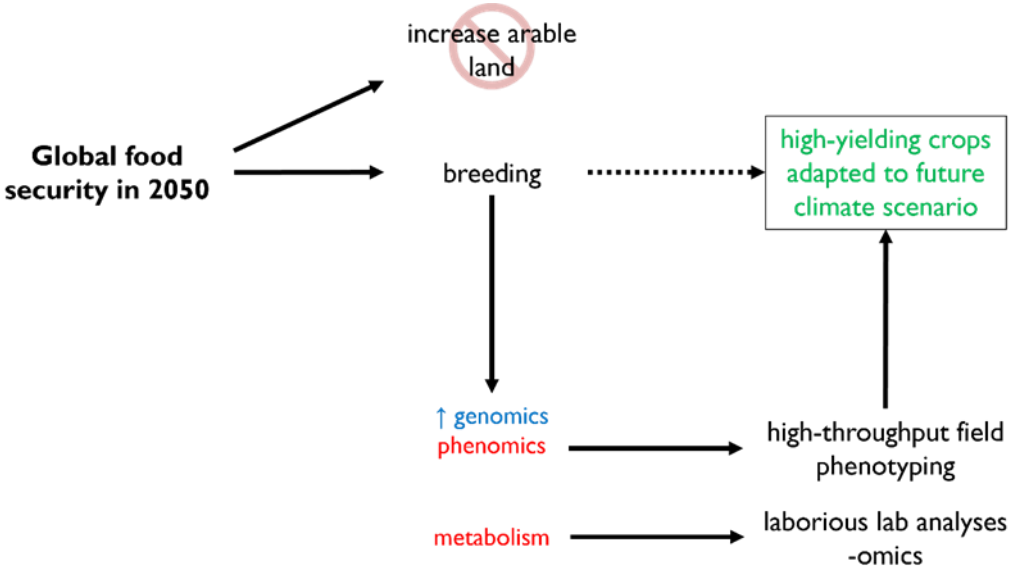


Figure 1.20. Outline showing the current problems faced in plant breeding.

1.5.1. Remote sensing and Phenotyping

Remote Sensing is the process of detecting and monitoring the physical characteristics of an object or area by measuring it is reflected and emitted radiation at a distance (NOAA, 2021). Therefore, due to its inherent characteristics, Remote Sensing of plant canopies allows non-intrusive (without contact), high-throughput monitoring of plant physiological characteristics, offering a rapid and non-destructive approach to plant screening (White *et al.*, 2012). As a result, it has become an essential tool in agriculture to control and monitor crop development and health. Results have shown that it is possible to observe and quantify changes in crop conditions using this tool throughout the entire phenological cycle of the crop. Furthermore, it is possible to generate high-quality quantitative data and effectively characterize traits during the growing season (Moreira *et al.*, 2020).

Remote Sensing approaches require several elements: a **platform** to transport the instrument, the object to be observed, and a **sensor** that captures the electromagnetic information coming from the thing (ESA, 2015).

1.5.1.1. Platforms

The platform is the object that transports the sensor. It can be terrestrial, typically used by a person or attached to off-road vehicles, tractors or similar. The platform can also be aerial, usually aircraft, but recently small unmanned aerial systems (UAS) are more used. Additionally, the platforms can be space-based, such as satellites. Depending on the trait evaluated and the phenotyping scale required, different combinations of sensors and platforms can be used to obtain different resolutions. Working with attributes that can be defined at the plant level is not the same as at the plot level (Guo *et al*., 2021). Therefore, the spectral information obtained in the experiments will be a direct function of the platform and sensor used. Thus, the phenotyping systems are classified into operating at the ground level or aerially.

Using ground-level systems, or proximal sensing, it is possible to obtain the highest resolution, while UAS-mounted sensors and satellite imagery will be the lowest (Mirik *et al*., 2011; Sadeghi-Tehran *et al*., 2017; Guo *et al*., 2021). Phenotyping requires a minimum spatial resolution; therefore, combinations of sensors and proximal sensing platforms or UAS are usually employed, taking into account that not necessarily higher proximity to the object and a higher resolution implies a higher accuracy in the estimation of traits for phenotyping, as some studies show that spectral information captured with UAS provides the best estimates (Tattaris *et al*., 2016). However, the lack of resolution is not critical, and it is also possible to use high-resolution satellite imagery. For example, Mercier *et al.* (2020) successfully used sentinel satellite imagery to predict wheat phenological stages. Futhermore, Mirik *et al.* (2011) employed remote sensing to detect and map wheat infected by the mosaic virus, demonstrating the potential of spectral vegetation indices in wheat monitoring, e.g. disease detection.

1.5.1.2. Sensors

Sensors used in phenotyping are devices that receive information from the surface of objects, which can be classified as active or passive. Active sensors emit energy to the object's surface, which interacts and returns to the instrument for analysis. Dualex would be an example of this kind (Goulas *et al*., 2004), consisting of an optical sensor for assessing flavanols, anthocyanin, N balance and chlorophyll contents in leaves. For example, it measures leaf chlorophyll content thanks to a transmittance ratio at two wavelengths: one in the far-red (700-720nm) absorbed by chlorophyll and one in the near-infrared (800-820nm) reference.

On the other hand, passive sensors collect the energy incoming from objects. This energy can be emitted by the object itself or reflected, in which case it comes from the sun or a light source. A standard digital RGB (red-green-blue) camera is an example of this type. Digital RGB cameras are the most common tool used for phenotyping due to their high-resolution, low cost and portable size, allowing the segmentation of vegetation in several environments with various illumination conditions from "simple" to "complex" images (Sadeghi-Tehran *et al.*, 2017). Other passive sensors are also employed in phenotyping, such as multispectral or hyperspectral cameras. They can be utilised to detect specific phenotypic traits, such as the responses to different N levels and the relationship with biomass, growth rate, and chlorophyll levels in wheat (Banerjee *et al.*, 2020) and, at the same time, to create models for the estimation of functional plant attributes.

The sun is the primary source of radiation for the passive sensors. Electromagnetic radiation can be divided into different types depending on the wavelength. For example, the visible region, approximately between 400 and 700 nm, can be perceived by the human eye (Lynch *et al.*, 2001). In addition, the near infra-red or NIR, the short wave infra-red or SWIR, etc. The set of all the wavelengths constitutes the object's spectral signature and is a function of the atoms that compose the object. Thus, in agriculture, the elements that compose the plants define the spectral information measured by the sensors, which can estimate their state or the concentration of these elements at a given time (Vergara-Díaz *et al.*, 2020a). The optical properties of plant structures, particularly the leaves that compose the canopy, are characterised by an abundance of chlorophyll and other pigments that have high absorption in blue (400-500 nm) and red (600-700 nm) but high reflectance in green (500-600 nm), and very high reflectance and transmittance in NIR (700-1100 nm). As a result, plants are green, as they primarily reflect light in this wavelength (Raven *et al.*, 2013). Remote sensing takes advantage of the relationships between plant composition and the spectral information emitted at specific wavelengths. In this way, the reflectance in the SWIR is affected due to absorptions by water, proteins, and other C constituents in the vegetation. The NIR is mainly affected by the gaps between cell walls and intercellular spaces in the internal leaf structure. Therefore, the chlorophyll degradation due to several factors, such as plant senescence, pests or lack of nutrients, causes high reflectance in the visible spectrum, low reflectance in NIR due to reduced green leaf area and senescence, and high reflectance in SWIR due to modified tissue chemistry (Mirik *et al.*, 2011) (Figure 1.21). The differences in the spectral responses of vegetation in different wavelength bands have led to the development of spectral vegetation indices.

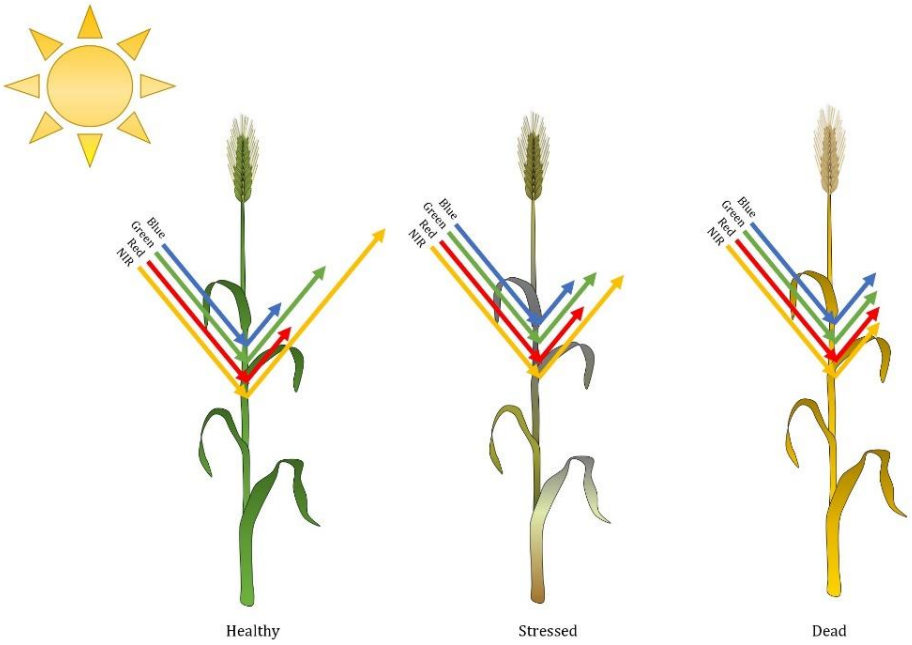


Figure 1.21. The reflectance of vegetation depends on its health condition.

1.5.1.3. Vegetation indices

Spectral vegetation indices are algebraic expressions that combine the reflectance values of other parts of the spectrum, aiming to increase the identification of plant characteristics. The most common procedure for extracting information about crops from remote sensing is estimating these spectral vegetation indices (Kyratzis *et al.*, 2017). Furthermore, the study of vegetation indices in phenotyping is significant because they can assist in identifying and predicting key characteristics, such as genetic variability (Babar *et al.*, 2006a). One of the most commonly used vegetation indices for high-throughput phenotyping is the Normalized Difference Vegetation Index (NDVI) due to its good results and the abundant literature on its application to all types of crops.

The NDVI is calculated using wavelengths within the NIR and RED (visible) regions of the electromagnetic spectrum (Rouse *et al.*, 1973). NDVI relates to chlorophyll content due to the molecule's absorption features, hence the photosynthetic capacity and status of the plant. It is based on healthy plants showing high NIR reflectance and very low RED reflectance (Lambers & Oliveira, 2019). Therefore, NDVI can estimate relative crop biomass at different growth stages, N deficiency, crop senescence rate, and thus grain yields (Babar *et al.*, 2006b; Olivares-Villegas *et al.*, 2007).

Some authors proposed other vegetation indices for phenotyping than NDVI, such as simple NIR/RED indices, whose performance is frequently better and not saturated as is the case of NDVI (Prey *et al*., 2020). Additionally, many other indices use different wavelengths, and each one is developed to detect certain phenotyping specific traits in plants, as listed in Table 1.4. However, selecting a particular vegetation index is not mandatory because they can be combined simultaneously or even according to the whole phenological cycle. Nevertheless, it is essential to consider that the ability of vegetation indices as proxies to identify plant functional traits is affected by plant phenology (Kyratzis *et al*., 2017).

Table 1.4. Summary of the most common vegetation indices, their spectral nature and possible traits targeted (Araus *et al.*, 2022).

Target trait	Spectral information	Vegetation indices	Applications
Vegetation cover and plot greenness	HIS color model,	GA, GGA, Hue (Casadesús et al., 2007)	Stress detection Canopy cover, LAI
		CSI (Zaman-Allah et al., 2015)	Shoot green biomass
	CIElab color model	NDab	Growth dynamics Senescence
	Red-Green-Blue (RGB, visible)	NDGRI (Hunt et al., 2005)	Canopy greenness
		GLI (Louhaichi et al., 2001)	Agronomic and yield traits
	Red, near infrared	NDVI (Rouse et al., 1973)	Plant emergence Phenology
		SAVI (Huete, 1988)	Stress detection Chlorophyll content
		OSAVI (Rondeaux et al., 1996)	Leaf nitrogen content
Chlorophyll content	Green, red, near infrared	MCARI (Daughtry et al., 2000)	Shoot green biomass Senescence
		TCARI (Haboudane et al., 2002)	Canopy greenness Stress detection
		TCARI/OSAVI (Haboudane et al., 2002)	Chlorophyll content Leaf nitrogen content
Anthodyanin and carotenoids content	Blue, red, near infrared	ARI2 (Gitelson et al., 2001)	Photosynthetic status Chlorophyll content
		CRI2 (Gitelson et al., 2002)	Anthocyanin and carotenoids content Leaf nitrogen content
Photosynthetic traits	Green, near infrared	PRI (Gamon et al., 1992)	Stress detection Photosynthetic status
		CCI (Gamon et al., 2002)	Senescence Chlorophyll content Stress detection
Water content	Water content	WBI (Peñuelas et al., 1993)	Plant water status monitoring
		NDWI (Gao, 1996)	

ARI2, Anthocyanin reflectance index; CCI, chlorophyll carotenoid index; CRI2, carotenoid reflectance index 2; CSI, crop senescence index; GA, green area; GGA, greener area; GLI, green leaf index; HIS, hue-intensity-saturation; MCARI, modified chlorophyll absorption ratio index; NDab, normalized difference a*b*; NDGRI, normalized difference green red index; NDVI, normalized difference vegetation index; NDWI, normalized difference water index; OSAVI, optimized soil adjusted vegetation index; PRI, photochemical reflectance index; SAVI, soil adjusted vegetation index; TCARI, transformed chlorophyll absorption in reflectance index; WBI, water band index.

1.5.1.4. Isotope analysis

To improve the knowledge about the genotypic adaptation to the environment in HTPP, the quantification of stable isotope signatures in dry matter is a well-established approach. This technique is indeed costly and time-consuming, but its use provides more reliable information (Araus *et al*., 2013) as it indicates the state of the plant throughout the entire crop cycle (Farquhar & Richards, 1984). Moreover, these analyses are appropriate when the target is phenotyping for crop adaptation to significant abiotic stresses such as drought, salinity, gaseous pollutants or nutrient deficiency (Araus & Cairns, 2014).

Isotopes are atoms of the same element that differ only in the number of neutrons and maintain identical chemical properties. Within each element there are radioactive (unstable) and non-radioactive (stable) isotopes. Most of the elements of biological interest are C, N, H and oxygen (O), and the analysis of their isotope composition in different plant materials allows us to obtain information on the plant functioning, e.g. the water status of the plant and the N metabolism by analysing the C and N isotopic compositions, respectively (Yousfi *et al*., 2012).

The isotopic composition is determined by mass spectrometry and is usually expressed in different notations. Equation (I) shows one of the ways of calculating the isotopic composition using the "delta" notation (Coplen, 2008)

$$\delta = \left(\frac{R_{sample}}{R_{standard}}\right) - 1 \quad \textbf{Eq (I)}$$

where R_{sample} and $R_{standard}$ are the molar ratios of the heavy and light isotopes (e.g., $^{13}C/^{12}C$) in the sample and the international standard, respectively. Since the differences in the ratios between the sample and the standard are very small, the values of δ are expressed per thousand (‰).

During physical and chemical processes, the isotopic difference between source and product is reflected in the isotopic fractionation, which is usually expressed by the alpha fractionation factor (equation II, the ratio of the product (R_p) divided by the source (R_r)) and the isotopic discrimination (Δ) (equation III: in some studies, isotopic fractionation refers to isotopic discrimination, without the negative sign):

$$\propto = \frac{R_p}{R_r} \quad \textbf{Eq (II)}$$

$$\Delta\ (‰) = \propto -1 = (\delta_r - \delta_p)/(1 + \frac{\delta_p}{1000}) \quad \textbf{Eq (III)}$$

where δ_r and δ_p are the isotopic composition of reactants and products, respectively.

In particular, $\delta^{13}C$, which is directly expressed as C isotopic composition ($\delta^{13}C$) or as CO_2 discrimination of the surrounding air ($\Delta^{13}C$), provides information on the efficiency of photosynthesis and transpiration during different environmental conditions (Yousfi *et al.*, 2016). In plants with C_3 photosynthetic metabolism, such as wheat, the $\delta^{13}C$ values found are lower than in the case of plants with C_4 metabolism (Farquhar, 1983). Generally, more unfavourable growth conditions, such as drought or salinity, affect $\delta^{13}C$, mainly by limiting CO_2 diffusion, decreasing stomatal conductance, and, to a lesser extent, mesophyll conductance, resulting in variations three to four times greater than the genetic components (Condon & Richards, 1992). Therefore, $\delta^{13}C$ has been a good indicator of how varieties adapt to drought and salinity conditions during growth (Araus *et al.*, 1997; Sanchez-Bragado *et al.*, 2020b).

On the other hand, the potential use of the natural abundance of different stable isotopes such as $\delta^{15}N$, which presented a higher analytical error (0.2) compared to C isotope analysis (0.1) (Handley & Raven, 1992), has as standard the relative composition of ^{15}N in atmospheric N_2 (Shearer & Kohl, 1986). It is closely related to N metabolism and has been studied to assess the performance of varieties under drought (Robinson *et al.*, 2000; Ellis *et al.*, 2002) or salt stress (Yousfi *et al.*, 2010). Furthermore, it allows us to study N dynamics in terms of the source-sink system, which encompasses the processes of N uptake, assimilation and redistribution within the plant (Choi *et al.*, 2002, Sanchez-Bragado *et al.*, 2017), with values closer to zero when the origin of the N fertiliser used and taken up by plant roots is synthetic (Bateman *et al.*, 2005). Nevertheless, the exact pathways behind the changes in $\delta^{15}N$ are still not well known.

Overall, the assessment of the isotopic composition of these two elements, C and N, as well as other promising features, allows the characterisation of different functional traits in the plants during their whole growth cycle, showing high heritability and genetic correlation with yield in several studies (Condon *et al.*, 2004; Araus *et al.*, 2013).

1.5.2. New traits

The link between fundamental plant science (in terms of plant genetics and physiology) and the applied science by transferring knowledge to farmers for breeding new crop varieties are not always easy. Some research spans the whole spectrum (laboratory, field and enterprise), but most operate only on one of the first two assumptions. So there is a big gap between research, mainly in the laboratory and greenhouse, and the subsequent validation and delivery to growers through commercial breeding companies (Passioura, 2012).

Identifying a candidate gene or quantitative trait loci (QTL) underlying a particular trait in a laboratory or greenhouse study does not necessarily translate into the expression or value of the trait in the field. So, when these new developments are translated into real-life scenarios, they often fail (Passioura, 2010). For example, genotype-by-environment interactions, which give rise to the plant phenotype, can change the ranking of varieties for traits such as specific leaf area and stem carbohydrate when assessed in controlled environments or the field (van Herwaarden & Richards 1999; Rebetzke *et al*. 2004).

Hence, the importance of study programmes that go in the opposite direction, making an appropriate selection of new traits through their preliminary characterisation in the field and subsequently analysing in the area the genetic and physiological mechanisms that define this behaviour without losing sight of the environmental conditions in which the plant has been grown.

1.5.2.1. The role of the ear

The ability to survive stress depends not only on the plant species but also on genotype, developmental stage and tissue (Ergen & Budak, 2009). In recent years, more and more studies have paid increasing attention to the role of inflorescences. Canopy photosynthesis depends not only on the photosynthesis of individual leaves but also on non-foliar green parts, including reproductive organs such as ears or panicles, when considering cereals. Furthermore, the ear appears to be a key factor in the grain filling stage as a C and N source under favourable or unfavourable growth conditions (Tambussi *et al*., 2007; Maydup *et al*., 2010; Sanchez-Bragado *et al*., 2017, Vicente *et al*., 2018b).

In wheat, non-foliar organs can represent between 27 and 62% of the total green area per stem. Specifically, the ear area is twice as large as that of the flag leaf. In the case of long growing cycles, the ear's surface area can increase by up to 60%. Consequently, the ears are responsible for approximately 50% of the total C exchange rate of the spike. This is also due to their proximity to the developing grains. Although spikelet bracts and awns pericarps can also fix atmospheric CO_2 (mainly glumes), a high CO_2 refixation capacity by PEPCase has been proposed (Sanchez-Bragado *et al*., 2020c) (Figure 1.22). This can reduce respiratory losses and increase the efficiency of water use in the ear as a process independent of gas exchange with the external atmosphere (Araus *et al*., 1992; Bort *et al*., 1996; Tambussi *et al*., 2002).

The ears also represent the first structure in direct contact with the sun's rays in the upper part of the canopy. Therefore, they are less exposed to possible shading due to their apical position than the rest of the organs, intercepting 30% of the incident radiation (Sanchez-Bragado *et al*., 2014a),

having temperatures several degrees higher than the flag leaves or the rest of the canopy (Vicente *et al*., 2018b). Moreover, ears are the youngest photosynthetic organs and the last to develop, showing a slower decline of the photosynthetic apparatus than leaves after the anthesis period, remaining photosynthetically active during the second half of the grain filling period, providing photoassimilates to the growing grains (Jia *et al*., 2015). There is less activity in ears than in flag leaves in terms of C and N metabolism (Lopes *et al.*, 2006; Vicente *et al.*, 2018b). However, it has been proven through the analysis of their isotopic composition that a significant part of the C and N accumulated in the grains comes from them, being especially relevant under abiotic stress, such as water and N limiting conditions (Sanchez-Bragado *et al*., 2014b, 2017). In contrast, the C assimilated by the flag leaf is proposed to be invested in the overall growth of the plant, including grain filling (Aranjuelo *et al*., 2011). This outperformance of ears under abiotic stresses is due to their improved water and N status, xeromorphic anatomy, photochemical efficiency and stability of their photosynthetic apparatus, lower diffusive conductance, higher expression of crucial genes for primary metabolism, drought stress responses, and delayed senescence (Vicente *et al*., 2018b). Moreover, the characteristic anatomy of the ear, a thick epidermis and a cuticle on the dorsal side of the bracts confer features of tolerance to water stress (Araus *et al*., 1992; Martinez *et al*., 2003). This allows osmotic adjustment by accumulating osmolytes to ensure turgor and gas exchange (Tambussi *et al*., 2005) (Figure 1.22).

Knowledge about the processes occurring in the ear concerning N metabolism is still scarce, but some results seem relevant. For example, it has been proposed that the glumes play a more prominent role than the leaves in N storage and supply as grain filling progresses (Lopes *et al*., 2006; Zhou *et al*., 2016). Furthermore, preliminary metabolome results showed that the content of respiratory, photorespiratory and amino acid intermediates were elevated under water stress or at least less negatively affected in the ear compared to the flag leaf, suggesting an active metabolism in terms of N assimilation and recycling under limiting conditions (Vergara-Díaz *et al*., 2020b), with a similar trend reported at transcript level (Vicente *et al*., 2018b) (Figure 1.23).

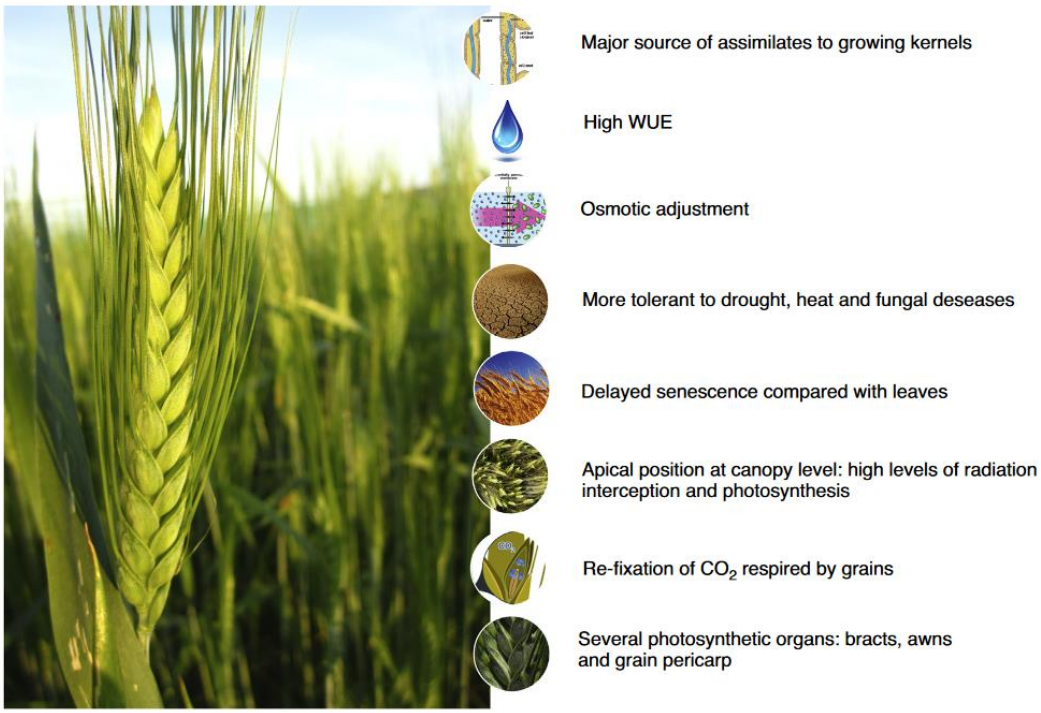


Figure 1.22. The ear's physiological and morphological characteristics make this organ especially advantageous under drought, heat and good agronomic conditions (Sanchez-Bragado *et al*., 2020c).

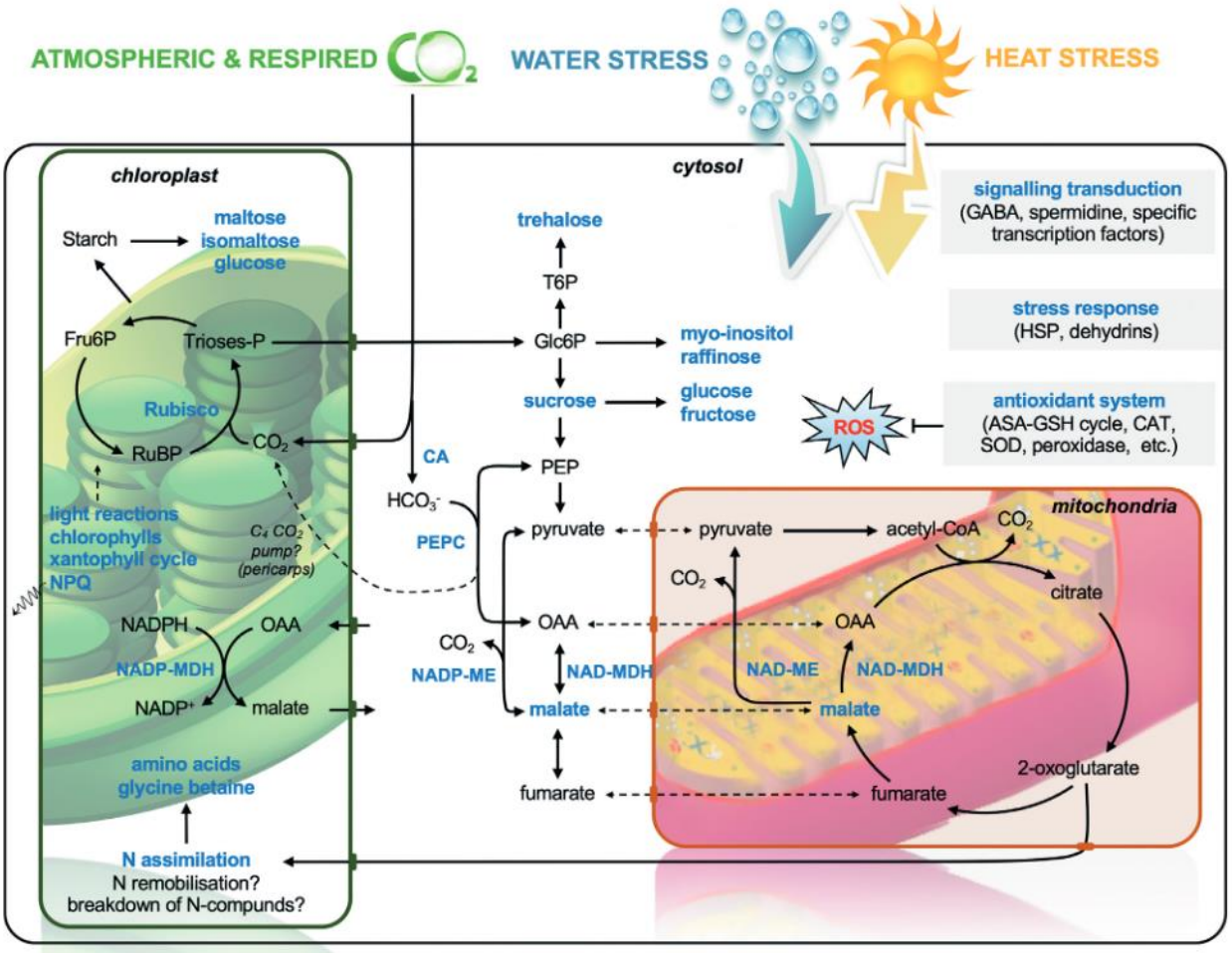


Figure 1.23. Overview of the most relevant changes observed in-ear organs under water or heated stress. The components and processes showed upregulation (higher content, activity or gene expression) and better performance (Sanchez-Bragado *et al*., 2020c).

Therefore, the study of the contribution to grain filling of other green organs, apart from leaf blades, could be crucial to understanding the nutrient supply in late growth stages. This is especially relevant under stress conditions like those in the Iberian Peninsula during the grain filling period or higher severity and duration in a climate change scenario. Likewise, this knowledge could be used to identify new traits as selection criteria in breeding programmes adapted to the Mediterranean basin.

2. CHAPTER 2:

Hypothesis and objectives

Due to the future and current challenges facing agriculture, wheat, particularly durum wheat, is presented as an essential crop for the human diet that will be necessary to continuously improve its yield and resilience to satisfy future food demands and meet the adverse effects of climate change. But the focus should be not only on improving its productivity but also on the grain quality and stability in response to expected changing climatic conditions without losing sight of obtaining a grain with good industrial and nutritional quality.

The working hypothesis of this doctoral thesis was based on the investigation of natural and physiological variation for tolerance to various factors, such as water and nutrient deficits, in a panel of durum wheat varieties registered after the Green Revolution and widely cultivated in Spain over recent years, to identify characters that may confer certain resilience to the variable climatic conditions that may occur throughout the growth cycle of the crop, with a particular focus on grain yield, quality traits and the potential key role of non-foliar organs providing nutrients to the developing grains. Our final aim was to propose the knowledge and the tools to contribute to the improvement of durum wheat in the Spanish region of Castile and León by describing new methodologies and traits that could make them more resilient the conditions predicted by climate change.

To this end, the main objective of this doctoral thesis was to perform a holistic approach to investigate the effect of the genotype, the environment and the genotype-by-environment interaction in durum wheat grown across field trials carried out in Castile and León to identify varieties best adapted to present and future climate conditions with high yield, industrial and nutritional grain quality, and stability, and novel traits that can explain complex traits (e.g. grain yield and quality) and contribute to developing more resilient durum wheat varieties. For that, we performed multi-environment experiments in field conditions to integrate the agronomic, physiological and biochemical mechanisms implicated in the response of the plants to the environment and the source-sink dynamics involved during grain filling, including different foliar and non-foliar photosynthetic organs, in the species *Triticum durum* L. ssp. *durum* (Desf.).

In order to achieve this objective, the following specific objectives are proposed:

(i) To evaluate the effect of environment, genotype and their interaction on grain quality parameters and to identify the most stable varieties for our region of Castile and León.

For this specific objective, a panel of 14 varieties registered after the Green Revolution and widely cultivated in our country were evaluated during five agronomic crop seasons, in 13 different environments. First, a wide variety of

treatments and annuities were carried out to simulate different optimal and stress growth conditions, followed by analyses of agronomic traits, the industrial and nutritional grain quality, and the performance through different statistical models.

(ii) To assess whether there has been a genetic improvement in nutritional quality of durum wheat grain over the last forty years of breeding and the effect of the environment on these changes to guide future breeding programmes.

In a retrospective study, 24 durum wheat varieties were grown under contrasting growth conditions over several crop seasons to evaluate the effects of the genotype × environment on grain nutrient composition and quality traits, to understand how the breeding has modified such factor and which agronomic traits are associated with changes in grain nutritional traits

(iii) To study the source-sink dynamics in durum wheat plants during grain filling, including investigating the metabolic processes that have taken place in different foliar and non-foliar photosynthetic organs, their contribution to the final grain yield as sources of carbon and nitrogen, and the effect of different nitrogen fertilisation in such responses.

To this end, four varieties within a panel of 24 durum wheat varieties, with contrasting grain yield and other agronomic traits, were selected and used to determine a series of physiological and biochemical parameters of primary carbon and nitrogen metabolism in six photosynthetic organs (leaf blade and sheaths, peduncles, awns, glumes and lemmas) during anthesis and grain filling and their relationship with yield under different nitrogen fertilisation regimes.

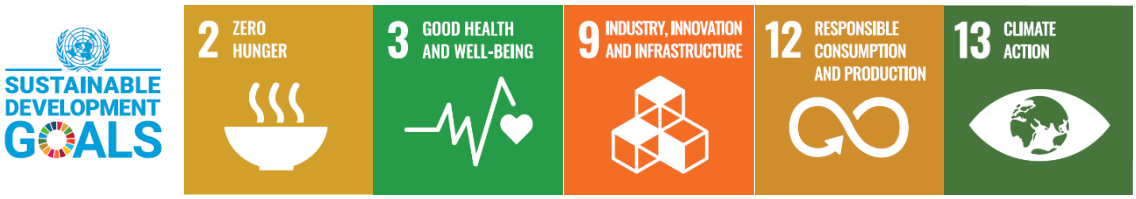
(iv) To assess the effects of water stress on durum wheat at the whole plant level, covering canopy growth, productivity, and primary carbon and nitrogen metabolism in foliar and non-foliar photosynthetic organs, in order to identify the organs and traits of interest in response to water stress that can contribute to design resilient crops adapted to future Mediterranean conditions.

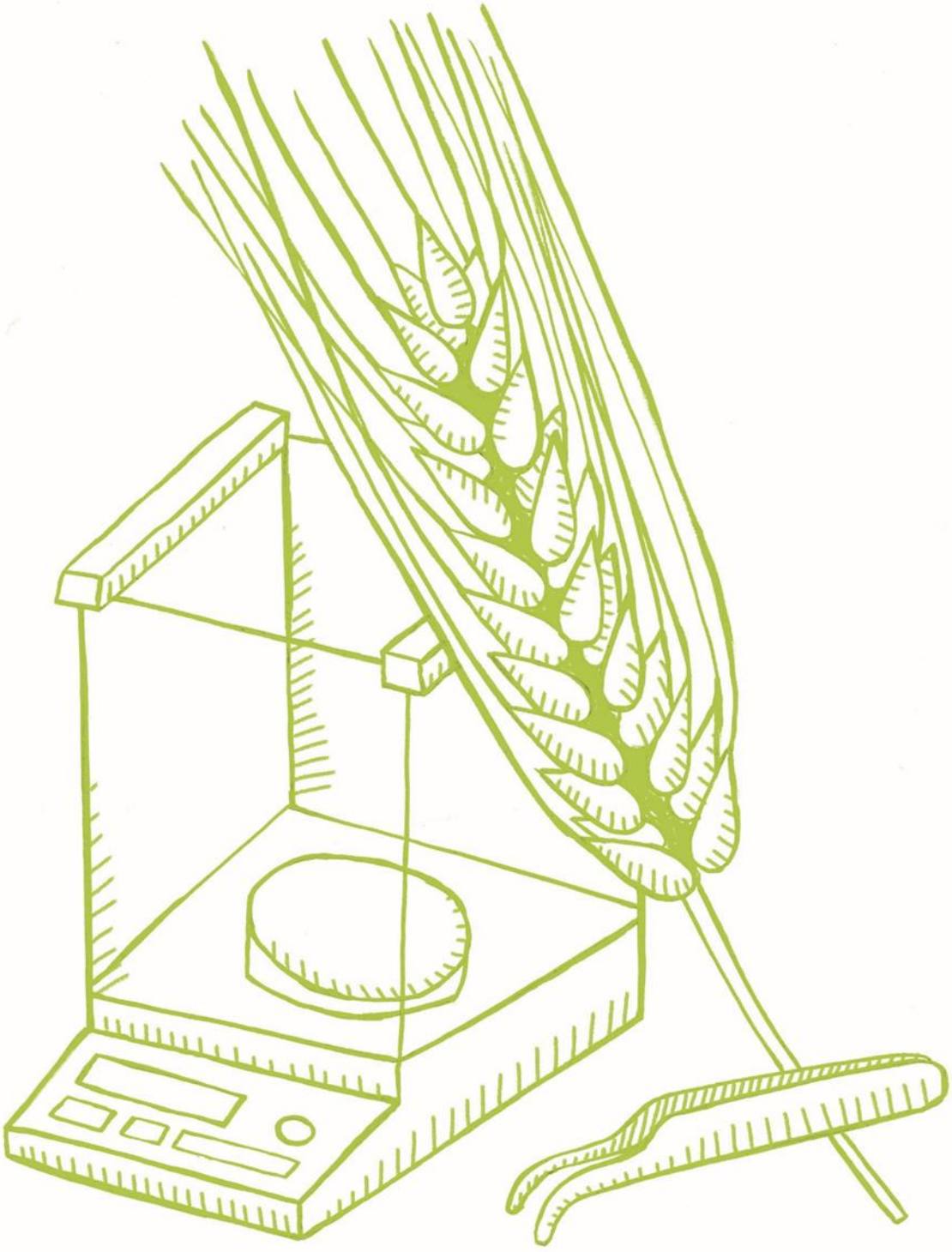
Five varieties within the panel of 24 durum wheat varieties were selected. The study was carried out over one crop season with a significant substantial effect of water stress to evaluate the plant growth performance at canopy and organ level. A particular focus was taken on the characterization of the water stress responses of six photosynthetic organs and their contribution to yield. In addition, an interesting novel approach consisted in quantifying and comparing the carbon and nitrogen metabolic pools expressed as concentration and total content per organ.

(v) The identification of new traits as selection criteria for breeding programmes involving high-yield field and molecular phenotyping.

Phenotyping techniques were used to a greater or lesser extent in all the studies carried out to decipher the effect of the environment on the plant phenotype and to define the key plant and organ-specific vegetation indexes or biochemical traits involved in the response of wheat to specific climatic conditions, as well as to their contribution to grain yield and quality.

The 2030 Agenda for Sustainable Development, adopted by all Member States of the United Nations in 2015, revolves around the 17 Sustainable Development Goals (SDGs), of which the ones covered in this doctoral thesis are:





Chapter 3

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Material and methods

3. CHAPTER 3: MATERIALS AND METHODS

3.1. Experimental design

3.1.1. Plant material

A panel of twenty-four durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.)) varieties released during the last 40 years (Post-Green Revolution) were selected based on their importance as the most representative cultivated varieties in Spain after 1980 (Table 3.1). The panel of varieties has been provided by the projects AGL2013-44147-R (years 2014-17) and AGL2016-76527-R (years 2017-20), in which they had been used to achieve the objectives proposed.

Table 3.1. List the varieties used with their corresponding year of release (ascending order), country, origin, or pedigree.

Variety	Year of release	Country	Pedigree/cross name or origin
Mexa	1980	SPAIN	GERARDO-VZ-469/3/JORI(SIB)//ND-61-130/LEEDS
Vitrón	1983	SPAIN	TURCHIA-77/3/JORI-69(SIB)/(SIB)ANHINGA//(SIB)FLAMINGO
Simeto	1988	ITALY	CAPEITI-8/VALNOVA[1620][1622][1623][1625][1666]
Regallo	1990	SPAIN	Diputación General de Aragón-CIMMYT
Pedroso	1993	SPAIN	Batle seeds
Gallareta	1994	SPAIN	RUFF/FLAMINGO//MEXICALI-75/3/SHEARWATER
Claudio	1998	ITALY	SEL.CIMMYT-35/DURANGO//ISEA-1938/GRAZIA
Iride	1998	ITALY	Altar 84 × Ares sib
Burgos	1999	SPAIN	SUDDEUTSCHE SAATZ
Dorondón	1999	SPAIN	Genética y Gestión,S.C.
Amilcar	2002	ITALY	ZEGZAG-1/LUNDE-5//GREENSHANK-32
Avispa	2003	ITALY	Limagrain-CIMMYT
Saragolla	2004	ITALY	Iride/0114
Solea	2005	SPAIN	Monsanto Agriculture Spain
Euroduro	2007	SPAIN	IRTA
Don Ricardo	2008	SPAIN	Agrovegetal-CIMMYT
Core	2009	SPAIN	Europgen PROSEME seeds
Kiko Nick	2009	SPAIN	SEL.CIMMYT-35/DURANGO//ISEA-1938/GRAZIA
Athoris	2011	ITALY	Limagrain Europe
Sculptur	2011	France	RAGT Semence
Don Norman	2012	SPAIN	Agrovegetal-CIMMYT
Olivadur	2013	SPAIN	RAGT 2N SAS seeds
Iberus	2014	SPAIN	Agromonegros
Haristide	2015	FRANCE	Caussade Semences S.A.

3.1.2. Growth environmental conditions

The field experiments were conducted over five consecutive growing seasons across six years (2014-2019) at Zamadueñas's Experimental Station, belonging to the Agro-technological Institute of Castilla y León (ITACyL) in Valladolid, Spain (41° 41′ N, 04º 42′ W, 700 m above sea level) (Figure 3.1). The plants grew under four contrasting growth conditions such as rainfed (R-), support irrigation (R+), late-sowing (L) and different levels of fertilization (N-) (Table 3.2). In the experimental field, the soil is xerofluvent with a sandy loam texture with alkaline pH.

Figure 3.1. Aerial picture and representation of the trail design done over the crop seasons.

The experimental design was an *alpha lattice* with three replications of 6 m long per 1.5 m wide (9 m^2) per plot, with six rows and 0.25 m of space between them, with 72 plots (Figure 3.1). The seeding rate was set up at 250 seed m^{-2} for each variety. The trials were sown approximately in November, and the emergence was close to December for every crop season. For the late sowing the sowing date was delayed to February. In July-August, harvesting was performed by a combine harvester about 30–35 days after reaching physiological maturity (Table 3.2). The climate in the study area is the continental Mediterranean, classified as Csb (temperate, dry summer, warm summer) according to the Köppen-Geiger classification, which is predominant in the northwestern and inland regions of Spain (Beck *et al*., 2018). In addition, the climate conditions were recorded with an automated meteorological station close to the field experiments. Environmental conditions during the growing seasons are detailed in Figure 3.2.

The similar fertilization protocol was followed in each season. Before sowing, all trials received a basal application of 300 kg ha^{-1} of 8-15-15 nitrogen, phosphorus and potassium (NPK). Further, the plants were dressed with nitrogen. During the first two seasons, all fertilizer was applied at tillering, using a dose of 300kg ha^{-1} of calcium ammonium nitrate (CAN 27%). The

following seasons, the nitrogen was applied at the beginning of tillering and jointing using a dose of 150 kg ha^{-1} of CAN 27% and ammonium nitrosulfate (SAN 26%), respectively. In the case of nitrogen deprivation (N-), a different level of fertilization was applied (Table 3.2). The plants relied exclusively on the soil's natural nitrogen availability before sowing last season. Weeds, insect pests, and diseases were controlled using the recommenced agrochemicals to avoid yield losses.

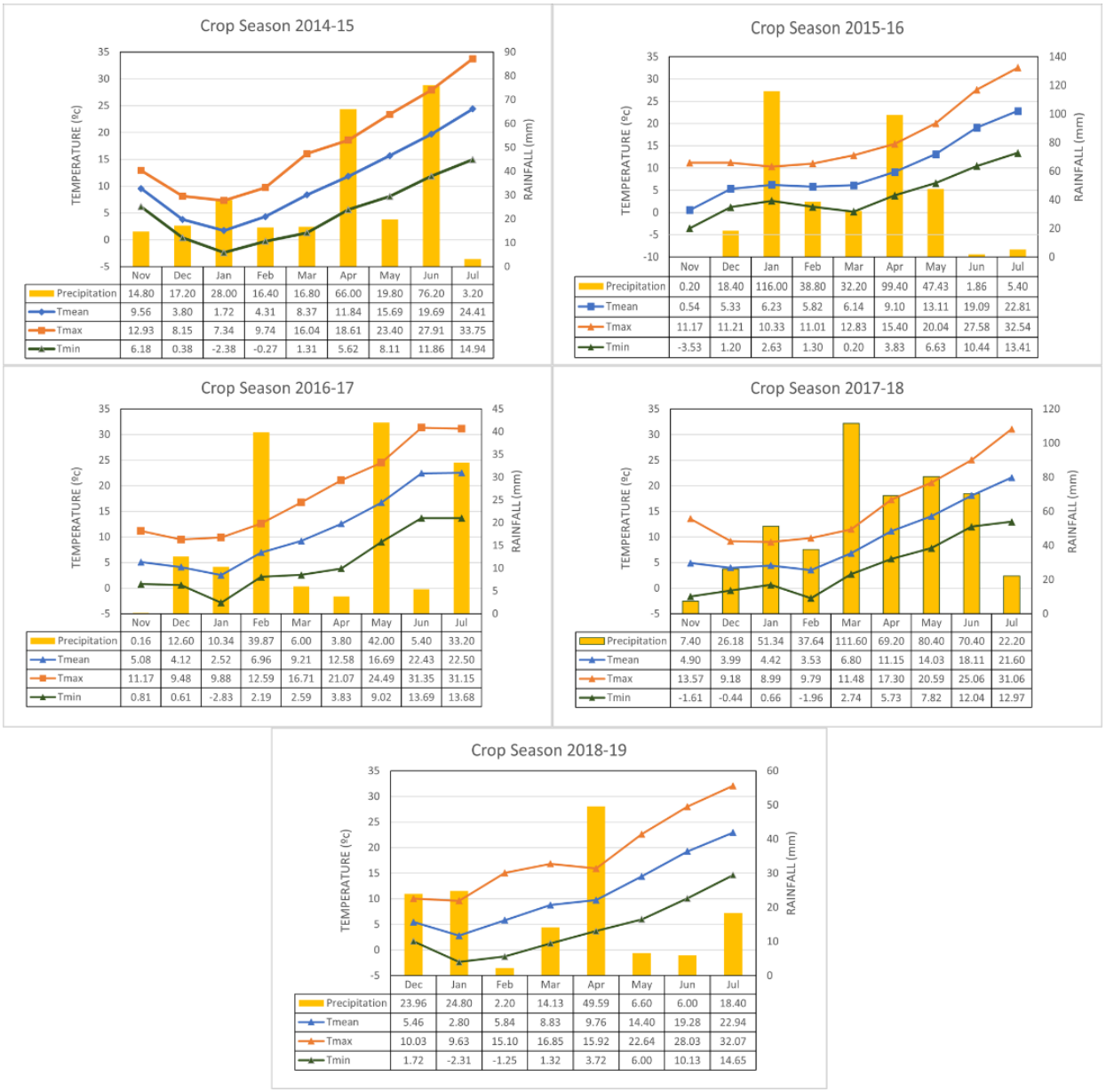


Figure 3.2. Monthly precipitation and temperature (maximum, mean, and minimum) during the five growing seasons at the Experimental Station of Zamadueñas (Valladolid, Spain) across the six years.

Table 3.2. Description of the field experiments carried out, including the year, the treatment (R+, irrigated; R-, rainfed; L, late sowing; N- low nitrogen supply), the sowing, heading and harvest dates, the climate conditions (mean temperature and humidity), the rainfall and support irrigation received during the growth cycle, and the fertilised applied (basal and top dressing). NPK, Nitrogen-phosphorus-potassium; CAN, Calcium ammonium nitrate; SAN, Ammonium nitrosulfate; UN, Units of nitrogen; UP_2O_5, Units of phosphorus; UK_2O, Units of potassium.

										Basal	Top dressing						
Season	**Treatment**	**Sowing date**	**Heading date**	**Harvesting date**	**Range of mean temperature (ºC)**	**Range humidity (%)**	**Rainfall (mm)**	**Irrigation (mm)**	**Total Water received (mm)**	**8-15-15 NPK (kg ha^{-1})**	**1º 27 % CAN**		**2º 26% SAN**		**U N**	**U P_2O_5**	**U K_2O**
2014-2015	**R+**	24/11/2014	29-Apr/08-May	22/07/2015	17.45 - 4.64	93.47 -46.62	258.40	125.00	383.40	300 (23-Nov)	300 kg ha^{-1}	20/02/2015			105	45	45
	R-	24/11/2014	29-Apr/08-May	22/07/2015	17.45 - 4.64	93.47 -46.62	258.40		258.40	300 (23-Nov)	300 kg ha^{-1}	20/02/2015			105	45	45
2015-2016	**R+**	30/11/2015	07-May/18-May	20/07/2016	16.50 - 4.32	97.52 - 51.89	360.69	70.00	429.69	300 (29-Nov)	300 kg ha^{-1}	22/02/2016			105	45	45
	R-	30/11/2015	07-May/18-May	15/07/2016	16.91 - 4.53	96.97 - 51.01	360.69		359.69	300 (29-Nov)	300 kg ha^{-1}	22/02/2016			105	45	45
2016-2017	**R+**	29/11/2016	25-Apr/4-May	06/07/2017	18.21 - 4.31	95.11 - 41.64	123.97	155.00	278.97	300 (7-Nov)	150 kg ha^{-1}	17/02/2017	150 kg ha^{-1}	21/03/2017	103.5	45	45
	R-	29/11/2016	22-Apr/28-Apr	06/07/2017	18.21 - 4.31	95.11 – 4.,64	123.97	55.00	178.97	300 (7-Nov)	150 kg ha^{-1}	17/02/2017	150 kg ha^{-1}	21/03/2017	103.5	45	45
	L	09/02/2017	10-May/20-May	20/07/2017	23.10 - 7.37	93.02 - 29.55	100.59	155.00	255.59	300 (22-Feb)	300 kg ha^{-1}	21/03/2017			105	45	45
2017-2018	**R+**	13/11/2017	11-May/19-May	25/07/2018	16.17 - 4.34	99.56 - 52.42	476.36	109.80	586.16	300 (12-Nov)	150 kg ha^{-1}	20/02/2018	150 kg ha^{-1}	17/04/2018	103.5	45	45
	R-	23/11/2017	11-May/19-May	20/07/2018	15.83 - 4.48	99.57 - 53.84	475.96		475.96	300 (22-Nov)	150 kg ha^{-1}	20/02/2018	150 kg ha^{-1}	17/04/2018	103.5	45	45
	N-	23/11/2017	11-May/19-May	20/07/2018	15.83 - 4.48	99.57 - 53.84	475.96		475.96	300 (22-Nov)					24		
2018-2019	**R+**	03/12/2018	1-May/14-May	15/07/2019	18.01 - 3.63	97.59 - 46.58	145.88	152.70	298.58	300 (16-Nov)	150 kg ha^{-1}	28/02/2019	150 kg ha^{-1}	12/04/2019	103.5	45	45
	R-	03/12/2018	2-May/13-May	03/07/2019	17.15 - 2.96	97.59 - 47.86	127.48		127.48	300 (16-Nov)	150 kg ha^{-1}	28/02/2019	150 kg ha^{-1}	12/04/2019	103.5	45	45
	N-	03/12/2018	2-May/10-May	03/07/2019	17.15 - 2.96	97.59 - 47.86	127.48		127.48								

3.2. Sampling procedure

3.2.1. Selection of a subset of varieties

We selected a representative subset of five durum wheat varieties from the panel for the physiological and biochemical analyses based on their phenology and grain yield (Figure 3.3).

Mexa (released in 1980) is one of the varieties from CIMMYT that was grown on almost 90% of the durum wheat area during the mid-1980s. It is characterised by a high yellow pigment, protein content, and gluten strength. It has a medium-large height.

Euroduro (2007) is a durum wheat variety released by The Institute of Agrifood Research and Technology (IRTA) in cooperation with the private sector (Guadalsem) and characterized by a high semolina quality (protein content, test weight, vitreousness and yellow pigment), adaptability and earliness heading, having an ear with average size.

Don Ricardo (2008) is a variety released by Agrovegetal company, with high yield recorded in the south of Spain and exceptional resistance to lodging. It has a large grain size and thousand kernels weight, combined with suitable grain quality parameters.

Kiko Nick (2009) is a variety obtain by LG Seeds with high technological, semolina and grazing quality. It has medium precocity of flowering and ripening with high resistance to lodging. It is recommended for dry and irrigated areas because of its productivity and resistance to the new race of leaf rust.

Haristide (2015) is a variety with a later cycle than the other varieties (more adapted to grow in winter conditions), similar to bread wheat. It has a delayed cycle with a spike length of medium to high. It has high productivity and suitable technological quality parameters (test weight, protein content, vitreousness and yellow pigment). It was released by Caussade (France).

Figure 3.3. Field picture of the different varieties evaluated.

3.2.2. Sample processing

The physiological and biochemical analyses of the subset of varieties of interest were performed during the reproductive phase of the durum wheat cycle, specifically at anthesis, early grain filling and late grain filling. The **anthesis** was recorded when 50% of the ears showed extruded anthers along their head lengths (Zadoks 65). The **early grain filling** stage was annotated when the grain was still medium milk and increased in solids of liquid endosperm, notable when crushing the caryopsis between fingers (Zadoks 75). Finally, **late grain filling** was considered when the kernel presented the hard enough to make a fingernail impression but not hold the grain, and the inflorescence still does not lose its greenery (Zadoks 85) (Zadoks *et al*., 1974). At these growth stages, five different plants were collected per plot (three biological replicates per variety and treatment). Then the different green photosynthetic organs were separated from each plant: the blade and sheath from flag leaves, the peduncle and the ears, which were immediately plunged into liquid nitrogen. These samples were stored at -80 °C. First, the laminar organs (leaf blades and sheaths) and peduncles were homogenized to a fine powder using a Mixer Mill MM300 (Retsch GmbH, Haan, Germany) (Figure 3.4). Secondly, other non-laminar organs belonging to the ears (awns, lemmas and glumes) were ground manually using a mortar and pestle with liquid nitrogen. To prevent thawing of the plant material, regular ice (frozen H_2O, ~0°C), dry ice (frozen CO_2, approx. -78.5°C) and liquid nitrogen (N_2, approx. -196°C) were used during the processing of the samples.

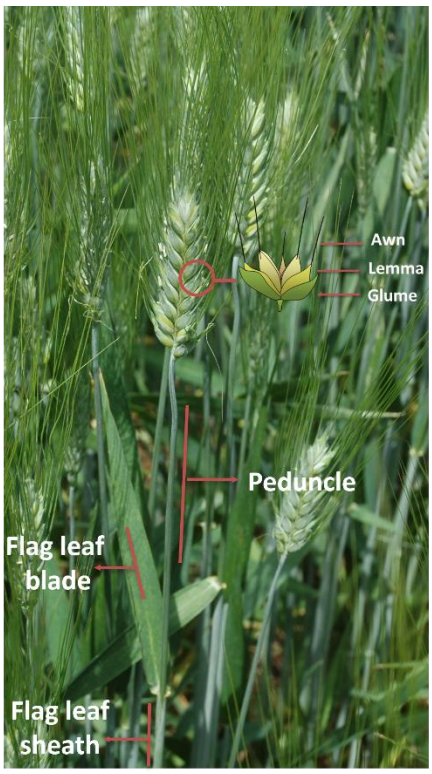


Figure 3.4. Different organs sampled and separated from *Triticum turgidum* L. ssp. *durum* (Desf.).

The separation of the photosynthetic organs under low temperatures to maintain intact the plant metabolism was repeated with the five plants per plot and different genotypes. Then, the samples were weighed to obtain fresh weight (FW) and be used for the subsequent laboratory biochemical analyses (see below). First, an aliquot of each sample was oven-dried at 70 °C for 48 h and weighed to obtain dry weight (DW). Then, the samples were finely ground for carbon/nitrogen isotope and nutrient content analyses (Figure 3.5). With these values obtained, the water content (WC) was calculated per organ, following the next equation:

$$WC\ (\%) = [(FW - DW)/FW] \times 100$$

Figure 3.5. Picture showing how the processing, grinding and weighing of the plants collected from the field trials were performed.

3.2.3. LRWC

The leaf relative water content (LRWC) was determined according to Estévez-Geffriaud *et al*. (2020). Firstly, the FW of the flag leaf blades (the fully expanded newest leaf from the main tiller) was measured. Secondly, the turgid weight (TW) was measured in the same leaf after the incubation for 24 h in deionized water in the dark at 4°C. Thirdly, the DW was obtained by drying the tissue for at least 48 h in an oven at 70°C until getting a constant weight. Then, the LRWC was calculated by using the following equation:

$$LRWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100$$

3.3. Phenotyping

The phenotyping measurements, including RGB images and GreenSeeker and DUALEX measurements, were taken on sunny days around noon (12–14 h, UTC+1).

3.3.1. RGB images 

For each plot of all the varieties studied in the different field trials, RGB images were taken throughout the whole crop cycle in the most representative stages of the plant. For that, a 20.1-megapixel camera (Sony ILCE-QX1, Sony Corporation, Japan) attached to a VCTMP1 monopod (Sony Corporation, Japan) was used at a distance of one metre above the canopy in a zenith plane and focused near the centre of the plot. The camera had a sensor size of 23.20 x 15.40 mm, a focal length of 35 mm and trigged and the exposure time was programmed in automatic mode.

Images were analysed with the JAVA8-adapted BreedPix 0.2 software (Casadesús *et al*. 2007), integrated as a plugin in the open-source image analysis platform FIJI (Fiji is Just ImageJ; http://fiji. sc/Fiji). This software allowed the estimation of RGB vegetation indices (VIs) related to different colour properties. The calculation of RGB indices in the canopy was based on the sum of frequencies of the histogram classes included in a specific Hue range in the image. In the HSI colour space (H, hue; S, saturation; I, intensity), the Hue component describes the colour traversing the visible spectrum at an angle between 0º and 360º, where 0º means red, 60º means yellow, 120º means green, and 180º means green cyan. Two of the most valuable indices were the relative green area (GA) and green plus green area (GGA), which are the percentage of pixels in the image (values between 0 and 1) in the hue range 60° to 180° (yellow to blue-green) and 80° to 180° (yellowish-green to blue-green), respectively (Vergara-Diaz *et al*. 2016; Casadesús *et al*. 2007). The GGA is more restrictive than the GA in excluding yellowish-green tones and, therefore, more accurately describes photosynthetically active biomass. In addition, those two indexes were used to formulate the Crop Senescence Index (CSI), which provides a scaled ratio between yellow and green vegetation pixels, which was calculated as follows (Zaman-Allah *et al*., 2015) (Figure 3.6).

$$CSI = \frac{(GA - GGA)}{GA}\ x\ 100$$

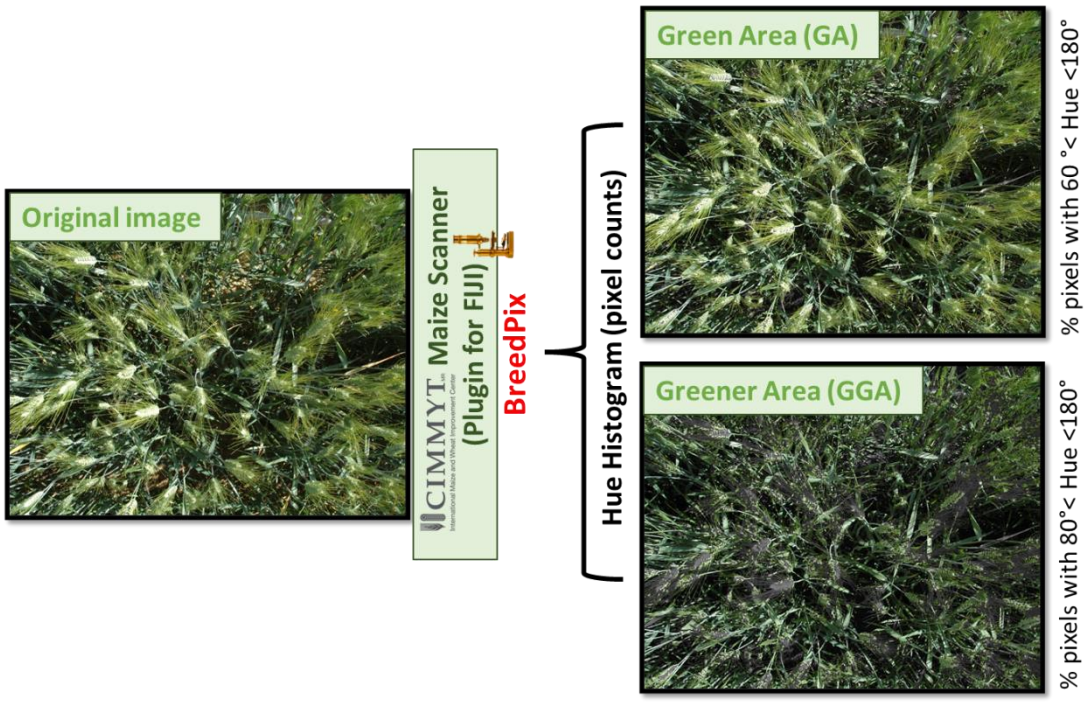


Figure 3.6. Representation of the analysis process to obtain the vegetation indices GA and GGA with the BreedPix software. The images for GA and GGA were focused on counting the green pixels and avoiding other colours, with GGA being more restrictive by excluding yellowish-green tones.

3.3.2. *In vivo* measurements

3.3.2.1. GreenSeeker

The canopy's normalized difference vegetation index (NDVI) (Rouse *et al*., 1974) was measured in each plot using a hand-held portable spectroradiometer (GreenSeeker, NTech Industries, USA) during all growing seasons, coincidently with the most relevant development stages such as tillering, jointing, anthesis and grain filling. The sensor has a self-illumination system that collects reflectance from the plant canopy in red (656 nm) and near-infrared (774 nm) bands. When the trigger is depressed, the sensor emits continuous bursts of light pulses while sampling the scanned area. Although the sensor's field of view is oval, its coverage increases the higher the sensor is above the ground, covering maximum a distance of 50 cm. The sensor automatically calculates NDVI as follows:

$$NDVI = \frac{(NIR - R)}{(NIR + R)}$$

where R is the reflectance in the red band and NIR is the reflectance in the near-infrared band. The NDVI measurements were made by walking through the plot at a speed of about 0.5 m s^{-1} and holding perpendicular to the sensor's canopy about 0.6 m above, avoiding the plot's border. The range of NDVI is -1 to +1. The value obtained is the average per plot, and the measurements were considered to end when GreenSeeker values went below 0.20.

3.3.2.2. DUALEX

In the case of the flag leaf, the relative content of chlorophylls (chl), flavonols (flav) and anthocyanins (anth), as well as the nitrogen balance index (NBI) as the ratio between chlorophylls and flavonols, were measured in the most crucial phenology stages with the leaf-clip meter DUALEX® (Force A SCIENTIFIC, France). The clip area is 19.6 $mm^{2,}$ and the samples cannot have more thickness than 1.5 mm. The measurements were done in the middle of the flag leaf blade (avoiding the veins) of five plants per plot selected randomly, and the values were averaged per plot. These plants were the same collected for the biochemical analyses described below. This portable device had five different light sources, which let it performs non-destructive measurements of transmittance ratio at two different wavelengths. One in the far-red (absorbed by

chlorophyll) and one in the NIR as reference. Chlorophyll fluorescence in the NIR is measured under a first reference excitation light pulse at which the polyphenols do not absorb it. This measurement is automatically compared with a second pulse of specific light absorbed by the polyphenols (e.g., green for anthocyanins or ultraviolet (UV) for flavonols). Only a fraction of this light reaches the chlorophyll in the mesophyll and can generate chlorophyll fluorescence in the NIR. This measurement process is called the 'screening effect' of polyphenols on chlorophyll fluorescence.

The values obtained were expressed as Dualex® units, which were recorded until the values went below 0.20.

3.3.3. Isotope analysis

3.3.3.1. Carbon isotope analysis

The stable carbon isotope composition ($\delta^{13}C$) and percentage in dry matter of the grain at harvest were determined in all the varieties of our durum wheat panel, and the developing grains at Zadoks stages 75 and 85. The samples were ground into a fine powder using a Mixer Mill MM300 (Retsch GmbH, Haan, Germany). In addition, flag leaf blades and sheaths, peduncles, awns, glumes, and lemmas collected at the Zadoks stages 65 and 75 were also grounded into a fine powder and analysed. Isotopic analyses were carried out by the Scientific-Technical Services of the University of Barcelona, Spain. For $\delta^{13}C$ analysis, approximately 1 mg of each sample was weighed into tin capsules, sealed and then loaded into an Elemental Analyser (EA; EA1108, Series 1, Carlo Erba Instrumentazione, Milan, Italy), coupled with an Isotope Ratio Mass Spectrometer (IRMS; Delta C with CONFLO III interface, Thermo Fisher Scientific, Bremen, Germany). The stable C isotope ratio was determined while operating in continuous flow mode. The $^{13}C/^{12}C$ ratio of the plant material was expressed in δ notation (Coplen, 2008) as follows:

$$\delta^{13}C = \frac{\left(^{13}C/^{12}C\right)_{sample}}{\left(^{13}C/^{12}C\right)_{standard}} - 1$$

where ‘sample’ refers to plant material and ‘standard’ to secondary international standards of known $^{13}C/^{12}C$ ratios (International Atomic Energy Agency, IAEA CH_7 polyethylene foil, IAEA CH_6 sucrose and the United States Geological Survey, USGS 40 l-glutamic acid) calibrated against

Vienna Pee Dee Belemnite calcium carbonate (VPDB) with an analytical precision (standard deviation) of 0.10‰.

The Beelemite Pee Dee (PDB) is usually the standard selected based on a Cretaceous marine fossil, *Belemnitella americana*, from the Peedee Formation in South Carolina. This material had an anomalously high ^{13}C:^{12}C ratio (0.01118) and is considered a $\delta^{13}C$ value of zero. However, since the original PDB specimen is no longer available, its ^{13}C:^{12}C ratio is currently calculated from a widely measured carbonate standard NBS-19, with a $\delta^{13}C$ value of +1.95 ‰ (Brand *et al.*, 2014).

3.3.3.2. Nitrogen isotope analysis

The stable nitrogen isotope composition ($\delta^{15}N$) and N concentration were analysed in all the organs as discussed above for the C isotope composition. To that end, 1 mg of fine powder was placed into tin capsules for analytical determinations. The measurements were carried out at the Scientific Facilities of the University of Barcelona (Spain). The samples were analysed using the EA-IRMS coupled in a continuous flow. The amount of ^{15}N atoms in the samples was calculated by atom% abundances (A) as described by Robinson *et al.,(*2000):

$$A = \frac{^{15}N}{^{15}N + {}^{14}N}\ x\ 100$$

where ^{15}N and ^{14}N are the numbers of ^{15}N and ^{14}N atoms present in the plant sample. IAEA N_1, IAEA N_2 ammonium sulfate and IAEA NO_3 potassium nitrate were used as secondary isotope standards of known $^{15}N/^{14}N$ ratios, referred to as atmospheric N_2, for calibration to a precision of 0.18‰.

The analyses of C and N concentration in the grains at harvest allowed us to calculate grain C yield (GCY, kg ha^{-1}) and grain N yield (GCY, kg ha^{-1}) as follows:

$$GCY = [grain\ C\ content\ (\%) \times GY(kg\ ha^{-1})]/100$$

$$GNY = [grain\ N\ content\ (\%) \times GY(kg\ ha^{-1})]/100$$

3.4. Nutrient composition

Approximately 500 mg of dry organ powder were weighed in 1.5 mL plastic containers to measure mineral nutrients. The aliquots of the plant material were transferred to a Teflon digestion tube and mixed with 8 mL of 65 % HNO_3 and 2 mL of 30 % H_2O_2. At controlled pressure, samples were heated at 200°C in an ETHOPS UP (Milestone) microwave digestion system and digested at different temperature ranges of variable duration. Soon after the digested solution was cooled, it was diluted to 25 mL with deionised water. The macro and micronutrients (K, Ca, P, Mg, Fe, Mn and Cu) were quantified by inductively coupled plasma optical emission spectrometry with an ICP-OES Varian 720-ES (Agilent) in the different photosynthetic organs studied.

In addition, the nutrient composition was also examined in developing grains (Zadoks stages 75 and 85) and mature grains at harvest. The grain nutrient concentration of macro and micronutrients (K, Ca, P, Mg, S, Fe, Mn, Na, Mo, Cu and Zn) and the total content of each mineral (obtained by multiplying by the yield) were determined. The analyses were performed at the Analysis and Instrumentation Service of IRNASA-CSIC (Salamanca, Spain).

3.5. Physiological and biochemical determinations

3.5.1. Ethanolic extraction

The ethanolic extraction was carried out to measure metabolites, as described by Stitt *et al.* (1989). Initially, 250 µL of a solution made of 80% ethanol and 10 mM HEPES-KOH pH 7.0 were added to each frozen aliquot (approximately 20 mg of fresh material) and mixed using a vortex shaker on a heat block for 30 min at 80°C. After heating, samples were cooled down on the ice and centrifuged at 14000 rpm and 4°C for 5 min. Then, the supernatant was added into a clean microtube, and the pellet was re-extracted again with another 150 µL of 80% ethanol and 10 mM HEPES-KOH pH 7. The samples were shaken again in a vortex, heated on a heat block for 30 min at 80°C and centrifuged under the same conditions described above. This new supernatant was mixed with the previous one, and the pellet was re-extracted an again with 250 µL of 50% ethanol and 10 mM HEPES-KOH pH 7, integrated on a vortex shaker, heated at 80°C on a heat-block for 30 min and centrifuged under the same conditions as described above. Again, the supernatants were mixed, adding up to around 650 µL. Finally, an extra extraction step with 350 µL of ddH_2O and 10 mM HEPES-KOH pH 7.0 was made exclusively to extract the high molecular weight

fructans, with are soluble in water but not in ethanol. The supernatants were covered from the light throughout the entire extraction to avoid chlorophyll degradation. The ethanol was removed before the following analyses to determine metabolites to avoid interferences with other compounds at 70ºC using a vacuum dryer Lyoalfa 60 (Telstar) coupled to a Savant Spead Var SPD 121P centrifuge (Thermo Scientific). First, the samples were used for chlorophyll determination (see next section), and after that, they were lyophilised and resuspended into the same volume with deionised water. The supernatants and pellets from each sample were stored at -80°C for the following analyses.

3.5.1.1. Chlorophyll content

Chlorophylls (chl) a and b were extracted and quantified using the ethanolic extracts described above. After a quick centrifugation of the supernatants to allow the precipitation of any particle, 50 µl of the extract was added to 120 µL EtOH 100% in each well of a 96-well plate. Each sample had two technical replicates. Next, the plate was vortexed, and, finally, the absorbance was measured at 645 and 665 nm wavelengths in an 800™ TS Absorbance Reader (Biotek). For chlorophyll determination, we used the following equations described by Lichtenthaler (1987):

$$Chl\ a\ \left(\frac{\mu g}{well}\right) = 5.48\ x\ A_{665} - 2.16\ x\ A_{645}$$

$$Chl\ b\ \left(\frac{\mu g}{well}\right) = 9.67\ x\ A_{645} - 3.04\ x\ A_{665}$$

$$Chl\ a\ or\ b\ \left(\frac{\mu g}{g\ FW}\right) = Chl\ a\ or\ b\ \left(\frac{\mu g}{well}\right) x\ \frac{extraction\ volume\ (650\ \mu L)}{sample\ colume\ (50\ \mu L)}\ x\ \frac{1}{FW\ (mg)}$$

where A_{665} and A_{645} are the absorbances at 665, and 645 nm, respectively, and FW is the fresh weight of the sample.

3.5.1.2. Soluble sugars

The determination of soluble sugars, such as free glucose (glc), fructose (fru), sucrose (suc) and fructans, was performed in the sample extracted with ethanol and water by a series of oxidation-reduction reactions (Jones *et al*., 1977; Stitt *et al.*, 1989). The reactions were monitored in the Synergy 2 Multi-Mode Microplate Reader (BioTek) spectrophotometer. For this, the commercial Test D-glucose Boehringer Mannheim/R-biopharm kit (Roche) was used. In the first step, an aliquot of the sample was transferred to a 96-well plate and mixed with 100 µL of a buffer (Boheringer buffer) containing 3 mM NADP, 10 mM ATP, Mg_2SO_4 and 86 mM TEA at pH 7.6. Each sample had two technical replicates. After adding ultrapure water to a volume of 200 µL, the plate was centrifuged at 12000 rpm for 10 sec in a Sorvall ST16R plate centrifuge (Thermo Scientific). Next, the plate was placed in the spectrophotometer Synergy 2. The initial absorbance was measured for approximately 5 min until the OD was stabilized, and an aliquot of the mixture of enzymes glucose-6-phosphate dehydrogenase (G6PDH) and Hexokinase (HK) was added. The latter enzyme catalysed the phosphorylation of glc and fru to glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P), respectively (reaction 1, Rc 1), while G6PDH catalysed the oxidation of G6P to 6-phosphogluconate in a reaction that releases NADPH (reaction 2, Rc 2). The reduction of NADP to NADPH can be monitored at 340 nm, using a wavelength of 400 nm as a reference. The increase in absorbance associated with NADPH formation in these reactions was equivalent to the amount of free glc in the sample. In a subsequent step, when the OD stabilized again after approximately another 20 min, the enzyme phosphoglucose isomerase (PGI) transformed F6P into G6P, which was, in turn, converted into 6-phosphogluconate (reaction 3, Rc 3). The increase in absorbance in this step was associated with the content of free fru in the sample. Each time the OD stabilized, the value was recorded for later calculations of sugar concentration in the plant material. The content of both carbohydrates was expressed in µmol g FW^{-1}.

The sequence of enzymatic reactions is shown below:

HK: Glc + Fru + 2 ATP → G6P + F6P + 2 ADP **(Rc 1)**

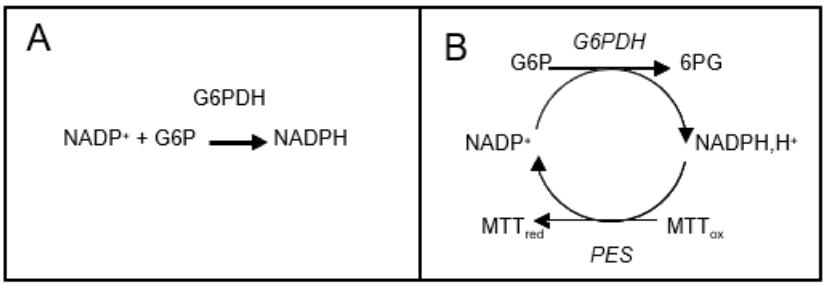
G6PDH: 2 G6P + 2 NADP+ → 2 6-Phosphogluconate + 2 NADPH **(Rc 2)**

PGI: F6P → G6P **(Rc 3)**

The determination of suc was carried out after the hydrolysis of the disaccharide into glc and fru by the action of the enzyme sucrase (McCleary *et al.*, 2000) and its subsequent calculation by the previous procedure to quantify monosaccharides. First, a new aliquot of the ethanolic-aqueous extract was transferred to a 96-well plate, and 4 µL of 100 mM sodium maleate buffer at pH 6.5 was added, together with 8 U of sucrase (Megazyme) and ultrapure water up to a volume of 54 µL. Next, the plate was covered with an aluminium adhesive foil to prevent the evaporation of the extracts during the incubation at room temperature for 30 min. Afterwards, the content of glc and fru was determined, and the values of free glc and fru were subtracted to obtain only those corresponding to suc. The final result of suc was expressed as the content of glc in µmol g FW^{-1}, since this and fru are equimolecular in suc.

Similarly, the determination of fructans was carried out by analysing the glc, and fru generated after their hydrolysis using the enzymes fructanases (Fructanase Mixture Purified-Liquid, Megazyme). The evaluation of the monosaccharides was as detailed above, measuring the extracts at 340 nm. First, an aliquot of the ethanolic-aqueous extract of the leaf samples was mixed with 2 µL of 100 mM sodium acetate buffer at pH 4.5, 2 U of fructanases and ultrapure water added to a volume of 54 µL. Next, the samples were incubated on a plate covered with aluminium foil for 30 min, and then the corresponding measurements were at 340 nm. Finally, the content of fructans was expressed as the content of glc plus fru as µmol hexoses g FW^{-1} after subtracting the free glc and fru contents and those associated with sucrose.

In the case of G6P, it was determined following the protocol of Gibon *et al.* (2002), based on the principle of these two reactions:



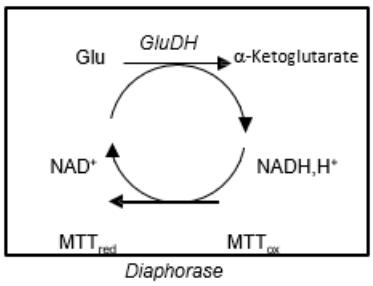
In a Sarstedt microplate, the two reactions were performed. Firstly, 5 µL of the ethanolic extracts were mixed and incubated at 25ºC for 20 min with the enzyme G6PDH and $NADP^+$ with

different standard concentrations of G6P. Immediately after, the reaction was stopped with the addition of NaOH. Then, the plate was sealed and heated to destroy the remaining $NADP^+$. Next, HCl was added to the plate to neutralise the pH. Finally, the catalyst reaction was prepared by adding methylthiazolyldiphenyl-tetrazolium bromide (MTT), G6P and the enzyme G6PDH and phenazine ethosulfate (PES) to the microplate (protected from the light during the process), and the absorbance was measured at 570 nm to quantify the NADPH generated, associated with the G6P content.

3.5.1.3. Total Amino acids and glutamate contents

Total amino acids were determined using the fluorescamine method described by Bantan-Polak *et al.* (2001). First, in a black microplate it was dispensed the following solutions in this order: 100 µL water, 15 µL sodium borate buffer 0.1 M pH 8.0, 2 µL extract/standard and 90 µL fluorescamine. Next, the plates were mixed and incubated at room temperature for 5 min in complete darkness before being read in the Synergy reader at 405 nm of excitation and 485 nm of emission. During the last step, the lights had to be switched off to protect the fluorescamine from degrading since it is light sensitive. Finally, the amino acid content (aa) was calculated using glutamate (glu) as standard from a calibration curve.

For glu determination, we followed the principle described by Cross *et al.* (2006):

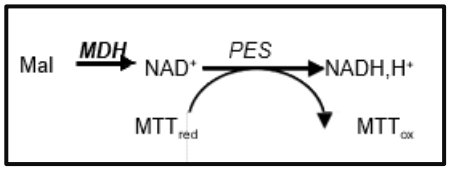


Firstly, 15 µL of extract or standard (using glu again as standard) was mixed with 10 µL tricine buffer 200 mM pH 8.5, 10 µl MTT 10 mM, 10 µL NAD^+ 30mM, 5 µL triton X-100 10%, 2 µL ADP 50mM, 1 µL Diaphorase and the rest of water up to a final volume of 185 µL in a Sarstedt microplate. Once the absorbance was stabilised (5-10 min) at an OD of 570 nm, 1 µL of glutamate

dehydrogenase (GluDH, Roche) was added, and the reaction was monitored for the calculation of glu content.

3.5.1.4. Malate content

The last components determined from the supernatants obtained during the ethanolic extraction were malate (malic acid), measured as described in Cross *et al.* (2006).



Standards were prepared in 20 mM NaOH pH 7.0 with the following concentrations in 70% ethanol: 500 µM, 250 µM, 125 µM and a blank with only 70% ethanol. In addition, 80 µL assay mix, containing 50 µL tricine-KOH 0.2 M pH 9.0, 10 µL thiazolyl blue tetrazolium bromide10 mM, 10 µL NAD^+ 30 mM, 5 µL PES 2.5 mM and 5 µL triton X-100 10%, was prepared and dispensed on a microplate. After adding 10 µL of standard or supernatant to each well containing the assay mix, the absorbance was measured at 570 nm. After OD-stabilization at around 20 min, 1 µL malate dehydrogenase (MDH) was added. Using the standards as a reference, the malate content was calculated.

3.5.1.5. Soluble protein and Starch contents

The resulting pellet from the ethanolic extraction contains molecules not soluble in ethanol, such as starch and proteins, which were quantified according to the following steps.

3.5.1.5.1. Soluble protein

The dye-binding assay determined the total protein content, as described by Bradford (1976). This assay used bovine serum albumin (BSA) as the standard. Firstly, the pellet from the ethanolic extraction was resuspended in 400 µL NaOH 0.1 M, shaken in a vortex for a few seconds and then heated at 95°C for 30 min on a heat block. After leaving the samples at room temperature to cool back down, they were shaken in a vortex and centrifuged at 14000 rpm and 4°C for 10 min. 3 µL

of the supernatant from this centrifugation and 180 µL of 1:8 diluted Bradford solution (Protein Assay Dye Reagent, Bio-Rad) were dispensed in each well of a 96-well microplate. After shaking on a vortex for around ten seconds and incubating for 5 min at room temperature, the OD was measured at 595 nm. The standard BSA was diluted with NaOH 0.1 M and used in the following concentrations: 50 mg mL^{-1}, 5 mg mL^{-1}, 1 mg mL^{-1}, 0.5 mg mL^{-1}, 0.25 mg mL^{-1} and pure NaOH 0.1 M as blank. The soluble protein content was calculated using the absorbances obtained and the BSA calibration curve.

3.5.1.5.2. Starch

The starch content was measured according to Hendriks *et al*. (2003). Firstly, 80 µL of a solution of HCl 0.5 M and acetic acid 0.1 M pH 4.9 was added to the remaining dissolved pellet from the previous step to determine proteins. Next, the sample was shaken in a vortex for a few seconds, and 100 µL of a starch degradation mix was added to each sample. This degradation mix was prepared by adding 1.5 mL amyloglucosidase (Roche), 15 µL α-amylase (Sigma) and 12.5 mL acetic acid 50 mM pH 4.9. Finally, the samples were incubated overnight at 37°C (10-16 h) to allow the degradation of starch granules into glc molecules.

The following day, the samples were vortexed for a few seconds, centrifuged at 14000 rpm and 4°C for 10 min and then put on ice. After some tests to determine the right aliquot volume, we dispensed 50 µL of the supernatant on a microplate with 160 µL of a glucose determination mix. This mix was prepared by adding180 µL G6PDH grade II (Roche), 35 mL HEPES 0.1 mM buffer, 1.083 mL 60 mg ml^{-1} ATP and 1,083 mL 36 mg ml^{-1} $NADP^{+}$. The plates were read at 340 nm for 20 min until the OD stabilized. After that, 1 µL HK was added. When the OD stabilized after approximately 30-40 min, the values were recorded, and the starch content was calculated by the production of NADPH molecules as described above to determine free glc.

The measurement of sugars underlies the following scheme (Stitt *et al.,* 1989):

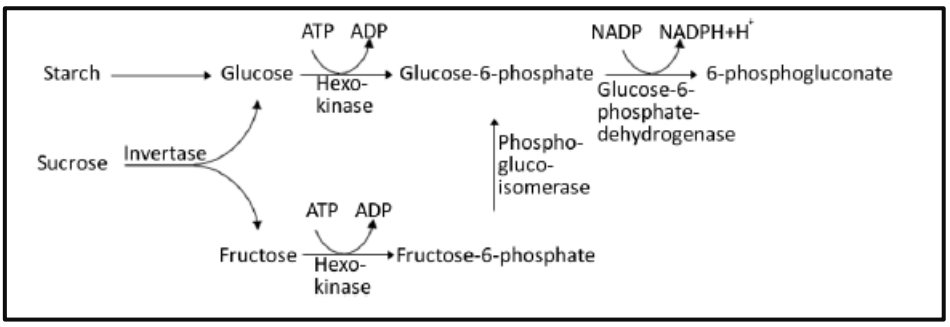


Figure 3.7. The scheme of reactions involved in the measurements of glucose, fructose, sucrose and starch in the supernatants (and the pellet for starch) were obtained from the ethanolic extractions.

3.5.2. Rubisco protein quantification

The Rubisco protein content of the flag leaf samples was measured from the soluble protein extracts. Polyacrylamide gels were prepared for SDS-PAGE electrophoresis (Laemmli, 1970). The protein content was quantified by densitometry from the protein bands corresponding to the large and small subunits of Rubisco (Pérez *et al.*, 2011). Aliquots of finely powdered material from each green organ at Zadoks stages 65 and 75 were used for the extraction of proteins by mixing with 10 volumes of extraction buffer that contains Tris/HCl 62.5 mM pH 6.8, glycerol 10% (v/v), sodium dodecyl sulfate (SDS) 2% (w/v), bromophenol blue 0.0125% (w/v), and β-mercaptoethanol 0.05% (v/v). Afterwards, the samples were heated at 95°C for 5 min and centrifuged at 13000 rpm for 5 min and kept at -20°C until loaded onto electrophoresis gels.

A 0.75 mm thick polyacrylamide gels were prepared using the Mini-PROTEAN Tetra System (Bio-Rad). The separator gel contained polyacrylamide 12.5% (w/v), Tris-HCl 375 mM pH 8.8, SDS 0.1% (w/v) and ammonium persulphate (APS) 0.05% (w/v). The loading gel contained polyacrylamide 5% (w/v), Tris-HCl 125 mM pH 6.8, SDS 0.15% (w/v) and APS 0.05% (w/v). N,N,N',N'-tetramethylethylenediamine (TEMED) was added to both the separator and the loading gel for polymerisation.

The molecular weight marker used was the PageRuler prestained protein ladder (10-180 kDa, Thermo Fisher Scientific, USA), while BSA (Protein Micro Standard, Sigma-Aldrich) was used as a concentration standard. Aliquots of the supernatants of the samples and the standards were loaded onto the gels. The electrophoresis buffer for the cuvette had the following composition: Tris 25 mM, Gly 0.2 M and SDS 0.001% (w/v). The electrophoretic process was carried out at 200 V and room temperature for approximately one hour using an EC 250-90 power supply (EC Apparatus Corporation). After completion, the gel was washed 3 times with water for 5 min, fixed

with a 5:4:1 (v/v/v) solution of water, methanol and acetic acid, respectively, for 15 min in a GelAir Dryer gel heater (BioRad), and kept for 1 h in agitation at room temperature, and then washed with water. The gel was stained with Coomassie brilliant blue R-250 dye (Thermo Fisher Scientific) 0.001% (w/v) for 1 h while shaking at room temperature. After washing off the excess staining solution, the gel was scanned on a ChemiDoc MP Imaging System (Bio-Rad) using Image Lab software. Densitometry with Image Lab software was used to determine the amount of Rubisco's large subunit as a proxy for Rubisco protein content (Pérez *et al.*, 2011; Vicente *et al.*, 2016) (Figure 3.8).

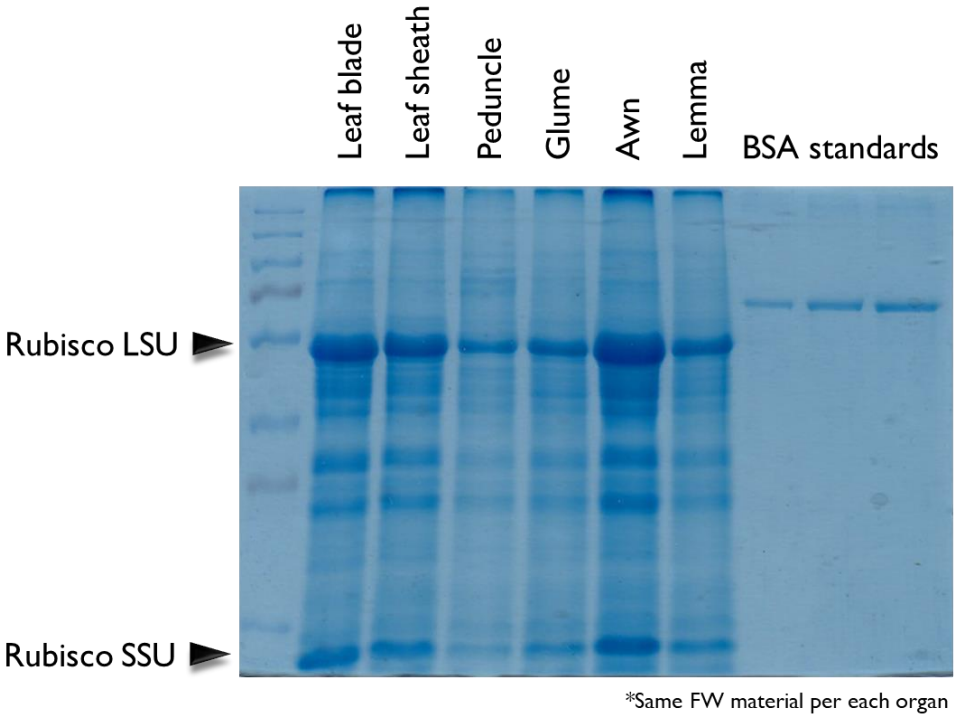


Figure 3.8. Example of a 12.5 % SDS-PAGE obtained using the scan ChemiDoc MP Imaging System (Bio-rad). The protein profiles of the different organs collected (leaf blade, leaf sheath, peduncle, glume, awn and lemma) and the BSA standards are shown in each lane. In addition, the exact amount of fresh weight was loaded in each lane to compare their Rubisco content and three different concentrations of BSA for the quantification. LSU, large Rubisco subunit; SSU, small Rubisco subunit; FW, fresh weight.

3.5.3. Enzyme activities

The enzyme activities of Rubisco (initial and total), phosphoenolpyruvate carboxylase (PEPCase), glutamine synthetase (GS), ferredoxin-dependent glutamate synthase (GOGAT), and NADH-dependent glutamate dehydrogenase (GDH) were determined in green organs at Zadoks stages 65 and 75 in the laminar and non-laminar organs sampled. Enzymes were extracted from

20 mg-aliquots of finely powdered material by adding 10 mg of polyvinylpolypyrrolidone (w/v) and 1 mL of ice-cold extraction buffer containing HEPES-KOH 50 mM pH 7.5, $MgCl_2$ 10 mM, (ethylenedinitrilo)tetraacetic acid (EDTA) 1 mM, ethylene-bis(oxyethylenenitrilo)tetraacetic acid (EGTA) 1 mM, benzamidine 1 mM, ε-aminocapronic acid 1 mM, BSA 0.25 % (w/v), leupeptin 20 mM, 1,4-dithiothreitol 0.5 mM, Triton X-100 1% (v/v), glycerol 20% (v/v), and phenylmethylsulfonyl fluoride 1 mM. After centrifugation at 14000 rpm and 4°C for 10 min, appropriate dilutions of the supernatants were rapidly used for the enzyme assays described in Sulpice *et al*. (2007) for Rubisco and Gibon *et al.* (2004) for the other enzymes. Assays were carried out in 96-well microplates using ELx800 microplate readers (Bio-Tek, USA) at the Max Planck Institute of Molecular Plant Physiology (Germany). Rubisco's activation state was calculated as the ratio between initial and total Rubisco activity. The following steps and volumes were followed and prepared, respectively, in 96-well plates:

Enzyme	***Rubisco (initial)***	***Rubisco (total)***	***PEPCase***	***GS***	***Fd-GOGAT***	***NAD^+-GDH***
Sample Extract	2µL	16µL+ 4µL Activation Buffer*	2µL	5µL	5µL	2µL
Assay Mix	8µL H_2O	8µL H_2O	11.4µL H_2O	20µL assay buffer 5X	5µL glutamine	11.6µL H_2O
	4µL assay buffer 5X	4µL assay buffer 5X	4µL assay buffer 5X	10 µL Glycerol	5µL methyl viologen	4µL assay buffer 5X
	4µL activation solution 5X*	4µL activation solution 5X*	2µL PEP 0 or 20 mM	5µL PEP	1µL 2-oxoglutarate	2µL 2-oxoglutaratem 0 or 150 mM
	2µL ribulose-1,6-bisphosphate 0 or 10 mM	2µL ribulose-1,6-bisphosphate 0 or 10 mM	0.4µL NADPH	5µL ATP	2.5µL HEPES buffer	0.4µL NADPH
			0.2µL malate dehydrogenase	6µL H_2O	2.5µL amino-oxyacetic acid	
				2µLpyruvate kinase	24µL H2O	
				1µL NADH		
				1µL LDH		

	Mix, incubate at RT for 90 sec + 20µL EtOH 80%	Incubate the activation plate for at least 15 min at RT	Incubate 20 min + 20µL HCl 0.5 M/ Tricine/KOH 100 mM pH 9	45µL H_2O or Glutamate	Incubate at RT for 5 min + 5µL dithionite starter	Incubate 20 min + 20µL HCl 0.5 M/ Tricine/KOH 100 mM pH 9
Extra step	Mix, incubate for 5 min at RT + 50µL H_2O	Run the same protocol as for the initial activity	Mix, spin down, incubate at 95°C for 5 min, cool and spin down + 20µL NaOH 0.5 M		Mix carefully, then incubate for 0 or 10 min at RT + 20µL NEM and mix vigorously	Mix, spin down, incubate 10 min at 90°C, cool on ice and spin down + 20 µL NaOH 0.5M
	Mix, then add 50µL of determination Mix		45µL determination Mix + 5µL PES 4 mM		Heat at 95°C for 10 min, cool and spin down + 100µL determination Mix	45µL determination Mix + 5µL PES 4 mM
OD	340 nm at RT	340 nm at RT	570 nm at RT	340 nm at RT	570 nm at RT	570 nm at RT
Procedure	Maintain samples at 4ºC until reading.	Maintain samples at 4ºC until reading.	Protect determination mix and assay plate from light	20-30 min stabilised	Read for 5 to 10 min and then add 2µL glutamate dehydrogenase (30-40 min stabilised)	Protect determination mix and assay plate from light

* Activation Buffer 5X: $MgCl_2$ 100 mM and $NaHCO_3$ 50 mM.
NEM, N-Ethylmaleimide.
RT, Room temperature (25ºC).

Determination Mix	***RubisCO***	***PEPC***	***Fd-GOGAT***	***NAD^+-GDH***
	10 µL tricine/KOH pH 8 buffer	18 µL H_2O	54.75 µL H_2O	18.5 µL H_2O
	16.8 µL H2O	10 µL tricine/KOH pH 9 buffer	20 µL Tricine buffer pH 8.5	10 µL tricine/KOH pH 9 buffer
	0.2 µL $MgCl_2$	10 µL MTT	10 µL MTT	10 µL MTT
	5 µL triose-phosphate isomerase	4 µL EDTA	10 µL NAD^+	2 µL ethanol 50%
	5 µL phosphoglycerate kinase	2 µL Ethanol 50%	5 µL Triton X100	1 µL alcohol dehydrogenase
	0.5 µL GDH	1 µL alcohol dehydrogenase	0.25 µL diaphorase	
	5 µL NAD-glyceraldehyde 3-phosphaste dehydrogenase			
	1 µL catalase			
	0.5 µL GPOX			
	1 µL NADH			
	5 µL ATP			

FW/dilution: Rubisco and GOGAT 500 and the rest 1000.

3.6. Agronomic traits

The phenology of each variety was monitored throughout the growing cycle using the Zadoks scale (Zadoks *et al.*, 1974). The days from emergence to heading were determined through frequent field observations. The heading was defined when approximately half of the spikes in the plot had already emerged, and the ear emerged about 50% on the main stem. Moreover, growing degree days at heading (GDDH) were calculated as follows:

$$GDDH = \sum \left(\frac{T_{max} + T_{min}}{2} \right) - T_{base}$$

where T_{max} corresponds to the highest daily temperature, T_{min} to the lowest, and T_{base} was set at 0°C.

At maturity, some days before harvest, the plant height (from the soil surface to the tip of the spike, excluding the awns) was measured in the field for every plot. Then, two 0.5 m-length samples were taken randomly from the central rows of each plot, which were processed as a dry

matter after drying at 74ºC for 48 h to determine the aboveground biomass (without the roots). The number of plants and spikes per m^2 was determined by counting the plants and spikes contained in this one m-length sample. In addition, the number of spikes per plant was also estimated. Then, in a random subset of ten main stems per plot of the previous sample, the peduncle length, measured from the last internode to the base of the spike, and the spike length, measured excluding the awns, were recorded. After that, kernels per spike were determined, and the thousand kernel weight after threshing the ears (Figure 3.9).

At ripening, each plot (9 m^2) was mechanically harvested, the grain obtained was weighted and the grain yield (GY, kg ha^{-1}) was determined and adjusted to a 10% moisture level, following the equation:

$$GY = \frac{grain\ weight * (100 - MC)}{(100 - 10)}$$

where MC represented the moisture content obtained with the Grain Moisture Tester PM-450 (Kett, US) for each plot. Finally, the harvest index (HI, g grains g $biomass^{-1}$) was calculated from the agronomic components obtained from the samples:

$$HI = grain\ weight/(grain\ weight + biomass)$$

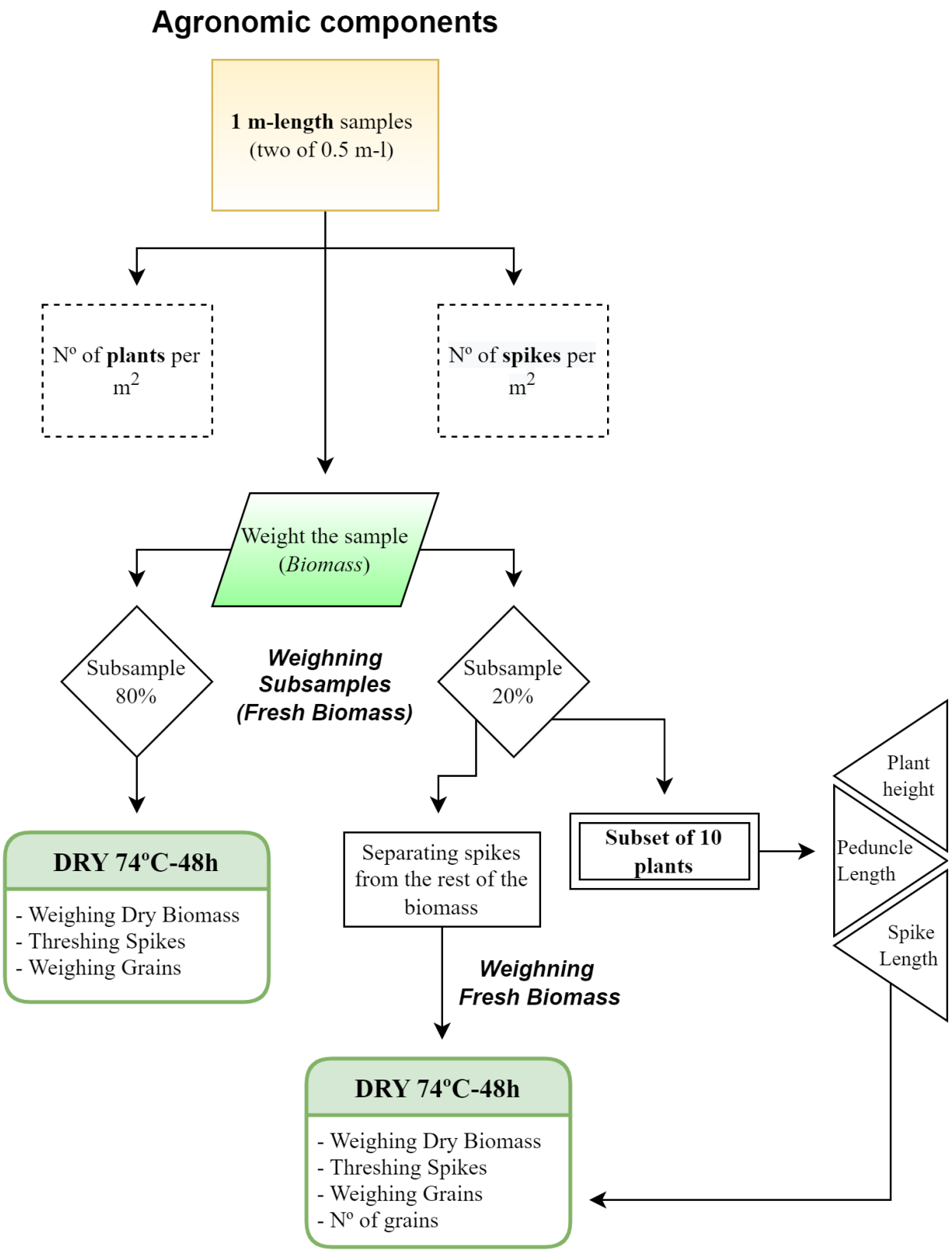


Figure 3.9. Description of the steps followed to obtain the different agronomic components from the one-meter length sampled.

3.7. Grain quality

The grain was cleaned before doing the quality analyses. The purpose of cleaning was to remove all impurities from the wheat. Samples of 300 g of durum wheat were passed through a Rationel Kornservice Sample Cleaner model MLN, which withdrew contaminants of different sizes and separated them based on particle diameter. This equipment consists of two sieves, slightly inclined and with a vibrating movement. With the larger perforations, the first sieve allows the wheat to pass through easily and retains the more significant impurities such as straws, twine, etc. The second sieve has smaller perforations than the wheat grain, keeping the wheat grain but allowing more minor impurities such as weed seeds and broken grains to pass through, while an airstream sucks in the dust and lighter particles.

Then, eight assessments were carried out to evaluate the technological quality of durum wheat in the varieties studied: test weight (TW), 1000-kernel weight (TKW), vitreousness percentage (VTR), moisture and protein content (PROT), colour index (b*), sedimentation volume (SDSS) and gluten characteristics (GI and WG). All of them were carried out with the small-scale equipment available at the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC) (Madrid-Spain).

3.7.1. Test weight

Once the clean grain was obtained, the test weight or hectolitre weight was determined using a Schopper Chondrometer, a 250 mL container. This method, which measures the space occupied by the grains in a specific volume, consists of filling the tube with them, removing the blade, letting the internal weight fall to the bottom and then moving the blade again, obtaining the weight of the grain (g) that fills the 250 mL container.

3.7.2. Thousand kernel weight

To obtain the thousand kernel weight or thousand seed weight, two batches of 1000 whole kernels without any visible damage (breakage, disease, malformation, empty grains) were counted with the Seed Counter PFEUFFER (0.3-15 mm) and weighted.

3.7.3. Vitreousness

The vitreousness percentage was estimated visually in two different batches of 100 grains of each sample, making a cross-section of the wheat grain with a Pohl grain cutter and using a tabletop magnifying glass with illumination, considering non-vitreous grains to be those that were not

translucent or had amylaceous dots in the endosperm. Good quality durum wheat kernels must be vitreous, with an amber colour and a translucent cut surface. The number of non-vitreous grains was counted to obtain the vitreousness percentage by the number of vitreous grains per 100 grains and the mean calculated (Figure 3.10).

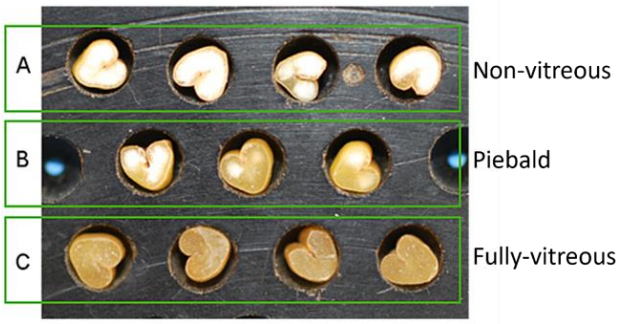


Figure 3.10. Visual classification of grain vitreosity (modified from Sieber *et al.*, 2014).

3.7.4. Milling

The mill used for milling was the Laboratory Mill 3100 (Perten Instruments AB, Sweden), equipped with a 0.8 mm sieve, obtaining wholemeal flour.

During milling, the grain was poured slowly to avoid overheating the rollers, and after milling each sample, the mill was carefully cleaned to prevent contamination with previous flours.

3.7.5. Moisture and grain protein content

The percentage moisture content was obtained by NIR reflectance spectrophotometry using the Foss Infratec 1241 Grain Analyzer spectrometer.

NIR technology is a fast, accurate and easy to use technique that can be applied to analyse the parameters of many products. The analysis is achieved using light in the NIR region (750-1250 nm). Data are expressed as a percentage of dry matter.

The protein percentage of the wholemeal flours was determined simultaneously with the moisture determination in the NIR spectrophotometer. Therefore, the data were expressed as % of dry matter.

3.7.6. Sodium dodecyl sulphate sedimentation

The method used to determine the settling volume in sodium dodecyl sulphate (SDS) medium was developed by Axford *et al.* (1979). The procedure is based on the swelling capacity of gluten in the lactic acid-SDS medium. For this, 5 g of wholemeal flour sample was weighed, and 50 mL of Bromophenol Blue 0.0009% w/v were added and shaken until homogenised. Then, 50 mL of 3% SDS and 0.35% lactic acid were added. The mixture was kept at room temperature to stand for a few min and afterwards read.

3.7.7. Gluten characterisation

The official methods of the International Association for Cereal Science and Technology (ICC 155 and 158) were used to determine the gluten characteristics. These methods determine the percentage of wet gluten (WG) and gluten index (GI). The WG estimates the total gluten in a sample, and the GI the dough strength.

Briefly, a salt solution (2% NaCl) was added to 10 g of flour and mixed to separate the gluten from the flour. Next, the gluten obtained was centrifuged to force it through a special standardised mesh. The sum of the gluten that passes through the mesh and the gluten that is retained is the WG. The percentage of WG that is contained in the mesh after centrifugation is the GI, so if the gluten is fragile, all of it must pass through the mesh (GI=0). If, on the other hand, all the gluten is retained, the gluten is strong (GI=100). The following formulas were used to calculate these parameters:

$$WG\ (\%) = [Gluten\ retained\ (g) + Gluten\ filtered\ (g)]\ x\ 10$$

$$GI\ (\%) = [Gluten\ retained\ (g) + Gluten\ wet\ (g)]\ x\ 100$$

For this purpose, the Glutomatic system (Perten Instruments AB, Sweden) was used, which includes:

(A) **Glutomatic 2100:** This is the central part of the system that washes the sample and kneads it to extract the gluten. It consists of two supports adapted to the equipment to deposit the samples, with 60 mm in diameter and two removable meshes, 88 µm for washing and another metallic one of 840 µm for kneading (Figure 3.11).

(B) **Gluten Index Centrifuge 2015:** Standardised centrifuge that includes two 22 cm diameter supports with a 600 µm metal mesh where the WG is placed to be centrifuged and subsequently collected from both sides using a spatula (Figure 3.11).

In more detail, the washing solution of sodium chloride (2% NaCl), kept at 22°C ± 2°C, is prepared daily to carry out this protocol. Then, 10 g of wholemeal flour sample was placed in the adapted holder of the Glutomatic 2100, and 4.7 mL of the saline solution was added and mixed to form a dough. After this time, the mixture was automatically washed with the saline solution for 5 min to obtain clean WG. Once this phase was finished, the gluten was transferred to another support with a metal mesh of 840 µm, and for another 5 min in contact with the saline solution, the kneading phase of the sample took place. The WG is then transferred to the Gluten Index Centrifuge 2015 support and centrifuged for 1 min at 6000 rpm. Finally, the fraction that passes through the 600 µm mesh is collected with a spatula and weighed on a precision balance. The fraction retained on the 600 µm mesh was then collected and weighed. Both fractions add up to the weight of the total WG. The gluten remaining on the mesh after centrifugation concerning the weight of the whole WG is the GI, expressed in %.

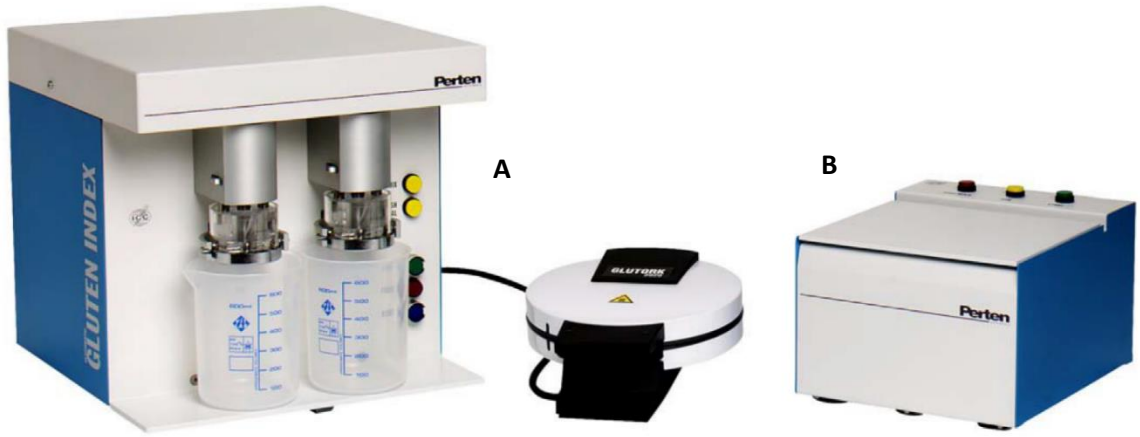


Figure 3.11. Equipment utilised for the determination of the gluten index. A) Glutomatic 2100 Gluten Index System; B) Centrifuge 2015.

3.7.8. Yellow pigment index

The colour index test was carried out with a Konica Minolta CR-310 colourimeter that performs chromaticity measurements in the colour system L* a* b*.

The CR-310 head uses a large illumination area, with a viewing angle of 0° to obtain readings that directly correlate with the colour as seen under typical lighting conditions. Inside a mixing chamber, a xenon arc lamp provides diffuse light illuminating the sample over 50 mm in diameter. Only the light reflected perpendicular to the surface is collected by the fibre optic cable and fed to the specially designed microprocessor to perform the analysis required for accurate colour determination of the sample.

The CIE L* a* b* colour space defined in 1976 has been adopted nationally and internationally as the standardised definition of colour. Various equations for colour differentiation and the assignment of tolerances have been agreed upon and accepted. Since the CIE L* a* b* Colour Space is a Cartesian system, a colour cannot simultaneously have a positive and negative value of a* or b*; a coordinate must be positive or negative (Figure 3.12).

In this colour space, there are three axes:

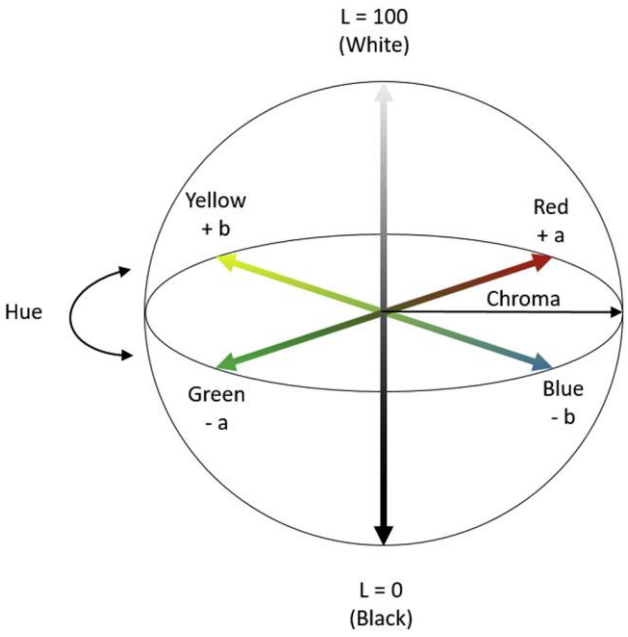


Figure 3.12. The CIELAB colour space diagram. The CIELab, or CIE L* a* b*, the colour system represents a quantitative relationship of colours on three axes: L* is the vertical axis and represents the measure of the lightness of a colour, varying from zero for a black to one hundred for a white (fluorescent colours can give a value of L* more significant than 100); a* is one of the two horizontal axes and represents a measure of the red or green content of colour. If a colour has red a*, it will be positive, while if a* is negative, then the colour will have some amount of green; b* is the other horizontal axis, perpendicular to the a* axis. Positive values of b* indicate yellow content, while negative values of b* indicate blue content.

To characterise the colour of durum wheat wholemeal flours, we use the data of b* (yellow index).

3.8. Statistical analysis

A sufficient number of biological replicates per analysis were used to obtain reliable and representative results. Plant material was collected from plots randomly selected from the pool of plants available for each treatment and developmental stage, while some phenotyping and agronomic traits were determined at the whole plot level as detailed in the methodology above. All the variables were subjected to *two-way* analyses of variance (ANOVA) using the general linear model to calculate the effects of the environment, the genotypic variability, different organs and their interactions by using RStudio (www.rstudio.com). Tukey's Honest Significant Difference (HSD) test was used to analyse differences between treatments and the means of the specific groups at the Studies I, III and IV. In the Study II, means were compared by the Least Significant Difference (LSD) test. Significance was accepted at $p<0.05$. This *p-value* considers significance with 95% accuracy to avoid a type I error (undue rejection of the null hypothesis).

3.8.1. Study I

A reaction norm defines a genotype-specific function that translates environmental inputs into a phenotype. The genotype x environment interaction (GEI) occurs when the reaction norms are not parallel, but intersect, diverge (linear increased tendency), converge (linear decreased tendency), cross (some genotypes diverge, and others converge) or not follow a trend (Figure 3.13). The occurrence of G x E forces phenotypic prediction models to become more elaborate and to contain genotype-specific parameters; intercepts, slopes and curvatures (van Euwijk *et al.*, 2016). These genotype-specific parameters are called sensitivity and adaptability parameters in the plant breeding literature. They facilitate the modelling of non-parallelism of reaction norms to account for G x E (Finlay & Wilkinson, 1963; Slafer *et al.*, 2014). Sensitivity applies to situations with single and well-identified explicit environmental gradients (i.e. drought stress, temperature), and adaptability to less concrete and non-explicit environmental gradients (environmental index based on average performance or all genotypes in a trial) (van Euwijk *et al.*, 2016) (Figure 3.13).

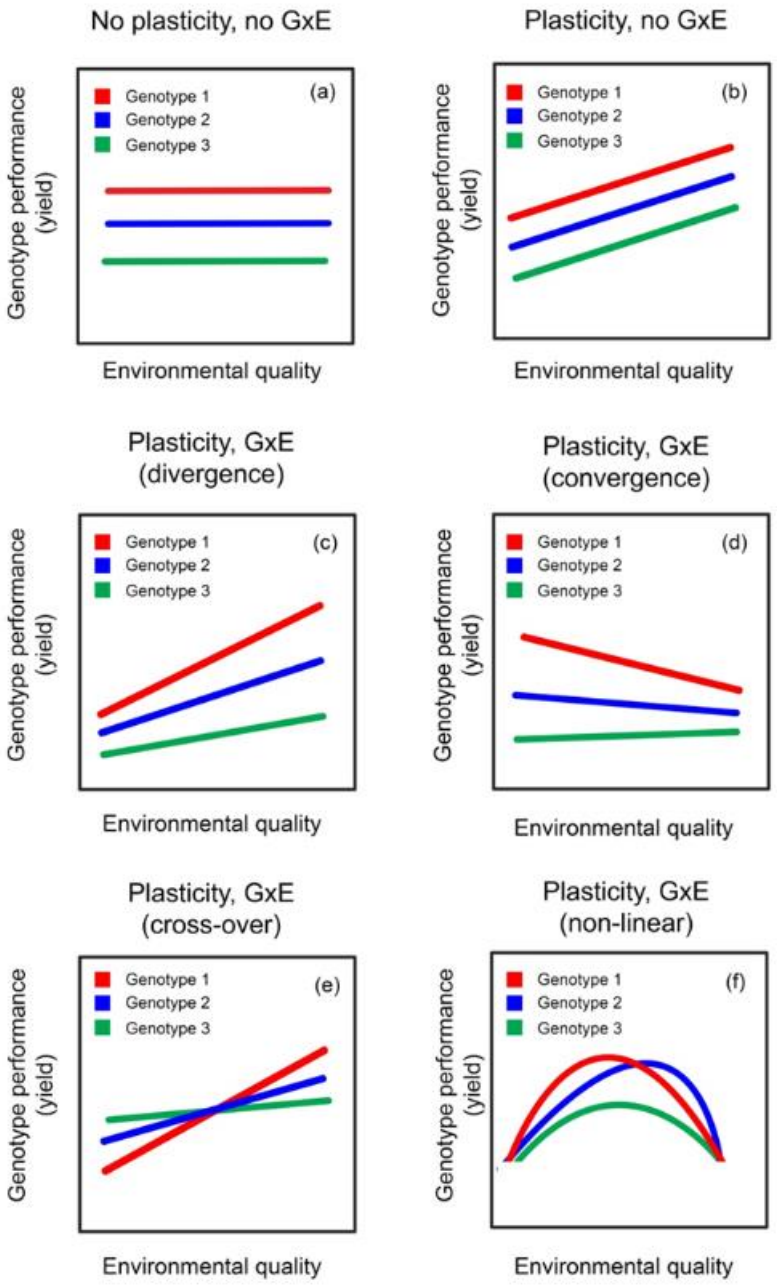


Figure 3.13. Reaction norms for three genotypes illustrate various plasticity forms and Genotype x Environment interaction (G x E). For example, interpreting such as no plasticity in (a) compared to b-f and representing the different forms of G x E interaction (c, d, e, f) (van Eeuwijk *et al.*, 2016).

We worked with three models to study the genotype-by-environment interaction for grain yield and quality-related traits. Finlay and Wilkinson analyses (1963) were performed with the *statgenGxE* package (Malosetti *et al.*, 2013) in RStudio. The Additive Main effect and Multiplicative Interaction (AMMI) triplot was constructed through the principal components generated by the interaction environment-genotype, which aims to explain the interaction associated with a two-factor ANOVA. It was performed with the *str(AMMI)* function included in

the *agricolae* package (Crossa, 1990). In addition, the GGE Biplot model was also presented as a complementary model to the AMMI. It works with a matrix combining the main effect of the genotype (G) and the interaction of the genotype with the environment (GE). The different GGE Biplots were developed through the interactive biplot implementation for modelling genotype-by-environment interaction (GEI) *GGEBiplot* function of the *GGEBiplotGUI* package (Frutos *et al.*, 2014), all packages implemented in the R environment. For generating the GGE Biplots, the data were not transformed or scaled, only centred by means of the environments. On the one hand, in the case of the "Ranking Genotype" and "Mean and Stability" biplots, the singular values were partitioned into the genotype eigenvectors for an appropriate visual comparison among varieties. On the other hand, the "Which Won Where/What" biplot was partitioned into the environment eigenvectors to better visualise the correlation among the environments studied.

To know the magnitude of the genetic variability present in our panel of studies related to agronomic and quality traits evaluated, we also estimated several genetic parameters, such as coefficients of variation and heritability, for being used in the future breeding programs. The heritability analyses were based on the following formulas. Genotypic variance (σ^2_G), the variance of interaction between G and E (σ^2_{GE}), error variance (σ^2_E), and phenotypic variance (σ^2_{PH}) (Steel *et al*., 1997; Sharma, 1998):

$$\sigma^2_G = (MS_G - MS_{GE})/r\,e$$

$$\sigma^2_{GE} = (MS_{GE} - MS_E)/r$$

$$\sigma^2_E = MS_E$$

$$\sigma^2_{PH} = \sigma^2_G + \sigma^2_{GE} + \sigma^2_E$$

where MS_G is the mean square of genotype, MS_{GE} is the mean squares of interaction, MS_E is the mean squares of error, r is the number of replications, and e is the number of environments. Broad sense heritability (h^2_{BS}) (Allard, 1960; Singh *et al.*, 1993):

$$h^2_{BS} = \sigma^2_G/\sigma^2_{PH}$$

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) (Burton, 1951; Kwon & Torrie, 1964):

$$PCV\ (\%) = \left(\sqrt{\sigma_{PH}^2}/\bar{x}\right) \times 100$$

$$GCV\ (\%) = \left(\sqrt{\sigma_{G}^2}/\bar{x}\right) \times 100$$

3.8.2. Study II

The Pearson correlation procedure was constructed to analyse the relationships between measured traits. Principal component analysis (PCA) was performed on the correlation matrix calculated on the mean across replications. In this study, statistical analyses and figures were performed using SAS (SAS institute 2011) and Excel (Microsoft 365, 2021), respectively.

3.8.3. Study III and Study IV

The line, scatter and bar plots were generated with SigmaPlot 12.0 (Systat Software Inc., USA) and the R package *ggplot2*. Stepwise regressions were performed using the *stepAIC()* function, while the proportion of variance explained by each predictor was calculated with the package *relaimpo*. The packages *factoextra* and *FactoMineR* extracted and visualised the multivariate data analyses (PCA). Finally, Pearson correlation matrices were built to analyse the relationships between trait pairs using the function *cor()* and visualised using the package *corrplot*.

Studies

4. CHAPTER 4:

First Study

Genotype-by-environment interaction for grain yield and quality traits in durum wheat: identification of ideotypes adapted to the Spanish region of Castile and León.

4.1. INTRODUCTION

Wheat is the world's most favoured staple food, which is nutritious, easy to store and transport, and can be processed into various types of food (Subedi *et al*., 2019). In particular, durum wheat accounts for 5% to 8% of total wheat production (33.8 millions of tons), with 13.5 M hectares globally (Martínez-Moreno *et al.,* 2022). Durum wheat is manufactured primarily for pasta production, and it is also an essential ingredient for couscous and bulgur, predominantly in countries from North Africa and the Middle East, where currently nearly 50% of global durum wheat acreage and production is (Xynias *et al.,* 2020). These products use the semolina resulting from the hard-textured durum wheat kernel milling. Moreover, it is considered a good source of proteins, minerals, B-group of vitamins, and dietary fibre (Kandel *et al*., 2018).

Test weight, thousand kernel weight and hardness are the main factors influencing milling performance and confer the physical properties necessary for obtaining the raw material required to produce the semolina (Finney *et al*., 2015). Also, vitreousness, associated with high protein content, must be considered, since this parameter gives the natural hard, shiny and translucent appearance to durum wheat grains. From semolina, pasta is produced by different processes, and a minimum of 12-15% protein content in grain is required. This ensures semolina with a uniform particle size that confers elastic, resistant, non-sticky and firm cooked pasta, offering the texture commonly referred to as "al dente" (Padalino *et al*., 2014). Furthermore, gluten strength confers less sticky dough with superior textural properties. This is because it grants the necessary tenacity to retain the gelatinised starch granules during cooking (Sissons *et al*., 2008). In addition, the high-quality dough is characterised by a uniform and bright golden yellow colour (given by carotenoids), without speckles and translucent, expected to the consumers (Subira *et al*., 2014). This importance resulted in the creation of specific regulatory standards to market these products. In particular, the quality classification system for durum wheat used in Spain ("Royal Decree 190/2013" by the Spanish Ministry of Agriculture, Food and Environment) classifies the durum wheat grain according to specific quality parameters. According to this regulation, four quality of durum wheat groups are defined, depending on the minimum requirements concerning three quality traits: protein concentration, test weight and vitreousness. Group 1 corresponds to the highest quality grain (≥13% protein, ≥80 kg hl^{-1} test weight, >80 vitreousness), followed by groups 2 (≥12% protein, ≥78 kg hl^{-1} test weight, >75 vitreousness), and 3 (≥11% protein, ≥77 kg hl^{-1} test weight, >60 vitreousness), while group 4 (the rest), is not suitable for obtaining semolina.

According to the data collected by FAO, agricultural production must be increased by 50% to meet food demand by 2050. Wheat products could account for 20% of protein and calories

consumed per capita for a global population of 9.7 billion in 2050 (CRP-WHEAT, 2016). Specifically, global wheat production needs to increase significantly to achieve this goal in a changing climate scenario that puts even current production rates at risk (Reynolds *et al.*, 2016). To increase wheat production, we rely on our ability to sustainably increase crop yields on actually cultivated land (Cassman, 1999). Unfortunately, climate change will also decrease the currently suitable regions where produced durum wheat can meet the high standards for end-use suitability (Ceglar *et al*., 2021). Nowadays, more than 90% of Spanish durum wheat is produced in Andalusia (Spanish Ministry of Agriculture, Fisheries and Food, www.mapa.gob.es). Nevertheless, the new climate scenarios may become suitable for cultivating durum wheat in new emerging areas, as is the case of Castile and León, currently the most relevant Spanish bread wheat region. This expected new scenario requires the development and future adoption of effective and sustainable strategies to stabilize production and adapt the entire food supply chain. This is a challenge to the agriculture community, and there are no simple solutions.

To overcome production and quality constraints, transformational research approaches are required for durum wheat agronomists, breeders, and producers. That includes developing robust production systems adapted to specific agro-ecosystems, which account for the myriad of interactions resulting from the genotype, crop management and fluctuating climate (Hatfield & Walthall, 2015). In the Mediterranean basin, durum wheat is mainly grown under rainfed conditions. Therefore, drought and its frequent association with heat are the stresses with the most significant impact on cereal yield. They usually occur together during the reproductive stages of the crop, particularly during the grain-filling period (Araus *et al*., 1998).

Moreover, the variability of thermo-pluviometric patterns results in large spatial and temporal fluctuations not only in yield but also in quality (De Vita *et al*., 2010; García del Moral *et al*., 2005). Indeed, grain quality traits in durum wheat are also greatly affected by diverse factors, such as water scarcity and high temperatures (Guzmán *et al*., 2016). For example, Li *et al*. (2013) reported that drought enhanced gluten strength, while heat stress reduced it. Flagella *et al*. (2010) also observed an increase in gluten strength under water stress, considerably influenced by glutenins composition, which was probably associated with a higher aggregation of glutenin subunits. Generally, protein content increased as grain yield decreased. Moreover, De Stefanis *et al*. (2002) studied the effects of high temperatures and showed that gluten, total and insoluble proteins increased whereas kernel and specific weight decreased.

The stability of quality parameters is becoming an essential requirement for the milling and pasta industries because of the potentially high annual variation observed, particularly under the

Mediterranean conditions. The high stability of raw material quality (grain or semolina) was defined as "economic stability" initially by Robert and Denis (1996). It is considered an economically stable genotype if its contribution to the GEI is low. Therefore, it is a desirable feature since it guarantees constant procedures and low product loss during processing (Grausgruber *et al*., 2000). In addition, the stability of grain quality characters is also essential in increasing variety selection efficiency for breeders in breeding programmes (Korkut *et al.*, 2007). Simultaneously, the success of targeted crop improvement is supported by estimating parameters such as variances, coefficients of variation and heritability, which provide insights into genetic variability in a population (Bartaula *et al*., 2019). Heritability, for a specific trait, is the ratio of genetic variance due to changes between genotypes divided by the total phenotypic variance for this trait. It is widely used in establishing breeding programmes and forming selection indexes (Falconer & Mackay, 1996).

Consequently, it is essential to test the performance of different varieties in specific environments to evaluate their adaptability and stability in terms of grain yield and quality traits, which can also provide helpful information for developing new varieties adapted to local, targeted environments. Moreover, considering the most important quality parameters for the semolina industry, this work aimed to study the effects of the GEI on durum wheat production. The objective is to identify an ideotype with high and stable yield potential and desirable stable superior quality characteristics for semolina production and widely adapted to prevailing and future environmental growth conditions in the northwest Mediterranean Spanish region of Castile and León. To this end, we have performed a multi-year study during five consecutive crop seasons under a wide range of environments (understood as the combination of a given season and the specific management conditions: rainfed, irrigated, late sowing and low nitrogen fertilisation in a set of fourteen durum wheat varieties widely grown in Spain. The genotype by environment interactions (GEI) for grain yield and diverse quality parameters were analysed using different statistical models. Finally, we have determined the potential of durum wheat in Castile and León, assessing the varieties best suited to this region to offer the most sustainable alternative to the cereal crop sector, implementing a production model oriented to quality, responding to the industry's internal demand.

4.2. MATERIALS AND METHODS

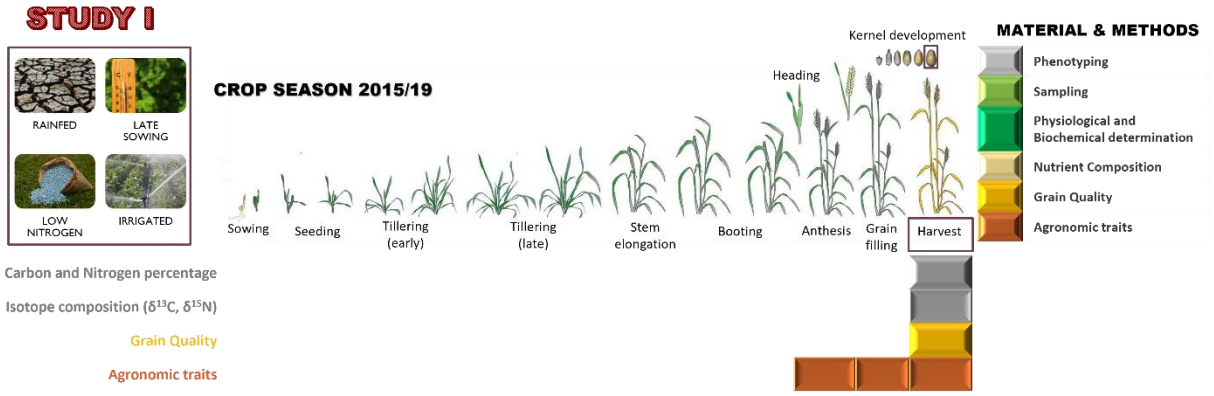


Figure 4.1. The field experiments were conducted over five consecutive growing seasons across six years (2014-2019). The plants grew under four contrasting growth conditions, rainfed (R-), support irrigation (R+), late-sowing (L) and low nitrogen fertilization conditions (N-). The combination of season and growth conditions resulted in a total of 13 environments (see Table 4.1 for further details). Fourteen varieties were selected: *Mexa* (MEX, 1980), *Vitrón* (VIT, 1983), *Simeto* (SIM, 1988), *Regallo* (REG, 1990), *Gallareta* (GAL, 1994), *Claudio* (CLA, 1998), *Burgos* (BUR, 1999), *Dorondón* (DOR, 1999), *Amilcar* (AMI, 2002), *Avispa* (AVI, 2003), *Don Ricardo* (DRI, 2008), *Kiko Nick* (KNI, 2009), *Sculptur* (SCU, 2011), and *Olivadur* (OLI, 2013) (Table S4.1). Grain quality and C-N% and isotope composition were evaluated in the harvest grain, together with the agronomic traits. See Chapter 3 for methodologies and specific analyses used.

Table 4.1. Description of the 13 environments used in the study, including the year, the treatment (R+, irrigated; R-, rainfed; L, late sowing; N- low nitrogen supply), the sowing, heading and harvest dates, the climate conditions, the rainfall and support irrigation during the period of the growth cycle, and the average values for each environment (including the fourteen varieties) for grain yield (GY), and carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition. The values for grain yield, $\delta^{13}C$ and $\delta^{15}N$, represent the mean ± standard deviation of 42 replicates per environment. Within columns, numbers followed by the same letter indicate non-statistically significant differences at $p<0.05$ as determined by Tukey's HSD test.

Environment	1	2	3	4	5	6	7	8	9	10	11	12	13
Season	2014-2015	2014-2015	2015-2016	2015-2016	2016-2017	2016-2017	2016-2017	2017-2018	2017-2018	2017-2018	2018-2019	2018-2019	2018-2019
Treatment	R+	R-	R+	R-	R+	R-	L	R+	R-	N-	R+	R-	N-
Sowing date	24/11/2014	24/11/2014	30/11/2015	30/11/2015	29/11/2016	29/11/2016	09/02/2017	13/11/2017	23/11/2017	23/11/2017	03/12/2018	03/12/2018	03/12/2018
Heading date	29/04-08/05	29/04-08/05	07/05-18/05	07/05-18/05	25/04-04/05	22/04-28/04	10/05-20/05	11/05-19/05	11/05-19/05	11/05-19/05	01/05-14/05	02/05-13/05	02/05-10/05
Harvest date	22/07/2015	22/07/2015	20/07/2016	15/07/2016	06/07/2017	06/07/2017	20/07/2017	25/07/2018	20/07/2018	20/07/2018	15/07/2019	03/07/2019	03/07/2019
Range of mean temperature (°C)	4.6-17.5	4.6-17.5	4.3-16.5	4.5-16.9	4.3-18.2	4.3-18.2	7.4-23.1	4.3-16.2	4.5-15.8	4.5-15.8	3.6-18.0	3.0-17.2	3.0-17.2
Range humidity (%)	46.6-93.5	46.6-93.5	51.9-97.5	51.0-97.0	41.6-95.1	41.6-95.1	29.6-93.0	52.4-99.62	53.8-99.6	53.8-99.6	46.6-97.6	47.9-97.6	47.9-97.6
Rainfall (mm)	258.4	258.4	359.7	359.7	124.0	124.0	100.6	476.4	476.0	476.0	145.9	127.5	127.5
Irrigation (mm)	125	0	70	0	155	55	155	109.8	0	0	152.7	0	0
Total water received (mm)	383.4	258.4	429.7	359.7	279.0	179.0	255.6	586.2	476.0	476.0	298.6	127.5	127.5
$\delta^{15}N$ (‰)	3.45 ± 0.44^{a}	3.49 ± 0.45^{a}	2.96 ± 0.41^{bc}	1.57 ± 0.35^{f}	2.53 ± 0.50^{cd}	0.93 ± 0.63^{g}	2.52 ± 0.42^{cd}	2.00 ± 0.59^{e}	3.25 ± 0.83^{ab}	2.39 ± 0.67^{d}	2.38 ± 0.53^{d}	1.19 ± 0.55^{fg}	2.31 ± 0.65^{d}
$\delta^{13}C$ (‰)	-25.85 ± 0.42^{f}	-24.25 ± 0.27^{c}	-26.55 ± 0.36^{g}	-25.49 ± 0.28^{ef}	-24.97 ± 0.82^{d}	-23.64 ± 0.48^{b}	-26.07 ± 1.04^{f}	-26.64 ± 0.56^{g}	-26.55 ± 0.49^{g}	-26.53 ± 0.42^{g}	-25.34 ± 0.78^{de}	-22.43 ± 0.75^{a}	-22.30 ± 0.65^{a}
GY (kg ha^{-1})	7750 ± 719^{b}	4182 ± 441^{f}	9622 ± 1262^{a}	7326 ± 836^{bc}	7042 ± 1072^{c}	2943 ± 977^{g}	5043 ± 1227^{e}	6337 ± 641^{d}	7023 ± 1097^{c}	5206 ± 1016^{e}	7822 ± 1331^{b}	2778 ± 662^{g}	2814 ± 743^{g}

4.3. RESULTS

4.3.1. Environmental conditions

The experiments were carried out at the same experimental station over five growing seasons. The annual variability in temperature and water input, together with the different management practices ensured a wide range of variability in environmental growing conditions (Figure 4.2, Table 4.1). As a consequence, grain yield and quality traits, together with the agronomical yield components and the stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope composition exhibited significant differences due to the environment were observed (Table 4.2). The highest yield was achieved in the environment (Env) 3 under irrigated conditions, with 9622 kg ha^{-1}, and the lowest under rainfed conditions, Env12, with 2778 kg ha^{-1}, with a difference of 6844 kg ha^{-1} between them (Figure 4.3). The range of variability in growing conditions also strongly affected crop water conditions and nitrogen metabolism as inferred from the wide range in $\delta^{13}C$ values across environments, ranging between -26.64 ‰ (Env8) and -22.30 ‰ (Env13), while $\delta^{15}N$ varied less, between 0.93 ‰ (Env6) and 3.49 ‰ (Env2).

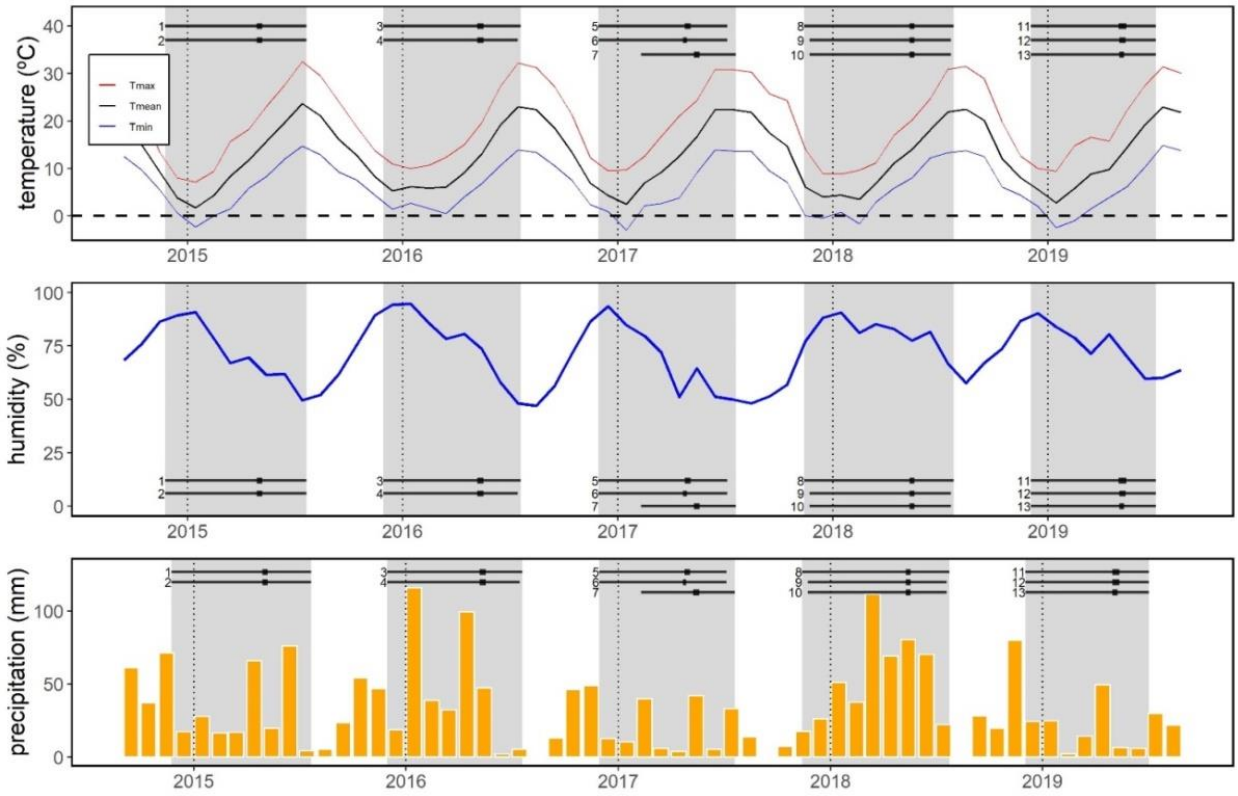


Figure 4.2. Monthly precipitation and humidity, together with the maximum, mean, and minimum temperature during the growing season at the Experimental Station of Zamadueñas (Valladolid, Spain) across the six years. Grey shadows illustrated the five different crop seasons. The horizontal solid lines show the specific period of the field trials performed for each environment, the small black box added to the line represents the range of days to heading for the fourteen durum wheat varieties, and the numbers refer to each environment (for a description, see Table 4.1).

The experimental site is representative of the Mediterranean climate characterized by an uneven distribution of rainfall during the growing cycle, accompanied by low temperatures in winter that rise sharply in spring and high temperatures continuing until the end of the crop cycle. But some differences were observed between the seasons (Figure 4.2). The total rainfall received by the panel of varieties (Figure 4.2) varied between 124 and 476 mm in the 2017 and 2018 seasons, respectively. The most unfavourable seasons for winter crops due to precipitation occurring across all crop cycles were 2017 and 2019, the driest ones. On the contrary, the 2016 and 2018 seasons were the most favourable because of evenly distributed precipitation. In 2016, 2017 and 2019, the rainfall after anthesis was low, which led to a dry harvest, whereas in 2015 and especially in 2018 still rained during all the spring and previous to harvest. Also, in the 2015 season, the spring rainfall was above average (Figure 4.2).

Concerning temperature, in 2015, the temperatures in the establishment of the crop were colder compared with the same period in the previous seasons. Also, in 2017 and 2019, the temperature in January was below average. Nevertheless, in 2016 and 2018, the colder period was later, in February. The most significant difference between the maximum and minimum mean temperature occurred during tillering stage (March-April) in 2019. The hottest temperature and lowest humidity at anthesis occurred in 2017. Therefore, the three first seasons showed the lowest values when analysing the moisture at harvest (Figure 4.2).

4.3.2. Environmental and genetic effects on grain yield and agronomic traits

The statistical analyses ANOVA showed that for the agronomic traits, including yield, the environment was the factor that had the highest contribution to the total sum of squares (SS, Table 4.2). The contribution to the SS of the environment reached the highest values for DH (98.5%), GY (83.1%), and GDDH (79.9%). Figure 4.3 shows the dispersion of GY values for each experiment and the environment ranking. The irrigated environments Env1, Env3 and Env11 were more productive, and the rainfed ones the less. Low nitrogen supply and late sowing conditions also contributed to reduce GY.

We observed a significant contribution to the SS of the genotypic variability for traits such as SL (32.4%) and NKS (20.0%), with a low impact for attributes such as DH (0.8%) and GY (1.1%). The GEI explained a high proportion of the variability observed for the traits HI (20.1%) and NSP (16.9%), while by contrast, DH was the least influenced by GEI (0.5%). Only for PL, the GEI was not significant. GY across varieties ranged between 5373 kg ha^{-1} (SIM) and 6255 kg ha^{-1} (OLI),

considering all the environments together (Table S4.2). DH varied slightly from 153 days in MEX to 159 in BUR, as well as GDDH from 1106ºC in MEX to 1181ºC in BUR. HI ranged from 0.35 in BUR to 0.43 in DOR, NSP from 342.1 spike m^{-2} in AVI to 423.7 in BUR. OLI presented the highest value for NKS (43.9 kernels $spike^{-1}$) and SL (7.6 cm) but the lowest value for PL (27.7 cm).

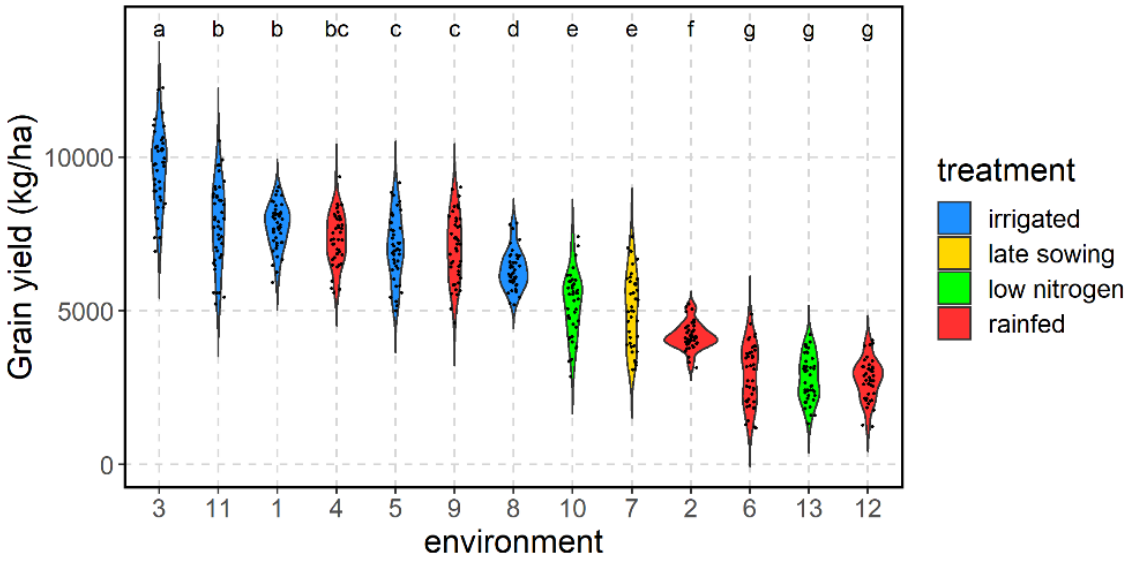


Figure 4.3. Violin plot of grain yield for the 13 environments studied. Each group colour represents a treatment according to the legend. The numbers on the x-axis refer to each environment (for details, see Table 4.1). Violins with the same letter on the top indicate non-statistically differences at $p<0.05$ as determined by Tukey's HSD test.

Table 4.2. Degrees of freedom (d.f), the sum of squares (SS) and contribution percentage to the total SS of the different factors (Contr.) from the combined analysis of variance of agronomic and grain quality traits, carbon and nitrogen content and isotope composition of 14 durum wheat varieties grown during five growing seasons in 13 environments. The SS of the GxE interaction was partitioned by AMMI analysis. G, genotype; E, environment; PCA, principal component of AMMI; GY, grain yield; DH, days from emergence to heading; GDDH, growing degree days at heading; HI, harvest index, NSP, number of spikes per m2; NKS, number of kernels per spike; PL, peduncle length; SL, spike length; PROT, protein content; TW, test weight; TKW, thousand kernel weight; VTR, vitreousness, b*, yellow pigment content; SDSS, SDS sedimentation; WG, wet gluten; GI, gluten index; %N, nitrogen percentage; %C, carbon percentage; $\delta^{15}N$, nitrogen isotope; $\delta^{13}C$, carbon isotope; C/N, carbon/nitrogen ratio; GNY, grain nitrogen yield; GCY, grain carbon yield. Significance codes: $p<0.001$, ***; $p<0.01$, **; $p<0.05$, *; $p>0.05$, ns (not significant).

Source of variation		GY		DH		GDDH		HI		NSP		NKS		PL		SL	
	d.f.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.
G	13	32930120 ***	1.1	1791.5 ***	0.8	304010 ***	9.5	0.3 ***	5.4	290950 ***	7.2	12631 ***	20.0	4080 ***	19.0	106 ***	32.4
E	12	2431391740 ***	83.1	227261 ***	98.5	2559324 ***	79.9	3.1 ***	53.4	1748399 ***	43.0	37186 ***	58.7	12619 ***	58.7	160.9 ***	49.1
Block	26	108881861 ***	3.7	82.9 ***	0.0	14140 ***	0.4	0.2 ***	3.7	184993 **	4.6	1124 ***	1.8	1363 ***	6.3	6.1 ***	1.9
G × E	156	15194692 6***	5.2	1241.4 ***	0.5	262748 ***	8.2	1.2 ***	20.1	685593 *	16.9	5623 ***	8.9	1196 ns	5.6	25.1 ***	7.7
PCA1	24	52165032 ***	34.3	513.3 ***	41.4	110839 ***	42.2	0.7 ***	56.5	273666 ***	39.9	1870 ***	33.3			9.6 ***	38.2
PCA2	22	33651120 ***	22.1	258 ***	20.8	66531 ***	25.3	0.2 ***	17.2	155220 **	22.6	1081 ***	19.2			4.7 ***	18.7
PCA3	20	23017755 **	15.1	220.1 ***	17.7	46078 ***	17.5	0.1 **	11.9	85767 ns	12.5	793.7 **	14.1			3.2 *	12.7
Residual	90	43113019 ns		250 ***		39300 ***		0.2 ns		170941 ns		1878 *				7.6 *	
Error	338	199118394		405.1		61303		1		1154567		6741		2261		29.2	
Total	545	2924269041		230782		3201525		6		4064503		63305		21518		327	

Source of variation		PROT		TW		TKW		VTR		b^*		SDSS		WG		GI	
	d.f.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.
G	13	272 ***	11.7	612.5 ***	15.7	6598 ***	16.5	12721 ***	7.1	394.5 ***	29.6	7327 ***	14.9	2457 ***	14.0	37486 ***	22.4
E	12	1302 ***	55.8	2343 ***	60.2	27719 ***	69.3	98489 ***	55.2	735.2 ***	55.2	32755 ***	66.8	8919 ***	50.7	75018 ***	44.9
Block	26	200 ***	8.5	106.6 ***	2.7	580.7 ***	1.5	8856 ***	5.0	10.6 *	0.8	1230 ***	2.5	1561 ***	8.9	7549 ***	4.5
G × E	156	267.4 ***	11.5	500.8 ***	12.9	2570 ***	6.4	22734 **	12.8	101 ***	7.6	4691 ***	9.6	2244 ***	12.7	33007 ***	19.8
PCA1	24	115.2 ***	43.1	259.1 ***	51.7	801.6 ***	31.2	13020 ***	57.3	42.8 ***	42.4	2552 ***	54.4	954.2 ***	42.5	11856 ***	35.9
PCA2	22	47.9 ***	17.9	87.3 ***	17.4	553.3 ***	21.5	4383 ***	19.3	18.8 ***	18.6	707.6 ***	15.1	311.2 ***	13.9	6464 ***	19.6
PCA3	20	31.3 *	11.7	53.2 ***	10.6	442.8 ***	17.2	2063 ns	9.1	15.3 ***	15.2	460.2 ***	9.8	277.7 *	12.4	5516 ***	16.7
Residual	90	73.1 *		101.3 **		772.2 **		3269 ns		24.1 ns		971.3 **		700.9 *		9171 ***	
Error	338	293.5		330.4		2503		35499		90.1		3047		2428		14051	
Total	545	2335		3894		39971		178299		1331		49050		17609		167110	

Source of variation		%N		%C		$\delta^{15}N$		$\delta^{13}C$		C/N		GNY		GCY			
	d.f.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.	SS	Contr.		
G	13	6.7 ***	5.4	19.9 ns	1.4	3.5 ns	0.7	11.6 ***	0.9	455.3 ***	7.4	5521 ns	0.4	6026801 ***	1.2		
E	12	70.4 ***	56.5	654.6 ***	46.3	325.5 ***	66.4	1124 ***	83.5	3040 ***	49.3	892585 ***	71.4	420435790 ***	82.1		
Block	26	9.6 ***	7.7	51 *	3.6	22.5 ***	4.6	46.3 ***	3.4	522.9 ***	8.5	66235 ***	5.3	18678417 ***	3.6		
G × E	156	16.1 ***	12.9	266.2 *	18.8	51.3 *	10.5	68.1 ***	5.1	895.6 ***	14.5	102749 ns	8.2	28531081 ***	5.6		
PCA1	24	6.4 ***	39.8	114.4 ***	43.0	15.4 ***	30.0	24.3 ***	35.7	433 ***	48.4			9843852 ***	34.5		
PCA2	22	2.9 ***	17.7	59.5 **	22.4	11.9 **	23.2	18.9 ***	27.8	162.1 **	18.1			5867751 ***	20.6		
PCA3	20	2.2 *	13.9	36.6 ns	13.8	9.6 *	18.8	7.9 ns	11.6	93 ns	10.4			4504645 **	15.8		
Residual	90	4.6 ns		55.7 ns		14 ns		17 ns		207 ns				8314833 ns			
Error	338	21.9		422.7		87.8		95.3		1256		183213		38136661			
Total	545	125		1414		491		1345		6170		1250304		511808750			

4.3.3. Environmental and genetic effects on quality traits

In the case of the grain quality traits, the effect of the environment, the genotype and the interaction were significant for all the cases. As in the previous section, the environment was also the factor that explained the highest proportion of the variability observed. The environmental effect on TKW was the highest among all quality traits (69.3%), followed by SDSS (66.8%) and TW (60.2%), while the lowest was GI (44.9%). The genetic effect on grain quality traits was relevant for traits such as b* (29.6%) and GI (22.4%), being VTR the least affected by genotypic variability (7.1%). The interaction was more significant for GI (19.8%), TW (12.9%), WG (12.7%), VTR (12.8%) and PROT (11.5%; Table 4.2).

Across all environments, SIM (15.9%) and BUR (15.8%) showed a higher protein content (Table S3) and, on the other hand, AVI (13.5%) and AMI (13.8%) were the lowest. VTR ranged from 78.1% (SCU) to 95.7% (SIM), b* ranged from 14.14 (VIT) to 17.0 (SCU), and SDSS from 37.8 (VIT) to 50.0 (BUR). Considering the environments, the rainfed ones achieved the upper values for protein. VTR varied from 51.0% (Env8) to 99.5% (Env2), WG from 20.3 (Env8) to 36.9 (Env6) , SDSS ranged from 57.6 (Env2) to 32.2 (Env8). The higher values of b* were associated with the 2019 trials; nevertheless, the previous year's trials showed the lowest values (Table S4.3).

4.3.4. Environmental and genetic effects on grain carbon and nitrogen content and isotope composition

The effect of the genotypic variability was not significant for traits such as %C, δ^{15}N and GNY, but it was significant for %N, δ^{13}C, C/N and GCY (Table 4.2). Nevertheless, the contribution to the SS of the genotypic variability was quantitatively low, only reaching a maximum of 7.4% for the C/N ratio. On the other hand, the effect of the environment was quantitatively much higher, ranging from 46.3% (%C) to 83.5% (δ^{13}C). Among the traits significantly affected by the genotypic variability, N content varied between 2.3% (SCU) to 2.6% (SIM), δ^{13}C from -25.4‰ (REG) to -24.9‰ (DRI and DOR), C/N from 16.5 (SIM) to 19.4 (SCU), and GCY from 2284 kg ha^{-1}(SIM) to 2666 kg ha^{-1}(OLI; Table S4.4). Considering the average values for the 14 varieties per environment, the highest value for δ^{13}C was observed in 2019 trials (Env12 with -22.5‰ and Env13 with -22.4‰), and the lowest for Env3 (-26.6‰; Table S4.4).

4.3.5. Genotype-by-environment interaction by the AMMI model

Analysing the decomposition of the GEI through the AMMI model (Table 4.2), we observed that the first, second and third principal components axes (PCA) explained most of the SS multiplicative effect for all traits evaluated. For the quality traits, these three axes explained a proportion of the variability from 68.8% (WG) to 85.7% (VTR), whereas for the agronomic traits, their sum varied from 64.5% (PL) to 85.6% (HI). For the traits related to the elemental and isotopic analyses, their sum ranged between 64.7% (GNY) and 79.2% (%C). All PCA axes were significant (p<0.05) for most traits, except the third axis for NSP, PL, VTR, %C, δ^{13}C, C/N and GNY.

The AMMI triplots were constructed by plotting the first three interaction PCAs (Figure S4.1). The varieties located closer to the centre of the plot showed a lower interaction. Therefore, we can visually identify varieties with a broad adaptation for a specific trait among the different environments studied. Still, we can also predict the environment (or group of environments) for which a variety performs best. For example, the varieties SCU and SIM showed stable values of GY among the environments studied, but this does not imply that their yield was the highest, as it did not occur for SIM (Table S4.2). Furthermore, we observed how some traits showed a significant GEI, such as GI and TW, while other parameters, for instance, b*, were modulated by the genotype (Figure S4.1). In addition, some traits were clearly influenced mainly by the environmental conditions, such as DH, GDDH and GY. Among these traits, we can identify some environments that greatly affected the plant performance, such as the Env7 (late sowing). Finally, due to its relevance, we showed that PROT was a trait greatly affected by the GEI, without a clear stable variety among all the environments; the environment determines which varieties are optimal for maximising grain protein content.

4.3.6. Genotype-by-environment interaction by regression coefficients

Furthermore, we evaluated in detail the varieties showing certain stability for each trait based on regressions coefficients, with a particular focus on GY, grain quality traits, and carbon and nitrogen isotopes compositions. To predict the stability performance of varieties and identify superior ones to varying environmental conditions, the coefficient or slope of regression (ß) was analysed for each trait evaluated, represented as “sensitivity” (Figure 4.4). Finlay-Wilkinson (1963) proposed this model in which regressions coefficients of 1 indicate average stability. We observed that varieties such as SIM and SCU, identified with the AMMI triplots as stable varieties for GY, were also recognised with the Finlay-Wilkinson model. Indeed, here we could propose that SCU, among others (DOR, AVI, etc.),

outperformed SIM quantitatively (Figure 4.4). OLI was one of the most susceptible varieties to environmental changes for traits such as GY, TW and TKW. Regarding quality traits, the slope for PROT varied from 0.68 (MEX) to 1.31 (OLI). It indicated that MEX produced an above-average protein concentration in low-protein environments and was less insensitive to environmental changes. However, OLI was one of the highest-protein concentration varieties under the most favourable conditions. Nevertheless, REG was a variety with a slope close to 1, meaning its protein concentration did not vary significantly with the environmental conditions, but additionally its average values were high compared to most of the other varieties. For the other traits, we can distinguish those that were clearly affected by the environment in a genotypic-specific manner, e.g. VTR and GI. In the case of VTR, AMI (1.05), GAL (1.01) and DOR (0.97) showed average stability and similar mean performance. SIM with the highest VTR mean (95.7%) and a slope of 0.28 indicated insensitive to environmental changes and adaptation to low VTR environments.

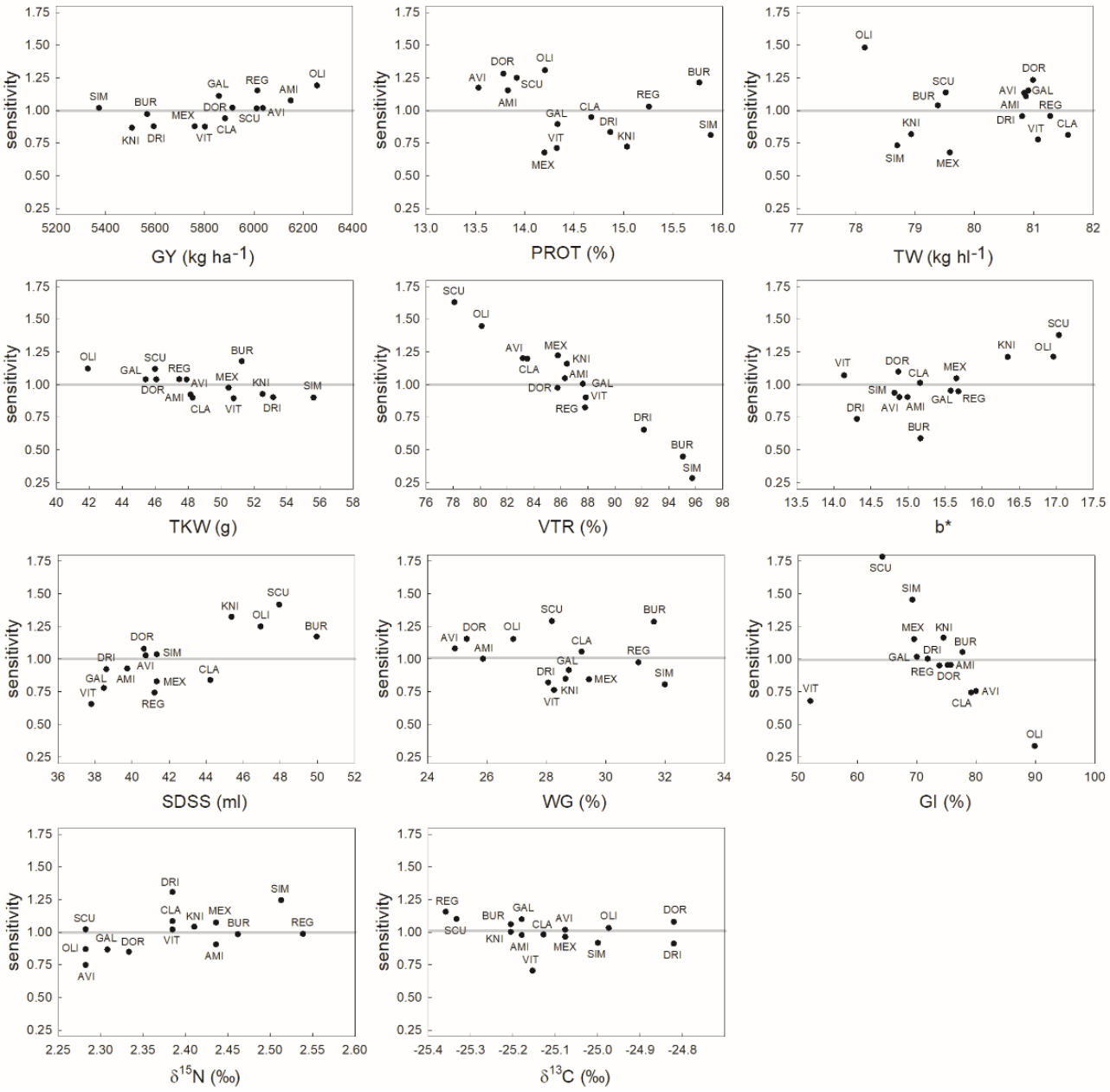


Figure 4.4. Representation of the Sensibility and Mean obtained by the Finlay-Wilkinson analysis for grain yield, grain quality traits and isotope composition. The abbreviations for the varieties are detailed in Table S4.1.

4.3.7. Genotype-by-environment interaction by GGE biplots

Genotype ranking based on their mean and stability. Ranking biplots were used to classify the varieties according to their performance and "stability" by using the average environment coordinate (AEC) for each trait evaluated (Figure 4.5 and S4.2). The single arrowhead line is the AEC abscissa (AEA), which points to the higher mean performance of the trait across the environments. The vector lengths (drawn by dotted lines in the figures) represent the stability of the varieties for each trait. In our study, according also to GGE biplots, GY was the highest in AMI and OLI among the different environments, while it was the lowest for KNI, SIM, and DRI. Though AMI and OLI showed a relatively low variability among environments (stability), most varieties were more susceptible to high changes due to the growth conditions. The variety SIM had the highest PROT mean value, followed by BUR and REG, whereas AVI had the lowest. OLI was highly unstable, and AMI and REG were highly stable for PROT stability. In the case of the VTR, again, SIM and BUR showed the highest mean average across all the environments, and AMI and CLA were the most stable varieties. The summary and the comparison of the performance and stability for the other traits evaluated are present in Table S4.2.

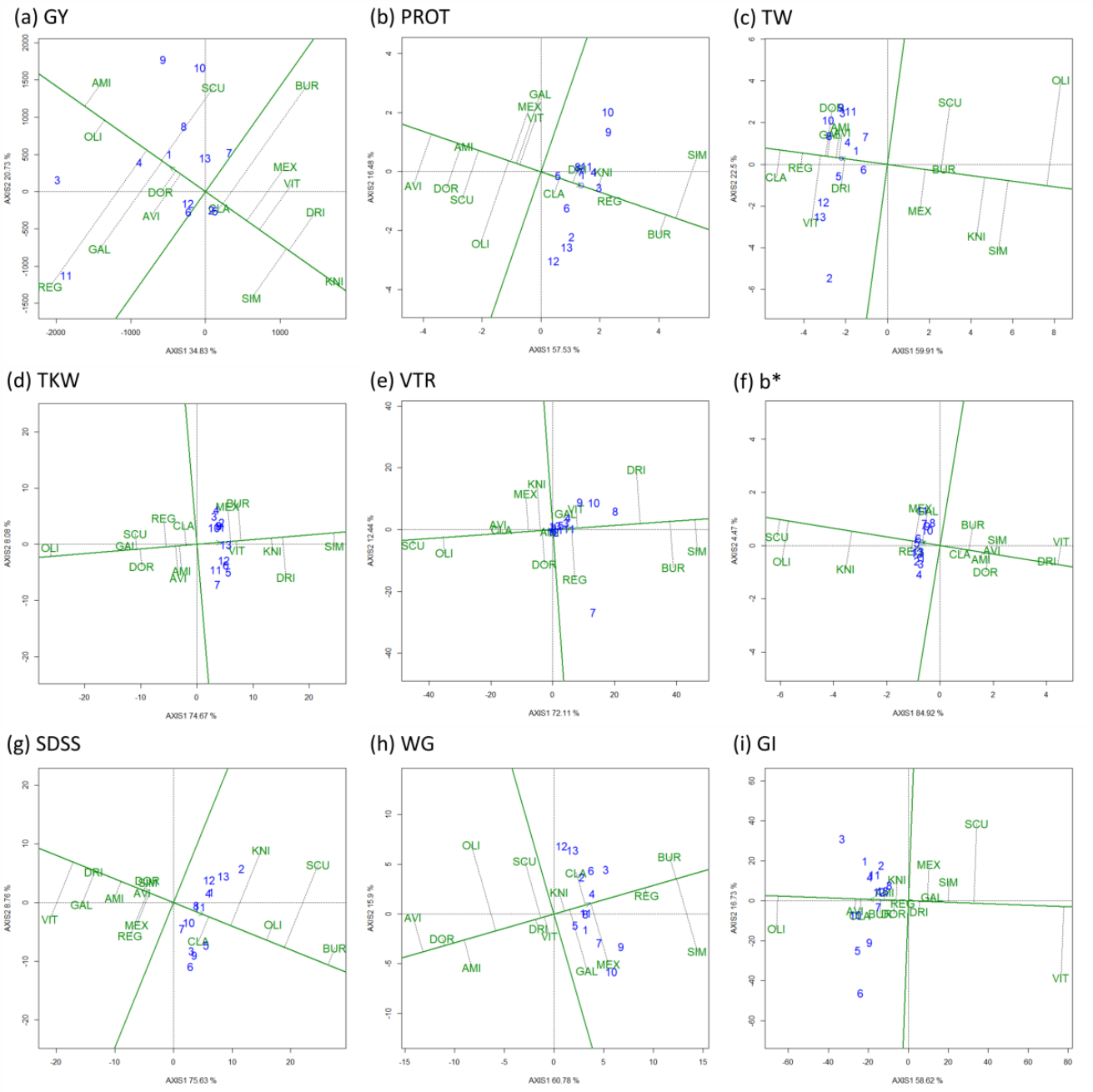


Figure 4.5. GGE biplots of "Mean and Stability" among 14 durum wheat varieties and the specific genotype × environment interactions evaluated across 13 environments during five growing seasons for: (a) GY (grain yield), (b) PROT (protein content), (c) TW (test weight), (d) TKW (thousand kernel weight), (e) VTR (vitreousness), (f) b* (yellow pigment content), (g) SDSS (SDS sedimentation), (h) WG (wet gluten) and (i) GI (gluten index). The average-environment coordination (AEC) solid green lines represented the average environment. The dotted lines show the stability of the varieties being. The shorter line, the higher the stability of each variety. The abbreviations for the varieties are detailed in Table S4.1 and for the environments in Table 4.1.

Ranking varieties relative to the ideal genotype. An ideal genotype should have high mean performance and high stability across environments. Figures 4.6 and S4.3 define for all traits evaluated the "ideal genotype" (the centre of the concentric circles), which should be as closer as possible to the AEA ("absolutely stable") and with a long vector length extended in the direction of the positive side of AEA ("highest mean performance"). The conclusions obtained were similar to the ranking biplots detailed above, for example, for GY, PROT and VTR. However, in these plots, we can clearly distinguish which varieties were at the same level of stability by the concentric circles. In the Table 4.3, we summarise the ideal and stable variety for each of the traits studied and the ones with the highest mean. It was interesting that some varieties outperformed for some traits, such as BUR, which was the ideal variety with a high mean for traits such as PROT, SDSS, WG and VTR (Figure 4.6). In the case of GY, AMI and OLI showed high stability associated with a high mean. Moreover, OLI was the ideal variety with a high mean for other traits such as HI, NKS, SL, b*, and GI. Furthermore, with this analysis we can also assess the most favourable environments for each trait according to their position in the concentric circle and proximity to the biplot origin. Due to either a favourable year in terms of rainfall or the support irrigation, the environments with high water input (Env3, Env4, Env8, Env9, etc.) achieved the highest yields. HI was a trait showing high variability due to the environment, according to the GGE biplot (Figure S4.3), with environments associated with stress conditions (i.e. rainfed and low nitrogen supply) far from the centre. The grain $\delta^{15}N$ and, notably, the $\delta^{13}C$ also showed high variability due to the environmental conditions. Nevertheless, for other factors, most of the environments were clustered in the same concentric circle, e.g. TKW, PROT, b*, SDSS, PL, SL, and NKS (Figures 4.6 and S4.3).

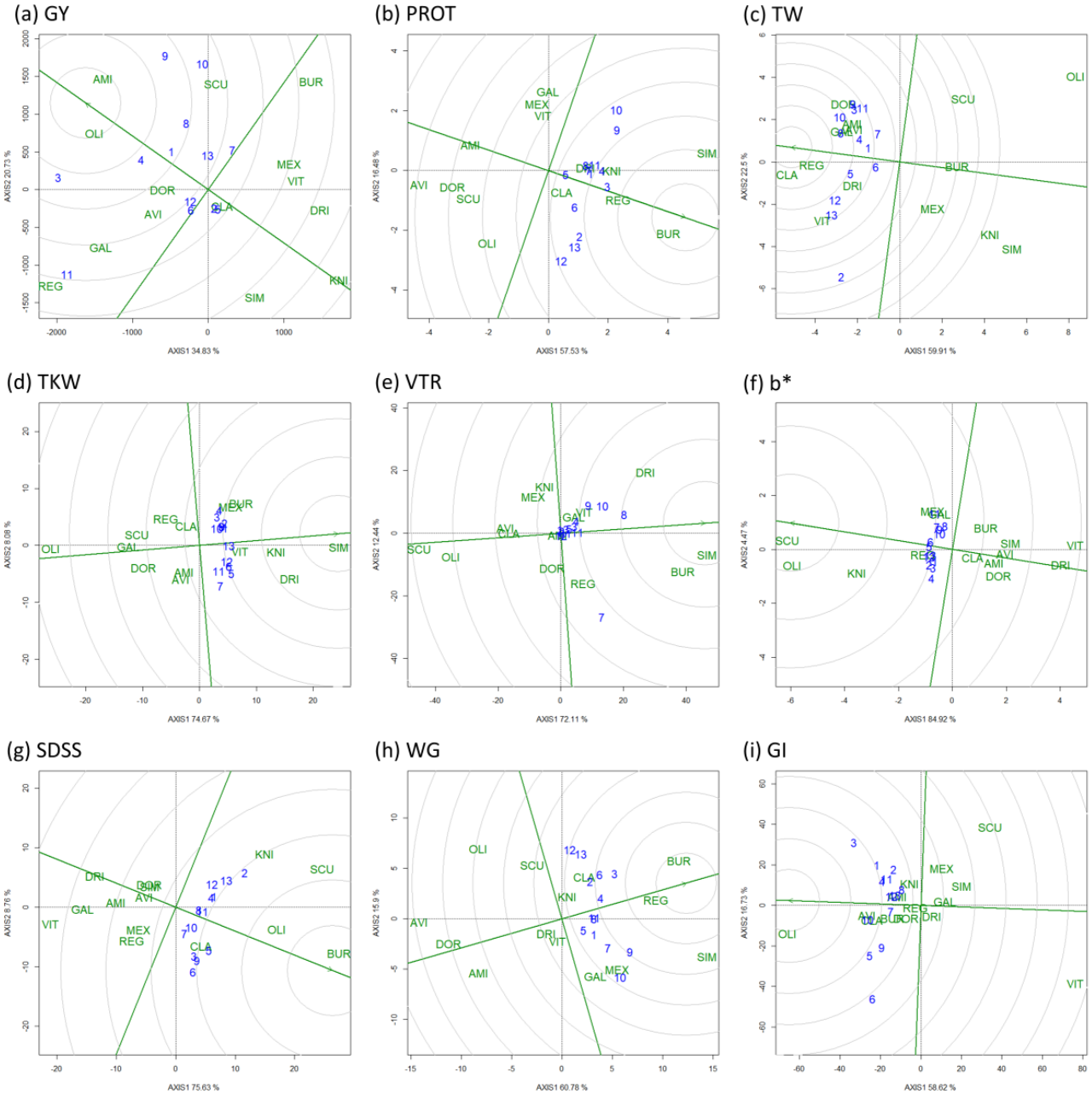


Figure 4.6. GGE biplots of "Ranking Genotype" (ideal genotype) among 14 durum wheat varieties and the specific genotype × environment interactions evaluated across 13 environments during five growing seasons for (a) GY (grain yield), (b) PROT (protein content), (c) TW (test weight), (d) TKW (thousand kernel weight), (e) VTR (vitreousness), (f) b* (yellow pigment content), (g) SDSS (SDS sedimentation), (h) WG (wet gluten) and (i) GI (gluten index). The average-environment coordination (AEC) view ranks varieties relative to an ideal genotype (the centre of the concentric circles). The abbreviations for the varieties are detailed in Table S4.1 and for the environments in Table 4.1.

Table 4.3. Summary of the ideal varieties and varieties with stable and high mean values for the agronomic and grain quality traits in five different seasons (2014-2019) and treatments (rainfed, irrigated, late sowing, and low nitrogen conditions). The varieties with high mean values are based on the Tukey's HSD analyses. The abbreviations are described throughout the text.

Trait	Ideal/near-ideal varieties	Stable variety	High mean
GY	**AMI, OLI**	**KNI, CLA, OLI**	**OLI, AMI**
DH	**BUR**	**OLI, DOR, REG**	**BUR**
GDDH	**BUR**	**OLI, AVI, AMI**	**BUR**
HI	**OLI**	**SCU, SIM, CLA**	**AVI, DOR**
NSP	**BUR, KNI**	**GAL, OLI, AVI**	**BUR, KNI**
NKS	**OLI**	**CLA, OLI, KNI**	**OLI**
PL	**MEX**	**SIM, GAL, MEX**	**MEX, DRI**
SL	**OLI**	**DOR, AMI, DRI**	**OLI**
PROT	**BUR**	**AMI, REG, DRI**	**SIM, BUR**
TW	**REG, CLA**	**BUR, REG, GAL**	**CLA, REG**
TKW	**SIM**	**GAL, OLI, VIT**	**SIM**
VTR	**SIM, BUR**	**AMI, CLA, SCU**	**SIM, BUR**
b*	**SCU, OLI**	**DRI, AVI, AMI**	**SCU, OLI**
SDSS	**BUR**	**AVI, DRI, SIM**	**BUR, SCU**
WG	**BUR, REG**	**DOR, REG, DRI**	**SIM, BUR**
GI	**OLI**	**REG, GAL, AVI**	**OLI**

Identification of "Which-Won-Where". The GGE biplot can also show the which-won-where pattern of a variety across the environments and visualize the best performing varieties (Figures 4.7 and S4.4). First, a polygon is drawn on varieties located furthest from the biplot origin and then perpendicular lines to each side of the polygon are drawn, dividing the biplot into several sectors (mega-environments, MGEs). The varieties in the vertexes were the most responsive to environmental interactions for each trait. For example, for GY, four independent MGEs were identified. The groups were mainly classified according to the water received during the growing period, among other environmental factors (Figure 4.7). One MGE included Env7, represented by high temperatures and low precipitations, where BUR was the best performing variety. The second, composed by Env2 and Env5, was characterised by a rainfall range of 258.4-279.0 mm, being KNI the most adapted to this scenario. The third MGE was composed of the contrasting environments Env3 and Env11 (non-stress, high yield) and Env12 and Env6 (stress, low yield), where REG was the best performing. Finally, the fourth MGE included environments with an average precipitation of 456 mm, being AMI the most representative variety. For PROT, only two MGEs were identified, one composed by the Env2, Env6, Env12 and Env13, being BUR at the vertex of this section, and the rest into the other MGE, with SIM at the vertex. Similar to the previous Figure 4.6, we can also distinguish traits where mainly only one or two MGEs were identified, such as TKW, DH, PL, and SL (Figures 4.7 and S4.4).

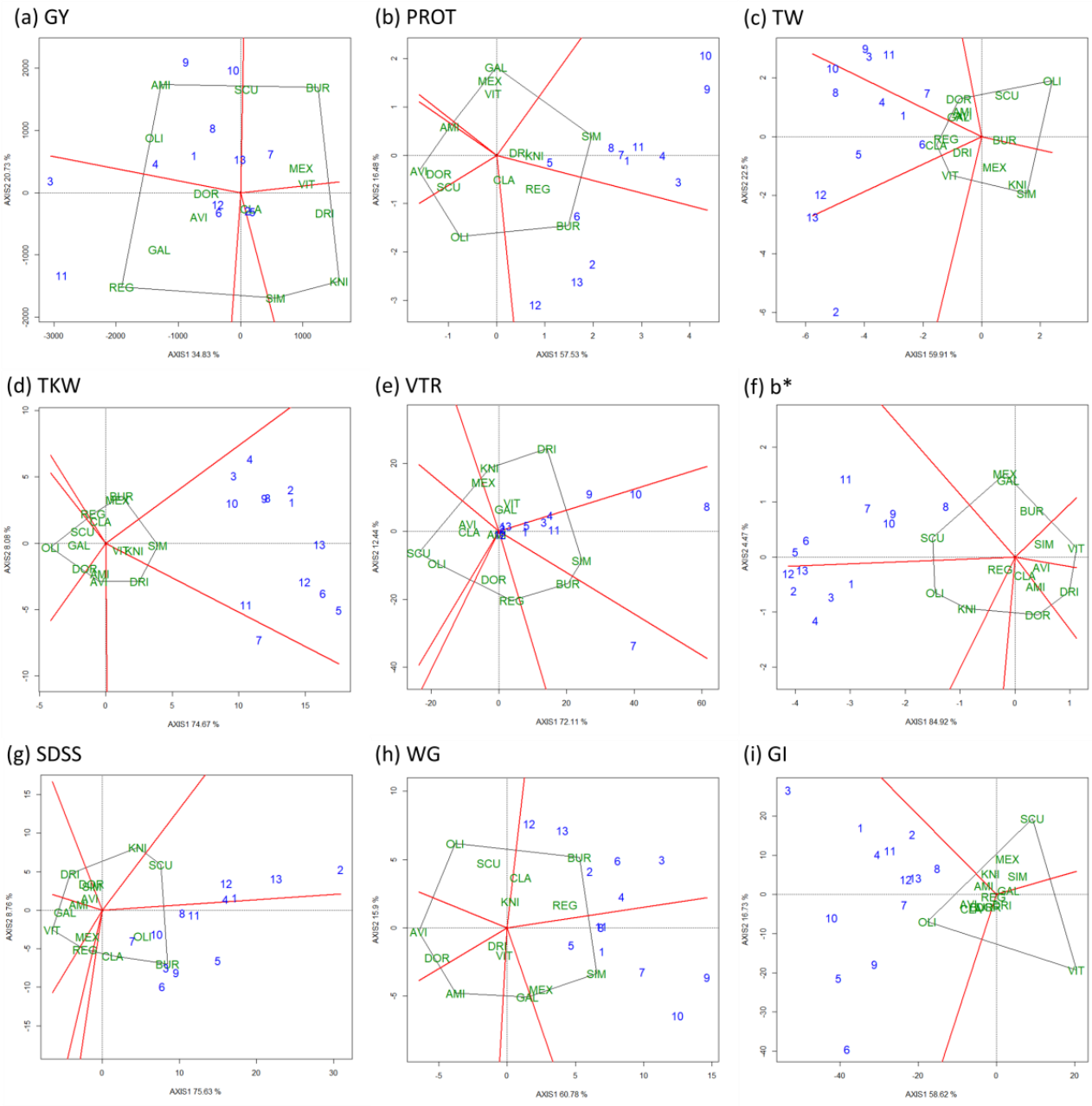


Figure 4.7. GGE biplots of "Which Won Where/What" among 14 durum wheat varieties and the specific genotype x environment interactions evaluated across 13 environments during five growing seasons for: : (a) GY (grain yield), (b) PROT (protein content), (c) TW (test weight), (d) TKW (thousand kernel weight), (e) VTR (vitreousness), (f) b* (yellow pigment content), (g) SDSS (SDS sedimentation), (h) WG (wet gluten) and (i) GI (gluten index). This plot consists of a polygon with perpendicular lines, called equality lines, drawn onto its sides. These lines divide the polygon into various sectors. Varieties located on the polygon's vertices are the best in each mega-environment from a particular sector. The abbreviations for the varieties are detailed in Table S4.1 and for the environments in Table 4.1.

4.3.8. Heritability

The results for the analyses of the genetic variability and heritability existing among the agronomic and quality traits and carbon and nitrogen isotope composition are shown in Table 4.4. The highest PCV was observed in $\delta^{15}N$ (22.2%), followed by NKS (20.0%), GI (17.7%), NSP (17.0%) and GY (14.9%). On the other hand, the lowest PCV was observed for DH (1.7%), TW (2.1%) and $\delta^{13}C$ (2.3%). GCV varied from 0.43% for $\delta^{13}C$ to 13.9% for NKS. The lowest differences between PCV and GCV were observed for the traits DH (0.50%), TW (0.79%) and b* (1.33%). In comparison, the highest differences were obtained for the traits GY (11.4%), NSP (11.3%) and VTR (8.3%). Therefore, they were associated with a higher environmental than the genotypic impact on the variation of these traits.

Table 4.4. Results for the coefficient of variation and broad-sense heritability (h^2_{BS}) of the agronomic and grain quality traits, and carbon and nitrogen isotope composition of 14 durum wheat varieties across five years of cultivation and 13 environments were obtained by ANOVA. The abbreviations of the traits are described throughout the text.

Traits	PCV	GCV	h^2_{BS} (%)
GY	**14.91**	**3.43**	**5.28**
DH	**1.67**	**1.17**	**49.09**
NSP	**17.00**	**5.63**	**10.97**
NKS	**20.04**	**13.98**	**48.69**
PL	**11.76**	**8.54**	**52.79**
SL	**8.76**	**6.97**	**63.40**
$\delta^{13}C$	**2.34**	**0.43**	**3.39**
$\delta^{15}N$	**22.16**	**-**	**-**
PROT	**8.87**	**4.83**	**29.60**
TW	**2.11**	**1.32**	**39.33**
TKW	**9.81**	**7.25**	**54.71**
VTR	**13.63**	**5.32**	**15.26**
b	**7.00**	**5.67**	**65.55**
SDSS	**12.84**	**8.71**	**46.05**
WG	**13.19**	**7.44**	**31.82**
GI	**17.68**	**11.33**	**41.08**

4.4. DISCUSION

Growing resilient crops with stable yield performance and technological quality characteristics amidst unexpected short-term climatic variations is fundamental to ensure food security. In Western

Europe, climate change accounts for 31-51% of yield variability (Ray *et al*., 2015) and food security is inextricably bound to this event. A low and erratic rainfall distribution characterises the Mediterranean climate which together with increases in temperature and decreases in precipitation towards the end of the crop cycle, compromise yield stability and grain quality (de Lima *et al*., 2021). Hristov *et al*. 2020 postulated that wheat yields are projected to decrease by up to 49% by 2050 in Southern Europe due to climate change, indicating insufficient adaptation of breeding programmes to this unpredictable situation. Therefore, assessment of durum wheat genetic diversity for resilience to environmental variation and searching for varieties with high performance and stable grain quality adapted to environmental variations remains an important consideration to meet the industry requirements and food demand of the increasing population worldwide. In the present study, agronomic and grain quality traits of 14 durum wheat varieties were studied under favourable and adverse conditions to assess the varieties suitable for growing under adverse conditions in the Spanish region of Castile and León.

4.4.1. Environmental conditions had a highly significant effect on the yield and quality traits of durum wheat in Castile and León region, which were also significantly affected by the effect of the genotype and the genotype-by-environment interaction as shown by the AMMI model

The environmental conditions were significantly relevant to durum wheat performance, affecting yield and quality-related traits. That variability affected the growth of durum wheat, showing remarkable differences for most traits across the 13 environments considered during the five consecutive growing seasons. Interestingly, the interannual climate variation, together with the treatments performed (contrasting water inputs, nitrogen fertilisation and late sowing), caused a difference of GY of 6844 kg ha^{-1} between the highest and the lowest yielding environments (Table 4.1, Figure 4.3), representing a range of more than three times the yield obtained in the most limiting environment. Previous studies have also reported similar values considering nine environments in the Mediterranean region of the Iberian Peninsula (Chairi *et al*., 2020). The area of study is frequently subjected to drought and high temperatures during the grain-filling period, characterised by a climate Csb (temperate, dry and warm summer) according to the Köppen-Geiger classification (Beck *et al*., 2018). Nevertheless, it is predicted to be more limiting by the end of this century (2071-2100), where a climate BSk (arid, steppe, cold) is likely to happen (Beck *et al*., 2018). Interestingly, water and nitrogen supply strongly influenced GY, NSP, NKS, PL and SL.

Previous studies proposed that the stable carbon isotope composition –expressed either as $\delta^{13}C$ or as its discrimination ($\Delta^{13}C$)– in plant dry matter (Farquhar & Richards, 1984), preferably in the grain at harvest, as in our case (Araus *et al.,* 2022) integrates crop water performance of wheat through its life cycle. It is considered a proxy for the diffusion of CO_2 mainly by stomatal conductance, and then it is used as an indicator of the level of water stress experienced by the crop. In our study, $\delta^{13}C$ matched with the water input of each environment (Table 4.1), which allowed us to corroborate that the different water regimes, due to the annual precipitation or the support irrigation, had an effect on plant performance. In general, lower access to water resulted in a higher $\delta^{13}C$ in mature grains (less negative), meaning that the plants adopted a conservative strategy by closing the stomata and then increasing water use efficiency (Farquhar & Richards; Araus *et al*., 2003). On the other hand, $\delta^{15}N$ is also used as a proxy of nitrogen metabolism through the life cycle, involving nitrogen uptake, assimilation and translocation. However, the exact factors affecting $\delta^{15}N$ are not entirely understood (Sanchez-Bragado *et al*., 2017). Thus, $\delta^{15}N$ is occasionally contradictory, decreasing or increasing under the same stresses, as it happened for salinity and drought (Lopes *et al*., 2004). In our study, some of the most limiting environments (e.g. rainfed treatments) had the lowest values of $\delta^{15}N$, which agree with the majority of reports on field wheat (Araus *et al.* 2013 but its pattern was not clear if it was influenced by, the environmental conditions or the treatments (Table 4.1) and further studies are needed to decipher its applicability. The decrease in $\delta^{15}N$ as response to rainfed condition (and thus to water stress) may be understood in the sense crop growth becomes less dependent on N fertilization (Yousfi *et al.,* 2012).

Then, we studied the AMMI partition of the GEI for the different traits. This model combines the analysis of variance for additive or main effects and then with principal component analysis for multiplicative or interactive effects (Yue *et al.,* 2022). The ANOVA analysis indicated that the variance due to the single factors independently (genotypic variability and environment) or together as interaction (GEI) were highly significant for most of the agronomic and grain quality traits evaluated, including the carbon and nitrogen-related traits (Table 4.2). Regarding the phenologic, the environment strongly affected DH and GDDH, which could be related to changes in the phenology shortening the life cycle and, especially, grain filling period and consequently GY, which was also highly affected by the environment (Tables 4.2 and S4.2). Although the contribution to the observed variability in agronomic parameters due to the effect of genotype was smaller than the environment, it was remarkable and significant for all cases. It suggests that there is a wide genotypic variability in durum wheat that needs to be evaluated to optimise GY and, as we will discuss later, grain quality. Our data indicated that DH was mainly affected by the environment, highlighting significant differences in

phenology due to the growth conditions. However, high genotypic variability was also reported for traits such as the ear attributes (SL) and sink strength (NKS), highlighting that ear architecture is a relevant trait to exploit in breeding programmes. The GEI was also significant for most cases, suggesting that adaptation to the environment depends on the selected variety. Therefore, studies adapted to particular agro-environments, such as those of the continental Mediterranean conditions of Castile and León, are essential to define ideotypes that contribute to durum wheat improvement.

We showed that the impact of the environmental conditions on grain quality, in the case of the PROT, depends significantly on the water regime, obtaining the highest values in the environments under rainfed conditions, in line with previous studies carried out (Flagella *et al.*, 2010). This pattern was also observed for the traits TW and TKW, which together with PROT, are some of the most important traits for commercialisation. In fact. PROT and TKW were negatively related through environments, which suggest PROT is affected by a "dilution" effect associated with the size of the grains (Ben Mariem *et al.*, 2021**)** Furthermore, the crop season, which is a factor integrating different growth conditions, significantly affected grain quality traits such as VTR, b*, SDSS, WG, and GI. The genetic effect and its interaction with the environment were also significant for some relevant grain quality traits, such as gluten properties (WG and GI) and PROT, among others (Taghouti *et al.*, 2010).

In the case of grain carbon and nitrogen content and the signatures of the C and N stables isotopes, the environment was again the main significant factor, but the genotypic or GEI effects were lower compared to yield and quality-related traits (Table 4.2). The grain N and C concentrations were affected positively by the water deficit. We observed clearly how the different environments affected the C concentration, obtaining the highest values in the years with less than 200 mm of total precipitation during the growth cycle. The genotypic variability was not significant in some traits, such as %C, $\delta^{15}N$ and GNY, highlighting that grain C concentration and total nitrogen harvested did not significantly vary between varieties. Nevertheless, other traits such as %N and $\delta^{13}C$ were remarkably affected between varieties. This highlights that grain nitrogen and protein concentration is genotype-specific, being an important factor conditioning grain protein, while $\delta^{13}C$ for wheat and other $C_{3\ crops}$ is a valuable tool that can be used in breeding programmes to search for genotypic variability since it usually correlates with yield (Araus *et al.*, 2022).

Overall, the environment, the genotypic variability and the interaction strongly affected the yield and quality traits of durum wheat in the Spanish region of Castile and León. This pointed out the relevance of exploiting the existing genotypic variability in durum wheat to select those varieties that

have hight values in specific environments (as those predicted for future climate scenario) or, on the contrary, those that are stable for the traits of interest to the industry, both at production and processing level (Grausgruber *et al*., 2000; Rharrabti *et al*., 2003; Sieber *et al*., 2015; Vida *et al*., 2021).

4.4.2. Evaluation of the genotype-by-environment interaction through Finlay-Wilkinson and GGE models to identify durum wheat ideotypes adapted to specific environments or with a broad spectrum in the region of Castile and León

Developing and selecting high-yielding durum wheat varieties with good grain quality and stability to diverse environmental conditions is important for food security worldwide and in the local private sector. In agro-environments like the Mediterranean region, where the climate conditions vary periodically and unpredictably, and more likely in the near future due to climate change (Beck *et al*., 2018), the stability of a variety is desired and reveals its consistency in performance, named resilience, for economically important traits such as grain yield and quality. In the Mediterranean conditions, with these erratic and unpredictable climatic variations, the assessment of stability requires the performance of multi-environments trials (Vida *et al*., 2021). Peterson *et al*. (1992) reported that the concept of optimal variety stability and response for quality parameters differs from what is conventionally used to describe yield stability. For end-users as millers, consistency in the quality performance of the durum wheat varieties is crucial regardless of changing genotypes ranks. Still, for breeders and farmers, the stability of quality attributes is linked to the ranks of genotypes across the environments and their effects on the selection process (Troccoli *et al*., 2000). Nevertheless, Grausgruber *et al*. (2000) indicated that the quality parameters react to environmental conditions like other quantitative characters. Therefore, a variety could be considered economically stable if its contribution to GEI is low.

Several statistical methods have been proposed to analyse GEI, providing information about the differential performance of the cultivars in different environments and having a key role in assessing performance stability or adaptability to specific environments (van Eeuwijk *et al*., 2016; Bustos-Korts *et al*., 2019). After studying the significance of the main effects and their interaction with the AMMI model, we applied other two methodologies to our data to explore in more detail the GEI: the Finlay-Wilkinson and the GGE models. The Finlay-Wilkinson model consists of a regression model for the performance of each variety on the environmental means, which aims to assess the performance of a

variety as a function of the environmental effects (Finley & Wilkinson, 1963; Lian & de Los Campos, 2015). The GGE model works with a data matrix combining the main impact of the geno genotype and the interaction of the genotype with the environment. It allows identifying the existing mega-environments, clusters of environments by the performance of the varieties under specific environmental characteristics. Therefore, it is an appropriate method for studying multi-environment trials to visualise the performance of the varieties studied (Yan & Tinker, 2006). Typically, the different models for the evaluation of the GEI are focused on grain yield, but we decided to apply these models for all our agronomic grain quality, and carbon and nitrogen isotope signature traits to study the behaviour and stability of these traits, with particular emphasis on grain quality.

According to joint regression analyses (Finlay & Wilkinson, 1963), the slope of each variety on the environment means for each trait provides information on stability and adaptation (Figure 4.4). If the slope value increases above 1, it describes varieties with increasing sensitivity to environmental changes and greater specificity of adaptability to high-yielding environments. Conversely, regression coefficients below one are associated with more resistance to environmental changes (above average stability) and, therefore, higher adaptability in low-yielding environments (van Eeuwijk *et al*., 2016). With these analyses, we are not only able to evaluate the most resilient varieties for a trait of study, but also those most adapted to a specific environment that may be of interest or even to select among the most stable varieties for all environments or adapted to a specific one, those with the highest values for the desired trait. It is the case of GY (Figure 4.4), where different varieties such as SIM, BUR, DOR, SCU, and AVI, among others, showed stability for the 13 environments. Moreover, we could easily visualise that SCU and AVI are the most stable varieties with high yields. Nevertheless, the varieties AMI and OLI showed even higher yield with relatively similar stability. Their slopes with values slightly above 1 for these two varieties suggested that they were more adapted to high-yielding environments. However, the varieties outperforming in terms of yield traits may not show the same trend for grain quality traits. With the highest GY, OLI presented an average PROT compared with the other tested varieties, being better adapted to high-yielding environments. If the purpose is to obtain a stable amount of PROT with a relatively high yield, REG was a good candidate with also stable values for TW and b*, which is relevant for the industry. In this sense, some key traits for the commercialisation of durum wheat and its products are VTR and GI (Fu *et al.*, 2018), which were strongly affected by the environment in a genotypic-specific manner. For example, VTR is a key trait in durum wheat because high vitreous grain content determines a higher semolina production, milling and cooking quality (Fu *et al*., 2018).

The GGE model has also been shown as an effective tool for assessing performance and stability (Yan *et al*., 2000; Enyew *et al*., 2021). However, GGE biplot analyses have been predominantly employed to determine yield and yield-related traits of crops. At the same time, there is a lack of studies regarding the mean performance versus stability of grain quality traits of interest for the food production chain. We considered, together with the results of GGE biplots of mean and stability, the biplots of ranking genotypes for the ideal performance of the varieties (Figures 4.5, 4.6, S4.2 and S4.3), in agreement with other publications (Chairi *et al*., 2020; Enyew *et al*., 2021; Vida *et al*., 2021). Similarly, to the conclusions obtained from the Finlay-Wilkinson model, AMI and OLI were among the best outperforming varieties achieving at the same time high grain quality and a significant yield independently of the climate conditions and abiotic stress. In the case of OLI, we can reach a high content of some very interesting parameters for the industry, such as b* and GI. However, the PROT of this variety was more susceptible to environmental changes. Another interesting variety was BUR, which achieved high PROT, SDSS, WG and VTR values compared to other varieties, demonstrating its high grain quality potential, but although its GY was stable, it was low. Other varieties also showed stable, and high values for several of the yield-related and quality traits studied, which are listed in Table 4.3 and can be used to support the selection of the most advisable varieties depending on the final objective, which may be the total production or some specific quality characteristics. These results were also corroborated with the Which Won Where/What biplots (Figures 4.7 and S4.4). They additionally provided information about the MGEs conditioning the agronomic and quality traits locally in our region and the best and the worst varieties performing in each MGE (Yan & Tinker, 2006). The results clearly suggested that water conditions in this region (determined by rainfall or irrigation) were a key factor for both yield and grain quality, largely defining the MGEs identified for each trait. Only a small number of MGEs were identified for certain traits, such as TKW, DH, PL, and SL. This may indicate that environmental conditions are not a key factor in these traits, which were associated with plant architecture, phenology and grain size, and may suggest a greater genetic dependence. Apart from selecting the varieties with broad stability, we can also exploit our data to search for varieties adapted to limiting environments, which will be the most likely scenarios in the future. In a pessimistic scenario, the challenge will not be to improve the yield and quality but to maintain current levels as far as possible. This is particularly urgent for durum wheat because the Mediterranean basin is a hotspot for adverse climate change predictions regarding decreased rainfall and increased temperatures (Lobell & Field, 2007).

4.4.3. High estimates of phenotypic and genotypic coefficients of variation and heritability indicated crop improvement through selection for agronomic and grain quality traits such as number of kernels per spike, gluten index, yellow pigment concentration, and spike length

The estimated PCV values were higher for $\delta^{15}N$, NKS, GI, NPS and GY (Table 4.4). It pointed out the existence of a greater scope of selection for these traits, while it is more limited for traits such as DH, TW and $\delta^{13}C$. For the estimated GCV values, NKS and GI were the highest, while the remaining traits reached low to moderate values. This suggests an outstanding presence of genotypic variability for these two traits that may contribute to selection. When the values of PCV and GCV are close, it indicates a narrow range of genotypic variability together with less influence from environmental factors (Ahsan *et al*., 2015). The low differences between PCV and GCV for DH, TW and b* were indicators of a low influence of the environment in the expression of characters or lower sensitivity of the varieties to the environment and a more significant role of genetic control governing the character. Opposite, the high differences between PCV and GCV for the traits GY, NSP and VTR were associated with a more significant environmental than the genotypic effect on the variation of these traits.

Heritability in a broad sense is a direct selection parameter that provide the repeatability of characters, which indicates the effectiveness of selection in their improvement. It measures the phenotypic variance attributed to genetic causes and is expressed as a percentage of the ratio between genotypic and phenotypic variances. According to Johnson *et al*. (1983), heritability can be classified as low below 30%, medium for 30-60%, and high above 60%. The estimated heritability for the traits in our study varied from 5.28% to 65.55%, being very low for GY (Table 4.4). In a previous study, Chairi *et al*. (2018) showed that the rate of genetic progress for GY in durum wheat in Spain after the Green Revolution has been low or even stopped during the last decades, while there is no clear trend in some grain quality traits (TKW and PROT). It highlights that the heritability for each trait can be different depending upon the genetic material, environment, and computation method (Blanco *et al*., 2012).

In our study, only for b* and SL, high heritability was observed. Several studies have been related to the detection of heritability in yellow pigment concentration in durum wheat, contributing to an early selection for this trait (Elouafi *et al*., 2001; Mares & Campbell, 2001; Clarke *et al*., 2006; Patil *et al*., 2008; Sieber *et al*. 2015). SL could be considered a key trait, as larger spikes will likely produce more

grains and increase yield. Baloch *et al.* (2016) reported highly significant variation for this trait among bread wheat varieties and high heritability. The number of kernels per spike (NKS) contributes considerably to grain yield by increasing sink strength. Our study showed a medium heritability (48.7%) for NKS (Table 4.4). High heritability wasalso reported for NKS by Mohammadi *et al.* (2011) and Gerema (2021) in durum wheat and by Baloch *et al*. (2016) in bread wheat. For PL, TKW, SDSS and GI, heritability was medium Similarly, in previous studies, it has been found that heritability for SDSS varied from moderate to high (Clarke *et al*., 2010, Taneva *et al*., 2019, Taghouti *et al*., 2010). Heritability was moderate for DH, TW and PROT. Oppositely, Mohsin *et al.* (2009) found high heritability for DH and TKW in wheat. In Taneva *et al*. (2019), VTR was the grain quality trait that had the lowest heritability, showing the substantial environmental effects on this trait, according to our results.

4.5. CONCLUSIONS

Achieving high yields with high grain quality, including most of the parameters of interest to the industry, continues to be a challenge facing agriculture. In particular, the Mediterranean continental conditions of, the Spanish region of Castile and León, show a large climatic variability between crop seasons that is expected will be intensified as climate change progresses. While areas in the south of the country, such as Andalusia, with warmer climates are currently the main producers of durum wheat in Spain, the Castile and León region is a strong candidate to increase durum wheat production, which the predicted new climate scenario would favour. Our study covering 14 of the Spanish most used commercial durum wheat varieties and a diverse panel of growth conditions, highlights the need to identify the durum wheat ideotypes for local adaptation. The effect of the environmental conditions was predominant in determining most traits, although some of them, such as TKW and yellow pigmentation, were also genetically controlled in a relevant proportion. We identified durum wheat candidates with high yield and quality standards for most of the environments considered in the study. However, we also note that we did not find varieties with high stability and high means for all the quality parameters of interest to the industry, so the use of one variety or another depends ultimately on the intended use of the grain. Considering the pool of varieties studied, we observed that some varieties such as AMI and OLI showed stability in the environments evaluated (rainfed, irrigation, high temperatures and nutrient deficiencies), having improved grain quality characteristics for several traits. The AMMI, Finlay-Wilkinson, and GGE models were used to study in detail the GEI and showed that they complemented each other. While we suggest that they can be used individually, using them

together allowed us to corroborate the results obtained and draw additional conclusions. Furthermore, the number of kernels per spike, gluten index, yellow pigment concentration, and spike length were the traits with high heritability estimates among the traits studied.

5. CHAPTER 5:

Second Study

Mineral elements concentration in a collection of durum wheat varieties in north Spain.

5.1. INTRODUCTION

Durum wheat (*Triticum turgidum* L. subsp. *durum*) is a significant staple food supplying both calories and nutrients in many parts of the world, playing an essential role in global food security (Shiferaw *et al.* 2013). Wheat provides vital components required for human nutrition, such as minerals, vitamins, beneficial phytochemicals, and dietary fibre (Shewry 2009). These components have a considerable impact on human health and well-being. With the projected population of 9.5 billion by 2050, agriculture faces the challenge of ensuring that crop production satisfies the increasing food demand. Thus, it is vital to focus on improving wheat's yield and nutritional properties (Alexandratos & Bruinsma 2012). The improvement of yield and grain quality of durum wheat is crucial when confronted with the increasing global population, changing climate environments, and the non-ignorable increasing incidence of wheat-related disorders (Yang *et al.*, 2022b). There is evidence that the number of people and the proportion of the global population suffering from micronutrient malnutrition have increased over the last four decades (Graham *et al.*, 2007; Welch & Graham, 2002). Iron (Fe) and Zinc (Zn) are the two most important mineral nutrients contributing to micronutrient deficiency (Velu *et al.*, 2014). One of the reasons is that durum wheat breeding programs carried out over the 20th century have been historically oriented toward high agronomic yield in combination with quality characteristics for pasta products rather than the nutritional quality and grain health-promoting components (Morris & Sands, 2006). The incidence of trace-element deficiencies appears to be increasing since the Green Revolution, which take place in the 1960s (Graham *et al.*, 2007). Increased grain yield may have resulted in a lower density of minerals in grain, although evidence for this available up to now is contradictory (Garvin *et al.*, 2006; Graham *et al.*, 1999; McGrath, 1985; Oury *et al.*,2006).

Durum wheat grain is a significant source of magnesium (Mg), manganese (Mn), Fe, Zn, copper (Cu) and molybdenum (Mo), and is less relevant of sodium (Na). As with vitamins, most minerals are located in the bran or germ because that milling reduces their concentration, especially in the case of Mn, Fe, Mg and Zn, due to their relatively low concentration in the endosperm. Then the consumption of whole-grain rather than refined products would increase the dietary trace element contribution from durum wheat, even though bioavailability is generally lower because fibre components bind the elements, mainly Fe and Zn.

In staple crops, the nutritional quality can be improved by different practices such as breeding, genetic engineering, agronomic practices and fortification (Bouis, 2003; Welch & Graham, 2004; Cakmak, 2008; Zhao & McGrath, 2009). Breeding of cereal crops with increased micronutrient concentration requires knowledge of the variation in the trait among the available germplasms. Several studies have screened wheat varieties for mineral concentrations, showing substantial variation in nutrient concentrations in grain (Graham *et al.*, 1999; Liu *et al*., 2006; Morgounov *et al*., 2007). The accumulation and distribution of trace elements in crops are affected by genetic factors. In the uptake and distribution of trace elements exist natural variation between harvests and varieties within species (Graham *et al.*, 2007; Welch & Graham, 2004). Then cultivar selection is a method for changing the trace element profile of durum wheat, therefore could reduce the requirement for other management practices such as fertilization. Also, must be considered the bioavailability of the nutrients. A reduction of grain phytate would be desirable for achieving higher mineral density (Cakmak, 2008).

Previous studies analysed the genetic variation for nutrients. For example, low variation was found for grain nutrient concentration within bread wheat (Oury *et al*., 2006; Zhao & McGrath, 2009; Bulut, 2022) or comparing *Triticum* species (Monasterio & Graham, 2000) or in a collection of Italian durum wheat cultivars (Ficco *et al*., 2009). However, studies that characterized variation in grain mineral concentration specifically within the Spanish durum wheat germplasm have not been published yet. Therefore, in this study, field experiments were conducted across three seasons (2017-2018-2019) and under different managements (irrigated, rainfed, late sowing and low nitrogen), using 24 varieties that were released in different decades after the Green Revolution, to evaluate the genetic variability of grain mineral concentration and to explore the relationships with agronomic and quality traits.

5.2. MATERIALS AND METHODS

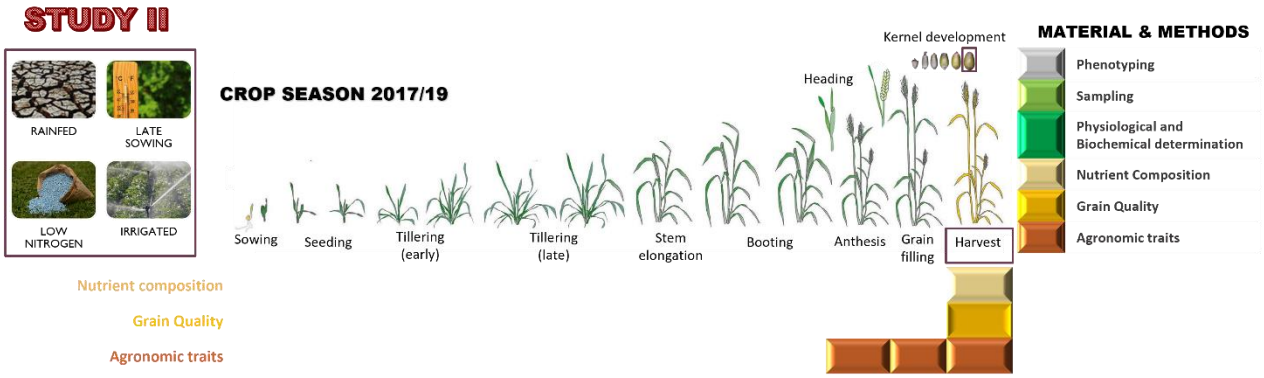


Figure 5.1. The field experiments were conducted over three consecutives growing seasons across four years (2017-2019). The plants grew under contrasting diverse growth conditions, such as rainfed (R-), support irrigation (R+), late-sowing (L) and low nitrogen fertilization conditions (N-), which, together with the different climate conditions over the four years, resulted in a total of 9 environments (see Table 4.1 for further details). Grain quality traits and nutrient composition were evaluated in the harvest grain, together with some agronomic traits (GY, TW and TKW) (see Chapter 3 for further details) in all the varieties presented in our panel of study, in which Iride was replaced for Arcobaleno respect to the previous panel presented in chapter 3 (Table 5.1).

Table 5.1. A representative set of twenty-four durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.)) varieties were study, representing the variability of varieties cultivated in Spain after 1970.

Nº	VARIETIES	ID	YEAR OF RELEASE	COUNTRY	PROVENANCE/PEDIGREE
1	Amilcar	AMI	2002	Spain	ZEGZAG-1/LUNDE-5//GREENSHANK-32
2	Arcobaleno	ARC	1996	Spain	Chen/Altar84
3	Athoris	ATH	2011	Italy	Limagrain Europe
4	Avispa	AVI	2003	Italy	Limagrain-CIMMYT
5	Burgos	BUR	1999	Spain	SUDDEUTSCHE SAATZ
6	Claudio	CLA	1998	Italy	SEL.CIMMYT-35/DURANGO//ISEA-1938/GRAZIA
7	Core	COR	2009	Spain	Europgen PROSEME seeds
8	Don Norman	DNO	2012	Spain	Agrovegetal-CIMMYT
9	Don Ricardo	DRI	2008	Spain	Agrovegetal-CIMMYT
10	Dorondón	DOR	1999	Spain	Genética y Gestión,S.C.
11	Euroduro	EUR	2007	Spain	IRTA
12	Gallareta	GAL	1994	Spain	RUFF/FLAMINGO//MEXICALI-75/3/SHEARWATER
13	Haristide	HAR	2015	France	Caussade Semences S.A.
14	Iberus	IBE	2014	Spain	Agromonegros
15	Kiko Nick	KNI	2009	Spain	SEL.CIMMYT-35/DURANGO//ISEA-1938/GRAZIA
16	Mexa	MEX	1980	Spain	GERARDO-VZ-469/3/JORI(SIB)//ND-61-130/LEEDS
17	Olivadur	OLI	2013	Spain	RAGT 2N SAS seeds
18	Pedroso	PED	1993	Spain	Battle seeds
19	Regallo	REG	1990	Italy	Diputación General de Aragón CIMMYT
20	Saragolla	SAR	2004	Italy	Iride/0114
21	Sculptur	SCU	2011	France	RAGT Semence
22	Simeto	SIM	1988	Italy	CAPEITI-8/VALNOVA[1620][1622][1623][1625][1666]
23	Solea	SOL	2005	Spain	Monsanto Agriculture Spain
24	Vitrón	VIT	1983	Spain	TURCHIA-77/3/JORI-69(SIB)/(SIB)ANHINGA//(SIB)FLAMINGO

5.3. RESULTS

5.3.1. Description of the environments or trials.

In this study, nine experiments (Env) were carried out at the same experimental station. The combination of treatment (R+, R-, L and N-) and season (2017-2018-2019) has been considered as environment (Table 5.2). High differences in yield across experiments were observed. The mean yield ranged from 7928 kg ha^{-1} (Env 7) to 2799 kg ha^{-1} (Env 9), it means a difference of 5099 kg ha^{-1}. Considering the rainfall plus the irrigation, the total water received fluctuated from 586 mm (Env 4) to 127mm (Env 8 and 9). The sowing date was between middle November to early December for all the trials except for Env 3, late sowing in February. All trails were harvested in July.

Table 5.2. Description of the 9 environments studied, including the season year, the treatment (R+, irrigated; R-, rainfed; L, late sowing; N-, low nitrogen supply), the sowing, heading and harvest dates, the climate conditions, the rainfall and support irrigation during the period of the growth cycle, and the average values for each environment for grain yield (GY).

Environment	1	2	3	4	5	6	7	8	9
Season	2016-2017	2016-2017	2016-2017	2017-2018	2017-2018	2017-2018	2018-2019	2018-2019	2018-2019
Treatment	R+	R-	L	R+	R-	N-	R+	R-	N-
Sowing date	29/11/2016	29/11/2016	09/02/2017	13/11/2017	23/11/2017	23/11/2017	03/12/2018	03/12/2018	03/12/2018
Heading date	25/04-04/05	22/04-28/04	10/05-20/05	11/05-19/05	11/05-19/05	11/05-19/05	01/05-14/05	02/05-13/05	02/05-10/05
Harvest date	06/07/2017	06/07/2017	20/07/2017	25/07/2018	20/07/2018	20/07/2018	15/07/2019	03/07/2019	03/07/2019
Range of mean temperature (°C)	4.3-18.2	4.3-18.2	7.4-23.1	4.3-16.2	4.5-15.8	4.5-15.8	3.6-18.0	3.0-17.2	3.0-17.2
Range humidity (%)	41.6-95.1	41.6-95.1	29.6-93.0	52.4-99.62	53.8-99.6	53.8-99.6	46.6-97.6	47.9-97.6	47.9-97.6
Rainfall (mm)	124.0	124.0	100.6	476.4	476.0	476.0	145.9	127.5	127.5
Irrigation (mm)	155	55	155	109.8	0	0	152.7	0	0
Total water received (mm)	279.0	179.0	255.6	586.2	476.0	476.0	298.6	127.5	127.5
GY (kg ha^{-1})	6937 ± 1045	2829 ± 1031	5208 ± 1268	6374 ± 734	6868 ± 1095	5230 ± 1031	7928 ± 1272	2832 ± 637	2799 ± 766

5.3.2. Yield, quality traits, and nutrient content effect of the variety, year, and interaction.

The statistical analyses ANOVA showed that the genotype, the environment and their interaction (GEI) had significant effects on all mineral elements concentration and also on yield and quality parameters determined, except for the Mg, P and Zn concentration in which the contribution of GEI to the Sum of Squares (SS) was not significant (Figure 5.2).

Except for the Test Weight (TW), for the other traits analysed, the environment was the factor that explains the highest variability observed and ranged from 21.3% (TW) to 77.8% (GY). In the case of

TW, the genotypic variability explained the highest portion of the variability (33.0%) followed by the environment (21.7%).

We observed that, in spite of been low the contribution to the SS, the genotypic variability was also significant for all the traits studied, ranging from 1.4% (GY) to 33.0% (TW), showing a high value for b* (23.5%). The contribution of the GEI ranged from 6.4% (b*) to 21.3% (TW).

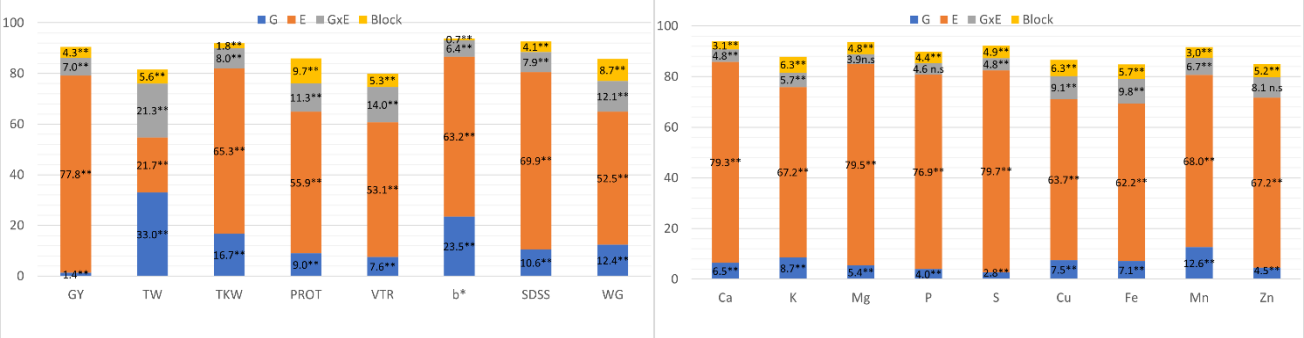


Figure 5.2. Contribution of the genotype (G) and environment (E) factors and their interaction (GxE) to the Sum of Square. .**, statistically significant at $p<0.001$; ns, no significant.

Considering the nutrient content, the contribution to the SS of the environment ranged from 62.2% (Fe) to 79.7% (S). The highest contribution of the genotypic variability was found for Mn concentration (12.6%) and the lowest for S (2.8%). The contribution of GEI varied from 3.9% (Mg), not significant, to 9.8% (Fe).

5.3.3. Variation in nutrient concentration

The mean of mineral element concentration in the grain and different indicators of their fluctuations among the 24 varieties in the 9 environments are presented in Table 5.3. Averaged across environments, in relation to macronutrients, grain K concentration ranged from 3.5 to 7.61 g kg $^{-1}$ (mean: 5.10 g kg $^{-1}$), Mg concentration from 0.90 to 2.27 g kg $^{-1}$ (mean: 1.39 g kg $^{-1}$) and Ca ranged from 0.23 to 0.80 g kg $^{-1}$ (mean: 0.43 mg kg $^{-1}$). Considering the concentration of micronutrients, grain Fe ranged from 22.5 to 79.46 mg kg $^{-1}$ (mean: 40.93 mg kg $^{-1}$), Zn concentration varied from 13.38 to 45.13 mg kg $^{-1}$ (mean: 22.02 mg kg $^{-1}$), Mn concentration from 20.61 to 72.94 mg kg $^{-1}$(mean: 36.49 mg kg $^{-1}$) and Cu ranged from 2.64 to 9.00 mg kg $^{-1}$ (mean 4.92 mg kg $^{-1}$).

Table 5.3. Grain concentration of macronutrients (K, P, S, Mg and Ca) and micronutrients (Zn, Fe, Mn and Cu) for 24 durum wheat cultivars across the nine environments.

		Mean	SD	CV	Min	Max
Macronutrients	**K** (g/kg)	5.105	0.939	18.39	3.534	7.608
	P(g/kg)	4.355	1.195	27.43	2.526	7.460
	S (g/kg)	1.944	0.545	28.02	1.068	3.230
	Mg (g/kg)	1.386	0.321	23.15	0.898	2.265
	Ca (g/kg)	0.434	0.133	30.71	0.229	0.797
Micronutrients	**Zn** (mg/kg)	22.022	6.597	29.96	13.376	45.130
	Fe (mg/kg)	40.928	10.944	26.74	22.492	79.590
	Mn (mg/kg)	36.495	10.490	29.52	20.613	72.940
	Cu (mg/kg)	4.924	1.273	25.85	2.640	9.001

The highest grain Ca concentration among all varieties was obtained for Mexa by more than 50 g kg^{-1} and the lowest for Sculptur and Burgos (0.38 and 0.39 g kg^{-1}) (Table 5.4). Sculptur, Gallareta and Avispa, showed the highest grain K concentration (5.59 to 5.47 g kg^{-1}), whereas Vitrón and Core (4.52 and 4.59 g kg^{-1}) showed the lowest K concentration. Concerning micronutrients concentration, Burgos, Don Ricardo and Simeto showed the highest value for Fe (46.80 to 46.30 g kg^{-1}); Iberus and Burgos for Mn (45.67 and 42.27 g kg^{-1}) and considering the Zn, Simeto, Euroduro and Claudio presented the highest concentration (25.60 to 24.08 g kg^{-1}). This indicates that the varieties Euroduro, Burgos, and Simeto were superior in terms of all nutrients considered, whereas Athorix, Haristide and Sculptur were inferior.

Table 5.4. Macro (Ca, K, Mg, P and S) and micronutrients (Cu, Fe, Mn and Zn) averaged per varieties (n=24) and environment (n=72, for each trial). Values are reported as means ± standard error. LSD' test at 5% indicated differences between groups statistically.

	Macronutrients (g/kg)					**Micronutrients (mg/kg)**			
	Ca	K	Mg	P	S	Cu	Fe	Mn	Zn
Varieties									
Amilcar	0.44±0.14	5.23±1.05	1.36±0.36	4.27±1.36	1.90±0.58	4.72±1.33	38.33±13.51	33.39±11.96	20.43±6.71
Arcobaleno	0.41±0.15	5.35±1.10	1.37±0.34	4.44±1.31	1.88±0.63	4.48±1.49	39.36±14.09	32.34±11.35	21.85±8.39
Athorix	0.43±0.14	4.78±0.85	1.29±0.29	4.09±1.07	1.75±0.56	4.25±1.12	39.57±11.57	34.30±9.51	19.24±5.89
Avispa	0.43±0.15	5.47±0.93	1.37±0.35	4.43±1.19	1.88±0.61	4.68±1.34	39.71±13.12	32.60±10.34	21.42±7.66
Burgos	0.39±0.12	5.06±0.95	1.52±0.34	4.47±1.12	2.16±0.62	5.24±1.44	46.80±11.12	42.27±13.45	23.06±7.21
Claudio	0.40±0.12	4.92±0.91	1.39±0.28	4.17±1.03	1.93±0.57	4.86±1.12	40.85±8.30	41.64±10.14	24.08±6.66
Core	0.47±0.14	4.59±0.79	1.34±0.33	4.32±1.31	2.10±0.59	4.67±1.38	41.13±8.86	37.07±9.88	21.15±7.04
D.Norman	0.41±0.15	5.04±1.12	1.49±0.34	4.58±1.37	1.92±0.56	5.09±1.10	40.43±10.43	39.17±10.78	22.59±7.75
D.Ricardo	0.35±0.12	4.82±0.85	1.49±0.26	4.72±0.94	1.84±0.57	5.25±1.21	46.73±8.25	39.29±7.76	22.59±5.78
Dorondó	0.41±0.10	5.09±0.95	1.37±0.36	4.20±1.35	1.85±0.53	4.71±1.37	37.92±11.97	34.63±11.36	21.13±8.19
Euroduro	0.41±0.11	5.23±1.06	1.45±0.39	4.79±1.58	1.99±0.56	4.92±1.06	42.20±10.95	38.02±11.08	24.44±7.53
Gallareta	0.42±0.14	5.54±1.05	1.39±0.38	4.49±1.40	1.89±0.53	4.95±1.49	40.50±13.54	36.28±13.44	22.86±8.29
Haristide	0.44±0.06	4.69±0.54	1.17±0.07	3.48±0.59	1.90±0.47	4.50±1.10	33.40±4.38	27.96±6.25	18.99±4.44
Iberus	0.45±0.14	4.99±1.08	1.53±0.43	4.43±1.58	1.97±0.66	5.02±1.43	42.99±15.21	45.67±13.31	22.72±8.19
Kiko Nick	0.42±0.13	5.01±1.02	1.45±0.36	4.37±1.33	2.06±0.58	5.14±1.17	43.08±11.95	37.35±10.44	21.81±6.91
Mexa	0.51±0.18	5.14±0.93	1.38±0.27	4.17±1.06	1.88±0.49	4.74±1.11	38.00±8.46	36.04±8.51	20.70±6.80
Olivadur	0.46±0.14	5.64±1.43	1.34±0.29	4.35±1.45	1.92±0.66	5.80±1.55	37.03±11.27	34.44±7.95	23.08±8.89
Pedroso	0.44±0.15	5.27±1.16	1.31±0.31	4.10±1.21	2.03±0.61	4.47±1.22	40.79±8.84	31.45±7.15	22.81±7.91
Regallo	0.48±0.15	4.84±1.11	1.39±0.37	4.32±1.28	2.07±0.65	4.63±1.53	41.63±9.34	41.37±13.27	22.07±7.40
Saragolla	0.47±0.14	5.34±1.00	1.37±0.37	4.37±1.45	1.89±0.56	4.58±1.29	40.38±12.51	36.97±11.98	21.35±6.74
Sculptur	0.38±0.12	5.59±1.01	1.25±0.32	3.98±1.27	1.85±0.59	5.19±1.41	34.89±10.42	31.01±8.61	19.00±5.89
Simeto	0.47±0.14	4.95±0.76	1.47±0.37	4.49±1.18	2.08±0.59	5.76±1.70	46.30±14.42	37.43±12.02	25.60±8.13
Solea	0.46±0.13	5.42±1.22	1.37±0.33	4.79±1.37	1.93±0.57	4.84±1.17	43.80±17.04	31.83±8.48	22.92±6.86
Vitrón	0.48±0.14	4.52±0.78	1.37±0.34	4.52±1.44	2.01±0.55	4.82±1.29	42.34±12.35	39.30±11.06	22.20±8.27
p-value	0.023	0.244	0.057	0.278	0.109	0.336	3,154	2,189	1,933
Environment									
1	0.50±0.08	6.24±0.60	1.49±0.12	5.06±0.56	2.23±0.30	5.54±0.96	38.59±5.57	42.25±7.25	25.53±3.53
2	0.60±0.09	6.07±1.00	1.89±0.31	6.09±1.22	2.36±0.42	6.29±1.05	63.31±13.04	58.04±11.53	22.12±4.35
3	0.66±0.10	6.46±0.71	1.97±0.16	6.36±0.66	2.83±0.34	7.07±1.05	52.44±8.05	45.18±6.21	37.60±5.08
4	0.32±0.05	4.54±0.38	1.22±0.11	4.44±0.40	1.30±0.14	4.42±0.65	32.96±5.43	32.80±4.41	20.78±4.75
5	0.30±0.04	4.19±0.35	1.15±0.10	3.99±0.28	1.44±0.18	4.53±0.71	35.08±5.92	33.05±4.36	17.00±2.63
6	0.30±0.04	4.10±0.29	1.15±0.11	3.85±0.35	1.29±0.17	3.75±0.58	32.30±5.44	30.86±4.42	18.29±3.61
7	0.38±0.05	4.72±0.39	1.35±0.13	3.51±0.42	1.66±0.23	3.99±0.76	40.07±8.45	33.78±6.47	16.96±3.57
8	0.41±0.06	4.66±0.60	1.11±0.11	2.90±0.45	2.15±0.22	3.86±0.68	38.03±5.63	26.34±3.91	18.36±4.13
9	0.45±0.06	5.18±0.70	1.22±0.10	3.28±0.62	2.31±0.27	4.82±0.79	36.91±4.89	27.92±3.47	22.55±5.55
p-value	0.014	0.149	0.035	0.17	0.067	0.204	1,925	1,336	1.18
TOTAL									
	0.43±0.14	5.11±1.03	1.39±0.34	4.36±1.29	1.94±0.58	4.89±1.35	40.86±11.94	36.46±11.21	22.04±7.37

Analyzing the environmentss, we observed that, in general, Env 1, 2 and 3 presented higher nutrient concentration, especially Envl 3 (late sowing). Otherwise, Env 6 (low nitrogen) showed the lowest concentration for all nutrients.

5.3.4. Variation in agronomic and quality traits

Across the nine environments, in Table 5.5, the most productive varieties that showed the highest GY were Olivadur, Arcobaleno and Athorix, with yields upper to 5600 kg ha^{-1}. In contrast, Simeto and Solea varieties showed the lowest mean yield (4679 and 4793 kg ha^{-1}). The TW ranged from 77.15 (Olivadur) to 81.17 g hL^{-1} (Athorix) and the TKW from 41.39 (Olivadur) to 55.64 g (Simeto). The protein content ranged from 13.33 to 16.09 % (Athorix and Simeto), whereas Don Ricardo showed the lowest values for b* and SDSS (14.27 and 37.26 mL, respectively) and Sculptur the highest for b* (17.00) and Burgos for SDSS (48.24 mL). Finally, Simeto was the variety with superior vitreousness (95,54 mL) and WG (32,74%).

Env 8 and 9, corresponding both to experiments carried out in 2019, presented the lowest values for GY and TKW. However, these trials showed the highest values for quality parameters such as VTR, b* and SDSS. The environmental conditions that characterized the Env 4 conditioned it to a worse quality and presented the lowest values for all quality traits.

Table 5.5. GY (grain yield), PROT (protein content), TW (test weight), TKW (thousand kernel weight), VTR (vitreousness), b* (yellow pigment content), SDSS (SDS sedimentation), and WG (wet gluten) averaged per variety (n=24) and environment (n=72 for each trial). Values are reported as means ± standard error. LSD' test at 5% indicated differences between groups statistically.

	GY	PROT	TW	TKW	VTR	b*	SDSS	WG
Varieties								
Amilcar	5537±2386	14.1±2.3	80.3±1.3	48.5±7.3	84.5±20.9	14.9±1.4	38.4±7.7	26.4±5.5
Arcobaleno	5633±2255	13.9±2.2	80.5±2.1	46.4±7.9	85.6±16.8	15.0±1.7	39.5±6.5	26.3±6.0
Athorix	5621±2319	13.3±1.9	81.2±1.5	47.2±7.5	77.1±22.0	16.7±1.5	42.1±8.7	24.5±5.4
Avispa	5377±2163	13.8±2.4	80.1±1.6	48.2±7.0	79.6±21.1	14.9±1.3	39.4±8.3	25.4±6.3
Burgos	4955±2284	15.8±2.4	78.7±1.7	50.3±7.4	93.4±8.9	15.2±1.0	48.2±9.8	31.6±6.7
Claudio	5305±1941	14.5±1.9	80.9±1.5	47.6±6.7	79.7±22.5	15.2±1.5	43.0±7.7	28.7±6.1
Core	5149±2146	14.9±1.7	79.1±1.9	51.3±7.9	79.2±18.8	14.8±1.5	41.0±8.5	29.9±5.4
D.Norman	5097±2119	14.7±1.9	80.7±1.7	45.8±6.7	83.6±19.4	16.6±1.2	42.9±7.4	29.3±5.5
D.Ricardo	5054±1899	14.9±2.2	80.1±1.7	53.6±7.0	90.1±17.3	14.3±1.5	37.3±8.5	28.5±5.8
Dorondó	5150±1947	13.8±1.7	80.5±1.1	46.6±5.4	83.5±15.4	14.8±1.1	39.2±7.8	25.7±5.3
Euroduro	5246±2159	14.9±2.4	80.6±1.5	49.0±6.0	86.6±15.3	15.6±0.9	44.4±7.8	29.3±6.7
Gallareta	5121±2317	14.7±2.0	80.3±1.5	44.2±6.1	86.1±20.1	15.8±1.3	37.6±6.8	29.4±5.6
Haristide	5553±2574	13.8±2.1	79.5±0.8	49.8±8.5	77.0±24.6	16.9±1.9	42.9±10.6	27.2±5.8
Iberus	5168±2096	14.8±2.4	80.7±1.6	46.8±7.4	78.2±20.5	15.7±1.5	41.8±9.5	29.9±6.9
Kiko Nick	5028±2124	14.8±1.9	78.1±1.4	52.3±7.0	81.9±21.3	16.3±1.9	42.8±10.7	28.2±5.6
Mexa	5305±2003	14.3±1.6	79.0±1.8	49.2±7.6	83.8±21.6	15.9±1.5	39.7±7.1	30.1±5.7
Olivadur	5640±2353	14.4±2.6	77.2±2.2	41.4±8.4	77.9±23.9	16.8±1.9	45.6±10.2	27.5±7.1
Pedroso	5017±2029	15.3±2.3	77.3±2.1	52.3±7.8	89.8±11.5	16.2±1.0	45.6±8.2	27.1±6.3
Regallo	5298±2369	15.4±2.2	80.6±1.5	46.6±7.8	84.9±17.2	15.7±1.4	40.1±7.0	31.5±5.8
Saragolla	5193±1920	14.1±2.0	79.0±1.2	48.4±6.8	76.2±22.6	16.2±1.5	44.3±6.8	23.9±4.3
Sculptur	5388±2185	14.1±2.6	79.2±1.9	45.6±7.9	74.5±26.4	17.0±1.9	46.0±10.5	28.9±7.5
Simeto	4679±2136	16.1±1.8	77.9±1.5	55.6±6.1	95.5±5.4	14.9±1.3	41.1±9.9	32.7±5.2
Solea	4793±2074	14.5±2.1	79.3±2.0	45.6±6.4	86.9±14.5	15.2±1.3	40.0±7.6	28.9±6.0
Vitrón	5227±1861	14.4±1.5	80.2±1.4	50.6±6.4	85.5±16.0	14.3±1.6	39.2±7.5	28.2±4.4
p-value	444.7	0.55	0.571	1,478	5.89	0.275	1,615	1.59
Environment								
1	6937±1045	15.2±1.3	80.1±1.6	48.4±5.5	93.0±7.7	15.7±1.0	44.2±5.8	31.9±3.7
2	2829±1031	17.4±1.1	79.1±1.9	48.7±5.6	96.8±2.4	15.0±1.0	38.6±4.5	36.7±3.4
3	5208±1268	14.7±1.5	78.2±1.9	47.8±5.0	66.4±23.7	15.4±1.0	47.1±4.5	28.0±4.5
4	6374±734	11.7±1.0	80.2±1.4	51±3.9.0	53.8±16.4	14.4±0.8	33.1±3.7	20.3±3.1
5	6868±1095	14.3±2.0	81.3±1.5	54.5±3.7	88.3±13.0	14.6±1.0	36.5±3.7	28.1±6.3
6	5230±1030	13.6±1.6	80.2±2.3	55.0±3.6	78.2±15.7	13.5±0.8	34.3±3.5	26.5±4.7
7	7928±1272	13.3±1.5	79.7±1.7	54.5±4.9	83.0±16.1	16.9±1.1	36.3±5.2	24.8±4.1
8	2832±637	16.6±1.3	78.6±1.5	37.0±3.8	97.3±3.7	17.6±1.1	52.7±5.3	32.6±4.1
9	2799±766	14.5±1.5	79.1±1.9	39.1±4.4	94.6±5.8	17.3±1.1	53.0±6.5	26.5±3.9
p-value	271.5	0.336	0.349	0.902	3.6	0.168	0.986	0.971
TOTAL								
	5226±2128	14.6±2.2	79.6±2.0	48.4±7.7	83.5±19.4	15.6±1.6	41.7±8.8	28.3±6.2

5.3.5. Relationship between grain yield and quality traits and mineral concentration

The correlation coefficients between 9 different mineral concentration, grain yield and quality traits are given in Table 5.6. The associations between mineral concentration and GY and its components (TW and TKW) were negative, and more significant relationships were found with test weight. Protein content (PROT) and wet gluten (WG) showed a positive and significant correlation with all minerals analysed. Also, S was significative correlated with all traits, being positive with quality traits and negative with the characteristics related to grain yield. Fe and S content showed a significative correlation with the grain vitreousness.

Table 5.6. Pearson correlation coefficient of grain macro (Ca, K, Mg, P and S) and micronutrients (Cu, Fe, Mn and Zn) concentration with GY (grain yield), PROT (protein content), TW (test weight), TKW (thousand kernel weight), VTR (vitreousness), b* (yellow pigment content), SDSS (SDS sedimentation), and WG (wet gluten), among 24 varieties and nine environments .** and * are statistically significant at $p<0.001$ and $p<0.005$, respectively, while ns refers to no significant differences.

	Macronutrients (g kg^{-1})					Micronutrients (mg kg^{-1})			
Traits	Ca	K	Mg	P	S	Cu	Fe	Mn	Zn
GY (kg ha^{-1})	**-0.34****	-0.18*	-0.13 ns	-0.00 ns	**-0.48****	-0.18*	**-0.36****	-0.07 ns	-0.14*
PROT (%)	**0.49****	**0.38****	**0.41****	**0.25****	**0.65****	**0.42****	**0.62****	**0.45****	0.21*
TW (g hl^{-1})	**-0.43****	**-0.37****	**-0.25****	-0.12 ns	**-0.51****	**-0.35****	**-0.30****	-0.06 ns	**-0.35****
TKW (g)	**-0.27****	**-0.30****	0.07 ns	0.20*	**-0.46****	-0.06 ns	0.01 ns	0.20*	-0.13 ns
VTR (%)	0.13 ns	0.09 ns	-0.02 ns	-0.17*	**0.32****	0.01 ns	**0.26****	0.08 ns	-0.17*
b*	0.19*	0.22*	-0.11 ns	**-0.37****	**0.38****	-0.03 ns	-0.03 ns	**-0.25 ****	-0.04 ns
SDSS (ml)	**0.39****	**0.36****	0.07 ns	-0.14*	**0.68****	0.24*	0.12 ns	-0.12 ns	**0.34****
WG (%)	**0.46****	**0.36****	**0.41****	**0.30****	**0.56****	**0.43****	**0.58****	**0.52****	0.18*

5.3.6. Relationship between traits

The Principal Component Analysis (PCA) was performed to elucidate the relationship among the traits investigated across 24 varieties and nine environments (Figure 5.3). The first two principal components (PCA1 and PCA2) explained 68.7% of the total variance. PCA1 accounted for 46.5% of the data variation. It separated grain yield and its components (TW and TKW) positively, while nutrient concentration and quality traits were separated in the opposite direction. PCA2 accounted for 22.2% of the data variation, and the quality traits with S content were separated in its negative direction. Thus, concerning PCA1, the first and fourth quadrant points present the highest GY, TW and TKW. For PCA2, the points presented in the second quadrant showed the most elevated TKW and P concentration (Figure 5.3A).

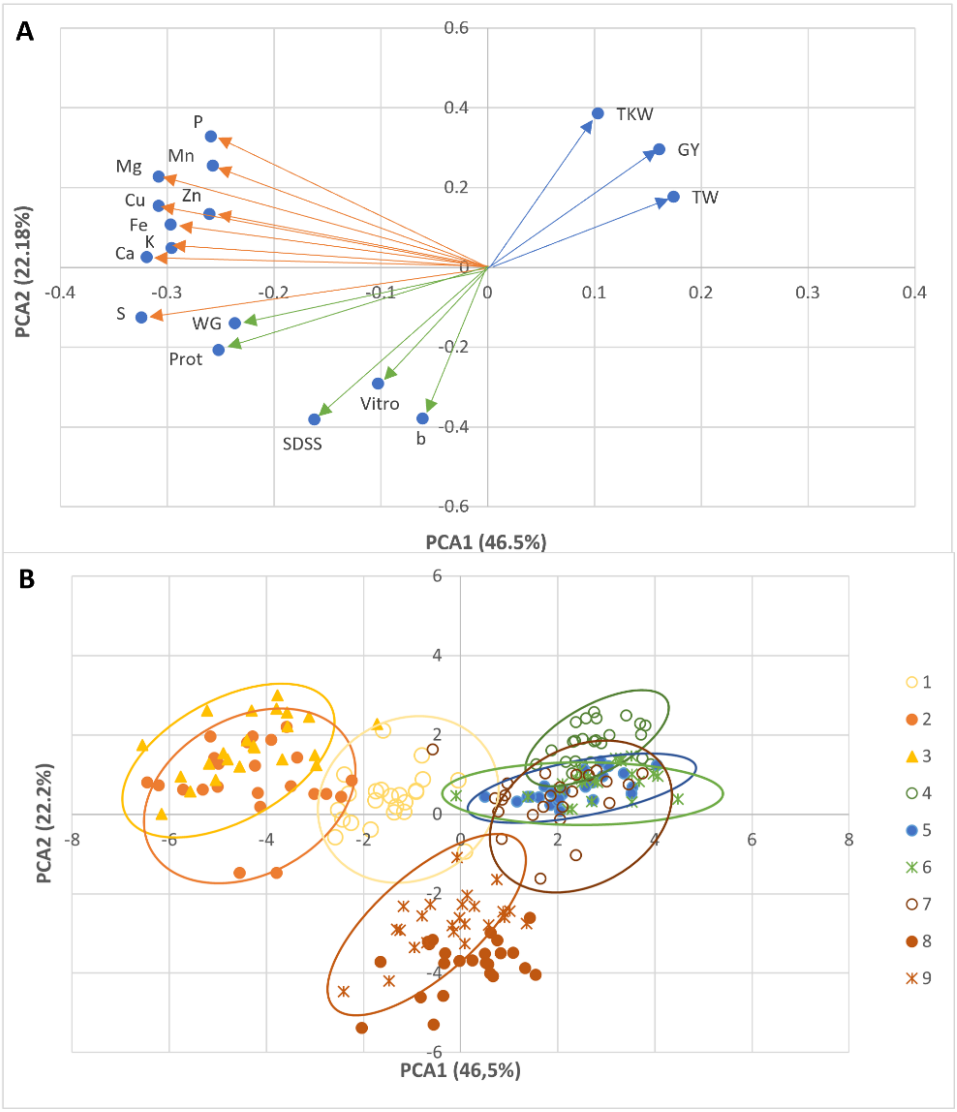


Figure 5.3. Principal component analysis (PCA) of 17 variables estimated for 24 varieties across nine environments (A) Projection of the model variables on the first two principal components (PCA1 and PCA2) and the arrows represent variables, coloured by their type of variables (orange, nutrient; green, quality traits; blue, grain yield). (B) Environment by variable biplots. The variables include grain macro (Ca, K, Mg, P and S) and micronutrients (Cu, Fe, Mn and Zn) concentration and GY (grain yield), PROT (protein content), TW (test weight), TKW (thousand kernel weight), VTR (vitreousness), b* (yellow pigment content), SDSS (SDS sedimentation), and WG (wet gluten).

Moreover, PCA indicated three trait groups in the plot. The first group was composed of grain yield, TW and TKW. The second group consisted of grain S concentration and all quality traits. The third group contained the rest of the nutrient concentration. The association among the traits could also be detected in PCA. For example, the S was strongly correlated with WG and PROT.

The differences in grain yields quality traits and grain nutrient concentration between the nine environments were analysed (Figure 5.3B). The results indicated that Env 4, 5, 6 and 7 have high values

of grain yield, TW and TKW but lower values for the nutrient concentrations and grain quality characteristics. However, Env 8 and 9 presented better quality characteristics and the worst yield. Furthermore, considering the nutrient concentration, the agroclimatic conditions prevalent across experiments 2 and 3 influenced positively to achieve a high level of nutrients in grains.

5.3.7. Trends of grain nutrient concentration of Durum Wheat Cultivars after Green Revolution

Regressions of mineral concentrations on the year of release were separated according to either macro o micronutrient (Figure 5.4). Slight decreases of Fe, Mn and Zn were shown among the 24 varieties. However, all other mineral nutrients concentration remained stable among the panel over the past 40 years. The same trends were observed if mineral content was considered instead of mineral concentration (Figure 5.4C,D).

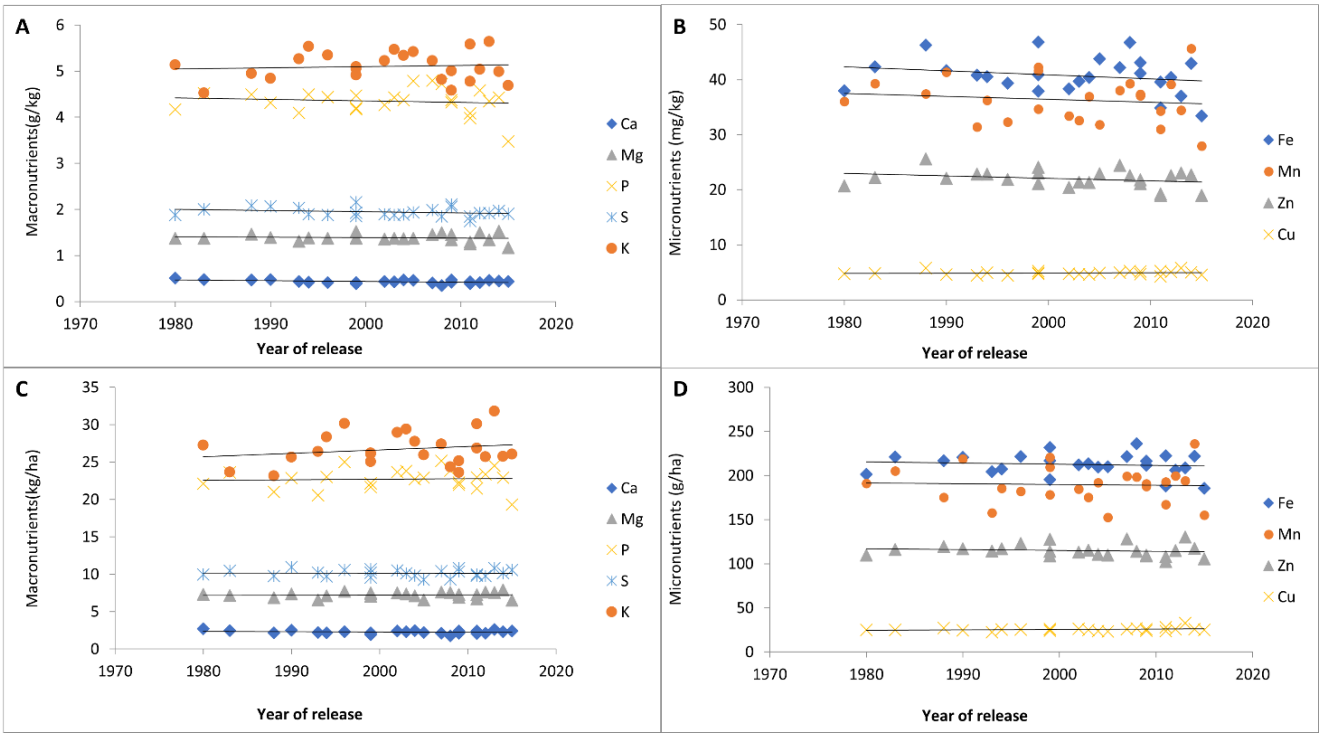


Figure 5.4.- Relationships between the year of release and (i) grain macronutrient and micronutrient concentration (A and B, respectively) and (ii) and grain macronutrient and micronutrient content (C and D, respectively).

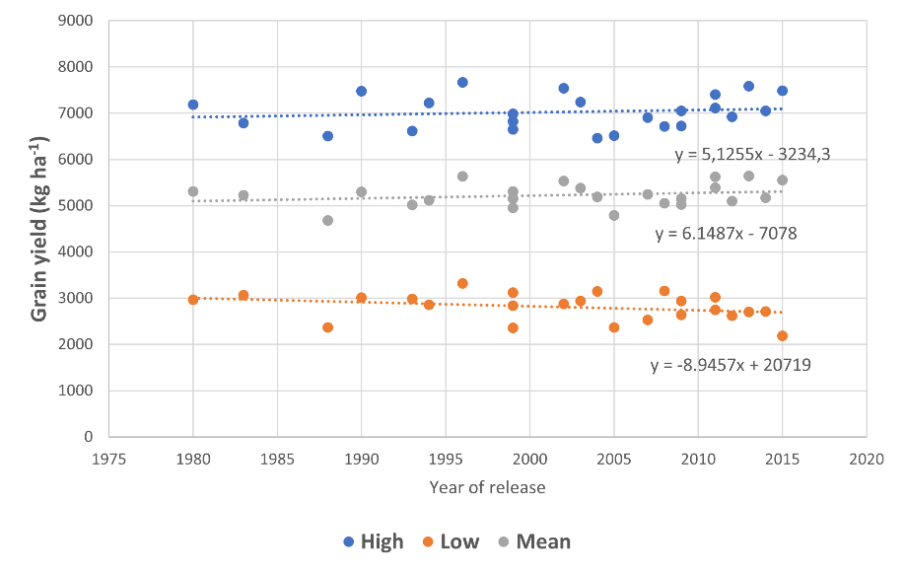


Figure 5.5.- Relationships between the year of release and grain yield, grouped by high (7671-6461 kg ha^{-1}), low (3317-2189 kg ha^{-1}) and mean (5640-4679 kg ha^{-1}) values obtained.

Related to the grain yield obtained during the 9 environments and correlated with the year of release of the varieties study, we observed that in the case of high-yielding environments, there has been a slight positive trend. On the other hand, in the case of low-yielding environments, a negative tendency of GY was found. This shows that the genetic gain in yield over the last decades is very poor in general and even slightly negative in low-yielding environments.

To identify varieties with high concentrations and contents of nutrient minerals, the amounts of these macro and micronutrients per grain versus the grain mineral concentration were plotted together (Figure 5.6). In this way, those varieties with high concentration due to "concentration effects" could be visualized at the bottom of the graph due to small seed size or weight.

Superior varieties were identified for each nutrient. For example, for Ca, Simeto and Mexa, followed by Vitrón, were the varieties with the highest Ca content and concentration and corresponded to varieties with the oldest year of release . Related to the quantity of P, it was higher in Don Ricardo and Euroduro. Simeto, Don Ricardo and Burgos presented the highest Fe content and concentration, one of the responsible for the most prevalent deficiencies of micronutrients. The other micronutrient that is related to current malnutrition is Zn. For it, Simeto and Euroduro presented the best performance.

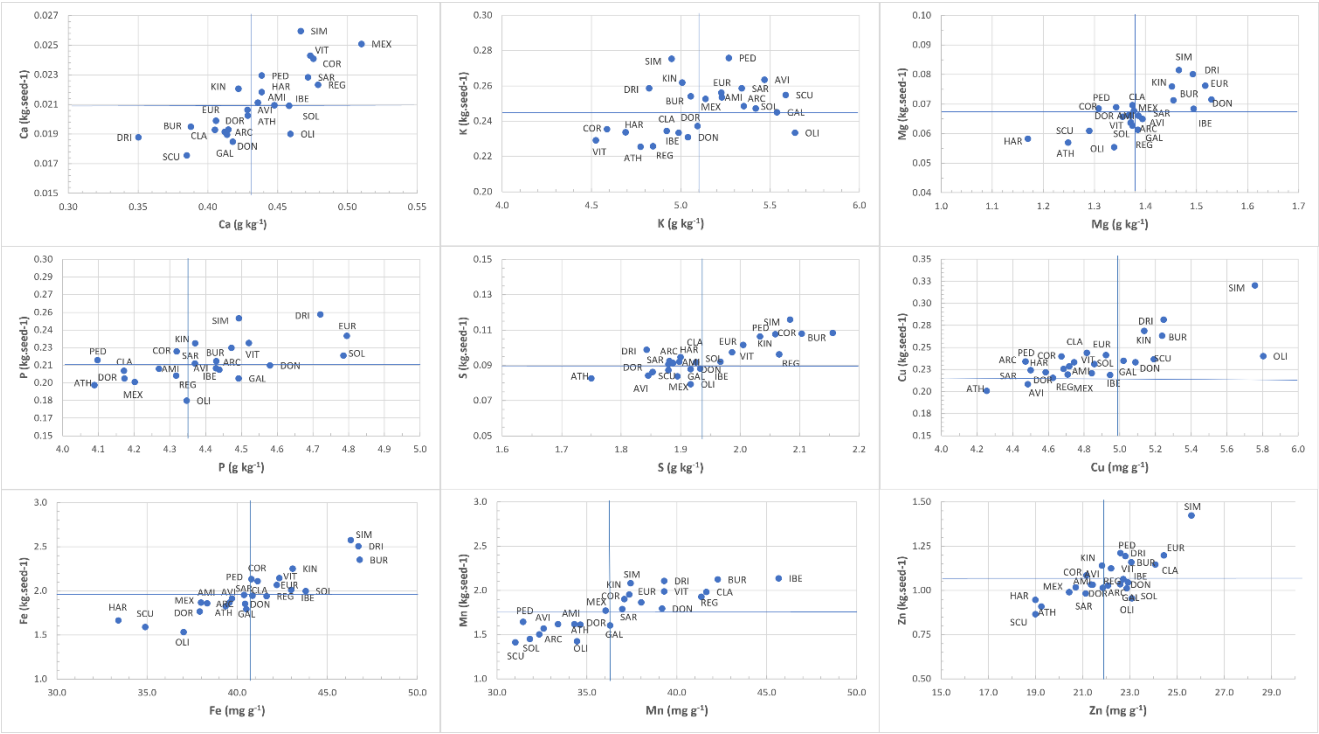


Figure 5.6. Graphical representation of concentration effects for mineral nutrient content. Dashed vertical lines represent averaged mineral concentration of the tested varieties. The abbreviations for the varieties are detailed in Table 5.1.

Only Olivadur presented the "concentration effect" for Cu, K and Zn. The mineral concentration was affected by the low grain weight.

5.4. DISCUSION

Durum wheat is one of the essential cereal species cultivated worldwide, with a global production of around 33 million tonnes. Furthermore, the countries of the Mediterranean basin are the largest consumers of durum wheat for both semolina milling and pasta production. Among European Union countries, Spain, with around 1 million tonnes (0.37 million ha) (MAPA, 2022), is the fourth in importance after Italy, France and Greece.

Cereals are an important source of proteins, macro and micronutrient minerals for humans, being essential in the daily diet. In countries with a high incidence of micronutrient deficiencies, cereal-based foods represent the most significant proportion of the daily diet (Cakmak *et al*., 2008; Bouis *et al*., 2011). While global cereal grain yields have increased dramatically since the Green Revolution, a cereal-based diet lacks sufficient protein and mineral nutrients, leading to an increased percentage of the population suffering from malnutrition (Peleg *et al*. 2009).

In the past 50 years, the main objective of modern wheat breeding programs has been to increase productivity by increasing yields. This has been achieved by selecting diseases, short plant height, and growing biomass and harvest index, among other essential traits (Velu *et al*., 2014). However, increasing the GY may have resulted in a lower density of minerals in grain, although published evidence for this is sometimes contradictory (McGrath 1985; Garvin *et al*, 2006). Further yield increases are necessary to feed the world´s population. Nevertheless, the nutritional composition of staple crops, especially micronutrients, is equally important but often overlooked.

The 24 varietiess studied represent some of the most important varieties that have been cultivated in Spain in the last 40 years. The Green Revolution had a considerable influence on Spain, and the impact of CIMMYT durum wheat germplasm from the 1970s forwards was significant (Royo & Briceño, 2011). Mexa was the second variety introduced and cultivated in almost 90% of the durum wheat area during the mid-1980s (Royo, 2005). In the early 1990s, Vitron and Jabato replaced Mexa and later Simeto, a competitive Italian line, spread in the south, whereas Regallo in the north (Royo, 2005). These lines represented more than 20% of the durum wheat area, and other CIMMYT-derived varieties such as Gallareta, Don Pedro and Sula are grown on more than half of the area. Subsequently, competitive Italian varieties (such as Claudio, Simeto and Colosseo) were introduced and represented more than 16% of the durum wheat area in 2005. In recent years, a picture of the current variety structure in Spain may be given by the percentage of certified seeds being sown from each variety. The use of durum certified seed reflects the variety structure very precisely across the country. In 2021, this ranking was started by Athorix, which represents 21% of total certified seed, followed by Don Ricardo (14%), Amilcar (9%), Avispa (7%) and Kiko Nick (7%). Except for five varieties, certified seed continues to be produced for the other lines.

Understanding the effects of varieties, environment and their interaction is required to make breeding efforts more efficient since both the genotype and the environment contributed to the wide range of variation in nutrient element concentrations in our study, which is in agreement with previous report (Zhang *et al*. 2010; Feil *et al*. 2005). Therefore, the responses of cultivars to production environments need to be well characterized. Furthermore, a thorough understanding of the variation among cultivars in their response to the environment would further improve the probability of identifying varieties with high nutrient concentration.

Fe and Zn are the two most important mineral nutrients contributing to micronutrient deficiency. Previous studies carried out on a wide range of *Triticum* germplasm to screen for Fe and Zn content

reported similar ranges (Batten, 1994; Graham *et al*., 1999; Monasterio & Graham, 2000; Liu *et al*., 2006; Oury *et al*., 2006; Özkan *et al*., 2007; Ficco *et al*., 2009), suggesting that enough genetic variation exists in durum wheat germplasm to largely increase mineral content, with special interest on Fe and Zn grain content. The values reported for Fe content in hexaploid wheats, wild wheats and landraces grown under field conditions, ranged from 28.8 to 56.5 mg kg^{-1} (Graham *et al*., 1999); 19.0–88.4 mg kg^{-1} (Oury *et al*., 2006); 22.9–67.6 mg kg^{-1} (Liu *et al*., 2006); 10–51 mg kg^{-1} (Cakmak *et al*., 2000) and 33.6-65.6 mg kg^{-1} (Ficco *et al,* 2009); while the ranges for Zn were 25.2–53.3 mg kg-1 (Graham *et al*., 1999), 16.4–39.5 mg kg^{-1} (Oury *et al*., 2006), 16.2– 32.4 mg kg^{-1} (Liu *et al*., 2006) and 28.5-46.3 mg kg^{-1} (Ficco *et al,* 2009). Graham *et al.* (1999) reported mean values sight inferiors to our results: particularly for Mn (44.7 mg kg^{-1}), Ca (416 mg kg^{-1}), Mg (1130 mg kg^{-1}) and K (3600 mg kg^{-1}).

This study also shows a significant environmental influence on the genetic variation for the grain concentration of mineral nutrients. Also, the effect of GEI was observed in Ca, K, S, Cu, Fe and Mn, which affected the rank of varieties across the environments. In contrast with other reported studies (Gomez-Becerra *et al*., 2010; Oury *et al*., 2006), no GEI was observed for Zn.

The high correlations between grain concentration of different mineral nutrients may indicate the existence of one or more common genetic-physiological mechanisms involved in mineral absorption or uptake by the root system, translocation and redistribution within the plant tissues, remobilization to the grain and accumulation in the developing grain (Chatzav *et al.* 2010).

Some studies showed no negative correlation between grain Zn and Fe with grain yield (Graham *et al*., 1999; Ficco *et al*., 2009; Velu *et al.*, 2014). On the contrary, other reports showed a slightly negative association between Zn and grain yield in wheat (Morgounov *et al.*, 2007; Peleg *et al.*, 2009; Zhao & McGrath, 2009; Gomez-Becerra *et al.*, 2010). In our study, the yield showed only a significant correlation with Ca, S, Fe and slightly with K.

The majority of the trace elements like Fe and Zn are localised in the aleurone layer and germ of the wheat grain, which are removed as the bran fraction during milling (Liu *et al*., 2007; O'Dell *et al*., 1972; Ozturk *et al*., 2006). Kernel weight is a component of grain yield that could negatively affect the relationship between grain yield and mineral concentration. Negative correlations of TKW with mineral concentration would be expected given the higher concentration of mineral in the aleurone and the increased-surface-to-volume ratio of smaller kernels. Because of this localisation pattern, it was thought that the concentrations of Fe and Zn in the whole grain might correlate negatively with kernel

size because a larger kernel would have a proportionally smaller bran fraction. However, the correlation between kernel size or bran yield and grain Fe and Zn concentrations was significant (Table 5.6), suggesting that coarse grain does not necessarily lead to smaller trace element concentrations. This finding is also consistent with the previous work of McDonald *et al.* (2008).

The strong positive associations found between grain protein and mineral content in durum wheat are similar to those found in various wheat germplasm (Zhao & McGrath, 2009; Gomez-Becerra *et al.*, 2010, Peleg *et al.*, 2008, Velu *et al.*, 2011a; Velu *et al.*, 2011b). The correlation of grain protein concentration with mineral concentrations could be simply a consequence of a "dilution effect" that similarly reduced grain protein and mineral concentrations as yield increased. Those high correlations indicate that grain mineral concentration and protein might have the same genetic base and could be improved by breeding (Velu *et al*., 2014). Some studies indicated that the plant nitrogen status is determined by root uptake and shoot transport, translocation from vegetative tissues into seed, and seed allocation of minerals (Aciksoz *et al.*, 2011; Kutman *et al*., 2011). Therefore, nutrient fertilization is clearly influenced by mineral uptake and/or translocation and then, special attention should be paid to the nutrient fertilisation management of the crops.

5.5. CONCLUSIONS

The environment has a great effect on the nutritional composition of durum wheat grain in our study area. However, the analysis of a collection of durum wheat varieties grown over nine environments pointed out to considerable genotypic differences in grain mineral concentration. This suggested some genetic potential to modify the levels of these components in durum wheat grains and thus the possibility of exploiting the wide genetic diversity to improve nutritional quality. In addition, a highly significant and positive correlation between protein content, grain yield and Fe concentration was observed, therefore suggesting the possibility of combining high Fe traits during wheat breeding. The retrospective study of the evolution of grain yield and nutritional quality highlighted that there has been no significant improvement in yield over the last forty years, except for a slight increase in highly productive environments, while nutrient concentration has not changed.

6. CHAPTER 6:

Third Study

Source-Sink Dynamics in Field-Grown Durum Wheat Under Contrasting Nitrogen Supplies: Key Role of Non-Foliar Organs During Grain Filling.

6.1. INTRODUCTION

Global crop production needs to double by 2050 to meet the rising population demands, nutritional requirements and increasing biofuels consumption (Ray *et al.*, 2013). Boosting crop yields to meet these rising demands is the ideal solution to meet this goal. Durum wheat is an economically and culturally important crop widely cultivated in the Mediterranean basin, used mainly to produce pasta and other non-baked products, as bulgur and couscous. It provides 18% of the daily intake of calories and 20% of proteins in the human diet (Royo *et al.*, 2017). Global durum wheat production achieved around 38-40 million tonnes (approximately 5% of total wheat production) and is concentrated in Mediterranean areas, being the European Union, North Africa and Middle East countries the primary producers and consumers (Beres *et al.*, 2020; Xynias *et al.*, 2020). In Spain, durum wheat was grown in 266644 ha, producing 704086 tonnes, which represented a 14% and 12% of the total wheat area and production in the country, respectively (Ministry of Agriculture, Fisheries and Food of Spain, 2019; www.mapa.gob.es).

To meet future food demands, a worldwide crop yield increase of 2.4% per year is required (Ray *et al.*, 2013), although the genetic advance in the last decade for durum wheat has been much lower or even stagnated in different Mediterranean agro-environments (Chairi *et al.*, 2018; del Pozo *et al.*, 2019). Moreover, climate change will increase the vulnerability of durum wheat production to the impact of abiotic stresses in the Mediterranean countries, where a rise in mean temperatures and lower precipitations is predicted (IPCC, 2013a), which will limit grain number and further grain filling by inhibiting C fixation and N assimilation (Vicente *et al.*, 2015; Medina *et al.*, 2016; Vicente *et al.*, 2018b; Vicente *et al.*, 2019a). Therefore, it is of strategic importance for Mediterranean agriculture to develop new varieties with more significant production potential, better adaptation to increasingly adverse environmental conditions and better grain quality (GQ). However, genetic advance is constrained by the lack of exploring the available genetic diversity in terms of traits to select, high-throughput phenotyping techniques to implement and the better understanding of key molecular mechanisms behind crop adaptation to stress conditions.

Firstly, the development and implementation of high-throughput phenotyping approaches are necessary for the provision of information about the genotype-by-environment interaction and selection criteria for breeding programmes, but further efforts are also needed for selection towards

adaptation to abiotic stresses (Kefauver *et al*., 2017; Vicente *et al*., 2019b; Prey & Schmidhalter, 2020). Secondly, most of the breeding efforts during the last century were focused on improving wheat yields. Selection towards high-yielding cultivars has been done using a few agronomical and physiological traits (del Pozo *et al*., 2016). However, other factors such as the nutritional grain quality and pasting behaviour, relevant for human diet and industrial processing, were considered secondary (Sanchez-Garcia *et al*., 2015). In the Mediterranean basin, where yield gaps are high and environmental stresses may prevent progress, selection for increased adaptation to abiotic stresses is a potential strategy to support future yield progress. However, its complexity has been a challenge for crop improvement as the study of local adaptation requires multidisciplinary studies with multiple environments. Hence, the success in future breeding strategies may reside in novel holistic approaches integrating agronomy, field phenotyping, metabolism and molecular biology in extensive wheat collections to identify attributes controlling complex traits, i.e. grain yield (GY) and GQ under various stresses (Araus *et al*., 2021).

Canopy photosynthesis, understood as the photosynthesis of foliar and non-foliar photosynthetic green organs, is a key target for improving crop yield and resilience (Sanchez-Bragado *et al*., 2020c; Araus *et al*., 2021). Traditionally, it has been thought that the key contributor for canopy photosynthesis during the grain filling stage was the flag leaf blade (the last fully developed leaf in cereals), while the reserves stored in the stems before anthesis were also involved providing C and other nutrients (Sanchez-Bragado *et al*., 2020c). However, it has been recently shown experimentally that the photosynthesis of non-foliar organs, including the whole ear, may significantly contribute to canopy photosynthesis and, then, GY (Gámez *et al*., 2020; Molero & Reynolds, 2020; Shokat *et al*., 2020; Araus *et al*., 2021). This can be particularly relevant under abiotic stresses, e.g. water stress, but may also contribute to GY under good agronomical conditions (Sanchez-Bragado *et al*., 2014a; Sanchez-Bragado *et al*., 2014b; 2016). However, the methodologies for studying the contribution of ears or other non-foliar organs to GY are frequently intrusive or cause compensatory effects (Sanchez-Bragado *et al*., 2016; Rivera-Amado *et al*., 2020). Ears exhibit a higher tolerance to limiting stress conditions compared to leaves, with minor or even insignificant negative impacts on photosynthetic and electron transport rates, and N and water status, including a higher content and expression of primary metabolism intermediates and genes, respectively (Sanchez-Bragado *et al*., 2014b; Sanchez-Bragado *et al*., 2017; Vicente *et al*., 2018b; Vergara-Diaz *et al*., 2020b; Tambussi *et al*., 2021). Ears are the latest photosynthetic organ to develop in wheat, therefore being the youngest organ and potentially the

last to show symptoms of senescence during the grain filling period (Vicente *et al*., 2018b). Moreover, ears are by nature more exposed to direct sun rays and less exposed to shadows due to their apical position, with a smaller physical distance to the grain than any other organ. Awns, which are not always present in wheat varieties, seem to be a major contributor to ear photosynthesis (Sanchez-Bragado *et al*., 2020a). Ear bracts (glumes and lemmas) have closer contact with grains and, moreover, access to the respired CO_2 released by grains, which could be relevant in a possible refixation of CO_2 (Sanchez-Bragado *et al*., 2020c). Regarding sheaths and peduncles, they have been associated with storage and nutrient transport functions (Scofield *et al*., 2009; Cimini *et al*., 2015). Overall, the precise pathways associated with the metabolism operating in the ears and other non-foliar organs are still poorly understood.

N metabolism is a key factor for plant growth, with a crucial impact on GY and GQ traits, such as protein content, dough quality, and processing characteristics (Zörb *et al*., 2018; Wang *et al*., 2021). It is relevant for the wheat research to understand the N assimilation and remobilisation taking place in the different green organs, especially during grain filling. For example, under water stress the ear bracts showed an active biosynthesis of organic and amino acids that was not observed in flag leaves, thanks to a coordination between C and N metabolism, including N assimilation, photorespiratory N cycle and TCA cycle (Vergara-Diaz *et al*., 2020b). A preliminary study analysing the N content and isotope composition in different parts suggested that the potential contribution of the ear providing N to the growing grains was around 42% (Sanchez-Bragado *et al*., 2017), which makes this topic of interest for addressing new avenues for crop improvement.

This study aims to perform a holistic approach integrating agronomic, physiological and biochemical traits to identify novel components involved in the control of complex traits in response to different N levels as a selection criterion for breeding programmes. Our specific objectives are to understand (i) the phenotypic traits that are related to GY and GQ at canopy and organ level, (ii) the potential contribution of foliar and non-foliar photosynthetic organs to developing grains and (iii) how N availability and genotypic variability modulate such factors. We evaluated a wide range of canopy vegetation indices, physiological, nutritional and metabolic traits in green organs (flag leaf blade, sheath, peduncle, awn, glume and lemma), and agronomic and GQ traits.

6.2. MATERIALS AND METHODS

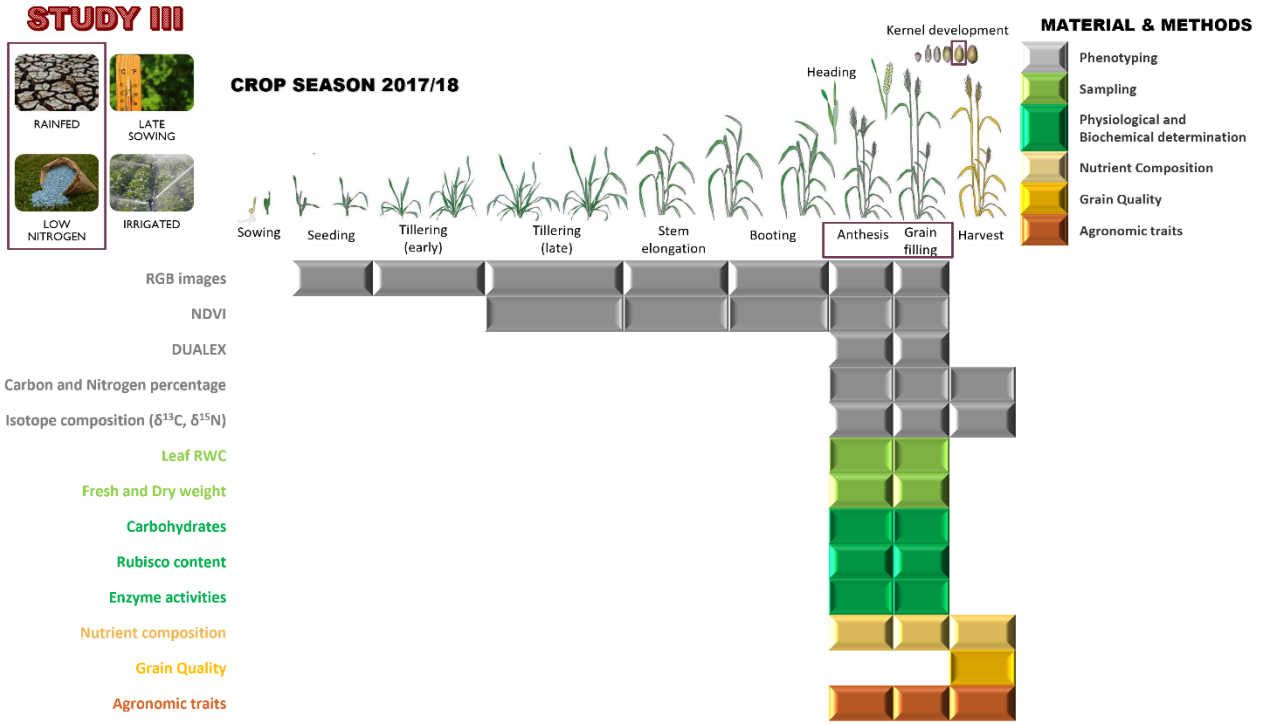


Figure 6.1. Four varieties were selected from the trial carried out during the crop season of 2017/2018: *Euroduro* EUR (2007), *Don Ricardo*, DRI (2008), *Kiko Nick*, KNI (2009) and *Haristide*, HAR (2015), which were subjected to two different nitrogen levels (105 and 24 kg ha^{-1}). Agronomic and GQ traits were evaluated at harvest, and the phenology was monitored throughout the growth cycle using the Zadoks scale. In addition, ground-phenotyping (RGB imaging, sensors NDVI and DUALEX) was performed during the crop cycle at the canopy level of the panel. At the same time, physiological and biochemical analyses were carried out in different foliar and non-foliar green organs (flag leaf blades and sheaths, peduncles, awns, glumes, and lemmas) at two specific stages; anthesis (Zadoks 65) and mid-grain filling (MGF; Zadoks 75). For these stages, the phenology of each variety was considered. Therefore, the samplings at anthesis for Kiko Nick and Don Ricardo were performed at 181 days after sowing (DAS) and for Haristide and Euroduro at 187 DAS, while at MGF Euroduro, Kiko Nick and Don Ricardo were sampled 195 DAS and, Haristide, 207 DAS.

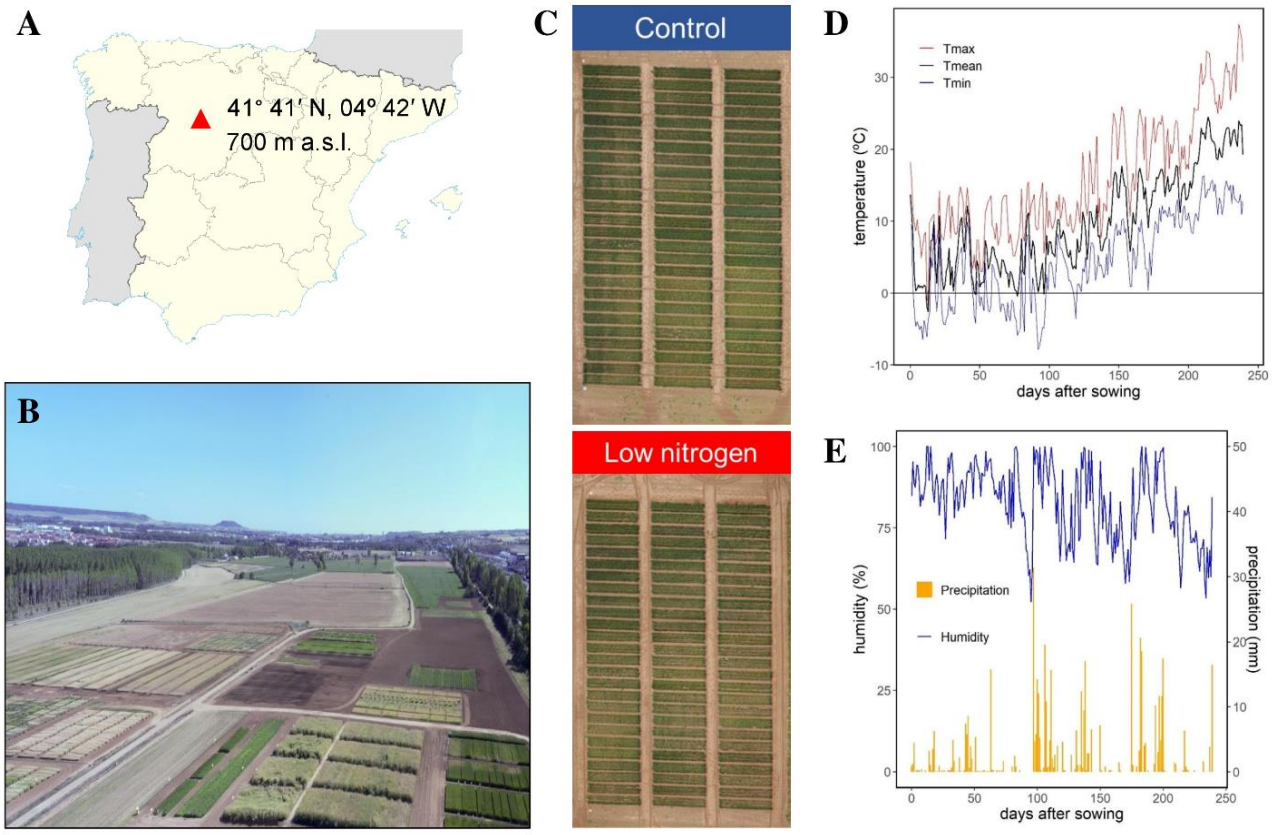


Figure 6.2. Location (A) and aerial images (B, C) of the field trials, and daily mean (Tmean), maximum (Tmax), and minimum (Tmin) temperatures (D), relative humidity, and precipitation (E) during the crop season from 23rd November 2017 to 20th July 2018 (D).

6.3. RESULTS

6.3.1. Effect of N and genotypic variability on durum wheat agronomic components, grain quality and physiology

Low N reduced GY (22%), biomass (25%), and plants per unit area (29%), as well as peduncle length and plant height, compared to control N, while TGW slightly increased (Figure 6.3A, Supplementary Table 6.2). For GQ, low N significantly reduced sedimentation index and increased moisture content and yellowness index (Figure 6.3A, Supplementary Table 6.2). The different N supplies were undoubtedly separated in the PCA by X-axis, representing a 34.7% of the variability. The four varieties clearly showed differences in agronomic and GQ traits, with a similar trend at each N supply as evidenced by the low number of significant G×N interactions and their distribution in the PCA (Figure 6.3). The variety Haristide had the highest GY regardless of N supply, followed by Euroduro, Don Ricardo, and Kiko Nick (Figure 6.3A). Most changes in agronomic components

followed the trend observed in GY, being Kiko Nick the one with lower values and Haristide with higher ones. These differences were slightly more pronounced under low N than control N. In the PCA, Y-axis explained 17.2% of the variance in the data, which was partially related with differences among varieties (Figure 6.4B). The most relevant GQ traits in this axis were grain protein content, WG, GI and SW.

A

Trait	Control				Low nitrogen				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
GY	ad	abc	ab	a	d	cd	bd	abc	***	**	
biomass	a	ab	ab	a	c	ab	bc	ac	***		*
HI	b	ab	a	ab	ab	b	a	a		**	**
plants.m2	a	ab	ac	a	ac	c	bc	ac	***	*	
ears.plant	b	b	b	b	b	a	ab	b	**	**	*
grains.ear	ab	ab	ab	a	b	ab	ab	a		**	
TGW	ab	ab	ab	b	ab	ab	ab	a	**		
ped.length	ab	a	ab	ab	ab	ab	ab	b	***		
ear.length	b	b	b	a	b	b	b	a		***	
height	a	a	ab	ab	b	b	ab	b	***		
prot.grain											
moisture.grain	ab	ab	ab	b	a	ab	ab	a	**		
SW.grain	ab	ab	a	ab	b	ab	ab	ab		**	
vitreo.grain											
sedim.grain	ab	b	a	a	c	c	c	c	***	**	**
b.grain	c	c	c	c	a	b	a	a	***	***	**
WG.grain											
GI.grain	ac	ab	a	c	ac	ac	a	bc		***	

min max

B

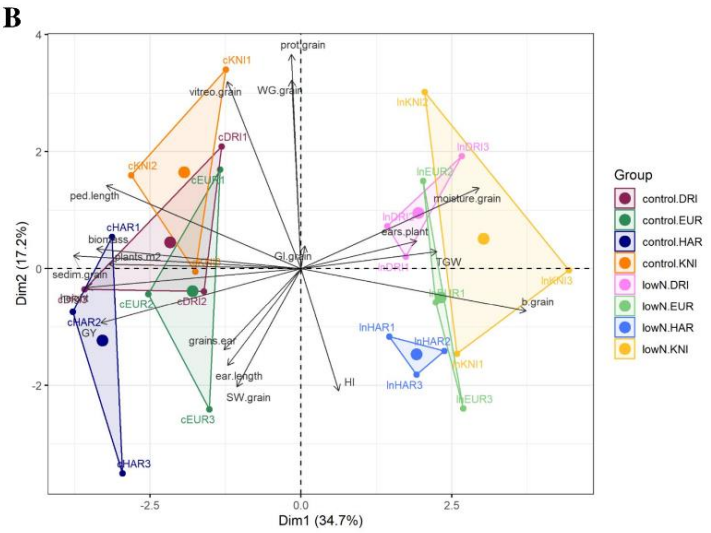


Figure 6.3. Effects of N supply and genotypic variability on agronomic components and grain quality traits (A) and principal component analysis (B) in four varieties of field-grown durum wheat (Kiko Nick, Don Ricardo, Euroduro and Haristide) at two N levels (control vs. low N). In (A), the different letters differ statistically (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). The abbreviations are described in Supplementary Table 6.1.

Ground-phenotyping was performed to monitor plant growth, pigment content and senescence at canopy and leaf levels. Low N supply significantly decreased GA, GGA and NDVI, and increased CSI from early stages to maturity (Figure 3). Minor changes, but albeit significant, were observed among varieties, mainly at late growth stages where Haristide showed a better performance regardless of the N supply (Figure 6.4, Supplementary Table 6.2). Leaf flavonols content was increased under low N compared to control, being significant at anthesis (Supplementary Figure 6.1). The flavonols content was higher in Kiko Nick, followed by Don Ricardo, Euroduro and Haristide at both growth stages independently of the N supply, while NBI tended to be higher in Haristide and Euroduro.

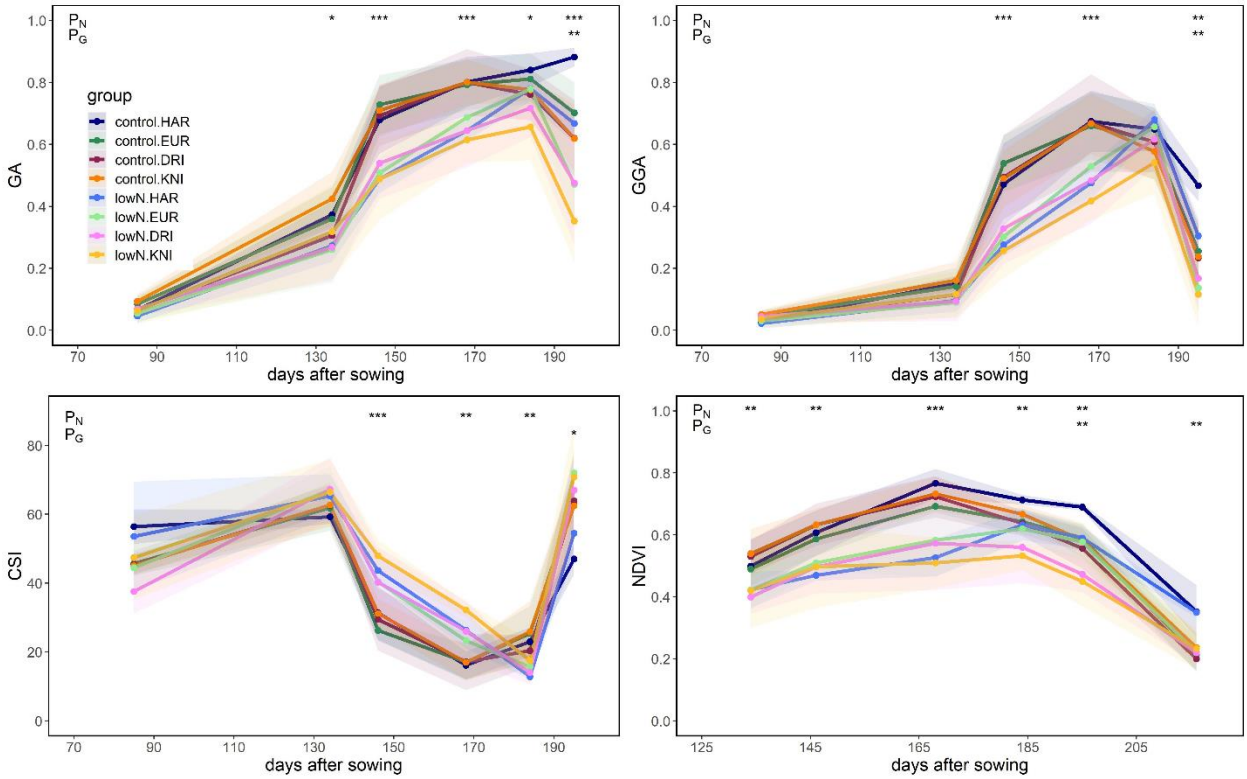


Figure 6.4. Green Area (GA), Greener Green Area (GGA), Crop Senescence Index (CSI), and Normalized Difference Vegetation Index (NDVI) in four varieties of field-grown durum wheat (Kiko Nick, Don Ricardo, Euroduro and Haristide) at two N levels (control vs. low N) measured at canopy level. Asterisks indicate a significant difference between varieties (G) and N levels (N) according to the two-way ANOVA (*, P<0.05; **, P<0.01; ***, P<0.001). The interaction G×N did not reach significance for these parameters.

Low N decreased LRWC significantly at anthesis and MGF, while Haristide and Euroduro had higher LRWC at anthesis than Kiko Nick and Don Ricardo (Supplementary Table 6.2). Regarding the effect of N on organ weights, low N decreased FW at anthesis and MGF and DW at Zadoks 85 in blades, and DW at Zadoks 85 in sheaths and peduncles, but not in ears (Supplementary Table 6.2). Genotypic variability affected organ weights, mainly blades, sheaths and peduncles at anthesis and MGF, while ear DW was the lowest in Kiko Nick at late-grain filling regardless of N supply.

6.3.2. Correlations between agronomic components and grain quality and physiological traits

GY was positively correlated with several agronomic components, such as biomass, plants per unit area, grains per ear, peduncle and ear lengths, and plant height (Figure 6.5). GY also correlated with

the GQ traits sedimentation index, positively, and with yellowness index, negatively. In addition, significant positive correlations were also observed between GY and traits such as LRWC and blade FW at both anthesis and MGF, and blade, sheath and peduncle DW at late-grain filling. The most interesting significant correlations between agronomic components and GQ traits, where those linking grain moisture content, and sedimentation and yellowness indices with GY. Grain protein correlated positively with vitreousness, WG, leaf chl and N contents, and negatively with SW, leaf anthocyanins and some organ weights (Figure 6.5). Biomass was highly correlated with GY and, therefore, the NDVI and RGB canopy indices with higher correlation coefficients as growth progressed (Figure 6.5). The leaf spectral indices were good proxies for GY, yield-related traits and biomass, particularly flavonols and NBI. These indices were also correlated with several GQ traits such as grain protein content, vitreousness, sedimentation index, WG and GI.

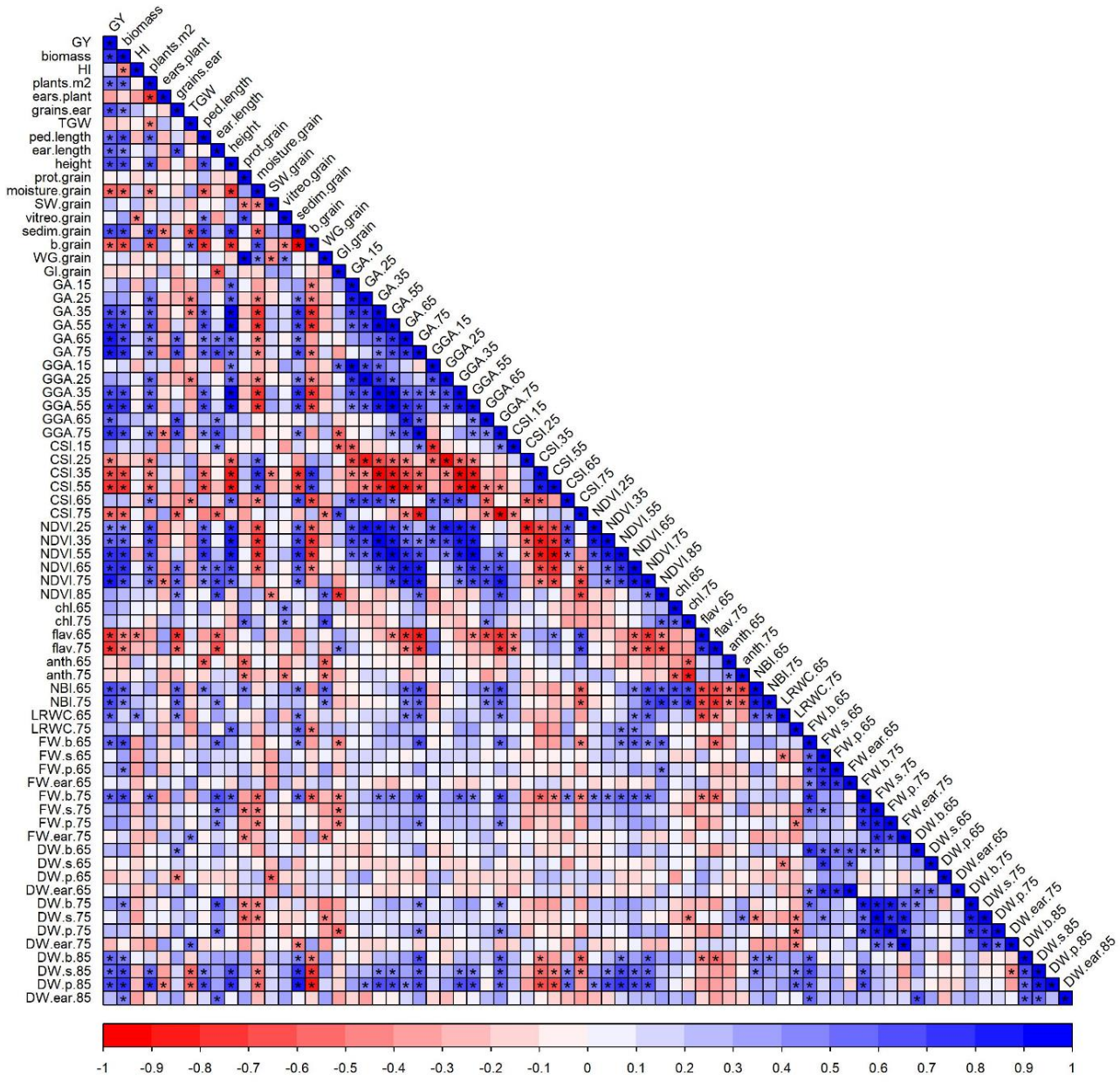


Figure 6.5. Correlation matrix of agronomic components and grain quality and physiological traits. Each point of the matrix is a Pearson correlation coefficient between two traits (blue, positive correlation; red, negative correlation). Asterisks indicate a significant correlation (P<0.05). The abbreviations are described in Supplementary Table 6.1.

6.3.3. Effect of N and genotypic variability on grain nutrient compositions in field-grown durum wheat

We built a PCA with grain and protein yields and 13 grain minerals expressed as concentrations and yields measured at harvest, to further characterise GQ under G×N interaction (Figure 6.6). X-axis and Y-axis explained 47.4% and 17.2% of the variance in the data, respectively, and they were associated with changes due to both N treatment and genotypic variability. N effect was clear, while the differences between varieties were similar under control or low N supply. Control N was certainly associated with grain protein and N yields, and nutrients such as C, S, Fe, P, Cu and Mn. The high-yielding Haristide had the highest concentrations of nutrients such as Ca, K, and Na, and the lowest of Zn (Figure 6.6), being statistically significant Ca and Zn by Tukey's HSD test (Supplementary Table 6.2). Considering the nutrient amounts by yields, low N undoubtedly decreased the uptake of most of them, while Haristide was the variety that uptake highest levels of most nutrients and Kiko Nick the least regardless of N supply.

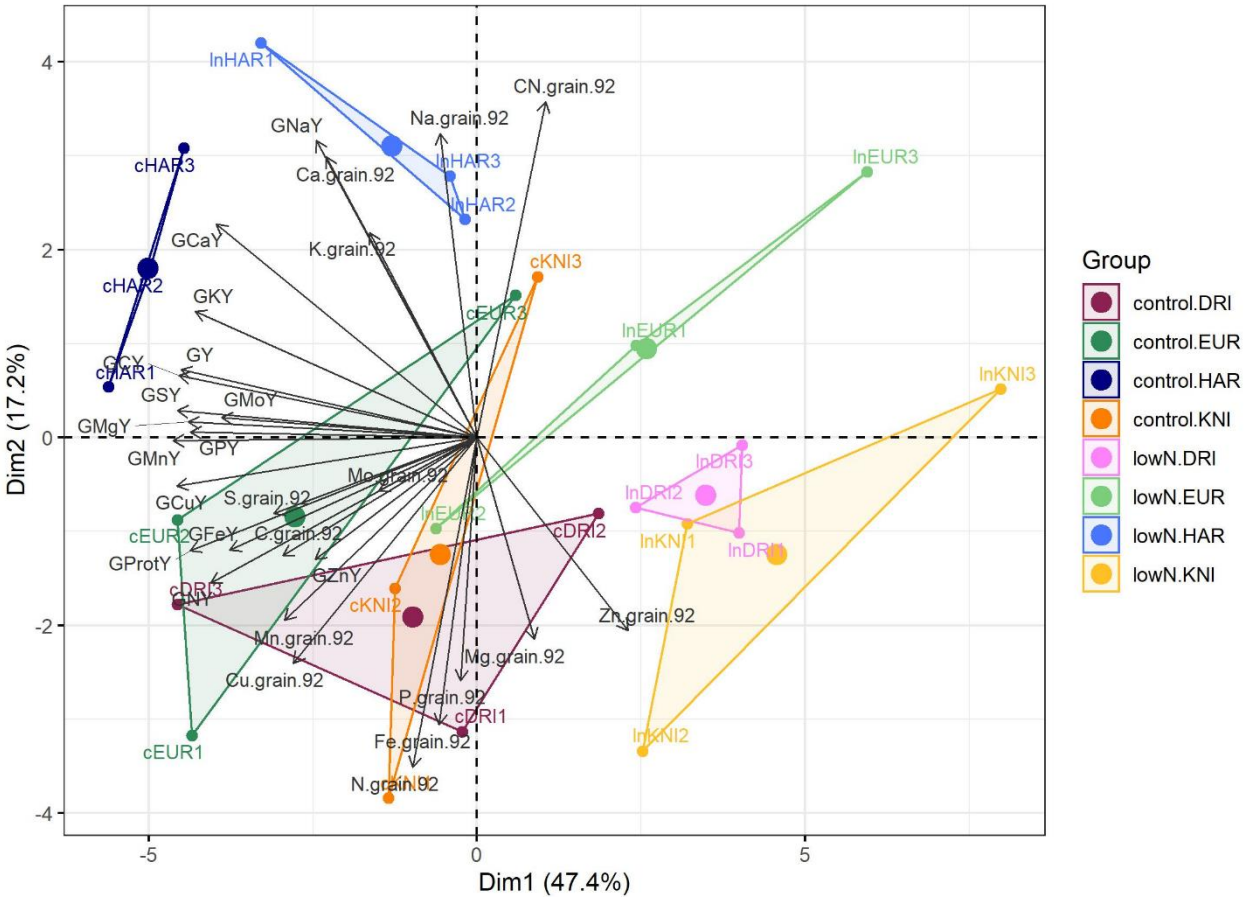


Figure 6.6. Principal component analysis of grain nutrient compositions and yields at harvest in four varieties of field-grown durum wheat (Kiko Nick, Don Ricardo, Euroduro and Haristide) at two N levels (control vs. low N). The abbreviations are described in Supplementary Table 6.1.

6.3.4. Primary metabolism and nutrient composition in green organs of durum wheat during grain filling under contrasting N supply

We examined the metabolism of photosynthetic green organs by determining carbohydrates and Rubisco protein contents, C-N metabolism enzyme activities, and nutrient and isotope composition. The blade was significantly separated from the other organs in the PCA due to its higher values for most of these traits, e.g. Rubisco protein, Rubisco, PEPCase, GS and GOGAT activities, and nutrients such as N, Ca, Mg, Mn and Cu (Figure 6.7). Initial Rubisco activity was 42-56%, 23-39%, 14-18%, 11-18% and 11-14% in awns, sheaths, peduncles, glumes and lemmas, respectively, compared to blades depending on N supply and growth stage (calculated from Supplementary Table 6.2). Similar patterns were observed for total Rubisco activity, which correlated with protein level (r = 0.80), as well as for the other enzymes, except GDH whose values were not so low in the non-foliar organs.

Considering X-axis (45.3% of the variability), the closer organ to the blade was the awn. Ear bracts, glumes and lemmas, almost overlapped and were close to awns, being separated from sheaths and peduncles mainly by Y-axis (11.9% of the variability). Ear organs had high levels of GDH activity, Rubisco activation state, and fructose, glucose and Fe levels at MGF. Sheaths and peduncles were characterised by the highest fructans levels and free carbohydrates (glucose and fructose) at anthesis.

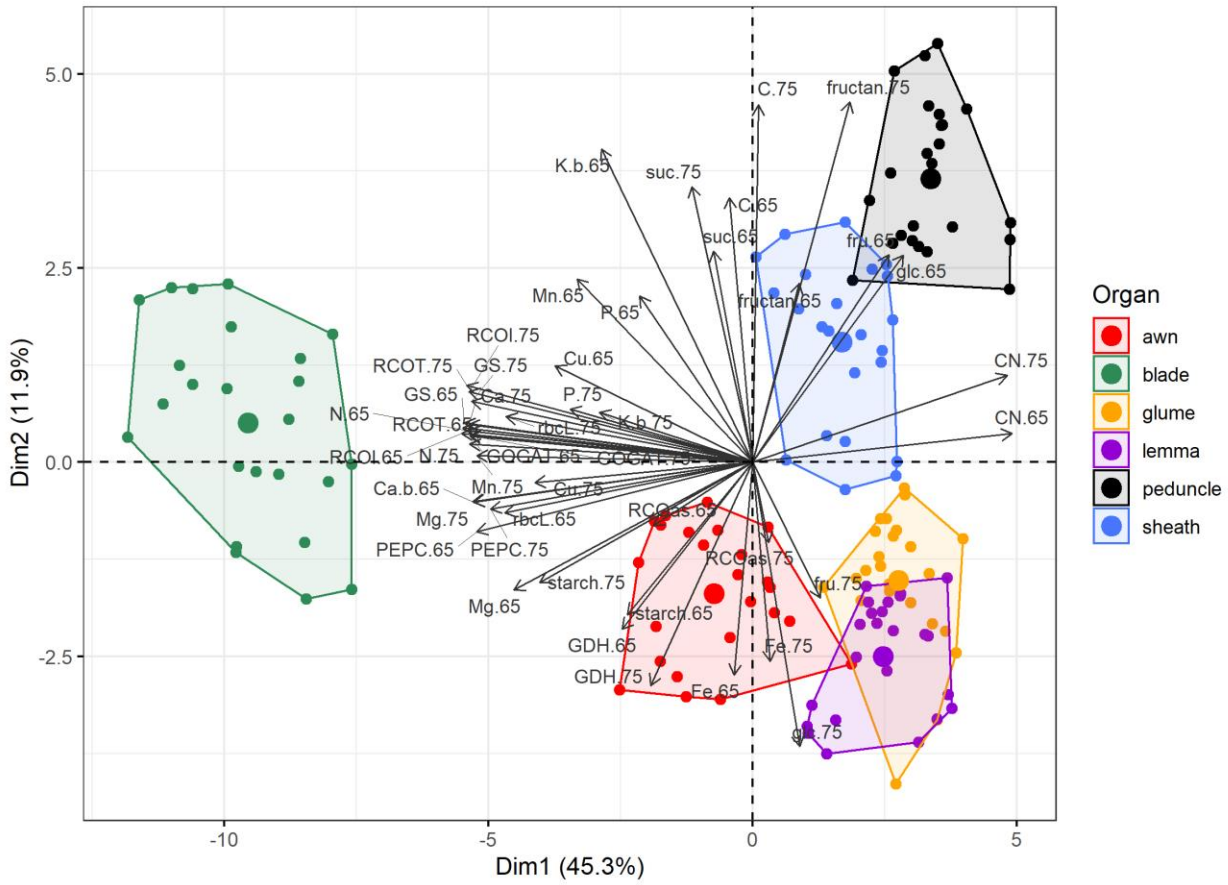


Figure 6.7. Principal component analysis of metabolites, enzyme activities and nutrient composition in different organs (blade, sheath, peduncle, awn, glume and lemma) in field-grown durum wheat. The abbreviations are described in Supplementary Table 6.1.

The N effect was relatively similar between organs, but when the position of the centroids was examined per organ and N supply, we observed slightly stronger effects on blades, while in other organs such as the ear bracts it was smaller or almost negligible (Supplementary Figure 6.2). Low N increased the levels of fructose, fructans and starch in the peduncle and sucrose in blades at anthesis compared to control N (Supplementary Table 6.2). Interestingly, low N decreased sucrose levels in blades, sheaths, and, more significantly, in ear organs at MGF together with starch. Low N increased Rubisco protein in peduncles and lemmas at anthesis, but reduced it in sheaths and peduncles at MGF

and in blades at both stages (Supplementary Table 6.2). Low N tended to decrease both initial and total Rubisco activities in most organs, except for a small increase in blades and a stronger increase in peduncles at anthesis. Low N also decreased activities of PEPCase (e.g. in glumes, sheaths and peduncles), GS and GOGAT, while GDH was less affected, with an interesting strong increase in blades in low N compared to control N. Although the N effect on grain nutrient composition at harvest was clear (Figure 6.6), its effects were not so evident in the organ-specific nutrient concentrations (Supplementary Table 6.2). Among the most relevant data, lower N supply increased Fe content, especially in glumes, and reduced C content at late stages and, non-significantly, N.

The genotypic variability greatly influenced primary metabolism and nutrient composition in green organs (Supplementary Figure 6.3, Supplementary Table 6.2). Fructose levels were higher in most green organs of high (Haristide and Euroduro) vs. low-yielding (Don Ricardo and Kiko Nick) varieties (Supplementary Table 6.2). Sucrose content was strongly reduced in Haristide in all organs at anthesis, but at MGF it was higher in blades, awns, glumes and lemmas compared to the other varieties, regardless of N supply. The pattern of changes in starch was very similar to sucrose, while overall fructans decreased in Haristide, except for an increase in peduncles at MGF. Rubisco protein content and activities were organ-specific and highly variable between varieties. Due to the amount of traits, treatments and significant results obtained for the other enzyme activities and nutrients, we focused on their correlations with agronomic components and GQ traits detailed in the following sections. An overview of the most relevant results for each variety and organ is shown in Supplementary Figure 6.3. In summary, the metabolism and nutritional composition of the high-yielding variety Haristide was markedly different from the others at the whole plant.

6.3.5. Changes in primary metabolism and nutrient composition in green organs between anthesis and mid-grain filling

Metabolic changes between anthesis and MGF were studied by organ (grouping all varieties as their differences were similar for each N regime) to understand their metabolic evolution during grain filling (Figure 6.8). Glucose content at both N supplies and starch at control N increased in blades and decreased in other organs at MGF compared to anthesis. Fructose content increased in blades, particularly under low N, while decreased in peduncles compared to other organs. Sucrose content was slightly higher in all organs under control N at MGF compared to anthesis, while it was similar under

low N, except for the high increase in peduncles at both N levels. A marked increase in fructans was observed in peduncles, higher under control N. The amount and activity of Rubisco in peduncles increased at MGF under control N, but decreased under low N, while they increased in sheaths. In ear organs, i.e. glumes and lemmas, Rubisco protein content tended to decrease, but the initial activity increased, partly due to a better activation state (Figure 6.8). By contrast, Rubisco activity was significantly decreased in blades at MGF under low N. PEPCase activity tended to increase at MGF, being negatively affected by low N, higher in peduncles under control N and lower in glumes. GS activity increased remarkably in glumes under control N and decreased in awns under low N. GOGAT activity was stable in blades, increased in sheaths under low N and glumes under control N, or decreased in peduncles and glumes under low N. In general, GDH activity was higher at MGF. Nutrients exhibited significant differences between organs but limited N effects (Figure 6.8). MGF led to a decrease of C in blades and more strongly in ear organs, N, P (more in peduncles), Cu, K and Mn in peduncles, Ca in ear bracts, and Mg in peduncles and ear bracts, and an increase of K in glumes, Ca in blades, peduncles and sheaths, Mg in blades and sheaths, Fe in ear bracts, and Mn in blades, sheaths and awns.

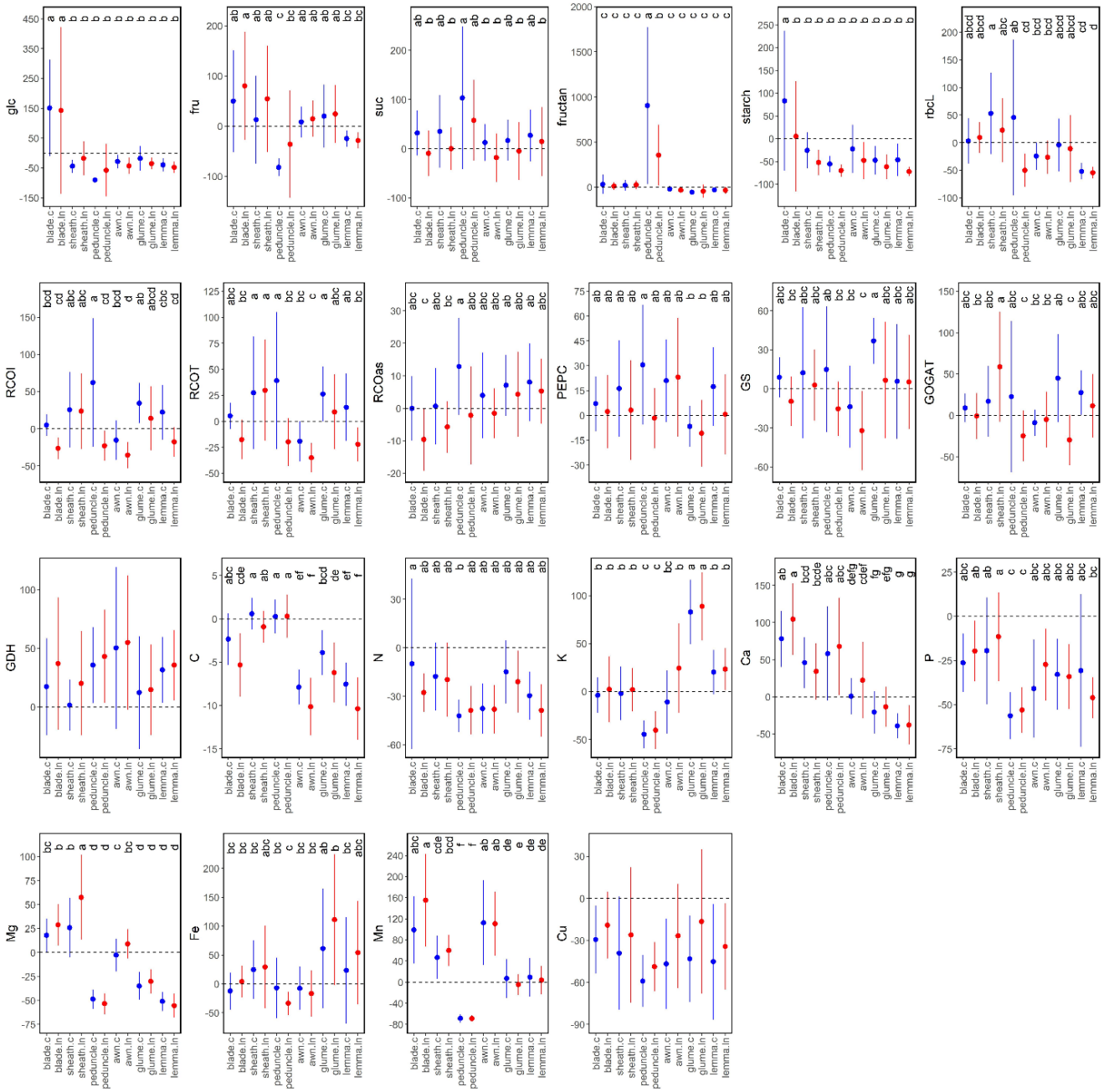


Figure 6.8. Percentage (%) of variation between anthesis (Zadoks 65, blue) and mid-grain filling (Zadoks 75, red) for the metabolite contents, Rubisco large subunit protein, enzyme activities, and nutrient contents. Each dot is the average of four field-grown durum wheat varieties per organ (blade, sheath, peduncle, awn, glume and lemma) and N supply (c, control; ln, low N). The different letters differ statistically ($P < 0.05$). The abbreviations are described in Supplementary Table 6.1.

6.3.6. Correlations between agronomic components and grain quality traits with the metabolic status of green organs

In blades, GY correlated positively with free carbohydrates and negatively with sucrose, fructans and starch, also observed for biomass (Figure 6.9). Grain protein content was positively associated with total Rubisco and PEPCase activities at MGF, and negatively with Rubisco activation state at anthesis. In sheaths, GY correlated positively with PEPCase activity at anthesis and glucose content at MGF, and negatively with sucrose and starch contents at anthesis. Biomass correlated with Rubisco, PEPCase and GS activities at MGF, while grain protein content correlated negatively with Rubisco activation state at anthesis. In peduncles, sucrose and starch contents at anthesis, GS activity at MGF, and GOGAT activity at both stages correlated negatively with GY. Negative correlations were also observed for biomass and the peduncle biochemical related traits, i.e. fructans, initial and total Rubisco and GS activities at anthesis. Grain protein content only correlated with starch and PEPCase activity at MGF. In awns, glumes and lemmas, GY generally correlated negatively with sucrose and starch at anthesis and positively with sucrose and starch, initial and total Rubisco and GOGAT activities at MGF, and PEPCase activity at both growth stages. GY also correlated positively with free carbohydrates in awns and lemmas, and negatively with Rubisco activation state and GS activity in awns at anthesis. Correlations between metabolic traits and biomass in ears were similar to those found for GY, with a remarkable correlation between biomass and Rubisco, PEPCase and GOGAT activities. Grain protein content predominantly correlated with lemma metabolic traits, such as Rubisco protein and Rubisco and PEPCase activities.

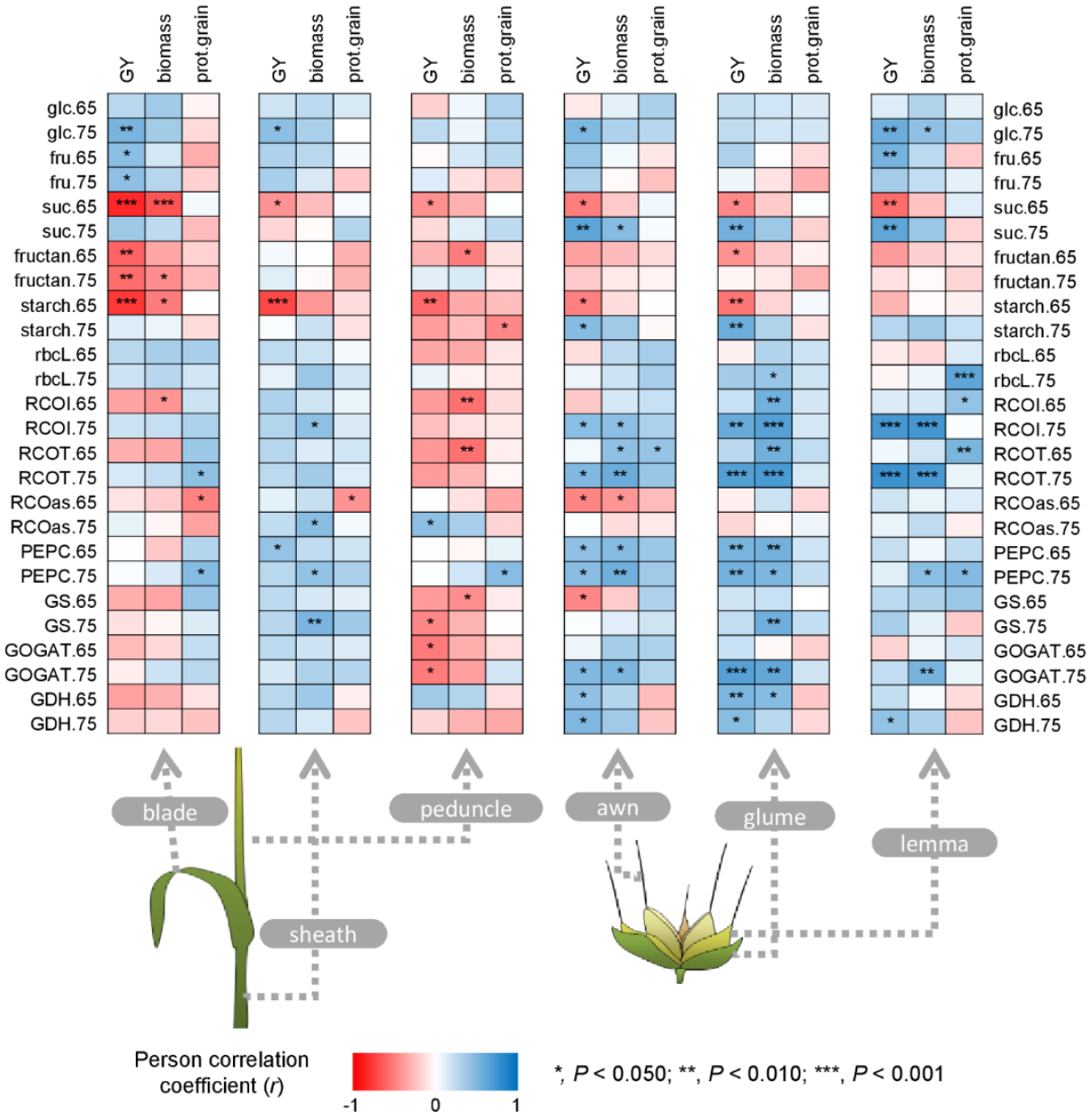
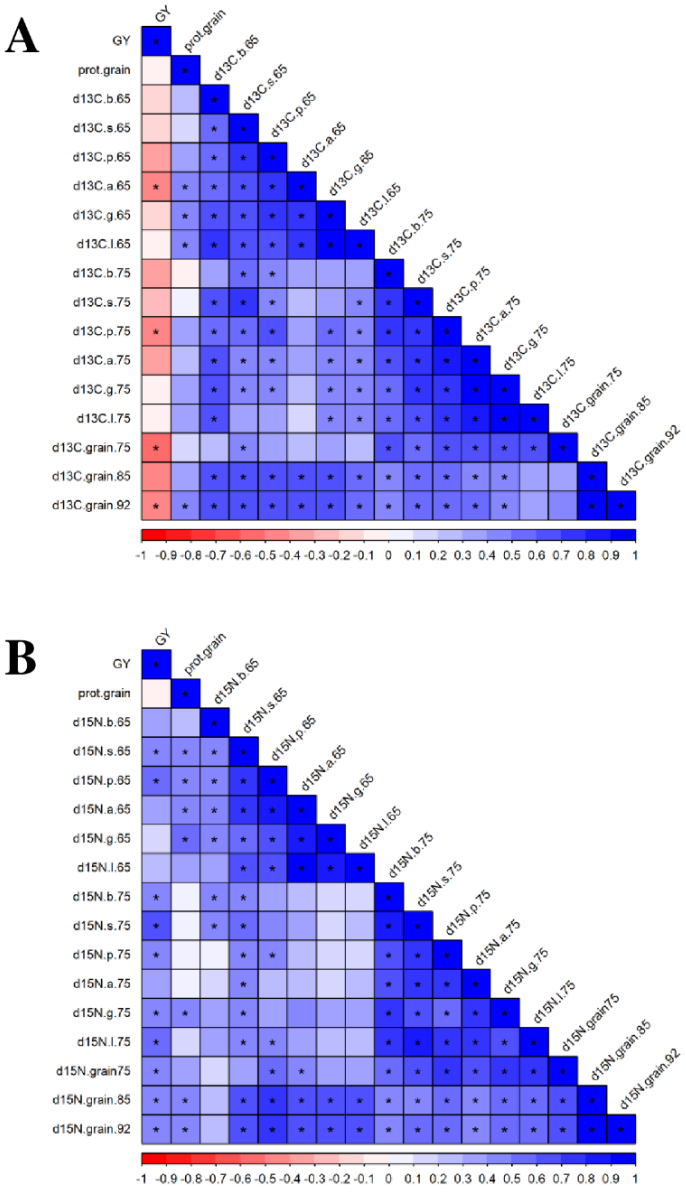


Figure 6.9. Correlations between metabolic traits in the different green organs at anthesis (Zadoks 65) and mid-grain filling (Zadoks 75) and agronomic components and grain quality traits at harvest. Each point is a Pearson correlation coefficient between two traits (blue, positive correlation; red, negative correlation). Asterisks indicate a significant correlation according to the legend. The abbreviations are described in Supplementary Table 6.1.

We used the isotope signatures of green organs to predict their contribution to grain filling and GY. A high number of significant correlations were observed between the $\delta^{13}C$ of organs and grains (Figure 6.10A). GY correlated negatively with $\delta^{13}C$ of awns at anthesis, peduncles at MGF and grains at MGF and harvest, while grain protein content correlated with $\delta^{13}C$ of awns, glumes and lemmas at anthesis and grains at harvest. Similar to $\delta^{13}C$, there was a high number of correlations between $\delta^{15}N$

of the organs with the one in grains, GY and grain protein content (Figure 6.10B). Moreover, we used regression models with the organ-specific isotope compositions to predict GY and grain $\delta^{13}C$, $\delta^{15}N$ and protein content (Figure 6.10C). According to the proportion of variance explained by each predictor (r^2), GY was mainly predicted by $\delta^{13}C$ of grains, awns and glumes, and grain $\delta^{13}C$ at harvest by $\delta^{13}C$ of ear organs and sheaths. Using the $\delta^{15}N$ values, GY was predicted by $\delta^{15}N$ of sheaths, peduncles, grains, blades and glumes, $\delta^{15}N$ of grains at harvest by $\delta^{15}N$ of peduncles, glumes and blades, and grain protein content by $\delta^{15}N$ of many organs, predominantly the glumes.



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Predicted trait (database)	Multivariate model	Estimate	*P*-value	r^2
GY			**0.007**	**0.869**
(d13C)	(Intercept)	-13752		
	d13C.grain.75	-2031	0.002	0.210
	d13C.a.65	-1654	0.003	0.158
	d13C.g.75	2157	0.005	0.088
	d13C.a.75	-2019	0.037	0.062
	d13C.grain.92	8590	0.049	0.061
	d13C.grain.85	-8537	0.041	0.055
	d13C.s.65	1189	0.042	0.047
	d13C.p.65	-932	0.042	0.045
	d13C.b.75	2527	0.023	0.037
	d13C.g.65	1705	0.048	0.035
	d13C.s.75	-2043	0.056	0.028
	d13C.l.65	-1071	0.209	0.024
	d13C.b.65	1081	0.094	0.018
d13C.grain.92			**0.001**	**0.75**
(d13C.organ)	(Intercept)	-7.28		
	d13C.a.65	0.36	0.052	0.182
	d13C.g.65	0.57	0.040	0.169
	d13C.s.75	0.79	0.018	0.129
	d13C.l.65	-0.55	0.086	0.091
	d13C.a.75	0.43	0.141	0.080
	d13C.g.75	-0.47	0.110	0.051
	d13C.b.75	-0.44	0.212	0.049
GY			**0.009**	**0.641**
(d15N)	(Intercept)	5346		
	d15N.s.75	1044	0.119	0.163
	d15N.p.65	1064	0.025	0.157
	d15N.grain.92	3905	0.072	0.089
	d15N.grain.85	-4176	0.073	0.078
	d15N.b.75	-768	0.248	0.069
	d15N.g.75	639	0.229	0.048
	d15N.g.65	-643	0.133	0.038
d15N.grain.92			**0.000**	**0.71**
(d15N.organ)	(Intercept)	0.47		
	d15N.p.65	0.66	0.013	0.308
	d15N.g.65	0.56	0.037	0.212
	d15N.b.75	0.64	0.010	0.143
	d15N.b.65	-0.52	0.034	0.047
prot.grain			**0.001**	**0.736**
(d15N)	(Intercept)	13.48		
	d15N.g.65	2.12	0.006	0.222
	d15N.g.75	1.52	0.024	0.116
	d15N.s.65	1.54	0.009	0.108
	d15N.l.65	-2.25	0.004	0.103
	d15N.grain75	0.69	0.103	0.082
	d15N.b.75	-1.74	0.012	0.069
	d15N.p.75	-1.18	0.102	0.036

Figure 6.10. Correlation matrix of C (A) and N (B) isotope composition per organ with grain yield and grain protein content. Asterisks indicate a significant correlation (P<0.05). Multivariate regression models (C) explaining grain yield and C and N isotope composition in grain at harvest across varieties under different N supplies. The abbreviations are described in Supplementary Table 6.1.

6.4. DISCUSSION

We performed a holistic study integrating phenotyping measurements of canopy and flag leaves and biochemical analyses of six foliar and non-foliar photosynthetic organs to identify the traits at the whole plant level that are related to plant growth, GY and GQ in field-grown durum wheat. A total of 426 traits were studied in four varieties grown under contrasting N fertilisation conditions. The pattern of changes among varieties was similar under both N conditions, as shown by the low G×N interaction. Therefore, we focused our attention mainly on the effects of N and genotypic variability separately.

6.4.1. N fertilisation has a significant effect on grain yield and quality, while agronomic differences between varieties were not affected by N availability

An efficient use of N fertilisation that meets sustainability is necessary since it is the nutrient that most affects crop production and quality, but it is costly and its excessive use can cause soil and water pollution (Vicente *et al.*, 2019b; Wang *et al*., 2021). Then, it is important to identify those physiological and metabolic parameters affected by N with an impact on GY and GQ. In our study, lower N supply decreased plant biomass by reducing plant height, peduncle length and tillering, with a direct impact on GY (Figure 6.3). However, under N-limiting conditions there were less plants per unit area but they used efficiently their nutrients on producing more ears per plant with larger grains (i.e. higher TGW), as it has been previously shown in barley (Vicente *et al*., 2019b) and wheat (Liu *et al*., 2021). Grain protein content is frequently affected by N fertilisation (Wang *et al*., 2021), but our contrasting N levels were not enough to alter it (Figure 6.3). Nevertheless, low N modified moisture content, and sedimentation and yellowness indices, which indicated an impoverishment of grain processing and end-product qualities (Zörb *et al.*, 2018).

The variety with highest GY, Haristide, was characterised by shorter peduncles and longer and heavier ears capable of lodging more grains, parameters associated with higher sink strength (Figure 6.3, Supplementary Table 6.2). Genotypic variability did not alter grain protein content, but affected other traits related to grain processing and end-product qualities. The most productive varieties Haristide and Euroduro had higher milling potential (SW), baking quality of wheat flour (sedimentation

and yellowness indices) and, only for Haristide, lower gluten strength (GI). In Djouadi *et al*. (2021), durum wheat yield also correlated with several grain quality traits, but frequently a negative correlation is found with grain protein content.

6.4.2. Phenotyping approaches to assess the effect of N and genotypic variability on grain yield and quality

Phenotyping approaches are suitable for characterising plant performance and identifying key attributes for plant growth and production (Kefauver *et al*., 2017; Vicente *et al*., 2019b; Prey & Schmidhalter, 2020). We performed ground-based phenotyping to quantify canopy greenness and thus relate it to plant biomass and health (Casadesús *et al*., 2007; Vergara-Diaz *et al*., 2016). RGB and spectral indices were good predictors of the N fertilisation on green biomass in durum wheat from early stages to maturity (Figure 6.4). These indices tend to saturate at intermediate growth periods, so their use is more valuable at early or late stages, as it happened in our study. Haristide showed a stay-green phenotype compared to the other varieties regardless of the N supply, which implies longer standing photosynthetically active biomass (Figure 6.4). Spectral indices measured at flag leaf level suggested that Haristide had improved performance due to better N status (NBI index) and lower flavonoid content (Supplementary Figure 6.1), while higher LRWC at anthesis and grain $\delta^{13}C$ indicated a better water status and water use efficiency, respectively (Rebetzke *et al.*, 2002; Araus *et al.*, 2022).

High-throughput phenotyping has been used to predict yield-related traits in wheat, e.g. using unmanned aerial systems, multispectral cameras and spectroradiometers (Prey & Schmidhalter, 2020; Garriga *et al*., 2021; Vatter *et al*., 2021), but their high cost and user training are drawbacks for their expansion. The vegetative indices used here quantified the greenness by counting pixels in the green colour range or by the spectrum reflected by the vegetation, so it was not surprising their high correlation with biomass and hence GY (Figure 6.5). The correlations were higher as growth progressed, indicating that late stages are better for prediction, although this is not an advantage for the use of high-throughput phenotyping in early detection or breeding programmes. The best leaf spectral indices for prediction were those estimating flavonoids and N content, which highlighted the relevance of antioxidant capacity and N status for productivity. Thus, the lower flavonoid content of Haristide compared with the other varieties may indicate a reduced need to produce antioxidants to counter the detrimental effects of reactive oxygen species that often occur during stress conditions or senescence

(Agati *et al*., 2020). Canopy indices did not stand out for its prediction of GQ parameters, except for those traits that already correlated with GY (Figure 6.5). However, leaf spectral indices had potential to predict some key GQ traits. In short, our study highlighted the use of low-cost and affordable phenotyping devices (RBG imaging and leaf spectral sensors) to rapidly estimate the effects of N fertilisation, to select high-yielding varieties and to predict GY and GQ.

6.4.3. Nitrogen fertilisation affects the uptake and/or allocation of micro- and macronutrients to the grain, while Ca and Zn could play an important role in yield

The concentration of mineral elements in the grain is relevant for GQ and human diet, being determined by the genotype-by-environment interaction (Sanchez-Garcia *et al*., 2015; Guzmán *et al*., 2016). The nutrient concentrations varied between N supplies and varieties, with few significant G×N interactions (Figure 6.6). Higher N supply quantitatively increased grain N and thus protein yields, and the uptake and/or allocation of nutrients such as C, S, Fe, P, Cu and Mn to the grain. This may be associated with a promotion of root growth under higher N supply that favours the nutrients uptake, as suggested by Ben Mariem *et al*. (2020). Nevertheless, an assessment of its cost-benefit and associated environmental pollution is crucial when selecting the best application rate and timing (Kefauver *et al*., 2017; Vicente *et al*., 2019b).

The high-yielding Haristide had higher yields of most nutrients than the other varieties, indicating higher uptake of nutrients from the soil, regardless of nutrient concentration (Figure 6.6). Anyway, the concentration of nutrients in the grain is an important factor affecting the quality parameters by which wheat flours are graded. Haristide was mainly distinguished from the other varieties by a significantly higher Ca and lower Zn concentration, being Ca mainly accumulated in blades (Supplementary Table 6.2). Brennan *et al*. (2007) reported that Ca application had a direct impact on wheat yields, what can suggest that its better uptake in Haristide could be a key factor to increase production. Indeed, Ca may modulate the absorption and translocation of several elements and maintain the integrity of selective ion transport proteins (Cobalchin *et al*., 2021). Lower Zn content in Haristide, irrespective of N supply, could suggest a poor root uptake or remobilisation from shoot to grains (Liu *et al*., 2019), which is relevant for human diet to avoid symptoms such as loss of appetite, growth retardation, rough and

peeling skin, and immune system dysfunction (Wang *et al.*, 2020). Uauy *et al.* (2006) showed that delayed senescence may decrease N, Fe, and Zn content in the grain. Haristide showed a stay-green phenotype, which might explain the lower Zn content found in this variety. In conclusion, N fertilisation is crucial to stimulate nutrient uptake, while higher GY was associated with better Ca status but lower Zn.

6.4.4. Canopy photosynthesis, N assimilation and C-N allocation to the grain are the result of a common effort of the green organs of the plant

After characterising wheat agronomy and canopy, we focused on the metabolism of green photosynthetic organs and their impact on GY and GQ. We hypothesised that non-foliar green organs have special physiological and metabolic features that make them suitable as source organs during grain filling, at least to complement the contribution of the flag leaf. This role has been predicted through other approaches under optimal and, more significantly, under stress conditions (Sanchez-Bragado *et al.*, 2014a; Sanchez-Bragado *et al.*, 2014b; 2016; Vicente *et al.*, 2018b; Sanchez-Bragado *et al.*, 2020c), although the precise metabolic pathways operating in each part are poorly understood. Multivariate analysis of metabolic and mineral traits indicated that the metabolism in the blades was undoubtedly the most active (Figure 6.7). It was followed by the awns, albeit by a wide margin. The different ear bracts, which were very similar to each other, had a similar behaviour to the awns. The peduncles and the sheaths were separated from the rest of the organs, suggesting they might have similar functions. Based on the PCA-centroids distribution of Supplementary Figure 6.3, it seems that N effect was more relevant on blades and less on bracts. Nevertheless, the differences were not very large, while Sanchez-Bragado *et al.* (2014a) found that the whole ear performance and contribution to grain filling improved under high N fertilisation.

We measure different metabolism traits, such as Rubisco protein and activity, and the amount of photoassimilates, as an alternative to previous approaches to characterise photosynthetic capacity of non-foliar organs to GY that were frequently intrusive or causing compensatory effects (Sanchez-Bragado *et al.*, 2016; Rivera-Amado *et al.*, 2020). The protein content and activities of Rubisco, directly involved in the fixation of atmospheric CO_2, were significantly higher in blades, but not negligible in other organs such as the awns, demonstrating active photosynthetic capacities at late stages, including a high degree of activation state in ear organs (Supplementary Table 6.2). Higher

PEPCase activity were shown in blades and awns, which was associated with their higher photosynthetic capacity and the need to process the C fixed, but the activities in the other organs were remarkable (Supplementary Table 6.2). This enzyme is involved in the balance of C and N metabolism by regulating the synthesis of C skeletons for the synthesis and nitrogenous compounds and its possible role in the re-assimilation of CO_2, such as grain respiration (Jia *et al*., 2015; Shi *et al*., 2015; Sanchez-Bragado *et al*., 2020c). Sucrose, which is the main compound used to transport C in cereals (Vicente *et al*., 2016; Al-Sheikh Ahmed *et al*., 2020), was highly abundant in all the green organs studied, which could be explained more by their photosynthetic capacity than by sucrose transport. The peduncles and the sheaths were clearly the organs where fructans accumulated (Figure 6.7), suggesting their predominant storage function. Takahashi *et al*. (2001) proposed a long-term storage function in peduncles and short-term in sheaths, involved in diel fluctuations. Starch, a minor storage carbohydrate in wheat (Scofield *et al*., 2009), is accumulated mainly in blades and later in ear organs. Glucose and fructose were predominantly abundant in sheaths and peduncles at earlier stages and in ear organs at both growth stages. The free carbohydrates are frequently derivate from the breakdown of other carbohydrates to transport C through the plant (Cimini *et al*., 2015), which could indicate that sheaths and peduncles provided C at anthesis (e.g. C from blades), and ears at grain filling. The ear is the youngest organ in the plant, so its delayed senescence (Jia *et al*., 2015; Vicente *et al*., 2018b; Tambussi *et al*., 2021) may indicate that ear organs play a more active role at later stages. According to Takahashi *et al*. (2001), from late grain-filling any new assimilate is used for grain growth. These results indicated that not only the blades, but any of the green organs are actively contributing to canopy photosynthesis with an impact on yield. Previous studies pointed out that the photosynthesis of non-laminar organs, mainly the ears, significantly contributed to canopy photosynthesis and, then, GY (Maydup *et al*., 2010; Jia *et al*., 2015; Gámez *et al*., 2020; Araus *et al*., 2021). Gross ear photosynthesis was approximately 56% of leaf photosynthesis on an area basis (Molero & Reynolds, 2020), while net photosynthesis may be much higher if we subtract the high ear respiration (Gámez *et al*., 2020; Tambussi *et al*., 2021) or consider the larger ear area (Olszewski *et al*., 2018; Sanchez-Bragado *et al*., 2020c), making ear photosynthesis a promising target for crop improvement.

A previous study suggested that 42% of the N in grains was coming from the ears using N isotope signatures (Sanchez-Bragado *et al*., 2017). We combined measurements of N content, isotope composition and enzyme activities to deepen into N metabolism at the whole plant level. The enzyme profiles revealed active N metabolism functioning in every organ, with higher levels of GS and

GOGAT in blades and awns, and GDH in blades and lemmas (Figure 6.7). It may indicate that an important part of N metabolism takes place outside the blades, corroborating at biochemical level previous results (Lopes *et al*., 2006; Sanchez-Bragado *et al*., 2017). The high GDH activities in ears, and particularly, in lemmas may suggest an important role in plant glutamate homeostasis, involved in C-N signalling (Labboun *et al*., 2009) and, given their proximity to grains, in the N supply for grain filling at late stages. Organ-specific N levels followed a similar trend that Rubisco traits (high in blades and awns), mainly due to the fact that Rubisco and other photosynthetic structures require a high N budget (Evans & Clarke, 2019). The rest of the nutrients also had higher levels in blades, but very high levels of Fe in the glumes were observed. Fe is essential for photosynthetic processes, heme biosynthesis and Fe-S cluster assembly (Morrissey & Guerinot, 2009), but its specific role in glumes remains unclear and should be further investigated.

Lower N fertilisation significantly inhibited photosynthetic capacity and N assimilation at the whole plant level, except for an upregulation in the peduncle during anthesis (Supplementary Table 6.2). It also promoted the storage of C in peduncles, as reported previously in bread wheat (Scofield *et al*., 2009), while the high decrease of sucrose and starch levels in ear organs at MGF may suggest that either (i) the ears decreased their capacity to supply C to the grain or other organs under low N, or (ii) most of the C produced is sent out due to the high demand of heterotrophic tissues. Our isotopic results and those of Sanchez-Bragado *et al*. (2017), together with the better activation state of Rubisco at late stages (Figure 6.8) pointed to the latter.

6.4.5. Metabolic and nutrient changes between anthesis and mid-grain filling point to the specialisation of each green organ in the later growth stages

The clear increase in free carbohydrates at MGF in blades may suggest that different C-rich cellular components are degraded to provide nutrients to other organs (Figure 6.8). The decrease of fructose and the drastic increase of fructan levels in peduncles at MGF may indicate that this organ is actively accumulating C which will be probably used when plant photosynthesis ceases at the end of grain filling (Takahashi *et al.*, 2001). These changes were not observed in sheaths, which could support the hypothesis that they participate more in the diurnal accumulation of fructans (Takahashi *et al*., 2001). Furthermore, CO_2 fixation by Rubisco was improved at MGF in sheaths, glumes and, only at control N, in peduncles and lemmas, suggesting a relevant photosynthetic contribution at late stages. The

increased Rubisco activity in ears was due to an increase in its activation state, even though protein levels decreased. We hypothesise that these organs may have redistributed efficiently the N stored in this enzyme to other limiting processes. Interestingly, Kanno *et al.* (2017) observed that rice mutants with lower Rubisco content improved N use efficiency and photosynthesis. While N decreased at MGF in every organ, GDH tended to increase, suggesting that it may act predominantly deaminating glutamate at late stages and, then, reallocating N to the developing grains (Labboun *et al.*, 2009). Low N supply had a clear effect on reducing C assimilation through the observed changes in sucrose levels and Rubisco activation state at the whole plant level, reflecting the strong coordination between C and N metabolism (Vicente *et al.*, 2018a). In general, low N also inhibited PEPCase, GS and GOGAT activities, probably by limiting their substrate concentrations. In parallel to N, P and Cu also decreased at MGF. The changes in K, Ca, Mg, Fe and Mn were organ-specific. Meanwhile, C decreased in blades and, more significantly, in ear organs, which may suggest a high C contribution of ears at late stages to the developing grains. Overall, the pattern of changes between anthesis and MGF suggested that each organ evolves in a different way, indicating diverse but complementary roles for the control of starch and protein deposition to the grain during the grain filling phase.

6.4.6. Linear and stepwise regressions highlight the key role of ear metabolic traits and blade carbohydrates for durum wheat growth and productivity

Although correlations do not imply cause-effect relationships, we used them to determine the possible contribution of the different photosynthetic organs to grain filling and to identify key traits (Figure 6.9). Accumulation of free carbohydrates and lower sucrose, starch, and fructan contents were positively associated with GY, mainly in blades and the different ear organs. It clearly highlighted that higher productivity is linked to a rapid translocation of photoassimilates, predominantly for grain filling since plant growth is ceased at late stages (Figure 6.4). Oppositely, sucrose was positively correlated with GY in ear organs at MGF, suggesting again ears as key C sources for grains. Apart from carbohydrate metabolism, it was surprising that other metabolic traits in blades were not associated with GY (Figure 6.8). However, GY and biomass were linked to a more active C and N metabolism in awns, glumes and lemmas, as observed with the concomitant association of Rubisco, PEPCase and sucrose at late stages with GY. The high contribution of ears to grain filling may be related to its proximity to the grain, delayed senescence, higher light harvesting at the top of the canopy, or even its

putative capacity to reassimilate respired CO_2 (Sanchez-Bragado *et al*., 2020c; Tambussi *et al*., 2021). Whether awn metabolism or, particularly, its photosynthetic capacity is relevant for GY has been controversial (Sanchez-Bragado *et al*., 2020a). Our results do suggest this at the biochemical level. Based on the correlations, the sheath appeared to be an organ that performed functions more oriented for plant growth, while the metabolic traits of the peduncle did not have a considerable impact on yield or biomass, even negative correlations between these parameters were observed (Figure 6.8). This may be associated with the advantage of shorter varieties (i.e. peduncles or stems) that favours the contribution of ears to grain filling (Tambussi *et al*., 2021). The only study to our knowledge comparing leaf and whole-ear photosynthesis with GY suggested that the latter correlated better than the former (Abbad *et al*., 2004). We previously found that Rubisco gene expression in durum wheat ears and leaves, as well as several N-metabolism related genes, were correlated with higher productivity (Vicente *et al*., 2018b). Moreover, Vergara-Diaz *et al*. (2020a) proved that leaf, glume and lemma metabolomes were determinant for GY in durum wheat. Lastly, Shokat *et al*. (2020) also reported that antioxidant and C metabolism enzymes in leaves and whole-ears correlated with yield-traits in bread wheat.

The similarity of the isotope compositions between green organs and grains at harvest has been used as a non-intrusive technique to estimate the relative organ contribution to grain filling (Sanchez-Bragado *et al*., 2014b; Sanchez-Bragado *et al*., 2017; Tambussi *et al*., 2021). Our models suggested that the supply of C and N to the grains was to some extent due to the contribution of the different organs (Figure 6.10). Moreover, the relative contribution of C from non-foliar organs, in particular the ear organs, stood out above the rest, while for N the contribution was more varied in terms of plant parts. Protein content, considered the most important GQ trait, was mainly associated with the metabolism of lemmas and blades (Figure 6.9), while the isotope signatures suggested a key role also for glumes (Figure 6.10).

6.5. CONCLUSIONS

We highlight that our novel characterization of key enzymes activities in six different green organs, together with carbohydrate profiles, mineral compositions, natural isotope compositions and plant canopy monitoring, was an integrative approach to identify metabolic and physiological targets involved in grain filling. The primary metabolism of green organs suggested that all have important

functions in contributing to early and late grain filling. Although in absolute terms the blades presented the greatest metabolic activity among the green organs, only their carbohydrate metabolism was associated with GY. The pattern of correlations between key enzyme activities and sucrose in ear organs with GY emphasise about the key role of ears during grain filling at metabolic level (Sanchez-Bragado *et al*., 2014b; Sanchez-Bragado *et al*., 2017; Vicente *et al*., 2018b; Shokat *et al*., 2020; Vergara-Diaz *et al*., 2020b). Our results showed that, regardless of the N supply, high yield was associated with plants with shorter peduncles and longer ears (high sink strength), stay-green phenotype with more photosynthetically active biomass at late growth stages, better leaf water and N status, and a more active ear metabolism, particularly at MGF (i.e. higher Rubisco, PEPCase, GOGAT and GDH activities). This study opens the doors to investigate on a larger population of varieties the molecular and morphological mechanisms operating in non-foliar photosynthetic organs that impact on GY and GQ. We predict that advances in organ-specific high-throughput phenotyping and metabolic regulation of source-sink dynamics will strongly contribute to crop improvement under optimal and unfavourable environments, highlighting the need of including ear photosynthesis in the breeding programmes as a new target for crop improvement.

7. CHAPTER 7:

Fourth Study

Analysis of durum wheat photosynthetic organs reveals a lower impact of water stress on ears metabolism as compared to flag leaves and a high accumulation of primary metabolites in peduncles during grain filling

7.1. INTRODUCTION

For decades, scientists have warned about global climate change and its impact on agro-ecosystems functioning and stability. At present, there is already evidence of a production fall in major crops such as wheat and maize in many regions like the Mediterranean basin due to climate change, i.e. increases in temperature, severe droughts, and extreme events occurrence (Lobell *et al.*, 2011). This is particularly relevant when the temperature increases take place in May-July, since it is negatively associated with wheat yields in Europe (Pinke *et al.*, 2022). Moreover, future exacerbation of climate change effects, together with growing population and changes in dietary habits, will further endanger crop yield stability particularly in the countries of the Mediterranean basin. Thus, plant scientists are called to provide new methods and explore potential sources of crop stress resilience, leading to enhanced food security.

Many efforts have been dedicated to boosting field-based high-throughput phenotyping in the last decade (Araus *et al.*, 2022). Advances in this area undoubtedly contribute to a better understanding of the genotype-by-environment interaction, making it possible to evaluate the physiologic and yield performance on extensive collections of varieties. However, besides robust phenotyping systems, there is a need for new sources of variability and traits to assist crop breeding programs. This is particularly critical for important crops such as bread and durum wheat, whose genetic advances have stagnated in the last decades, particularly in Southern European countries (Chairi *et al.*, 2018; Pinke *et al.*, 2022).

Durum wheat is one of the most relevant food crops in terms of cultivated area (about 16 M hectares) and nutritious importance, e.g. production of pasta, couscous and bulgur (Guzmán *et al.*, 2016; Beres *et al.*, 2020). Its production is concentrated in the south and east of the Mediterranean basin, the North American Great Plains, Russia, and Kazakhstan (Tidiane Sall *et al.*, 2019; Royo *et al.*, 2021). In these producer regions, durum wheat is usually subjected to terminal drought during the highly sensitive stages of anthesis and grain filling, which comprises yield production and its quality.

During water deprivation events, plant growth and development are inhibited as a result of the restricted CO_2 diffusion (i.e. stomatal closure) and a fall in photosynthesis and N assimilation (Tezara *et al.*, 1999; Xu & Yu, 2006). At the same time, water stress triggers major transcriptional

reprogramming of plant metabolism, including signalling, which leads to cell osmotic adjustment (e.g. accumulation of certain sugars and amino acids as osmoprotectants) and antioxidant response (Ergen *et al.*, 2009; Rybka & Nita, 2015; Ullah *et al.*, 2017; Vicente *et al.*, 2018b). Prolonged stress, mainly occurring at sensitive growth stages, may lead to metabolic disruption and collapse, affecting severely grain yield (Rybka & Nita, 2015).

Yield is a complex trait that depends on its agronomic components (e.g. ears per area, grains per ear, and grain weight) and physiologic components such as the efficient use of resources (water, nutrients and light). From a physiologic and metabolic point of view, grain yield can be limited by sink and source organs in terms of assimilation and storing capacity. In the absence of stress, grain filling is considered to be mainly limited by the ability of the developing grains to utilise the translocated assimilates (sink limitation), but also source and sink co-limitations may exist (Slafer & Savin, 1994; Rivera-Amado *et al.*, 2020). Oppositely, under water stress, grain filling is limited by source capacity due to the inhibition of C fixation and nitrogen assimilation, mainly reported in the flag leaves (Medina *et al.*, 2016; Vicente *et al.*, 2018b). Assimilates come from different organs and pathways, such as the photosynthesis of the leaves (blades and sheaths) and ears (mainly glumes, lemmas, and awns), as well as the translocation from internodes (Sanchez-Bragado *et al.*, 2020c; Tambussi *et al.*, 2021). The contribution of each of these compartments to grain filling is complex as it depends on the environmental conditions, the genotype and the phenology.

In this regard, the leaf blade has traditionally been considered the primary source of photoassimilates during optimal and stress conditions in wheat, especially the flag leaf at late developmental stages (Hu *et al.*, 2018; Tambussi *et al.*, 2021), whereas little attention has been paid to the performance of other photosynthetic foliar or non-foliar organs. For instance, peduncles and leaf sheaths are photosynthetic organs showing active photosynthesis, delayed senescence and playing an important role in sugar storage (Schnyder, 1993; Kong *et al.*, 2010; Martínez-Peña *et al.*, 2022 (see chapter 6)). In the last few years, far more attention has been paid to the metabolic performance of wheat ear and its contribution to grain filling (Sanchez-Bragado *et al.*, 2020c). The ear is made up of different photosynthetic tissues receiving even higher irradiance than the leaves due to its top position. It is worth mentioning that the significant role of this organ has been corroborated by using different methodologies such as organ removal and shadowing (Maydup *et al.*, 2012; Sanchez-Bragado *et al.*, 2016), isotopic and nutrient composition determinations in different organs (Sanchez-Bragado *et al.*,

2014a; Sanchez-Bragado *et al.*, 2014b; Sanchez-Bragado *et al.*, 2017), as well as transcriptional and metabolic approaches (Lopes *et al.*, 2006; Jia *et al.*, 2015; Vicente *et al.*, 2018b; Shokat *et al.*, 2020; Vergara-Diaz *et al.*, 2020b; Martínez-Peña *et al.*, 2022 (see chapter 6). Interestingly, some studies suggest that ear tissues contribution can be even more relevant under stress conditions (Hu *et al.*, 2018; Sanchez-Bragado *et al.*, 2020c).

Ears may be considered constitutively adapted-to-stress organs due to their xeromorphic anatomy, more stable N and water status and C and N assimilation as compared to other organs (Araus *et al.*, 1993; Araus *et al.*, 2003; Martinez *et al.*, 2003; Lopes *et al.*, 2006; Vicente *et al.*, 2018b; Sanchez-Bragado *et al.*, 2020c). While some studies reported that awns are the most relevant CO_2-fixing tissues in the ears, others highlight that glumes and lemmas may serve as essential refixation sites of the endosperm-respired C besides fixing atmospheric CO_2 (Araus *et al.*, 1992; Bort *et al.*, 1996; Sanchez-Bragado *et al.*, 2020c; Tambussi *et al.*, 2021). In addition, under water stress, N and respiration metabolism may be up-regulated and senescence delayed compared to leaves (Martinez *et al.*, 2003; Hu *et al.*, 2018; Vicente *et al.*, 2018b). Finally, respiration and photorespiration intermediates and specific amino acids have been reported to increase in ear bracts as compared to leaves, highlighting better coordination of C and N metabolism in these particular tissues (Vergara-Diaz *et al.*, 2020b). There is, however, some degree of uncertainty about the real activity and potential of ear organs, as summarised in Araus *et al.* (2021). For instance, methodological issues such as considering of whole organ vs. area basis for some of these traits may greatly affect the conclusions.

Plant organ specific responses to water stress are undoubtedly variable but also promising for yield stability acquisition. In this sense, the knowledge of the assimilation and translocation of C and N through the plant, particularly at the latest stages of grain development, is still limiting. The present work aims to study the source-sink dynamics of C and N metabolites in laminar (leaf blades and sheaths) and non-laminar (peduncles, awns, glumes and lemmas) organs in response to water stress. Special attention is paid to the phenology-dependent dynamics and the identification of new traits as selection criteria for developing climate-resilient crops in future breeding programs.

7.2. MATERIALS AND METHODS

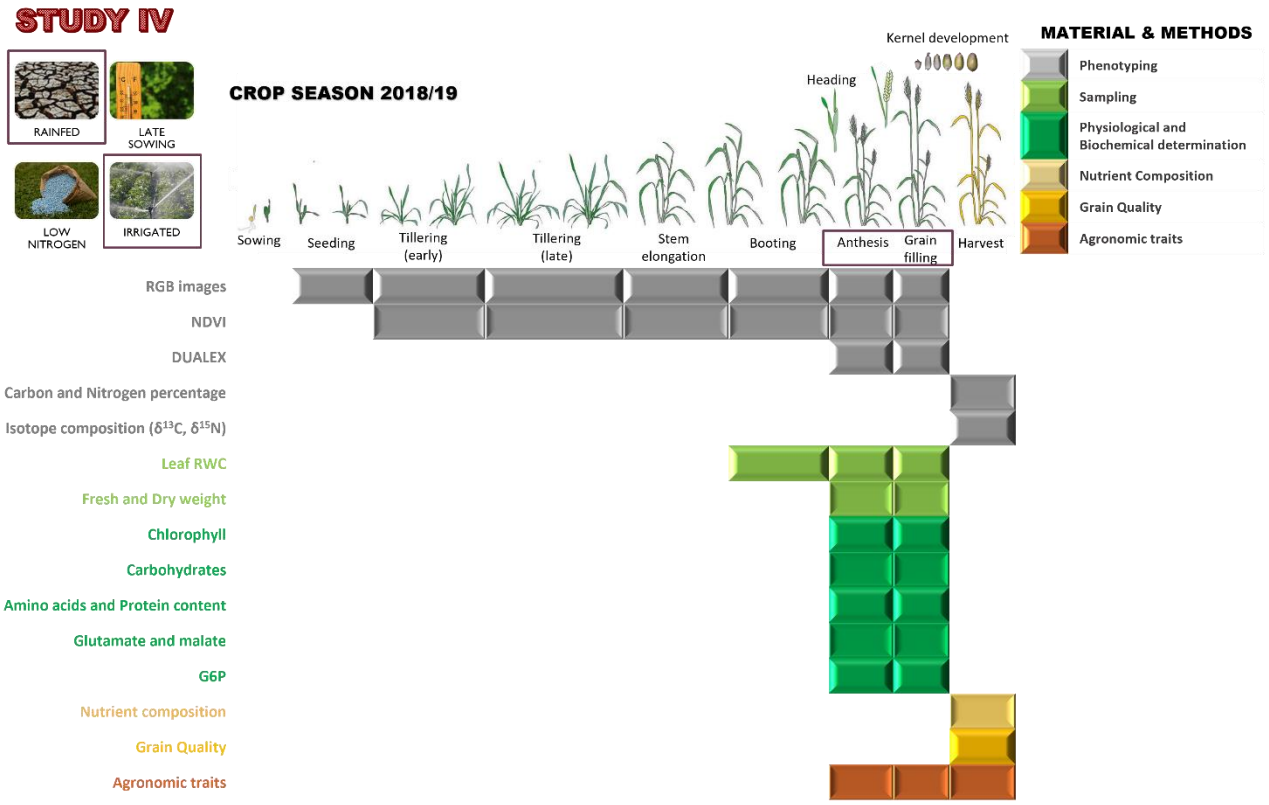


Figure 7.1. Five varieties were selected from the trial carried out during the crop season of 2018/2019: *Mexa*, MEX (1980), *Euroduro*, EUR (2007), *Don Ricardo*, DRI (2008), *Kiko Nick*, KNI (2009) and *Haristide*, HAR (2015), which were grown under two different water regimens. The control treatment (irrigated conditions) and the stress treatment (rainfed conditions). The control received supplemental irrigation that, together with the rainfall, accounted for a total of 298.6 mm during the life cycle, the stress treatment depended exclusively on the rainfall (127.5 mm). The phenology was monitored throughout the growth cycle using the Zadoks scale. In addition, ground-phenotyping was performed during the crop cycle at the canopy level of the panel. At the same time, physiological and biochemical analyses were carried out in different foliar and non-foliar green organs (flag leaf blades and sheaths, peduncles, awns, glumes, and lemmas) at two specific stages; anthesis (Zadoks 65) and mid-grain filling (Zadoks 75). For these stages, the phenology of each variety was considered. Agronomic and grain quality traits were evaluated at harvest, together with the grain carbon and nitrogen isotope composition.

7.3. RESULTS

7.3.1. Effect of the genotypic variability and water regime on the agronomic components and grain quality traits

The Mediterranean climate in the study area was characterised by low temperatures during the first months of durum wheat growth, with minimum temperatures below 0ºC, and high temperatures during the last stages of grain filling, with maximum temperatures above 30ºC (Figure 7.2a). Humidity also showed significant oscillations during wheat growth, with a tendency to decrease as temperatures increase. Rainfall was low this year during the crop season (rainfed=127.5 mm), so irrigation was an essential factor in observing changes in durum wheat growth and production (irrigated=298.6 mm).

Rainfed showed an overall yield reduction of 64% for the five varieties studied, from 7230 kg ha^{-1} under irrigation to 2576 kg ha^{-1} under rainfed conditions (Figure 7.2b). Although there are slight changes between varieties, there are no significant differences, so we can conclude that the five varieties showed similar yields in both water regimes, with a strong effect of water limitation in all of them. Likewise, biomass was also reduced by 52%, with similar results between varieties (Supplementary Table S7.1). The effect of water stress was also reflected in the C isotopic composition ($\delta^{13}C$) of the mature grain, which changed from -25.3‰ under irrigation to -22.3 under rainfed conditions (Figure 7.2c). Another parameter that is also strongly influenced by water conditions is the RWC. This was measured on the flag leaves in the heading, anthesis and mid-grain filling, showing how the effect of water limitation was progressive, being more pronounced at later stages (Figure 7.2d). There is no clear apparent effect between either water treatments or varieties at heading. Significant changes in water stress begin to be observed at anthesis, with some slight differences between varieties. During mid-grain filling the rainfed conditions clearly reduce the RWC in all varieties, being relevant in the five varieties studied but slightly more pronounced quantitatively in Mexa and Haristide.

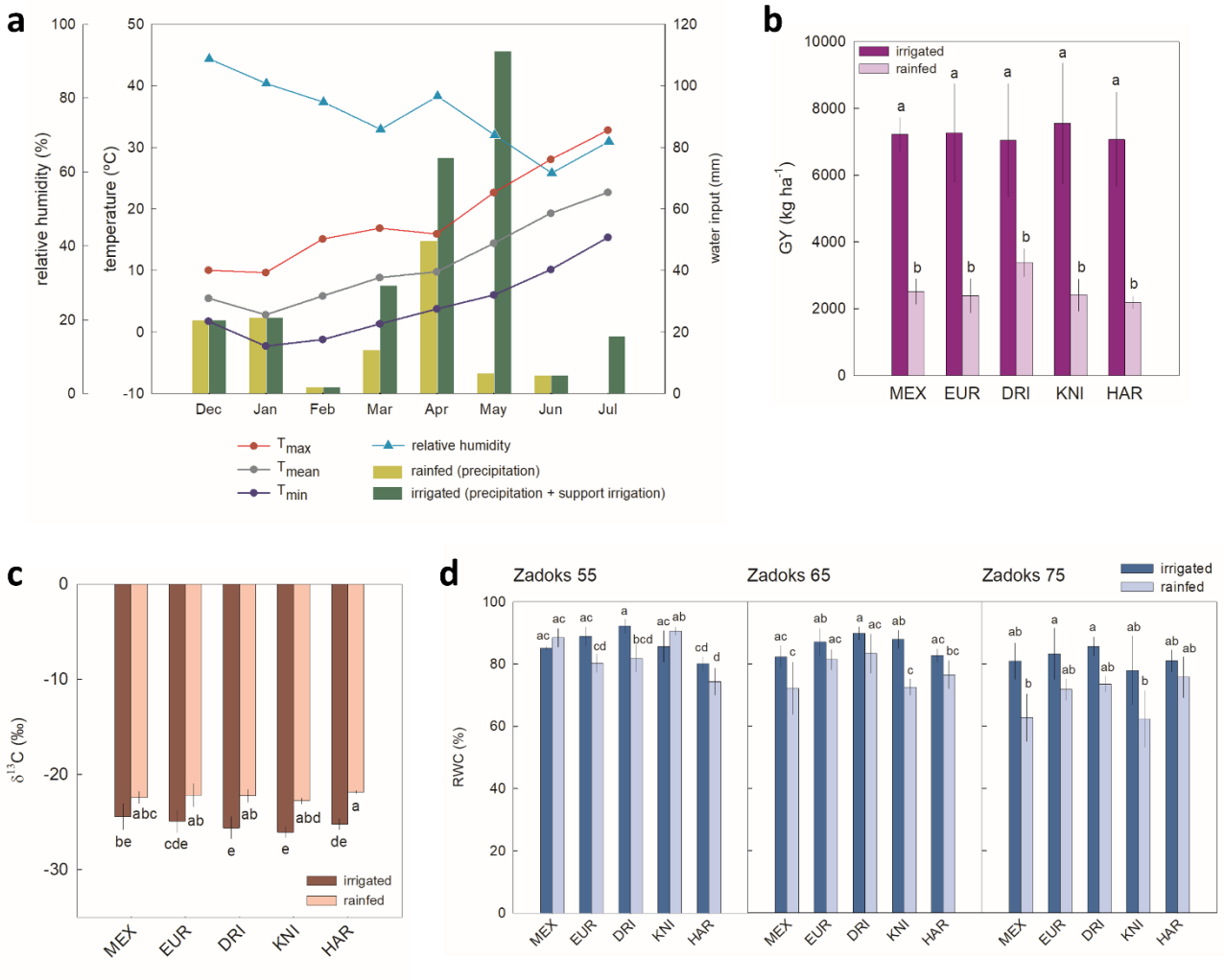


Figure 7.2. (a) Climatic conditions during the crop season of 2018/2019, and (b) grain yield (GY), (c) carbon isotope composition ($\delta^{13}C$) in grains at harvest, and (d) relative water content in flag leaves at Zadoks 55, 65 and 75, in five durum wheat varieties (MEX, Mexa; EUR, Euroduro; DRI, Don Ricardo; KNI, Kiko Nick; HAR, Haristide) under rainfed and irrigated conditions. The daily maximum (red line), mean (grey line), and minimum (dark blue line) temperatures, relative humidity (light blue line), water inputs (precipitations as light green bars, and with support irrigation as dark green bars) are shown in (a).

7.3.2. The impact of water regime on spectral vegetation indices throughout durum wheat growth

Two spectral sensors were used for phenotyping the growth of the five durum wheat varieties in response to contrasting levels of water supply. NDVI at canopy level and the four parameters measured by DUALEX at flag leaf level showed that the main effect on the vegetative indices was water input (Figure 7.3 and Supplementary Table S7.1). Rainfed conditions led to lower NDVI values than irrigation, which were significant from Zadoks stage 40 onwards. The later stages clearly show a substantial reduction of NDVI under rainfed conditions. While significant differences between

varieties were observed at the intermediate stages measured (Zadoks 35-55), mainly under drought conditions, these disappeared at the last sampled point, anthesis (Zadoks 65).

Rainfed conditions also negatively affected the indices measured on the flag leaves at two key stages for yield, anthesis and mid-grain filling (Figure 7.3b). A significant reduction in chlorophyll content was observed in rainfed relative to irrigation and was more evident at later stages (Zadoks 75; Figure 7.3b). The effect of variety was significant at this stage, where a strong reduction was observed mainly in Kiko Nick. Flavonoids content increased slightly at anthesis (p=0.041, Supplementary Table S7.1), and more intensely at mid-grain filling. This increase was slightly higher in the Kiko Nick variety. No significant changes in anthocyanins content were observed at anthesis, but at mid-grain filling the increase in rainfed conditions was noticeable for all varieties, again being notably higher in Kiko Nick (Figure 7.3b). Finally, the NBI index was strongly reduced in rainfed conditions, the effect being more pronounced in late stages and Kiko Nick.

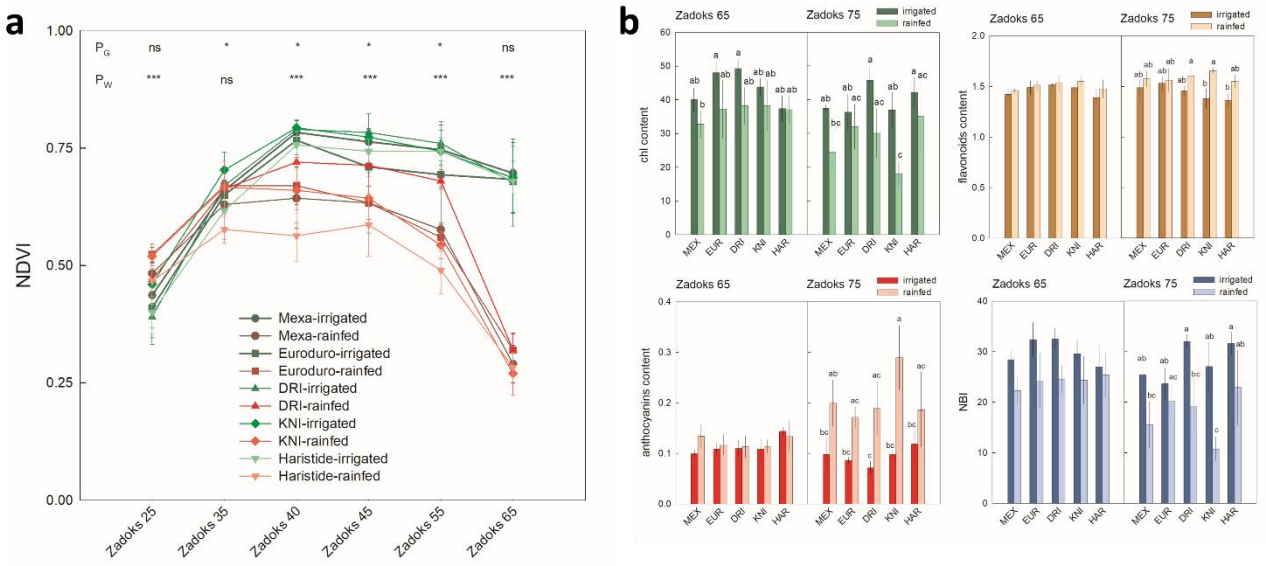


Figure 7.3. Spectral vegetation indices at canopy (a) and leaf (b) level in five durum wheat varieties (MEX, Mexa; EUR, Euroduro; DRI, Don Ricardo; KNI, Kiko Nick; HAR, Haristide) under rainfed and irrigated conditions. The spectroradiometer GreenSeeker was used to determine the Normalised Difference Vegetation Index (NDVI) at 6 growth stages (a), while the leaf-clip sensor DUALEX estimated the chlorophyll, flavonoids, antocyanins contents and NBI index at anthesis and mid grain filling stages (b). For each comparison of means, letters are significantly different (p<0.05; two-way ANOVA, TUKEY test).

7.3.3. Multivariate analysis of the performance of five durum wheat varieties on two contrasting water regimes

Differences in water input at the agronomic level were supported by multivariate analysis (PCA, Figure 7.4). Other agronomic data of interest, along with grain quality and physiological traits, were also included in this PCA. Dimension 1 of the PCA explained 44.1% of the variability and was clearly associated with the effect of water input. On the other hand, dimension 2 explained 12.2% of the variability in the data, which was associated with other factors, including the genotypic variability. The genotypic variability was less evident than water regime, but it was relevant in optimal conditions (irrigation), where the centroids of the different varieties were separated from each other mainly by dimension 2. The most remarkable difference was between the Kiko Nick and Don Ricardo varieties under irrigation, while Don Ricardo was slightly different from the rest of the varieties in rainfed conditions.

As we have highlighted, irrigation contributed significantly to increasing yield, biomass (both in terms of plant weight at harvest and NDVI throughout the plant cycle) and leaf greenness (chl content and NBI), but also increased other agronomic parameters such as GCY, GNY, HI, ears m^{-2}, ears $plant^{-1}$, and grains ear^{-1} (Figure 7.4 and Supplementary Table S7.1). While irrigation increased $\delta^{15}N$, rainfed increased $\delta^{13}C$. Regarding grain quality parameters, there was less homogeneity in the trends of the five varieties for each water condition, which showed that these parameters were influenced by the environment and the variety (Supplementary Table S7.1). However, protein content, vitreousness, SDS sedimentation, and WG under rainfed conditions increased independently of the variety. On the other hand, TKW decreased in rainfed conditions, while for the b* and GI parameters, the genotype × environment interaction was significant.

Furthermore, we analysed the nutritional quality of durum wheat at harvest by analysing the content of 11 micro- and macro-nutrients. Significant changes were observed depending on the water regime, the environment or the interaction (Figure 7.4 and Supplementary Table S7.1). Among the most significant changes, it was observed that in rainfed, compared to irrigation, the content of N and S in grain increased, while Mg, P, Cu and Mn decreased.

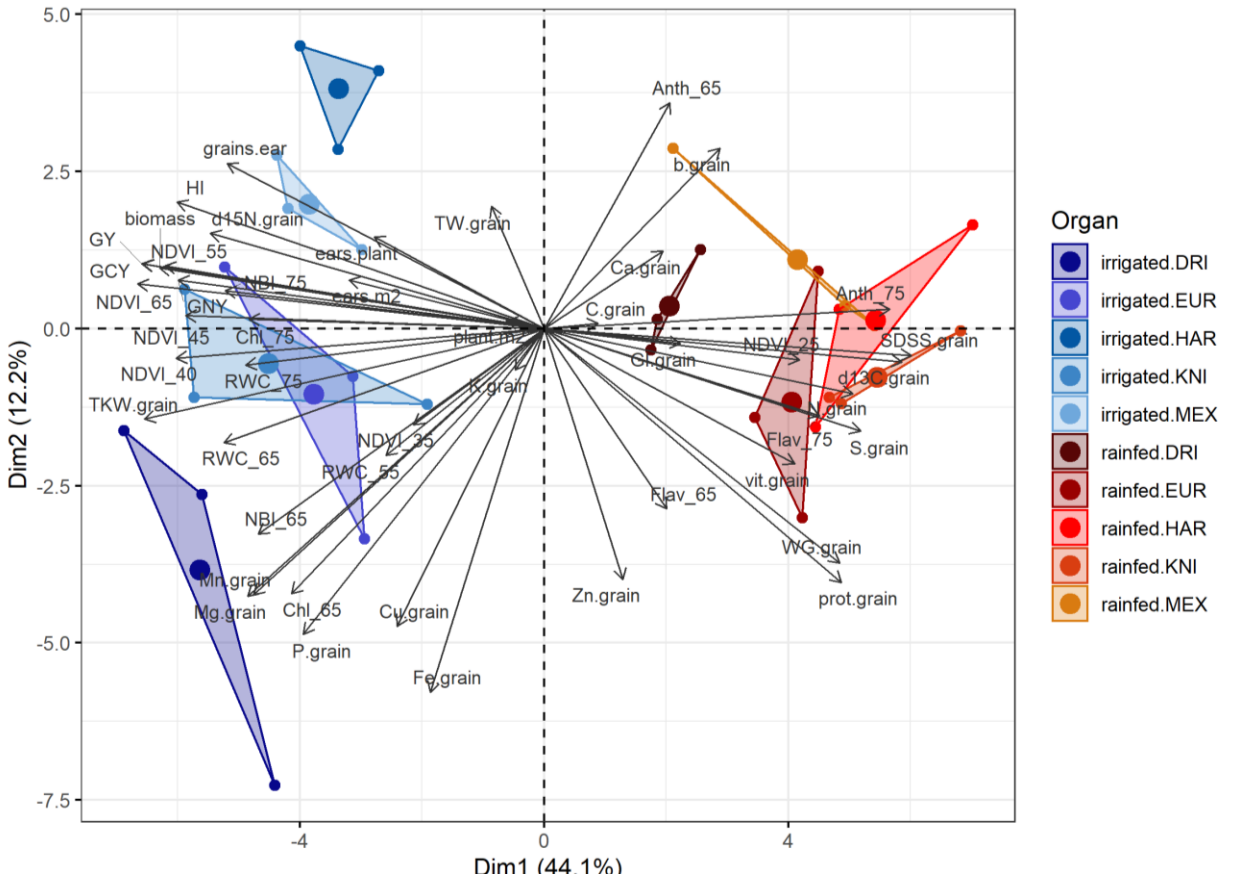


Figure 7.4. Principal component analysis (PCA) of agronomic, grain quality and physiological traits in five durum wheat varieties (MEX, Mexa; EUR, Euroduro; DRI, Don Ricardo; KNI, Kiko Nick; HAR, Haristide) under rainfed and irrigated conditions.

7.3.4. Differences in the concentration of carbon and nitrogen metabolites between the foliar and non-foliar photosynthetic organs under contrasting water regimes

Once the effect of water stress and genotypic variability at the agronomic and physiological levels on the plant canopy and grain production and quality were characterised, we focused on the physiological and metabolic changes that took place between various organs at anthesis and mid-grain filling. To do this, we performed a multivariate analysis of the results obtained for the DW, WC and diverse C and N metabolites in six photosynthetic organs, i.e. blades, sheaths, peduncles, awns, glumes and lemmas (Figure 7.5). In the first case, these results were expressed in concentration by DW, avoiding the use of FW to not introduce variations due exclusively or in part to changes in the water

content of the organs. PCA dimension 1 explained 26.8% of the variability, while dimension 2 explained 19.8%.

The PCA clearly showed that the organ is the main factor affecting the physiological and biochemical traits. The ear organs (awns, glumes and lemmas) were grouped together regardless of the water regime. The differences between irrigation and rainfed conditions were scarce in the awns and a little higher in the bracts due to the position of the centroids. These organs showed the highest concentrations of glucose-6-phosphate (glc6P) and starch in general. On the other hand, the peduncle was located in another area of the PCA, with relative proximity only to the leaf sheath. The peduncle tended to have a higher DW, glc and fru contents at anthesis. The effect of the water regime was remarkable. The leaf sheath exhibited little variability in response to the water regime, with high concentrations of chl b at anthesis and mid-grain filling, and suc at the latter. The blade was separated from the other organs and showed a tendency to have the highest concentrations of several C and N metabolites. However, it is the organ that showed the greatest effect of the water regime due to the distance between the irrigated and rainfed centroids. Among the metabolites with the highest concentrations in the blades, chl and suc at anthesis and proteins and malate in the two stages stand out.

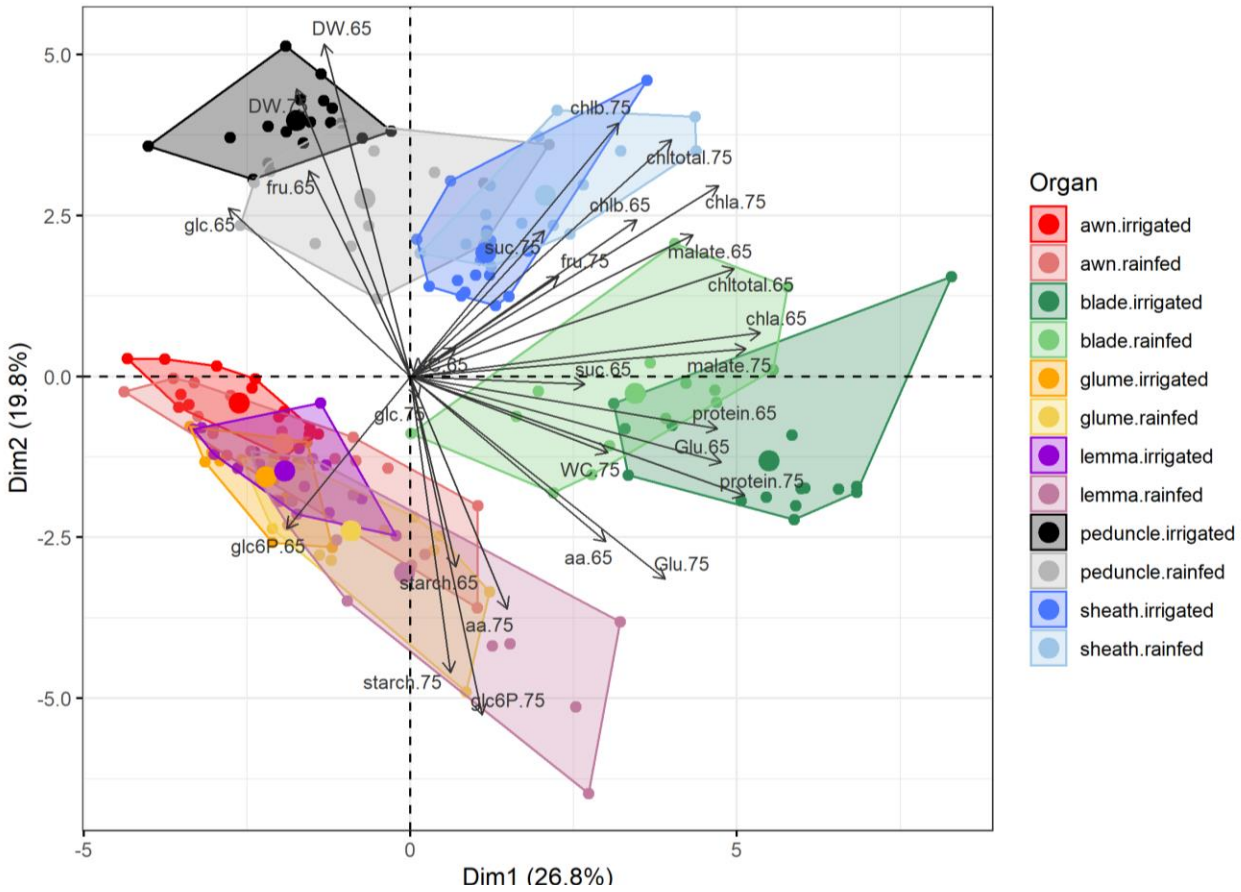


Figure 7.5. Principal component analysis (PCA) of dry weight, water content, and carbon (glucose, glucose-6-phosphate, fructose, sucrose, starch, and malate) and nitrogen (glutamate, total amino acids, proteins, chlorophylls a, b and total) metabolites. The traits were expressed as concentration and measured in six photosynthetic organs (leaf blades and sheaths, peduncles, awns, glumes and lemmas) of five durum wheat varieties (MEX, Mexa; EUR, Euroduro; DRI, Don Ricardo; KNI, Kiko Nick; HAR, Haristide) under rainfed and irrigated conditions. The measurements were carried out at anthesis (Zadoks 65) and mid-grain filling (Zadoks 75).

A more detailed analysis of the response to water limitation on physiological and biochemical traits by organ is shown in Figure 7.6, while the effects of the genotype × water regime interaction on these traits per organ are detailed in Supplementary Table S7.1. In general, FW and DW were significantly reduced under rainfed than irrigated conditions in the different organs. These traits also varied by variety, being higher in Haristide under irrigated conditions and lower in Kiko Nick under rainfed conditions. The trend in durum wheat, grouping the five varieties studied, was a strong reduction in DW in blades (30-35%), followed by peduncles (24-41%), awns (15-20%), lemmas (16-17%), sheaths (5-9%) and glumes (5-6%) under rainfed conditions. Under these conditions, the water

content decreased significantly in the blades, sheaths and peduncles, while in the three ear organs this reduction was smaller or even increased surprisingly at mid-grain filling (6-22%).

The concentration of free glc per DW increased dramatically in rainfed compared to irrigated conditions at anthesis (204%) but decreased at grain filling (Figure 7.6). Irrespective of the growth stage, glc decreased in the bracts (21-42%). Glc6P significantly decreased in all organs at anthesis. However, this reduction was only maintained in the laminar organs (blades and sheaths) at mid-grain filling and, in contrast, increased in the peduncles, glumes and lemmas. Fru tended to increase in blades and awns, but although quantitatively notable, it was not significant due to high genotypic variability (Supplementary Table S7.1). Only the decrease in fru in the peduncles and lemmas at anthesis was statistically significant. Suc content increased significantly in all organs regardless of the growth stage (except sheaths at mid-grain filling), but this increase was organ-specific. While the increase was 29-35% in blades, 6-25% in sheaths, and 11-35% in peduncles, in ear organs, the increase was higher (53-105%, 51-62% and 60-92% for awns, glumes and lemmas, respectively). Similar to suc, starch concentration increased markedly in all organs under rainfed conditions, but the increase was not as high or significant in blades at both stages. The greatest increases in starch occurred in the three organs of the ears (with the awns being the highest, 158%) and the peduncle at mid-grain filling. Malate decreased in blades and awns (significantly at mid-grain filling), peduncles at anthesis, and sheaths at both stages. However, rainfed conditions did not affect ear bracts; even a substantial increase in glumes was observed at anthesis (86%).

Regarding the N-rich metabolites, firstly, the glu response to the water regime was highly dependent on the green organ (Figure 7.6). It decreased at anthesis and mid-grain filling in blades (32-43%) and sheaths (23-24%) but was not altered in peduncles and awns under rainfed conditions. On the other hand, its concentration increased in glumes (30%) and lemmas (34%) at mid-grain filling. The aa concentration was not changed in blades and awns by water supply. However, it increased at mid-grain filling in the sheaths (26%) under rainfed compared to irrigated conditions and at both stages analysed in the peduncles (19-44%), glumes (57-59%) and lemmas (59-97%). Chl a concentration decreased in blades at both stages under rainfed conditions and increased in the sheaths and peduncles at anthesis. Chl b tended to increase significantly in all organs in response to water limitation, with the greatest increase in sheaths. These changes in chl a and b resulted in increased total chl levels in the sheaths and peduncles at anthesis and in the ear bracts at mid-grain filling.

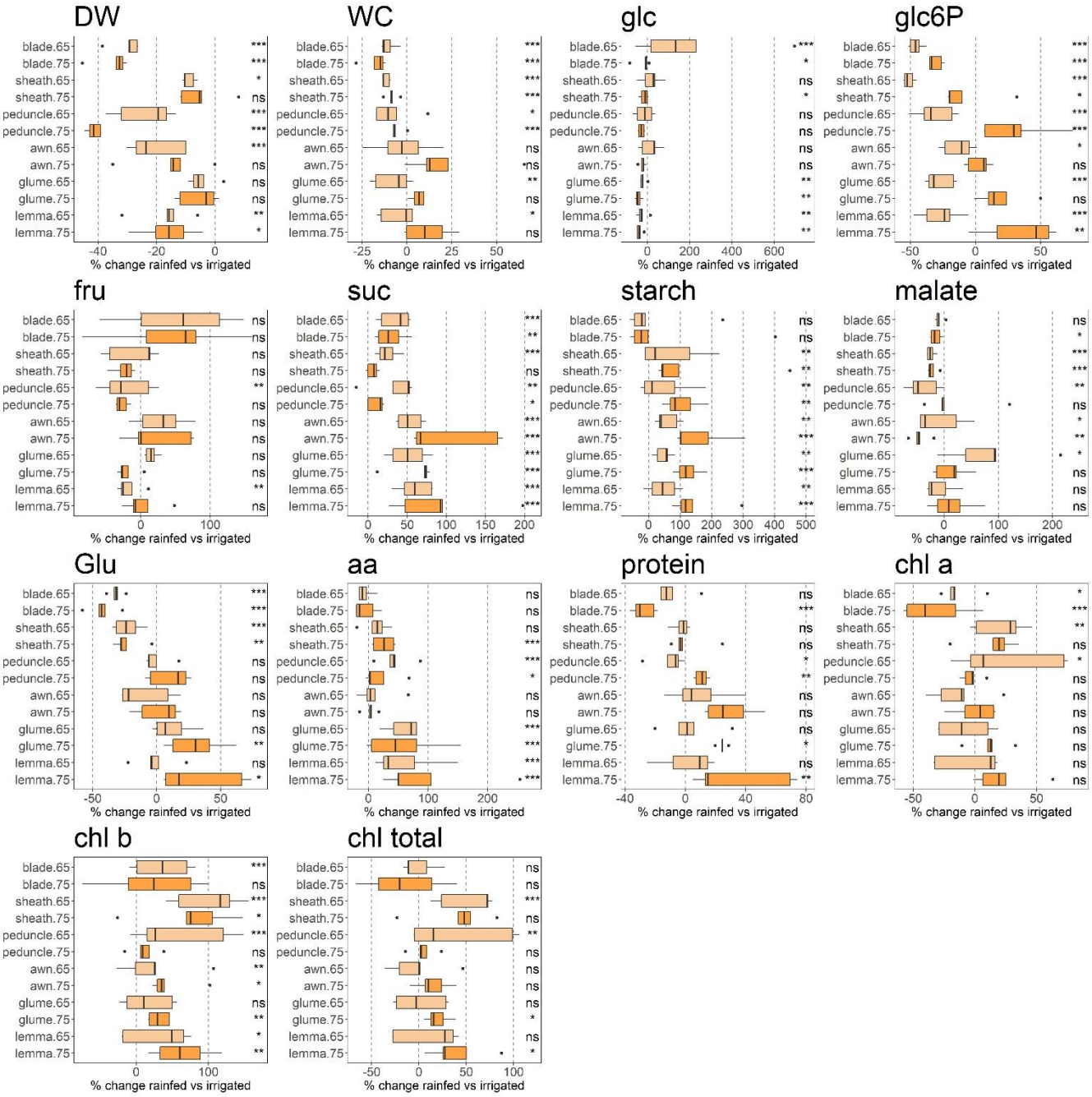


Figure 7.6. Effect of water regime on durum wheat physiological and metabolic traits in six photosynthetic organs during anthesis (Zadoks 65) and mid-grain filling (Zadoks 75). The boxplots were built per organ using the data from five durum wheat varieties by using the percentage of change in rainfed compared to irrigated conditions for every variety. Symbols on the right for each figure indicate the significance of the water regime effect according to the two-way ANOVA in Supplementary Table S7.1 (*, p<0.05; **, p<0.01; ***, p<0.001).

7.3.5. The total content of primary metabolites per organ shows significant differences in carbon and nitrogen pools throughout the plant

C and N metabolite content was also expressed as total metabolite content per organ at anthesis and mid-grain filling by multiplying by the DW of each organ. The results were evaluated statistically with a two-way ANOVA for the organ × water regime interaction (Supplementary Table S7.2) and visualised in a PCA (Figure 7.7) and bar plots (Figure 7.8). PCA dimension 1 explained 53.7% of the variability, while dimension 2 explained 12.8%. It was observed that a large part of the metabolites analysed and expressed by total organ content tend to accumulate in the peduncles. Only the starch content tended to present higher values in the ear organs and chl at anthesis and malate at both stages in the blades and sheaths (Figure 7.6). The differences between the photosynthetic organs were narrowed when expressing metabolites by total content as opposed to concentration, as we showed in Figure 7.5. The ear organs awns, glumes and lemmas were again located close to each other, with a shorter distance to the blade in this case. Interestingly, the sheath overlapped with the blade under irrigated conditions, whereas the two organs differed more markedly under rainfed conditions. While the effect of the water regime was less pronounced on sheaths and ear bracts, it was more relevant on blades, peduncles and slightly on awns.

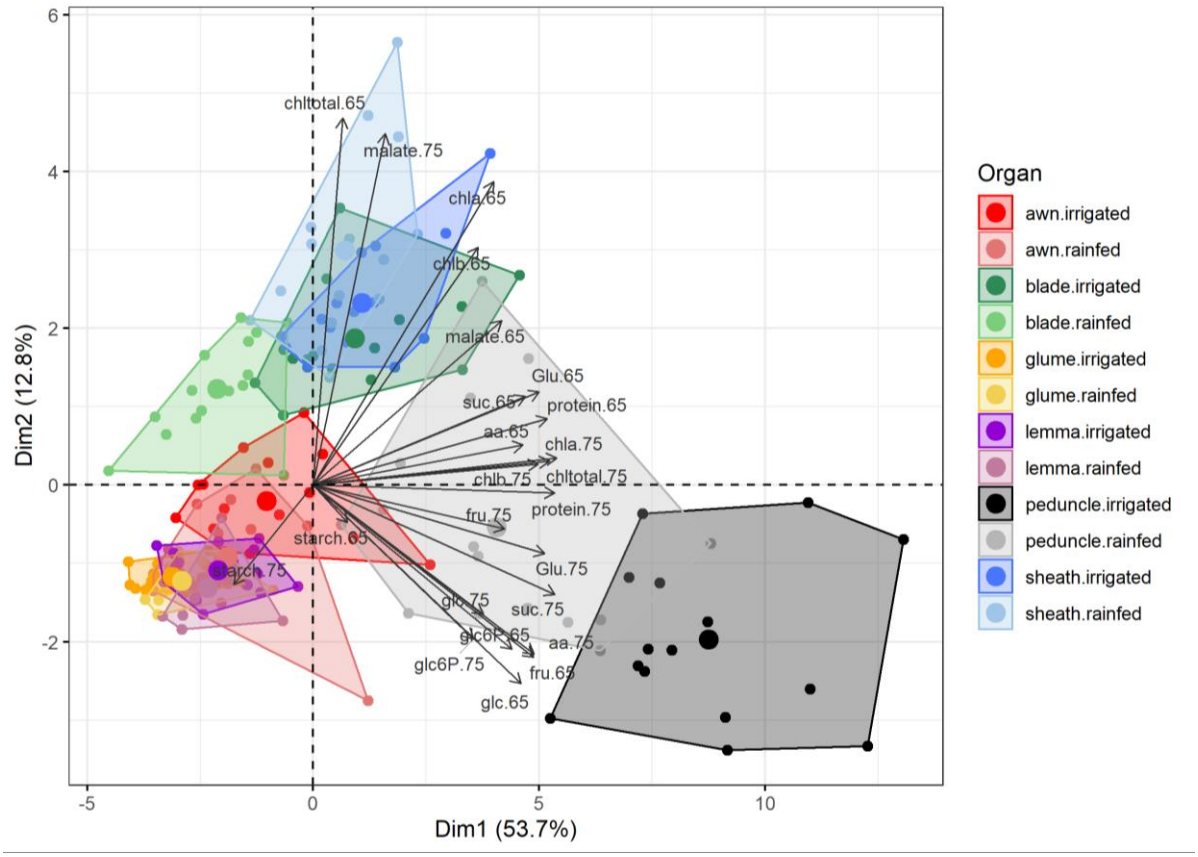


Figure 7.7. Principal component analysis (PCA) of carbon (glucose, glucose-6-phosphate, fructose, sucrose, starch, and malate) and nitrogen (glutamate, total amino acids, proteins, chlorophylls a, b and total) metabolites expressed as total content per organ. Six photosynthetic organs (leaf blades and sheaths, peduncles, awns, glumes and lemmas) of five durum wheat varieties (MEX, Mexa; EUR, Euroduro; DRI, Don Ricardo; KNI, Kiko Nick; HAR, Haristide) under rainfed and irrigated conditions were considered. The measurements were carried out at anthesis (Zadoks 65) and mid-grain filling (Zadoks 75).

The univariate analysis clearly revealed that the effect of water regime, organ and their interaction were highly significant for most of the metabolites analysed (Supplementary Table S7.2). Quantitatively considering the whole organ itself, the glc and fru contents were higher in peduncles under irrigated and rainfed conditions (Figure 7.8). While glc6P has a similar trend, with a higher content in peduncles, the differences between organs were attenuated with an apparent effect of water stress on all organs at anthesis. The negative impact of water stress on glc6P disappeared mainly in all three ear organs at mid-grain filling. Suc content was again higher in the peduncles, mainly during grain filling, where it was negatively affected by rainfed conditions. There was a clear tendency for the suc pool to increase in all the three ear organs at both growth stages, but this did not reach statistical significance. Starch content was slightly higher in ear organs such as awns and lemmas. While there is

a tendency to decrease under rainfed conditions in the blades, starch clearly increased under such conditions in the sheaths, awns, lemmas, and glumes. Malate content was markedly higher in the blades and sheaths at both stages and in the peduncles at anthesis, being significantly (and negatively) affected by rainfed conditions but not in the case of the ear organs. Glu followed a similar trend to malate, but levels between organs were not as marked. Interestingly, Glu content decreased in rainfed compared to irrigated conditions in blades, sheaths and peduncles, but not in awns, glumes and lemmas. The total free aa content was higher in the peduncles and similar in the rest of the organs, with only a slight decrease in the blades at both stages and the peduncle at mid-grain filling under rainfed conditions and a small increase in ear bracts. Soluble protein levels were highest in peduncles and lowest in bracts. Furthermore, water stress decreased their levels in peduncles and blades strongly, with no significant changes observed in the other organs. Total chl content was highest in blades, peduncles and awns at anthesis and in peduncles at mid-grain filling. Water stress increased its levels in sheaths at anthesis, while it reduced them in peduncles and blades at mid-grain filling.

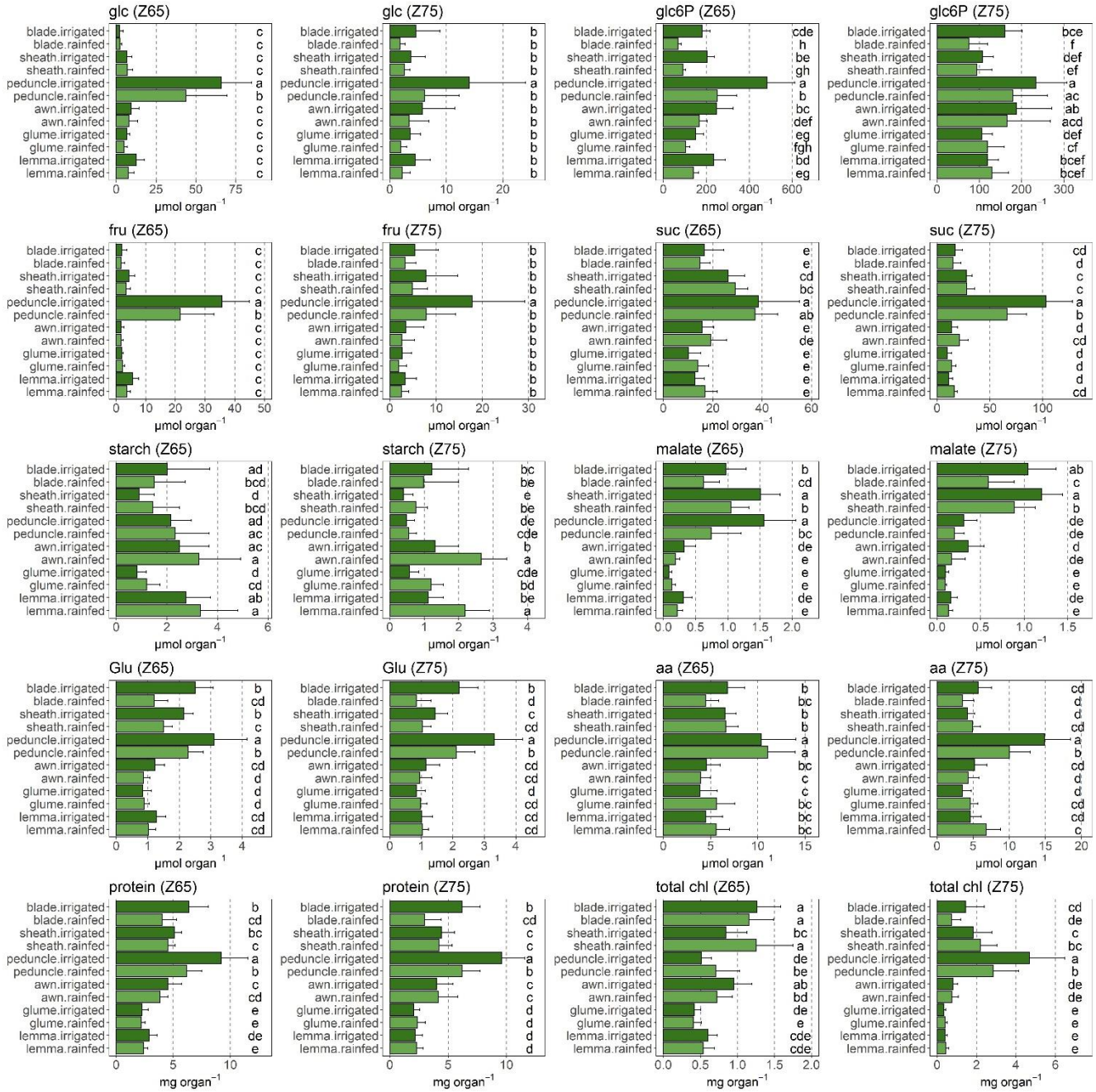


Figure 7.8. Differences in the content of metabolites related to carbon (glucose, glucose-6-phosphate, fructose, sucrose, starch, and malate) and nitrogen (glutamate, total amino acids, proteins, and total chlorophylls) metabolism per organ and water regime. The analyses were performed in six photosynthetic organs during anthesis (Zadoks 65) and mid-grain filling (Zadoks 75). For each comparison of means, letters are significantly different as detailed in Supplementary Table S7.2 (p<0.05; two-way ANOVA, TUKEY test).

7.4. DISCUSSION

7.4.1. Severe water stress in field-grown durum wheat led to a significant reduction in crop yield, biomass and changes in grain quality in five varieties

In the present study, five durum wheat varieties were grown under field conditions in a region with a marked continental Mediterranean climate. It is classified as Csb (temperate, dry summer, warm summer) according to the Köppen-Geiger classification (Beck *et al.*, 2018), characterised by low rainfall and high temperatures, mainly during grain filling (Figure 7.2a). In fact, the annuity of the study showed lower rainfall values compared to previous years (http://www.aemet.es/), which led to characteristic and strong water stress effects (Figures 7.21b-d, 7.3a-b, and 7.4). It has been predicted that by 2071-2100 the study area is very likely to have a Csb climate (temperate, dry and warm summer) (Beck *et al.*, 2018), which to some extent the climate of the present work serves to study the negative effects of climate change that will take place. Irrigation was concentrated in the months of March, April and May, when the crop is in the heading, anthesis and grain filling stages. This water supply was important because in these months, mainly April and May, rainfall was almost non-existent, precisely in the period of grain filling, which can quantitatively affect grain size and the final grain yield. In fact, irrigation can help alleviate the effects of high temperatures in those months that have been observed to have a negative effect on wheat yields (Pinke *et al.*, 2022). Optimal photosynthesis temperatures during anthesis and grain filling are around 21.3-23.0ºC (Farooq *et al.*, 2011), so in our study the plants were also subjected to heat stress as these temperatures were greatly exceeded in the last months of the crop during the central part of the day when T_{max} is reached (Figure 7.2a).

Despite the differences observed between varieties for agronomic components, physiology, and grain quality, the effect of the environment (irrigation vs. rainfed) was the main factor contributing to the variability of the results (Figure 7.4 and Supplementary Table S7.1). The effect of water stress was evident on durum wheat growth and production, leading to a substantial reduction in yield and biomass in agreement with previous studies (Sanchez-Bragado *et al.*, 2014a; Vicente *et al.*, 2018b). The varieties used showed certain differences at physiological and agronomic level (Figure 7.4), but the yields were not significantly different between varieties at both water regimes (Figure 7.2b), so we can conclude that they have a similar behaviour in response to water supply but a slightly different strategy to cope with the environment. Nevertheless, it was the ideal starting point to study a general response

of the different photosynthetic green organs to contrasting water conditions in field-grown durum wheat. The main differences between varieties were observed under irrigated than in rainfed conditions, indicating that stress conditions tend to attenuate the differences between varieties. The decline in yields under rainfed conditions was associated with lower atmospheric C sequestration and soil N uptake per area (GCY and GNY), which suggested that water limitation inhibited C and N assimilation at the canopy level and/or their translocation to the grains (lower HI). The decrease in yield was not due to a decrease in plants per area, but because fewer stalks were able to produce ears and these had fewer grains. Indeed, these parameters have been argued to be the most important in determining yield under contrasting water inputs (De Santis *et al.*, 2021).

The $\delta^{13}C$ is an interesting trait that, when is analysed in plant dry matter, integrates the plant performance during the crop cycle. It indicates the biochemical discrimination that takes place during CO_2 assimilation, highly dependent on environmental conditions, which is intimately related to water use efficiency (WUE) and an indicator of varieties adapted to stress conditions (Araus *et al.*, 2022). In our case, it demonstrated that a severe water stress took place in all the varieties leading to an improvement of WUE in rainfed conditions as a water-saving strategy (Sanchez-Bragado *et al.*, 2014a; Chairi *et al.*, 2018) (Figure 7.2c). Regarding differences between genotypes, the $\delta^{13}C$ highlighted slightly lower WUE in Kiko Nick under both growth conditions. The decrease in the water content of the leaves, measured with the RWC trait, is one of the most well-documented symptoms that leads to a loss of turgor in the tissues that affects the metabolic capacities of the plants (Bowne *et al.*, 2012; Estévez-Geffriaud *et al.*, 2020). At a very early stages, when irrigation differences were not yet very evident (Zadoks 55), this index did not show a great effect of water stress. However, as the differences in water supply increased, the negative effects of water stress were significant indicating a loss of water (Figure 7.2d). The natural abundance of $\delta^{15}N$ in plant organs integrate the isotopic fractioning taking place during soil N cycle, plant uptake, assimilation and remobilization to the grains, what complicates the interpretation of this trait (Sanchez-Bragado *et al.*, 2017; Fuertes-Mendizábal *et al.*, 2018). In our study, the values of GNY, spectral indices associated with greeness/N, and grain N suggested that N assimilation was promoted under irrigated conditions at canopy level but it was not enough to increase N concentration in mature grains, probably due to a dilution effect by the high yields obtained. Moreover, according to the PCA, the grain $\delta^{15}N$ was negatively associated with grain N concentration. Water regime also modified other grain quality and nutritional traits in durum wheat. Yield decline under water stress was also associated with smaller kernel size in agreement with De Santis *et al.* (2021), although a correlation between these parameters is not always observed (Chairi *et al.*, 2018).

The increase of grain N concentration in rainfed conditions was associated with higher protein content (De Santis *et al.*, 2021), as the former is a major component of the latter. Moreover, S and other quality traits relevant for the industry such as SDSS, vitreousness and WG increased under rainfed conditions, but an impoverishment of Mg, P, Cu and Mn was also shown.

In order to monitor the growth of the plants during their life cycle non-destructively, two low-cost spectral sensors were used to collect data on plant health and growth at canopy and leaf level. The NDVI has been widely used to for research and commercial agronomy applications since it is well correlated with wheat grain yield, N content and leaf area index, as well as to estimate the stress status (Duan *et al.*, 2017). According to the previous literature, NDVI at mid and late stages was positioned in the PCA close to yield-related traits and indices related to water and N status (Figure 7.4). The greatest differences in NDVI were observed under drought conditions (Figure 7.3a), probably because this spectral index tends to saturate under optimal conditions (irrigation) and lose their effectiveness in capturing differences between varieties (Duan *et al.*, 2017). Whereas under non-optimal conditions, such as rainfed conditions, these indices were far from saturated and allowed better differentiation of changes between varieties. Although some inter-variety changes were observed for NDVI during intermediate growth stages, these did not lead to notable changes in biomass at the end of the trial, while the main effect was water regime. In the anthesis stage (Zadoks 65), a strong decrease in NDVI was observed in rainfed conditions, which could indicate that senescence at the plant canopy level began prematurely due to water limitation, being similar in the five genotypes, probably due to the high temperatures and the absence of precipitations that took place at this time (Figure 7.2a). This shortening of the life cycle and/or reduction of source organs that can provide nutrients may have been associated with the lower yields under rainfed conditions due to shorter grain filling periods (De Santis *et al.*, 2021). The spectral indices measured in flag leaves were more evident in late stages, when the effect of water stress was greater (Figure 7.3b). This may be because the symptoms of water limitation could be more evident in other parts of the plant, such as mature, senescing leaves, or secondary stems, which would clearly be observable at the canopy level (e.g. NDVI) but not at the flag leaf level. Nevertheless, the estimation of chl content in flag leaves was a good proxy for water stress symptoms at the both anthesis and mid-grain filling stages. Even this spectral index showed the differences between varieties that were also observed in the PCA with all the agronomic, physiological and quality traits together (Figure 7.4), with Don Ricardo and Kiko Nick at the extremes. This index was proposed to be a better solution for leaf N content than the NBI index, since the latter is calculated as Chl/Flav ratio being more associated to changes in the C/N allocation (Cerovic *et al.*, 2012). Anyway, both indices

estimated a decrease of foliar N content under water stress. The increase in flav and anth indices at mid-grain filling in rainfed compared to irrigated conditions highlighted the higher production of antioxidants under stress conditions to function as sunscreens and counteract the negative effects of reactive oxygen species (oxidative damage) that typically increase in drought (Landi *et al.*, 2015; Agati *et al.*, 2020).

Overall, the different agronomic and physiological traits measured have allowed us to confirm and quantify the effect of water stress in our study. This effect was observable in the five varieties studied, and no variety was clearly more susceptible or tolerant to this stress than the others. Water stress led to a decrease in yield, biomass, and C and N assimilation, promoted WUE and modified differentially grain quality traits of interest for the industry. Among the methodologies used to study the effect of the water regime, it is worth mentioning the use of *in vivo* vs. destructive techniques, quick vs. time-consuming measures, and measurements taken at any time of the crop vs. at harvest. While the $\delta^{13}C$ is one of the most widely used measures to assess water stress that encompasses all the constraints that have taken place during plant growth (Araus *et al.*, 2022), the use of phenotyping approaches (e.g. spectral indices) offers a great advantage in terms of speed and cost to obtain similar results at any moment of the life cycle.

7.4.2. Metabolite profiles in foliar and non-foliar photosynthetic organs suggest that blades and peduncles are the most susceptible organs to water stress, while ear organs show high stability and even better status at later grain filling stages

The complexity of these studies covering agronomical, physiological, and metabolic analyses, different growth conditions and several plant organs has been a limitation in previous studies to work with a high number of varieties (Hu *et al.*, 2018; Sanchez-Bragado *et al.*, 2020c; Tambussi *et al.*, 2021). Our study included five varieties widely used in Spain in the last decades that showed similar absolute yield values in both water regimes with a certain degree of agronomic and physiological contrast. Then, the pool of durum wheat varieties selected allowed us to predict a general response of the foliar and non-foliar photosynthetic green organs to contrasting water conditions in field-grown durum wheat.

In the multivariate analysis of metabolites (Figure 7.5), we observed that the organ was the main factor explaining the variability in the data, as previously reported in durum wheat (Vergara-Diaz *et*

al., 2020b; Martínez-Peña *et al.*, 2022 (sse chapter 6)) and rice (Lawas *et al.*, 2019; Yang *et al.*, 2022a). However, the contrasting water regimes of our study were also significantly affecting most of the metabolites in an organ-specific manner (Supplementary Table S7.2). Overall, the blades were the organs that presented a higher content of several primary metabolites, such as total chl, protein, malate and glu. It highlights that blades are key source organs to provide nutrients to other parts of the plant, with a strong coordination of light reactions and C fixation to provide C skeletons for the synthesis of N compounds (Sun *et al.*, 2013; Vicente *et al.*, 2018a). Nevertheless, the peduncles and the blades were the organs more susceptible to water stress, with little effects in sheaths and awns. Although the multivariate analysis also showed remarkable changes in ear bracts, these changes were not associated with negative effects on metabolite content in most cases (Figures 7.5 and 7.6), as we describe below.

The peduncles were the photosynthetic organ of the six considered in this study with the highest DW (Figure 7.5), which will influence the total metabolite content per organ that we will describe in the next section. However, we showed lower decreases of DW in ears, as well as in sheaths, than those observed for blades and peduncles under rainfed conditions, indicating a lower susceptibility to water stress morphologically. Peduncles and sheaths are shown close to each other, as previously reported under contrasting N conditions (Martínez-Peña *et al.*, 2022 (see chapter 6)). This is because these organs seem to exhibit similar functions, predominantly related to nutrient storage for later stages of grain filling as previously reported in durum wheat and barley (Cimini *et al.*, 2015; Torralbo *et al.*, 2019; Martínez-Peña *et al.*, 2022 (see chapter 6)) and as shown with the substantial increase in glc and fru detailed below.

The pool of metabolites analysed is related to processes occurring during the diurnal metabolism of the plant, associated with photosynthetic C fixation and N assimilation, which are not directly involved in structural components of the organs. We observed a tendency to higher levels of free monosaccharides (glc and fru) in blades and lower in few cases in ear organs. The free sugars glc and fru are frequently derivate from the breakdown of other more complex carbohydrates, such as sucrose, to act as C sources in other sink tissues or as C skeletons for other compounds such as starch (Cimini *et al.*, 2015; Živanović *et al.*, 2020). The strong increase of glc in blades at anthesis was not associated with starch biosynthesis or suc breakdown according to our results, so we suggest that it was linked to a role as osmoprotectant due to the magnitude of the change (Živanović *et al.*, 2020). Water stress usually limits amylose content and starch granules size in wheat endosperm (De Santis *et al.*, 2021),

so it is interesting to understand how the starch dynamics in different photosynthetic organs response to water stress. Starch increased markedly in the ears and peduncles, particularly at mid-grain filling (Figure 7.6). This storage sugar is not associated with an osmoprotective role under water stress since it forms granules, but may suggest an improved C status of these organs. According to these results, our data may support the idea that glc decreased as it was the substrate for starch synthesis, observing subsequently a strong increase in the intermediate of this pathway, glc6P at mid-grain filling. This metabolite has also a key role in energy metabolism and to generate NADPH for reductive biosyntheses (Schluepmann *et al.*, 2012). Moreover, the slight decrease of glc and fru in some ear organs could indicate their possible use in the synthesis of suc (or a lower suc breakdown) and/or a lesser need to act as osmoprotectants in these organs. The increase in suc in all organs in response to water stress indicates an osmoprotective role (Živanović *et al.*, 2020). Although its greater increase in ears could also indicate a relevant photosynthesis under stress conditions. This could be supported by the aforementioned increase in starch synthesis, which would be activated when the C status is optimal in the cell by high levels of suc which, through the signal molecule trehalose 6-phosphate, would lead to the posttranslational redox activation of ADP-glucose pyrophosphorylase (Kolbe *et al.*, 2005). Malate, another transient C reserve, also involved in respiration (Barros *et al.*, 2020), was reduced in several organs under rainfed conditions, including the awns. However, it was not affected or even increased in ear bracts, supporting their better C status. Takahashi *et al.* (2001) proposed that any new assimilate at late grain-filling stages is mainly used for grain growth. A better C status in ear organs and putative contribution to grain filling has been firstly proposed by Sanchez-Bragado *et al.* (2014a) analysing the $\delta^{13}C$ in its natural abundance. The comparison of $\delta^{13}C$ values in water-soluble fractions (of different organs last photoassimilates synthesised) and $\delta^{13}C$ of mature grains suggested that the ears played a key role under stress conditions.

Overall, the ear organs showed better water and N status than the other organs, particularly under mid-grain filling, where a most remarkable role has been proposed in our previous study (Martínez-Peña *et al.*, 2022 (see chapter 6)). There, we reported that under contrasting N fertilisation conditions, the activities of key enzymes in C and N metabolism, e.g. ribulose-1,6-bisphosphate carboxylase oxygenase (Rubisco), phosphoenolpyruvate carboxylase (PEPCase), glutamate synthase (GOGAT), and glutamate dehydrogenase (GDH), correlated with yield more than the activities from other organs such as the blades in durum wheat. Similarly, Shokat *et al.* (2020) showed that the activities of enzymes related with antioxidant capacity and carbohydrate metabolism in leaves and whole ears correlated

with bread wheat yield. This is interesting knowing that photosynthetic proteins content and enzyme activities are quantitatively lower in these organs (Martínez-Peña *et al.*, 2022 (see chapter 6)), which leads to a higher C/N ratio compared to blades (Vicente *et al.*, 2018b) partly because these proteins require a higher cost of N. Indeed, water stress did not lead to significant decreases in the levels of N-rich metabolites in the three ear organs, awns, glumes and lemmas. By tracking the dry matter $\delta^{15}N$ in the durum wheat photosynthetic organs, Sanchez-Bragado *et al.* (2017) suggested that a substantial portion of the N content in the grains came from the ears, comparable to the provision of N from the vegetative parts of the plants, and this was even more relevant as water and N stresses increased. Our data supports this study with stable isotopes showing that the pool of glu, free aa, soluble proteins, and chl were not affected by water stress as in the leaves, even with higher levels for some cases in the glumes and lemmas at mid-grain filling (Figure 7.6). In Vergara-Diaz *et al.* (2020b), we also reported higher levels of aa in a pool of ear bracts vs. leaf blades in durum wheat. We hypothesize that, although some aa have a role in the abscisic acid-mediated drought response, such as proline (Živanović *et al.*, 2020), the observed increases in many of the main aa in ears were not only justified by this role (Vergara-Diaz *et al.*, 2020b). The higher levels of N metabolites in ears was supported by our previous studies showing that the expression of N metabolism genes (nitrate reductase, nitrite reductase, and glutamine synthetase) were upregulated in durum wheat ears at early grain filling leading to reduce the negative effects on N content that were observed in leaves under water stress (Vicente *et al.*, 2018b). The better water status that we showed in ears, particularly at late stages, was suggested in our former study by using phenotyping approaches such as water spectral indices and $\delta^{13}C$ analyses (Vicente *et al.*, 2018b).

Finally, we observed a high variability in the content of some key C and N metabolites between varieties (Figure 7.6 and Supplementary Table S7.1), suggesting that mining natural variability in wheat in response to water stress at the whole plant level could be useful to develop resilient varieties in breeding programmes.

7.4.3. The total metabolite content per organ shows that the peduncles are the major reservoir of carbon and nitrogen during grain filling and that the ear organs may play an important role under water stress conditions

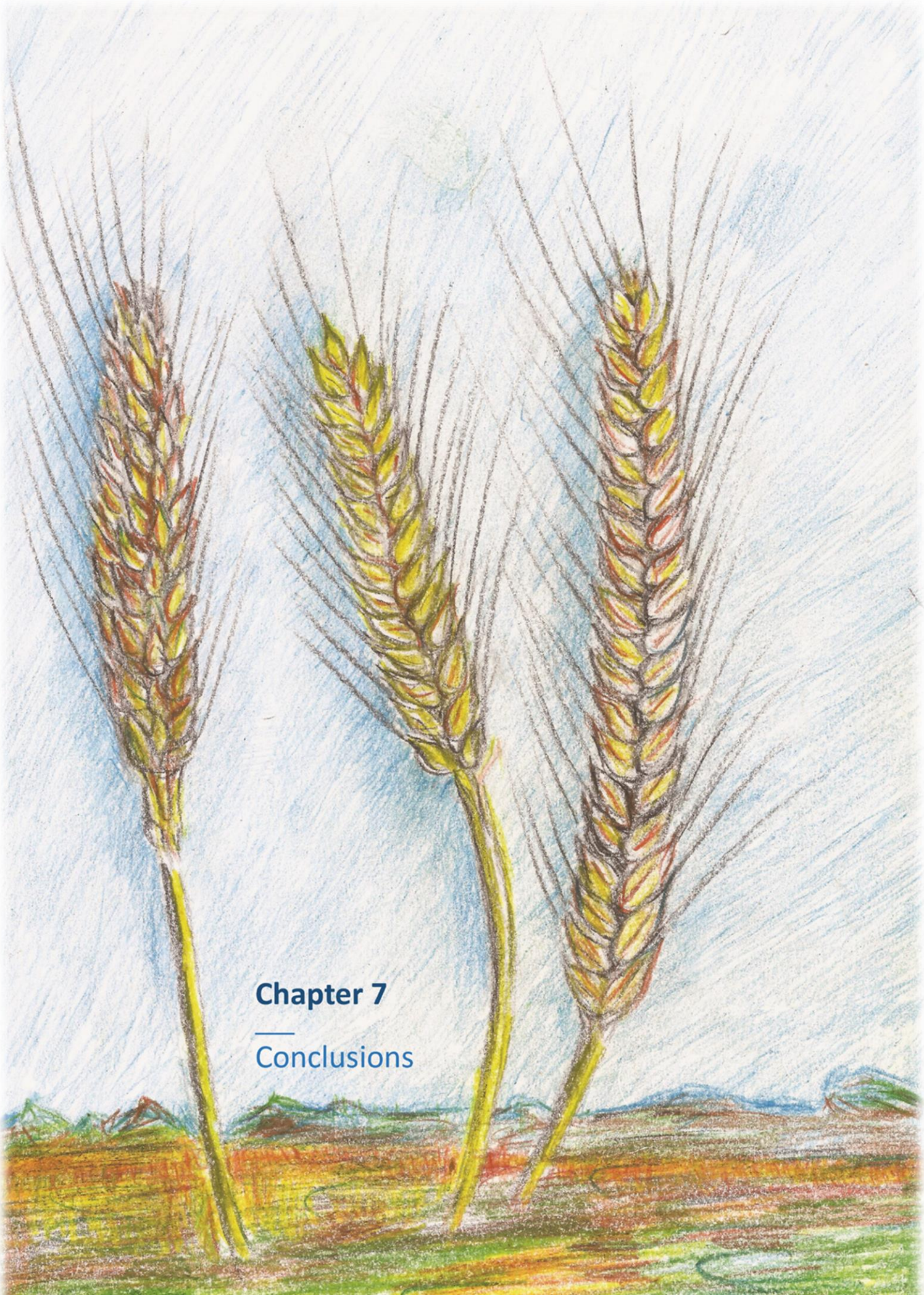
Peduncles accumulated remarkably high levels of fru compared to the rest of the organs. This is directly related to the synthesis of fructans in this organ, which is one of the main sources of C to nourish the growing grain, mainly when plant photosynthesis (in leaves and/or ears) ceases in the late stages of grain filling (Takahashi *et al.*, 2001; Cimini *et al.*, 2015; Martínez-Peña *et al.*, 2022 (see chapter 6)). However, fru levels, and probably indirectly fructan levels, were strongly decreased in this organ under rainfed conditions. This indicated that part of the decrease in yield under water stress was due to a reduced contribution of the peduncles to supply C at late stages. Consequently, the search for durum wheat varieties with peduncles more tolerant to water stress may contribute to breeding programmes and crop improvement. From a quantitative point of view, surprisingly no decreases in any of the metabolites analysed were observed in the three organs of the ears, apart from a slight decrease in glc6P in bracts during anthesis. Even the pool of certain metabolites increased in the ears, such as starch and slight, non-significant increases in aa and suc. In contrast, other metabolites did decrease under rainfed conditions compared to irrigation. Apart from the case of fru mentioned above for the peduncle, they accumulated large levels of other primary metabolites due in a large extent by their higher DW as mentioned above. In fact, the peduncles have not only been proposed as C storage organs, but also as photosynthetic active organs in late stages of grain filling with a high stomatal density and PEPCase activity (Kong *et al.*, 2010). Although they are not the most efficient organs at the photosynthetic level, their biomass, exposure to light and late senescence contribute to their being a relevant organ in the synthesis of photoassimilates.

Although leaf photoassimilates and sugars accumulated in the stems have traditionally been considered the main sources of nutrients for grain filling in cereals and more specifically in wheat (Tambussi *et al.*, 2021), there is a growing trend that highlights the photosynthetic and N assimilation or recycling role of non-foliar organs, being more relevant under limiting conditions (Sanchez-Bragado *et al.*, 2020c). Our study highlights this hypothesis and corroborates that the pool of C and N metabolites that nourish the grain could not be understood by the role of the leaf blade alone. The absolute values of soluble C and N compounds that are available during grain filling quantitatively demonstrate that all green photosynthetic organs have a contribution, to a greater or lesser extent, to

grain filling. Supported by the previous isotopic, transcriptomic, metabolomic, and enzymatic studies, we can conclude that the peduncles and the ears, the latter especially under water stress conditions, have an important role in providing nutrients for the grain, especially when the flag leaves cease its photosynthetic activity and the non-foliar organs, with a later senescence, increase their relevance.

7.5. CONCLUSIONS

Our results showed that the photosynthetic organs have different concentrations of primary metabolites and strategies in response to water stress. The ear organs have a greater stability in response to contrasting water regimes, while the blades and peduncles were more susceptible. Ear organs, especially bracts, seem to tolerate or even improve their pool of metabolites in response to water stress, showing better water, C and N status. The peduncle proved to be the organ with the largest pool of C and N during grain filling, mainly due to its higher dry weight. Although awns, glumes and lemmas presented lower absolute levels of metabolites, their stable concentration and quantity per organ in response to stress highlight their role in contributing to grain filling. However, it is noteworthy that there is a large genotypic variability for the parameters evaluated, highlighting that the response of C and N metabolism to water conditions is highly dependent of the variety. Breeding, together with agronomy, is one of the pillars to make agriculture more resilient to the increasing challenges imposed by climate change. This suggests that in durum wheat there is a large window for crop improvement under stress conditions given the possibility to exploit the existing natural variability.



Chapter 7

Conclusions

8. CHAPTER 8: CONCLUSIONS

First.- Obtaining a higher crop yield combined with a high quality of grain for the industry continues to be a current challenge for agriculture. In particular, in the Mediterranean region and, specifically, in the Spanish area of Castile and León we observed a high climatic variability between successive agronomic seasons, which will be even exacerbated due to the climate change, affecting the growth and development of durum wheat crop and making it more difficult to choose the suitable varieties for each condition. In this future scenario, Castile and León is a strong candidate to increase the production of durum wheat in Spain, requiring the identification of ideotypes that can contribute to improve the local adaptability of this crop.

Second.- The influence exerted by the environmental conditions on the durum wheat crop was the predominant factor in determining most of the agronomic and grain quality traits evaluated, although some of them were also influenced to a certain extent by the genetic factors, such as the grain weight and its carotenoids concentration. Among the agronomic and grain quality traits studied, the number of grains per ear, gluten index, yellow pigment concentration and ear length were the traits with the highest heritability estimates. This indicated that these traits could be identified as possible targets for future breeding plans.

Third.- Amilcar and Olivadur were identified as potential ideal durum wheat candidates for our area of study, Castile and León region, due to their stability with high grain yields and quality standards in most of the environments considered . However, due to the differences found between the distinct levels of stability, yield and grain quality, we recommended the use of one variety, or another depending on the intended end-use of the grain. It was also possible to identify the varieties that maximise their yield in more limiting environments, although their production and quality are not the highest in the most productive environments.

Fourth.- Despite the slight increase in grain yields over the last years, the concentration of minerals in the grain has stagnated. The environment was the most significant factor influenced on the final concentration of nutrients obtained in the mature grain. However, we were able to identify the varieties with a higher concentration of nutrients in our panel.

Fifth.- The correlations obtained between specific parameters, such as grain yield and protein content with the concentrations of Ca, K, S and Fe, could be of interest for crop improvement.

Sixth.- The combination of high-throughput field phenotyping at canopy level and organ-specific metabolic phenotyping, was an ideal approach to study the plant source-sink dynamics and, then, to identify the factors controlling certain complex traits, such as yield and grain quality, which can be implemented in crop improvement in optimal and unfavourable environments. Our studies showed that the contribution of non-laminar organs to grain filling, such as the ears, should be included in breeding programmes, especially under limiting conditions such as the Mediterranean environments, due to their constitutively stress-adapted metabolism.

Seventh.- The primary metabolism of photosynthetic organs suggested that all have important roles in contributing to early and late grain filling. Although flag leaf blades had the highest metabolic activity among the green photosynthetic organs in absolute terms, only their carbohydrate metabolism was associated with yield. In particular, the blades generally showed higher levels of Rubisco protein and key enzyme activities for the synthesis of assimilates for grain, but the metabolism of other non-laminar green organs was correlated to a greater extent with grain yield and quality in contrasting nitrogen fertilisation environments.

Eighth.- The analysis of enzyme activities related to the limiting steps of carbon fixation and nitrogen assimilation, together with the analysis of stable isotopes in dry matter of the different plant organs, confirmed the essential role of the metabolism of ear organs (awns, glumes and lemmas) during grain filling.

Ninth.- Respective to the nitrogen input, high yields were associated with plants with shorter stalks and longer ears, a phenotype with later senescence ("stay-green"), which maintains photosynthetically active biomass for longer, especially in late growth stages, a better water and nitrogen status of the leaves, and a more active metabolism of the ears, particularly at grain filling with higher enzyme activities such as Rubisco, phosphoenolpyruvate carboxylase, glutamate synthase and glutamate dehydrogenase, without affecting significantly the grain quality parameters.

Tenth.- Water stress led to yield reduction, biomass and grain quality changes in a similar way in the durum wheat varieties studied, while different phenotyping techniques, allowed predicting and characterising the effects of stress on the plants and at different plant levels, such as stable carbon isotope composition, leaf relative water content, and canopy and leaf greenness indices through the use of spectral sensors.

Eleventh.- The metabolic profile of different foliar and non-foliar photosynthetic organs in response to water stress suggested that leaf blades and peduncles are the most susceptible organs to water stress, with adverse effects on their carbon and nitrogen metabolism.

Twelfth.- Ear organs, including awns, glumes and lemmas, showed a certain degree of stability to limiting water conditions in contrast to other photosynthetic organs. As a result, these organs showed a better water status and carbon and nitrogen metabolite content, especially the glumes and lemmas. This suggests that ears had an active metabolism to provide nutrients to the developing grains, particularly at mid-grain filling phase and when water conditions are limiting for other organs.

Thirteenth.- The content of carbon and nitrogen metabolites was expressed per organ in a novel way to study the pool of nutrients available for the grain and we showed that the peduncle was an organ with a large amount of them, either for storing them as reserve carbohydrates for their use at later stages or for using them directly at earlier stages. However, the pool of metabolites in peduncles was significantly affected under stress conditions, while this was not observed in the ears.

8.1. CONCLUSIONES

Primera.- La obtención de un mayor rendimiento del cultivo unido a una alta calidad del grano para la industria, continúa siendo un reto actual para la agricultura. En particular, en la región del mediterráneo, y en concreto, en la comunidad de Castilla y León, existe una gran variabilidad climática entre las sucesivas campañas agronómicas, lo que se intensificará negativamente debido al cambio climático, afectando al cultivo y desarrollo del trigo duro y dificultando así la elección de las mejores variedades para cada condición en ese futuro escenario. Castilla y León se presenta como una fuerte candidata para aumentar la producción de trigo duro en nuestro país, necesitando para ello, la identificación de ideotipos que permitan mejorar la adaptabilidad local de este cultivo.

Segunda.- La influencia que ejercen las condiciones ambientales sobre el cultivo del trigo duro fue el factor predominante en la determinación de la mayoría de los parámetros agronómicos y de calidad evaluados, si bien algunos de ellos fueron también influenciados en cierta magnitud por factores genéticos, como por ejemplo el peso de mil granos y la concentración de los carotenoides en grano. Entre los rasgos agronómicos y de calidad estudiados, el número de granos por espiga, el índice de gluten, la concentración de pigmento amarillo y la longitud de la espiga fueron los rasgos con mayores estimaciones de heredabilidad. Pudiéndose identificar esos rasgos como posibles objetivos para futuros planes de mejora.

Tercera.- Las variedades Amilcar y Olivadur fueron identificadas como posibles candidatas de trigo duro, dentro de nuestra área de estudio, Castilla y León, por su estabilidad respecto a los estándares de rendimiento y calidad para la mayoría de los ambientes considerados. No obstante, debido a las diferencias halladas entre los diferentes niveles de estabilidad, rendimiento y calidad en grano, indicamos que el uso de una variedad u otra dependerá en última instancia del uso previsto que se quiera dar al grano cosechado. También fue posible identificar variedades que maximizan su rendimiento en ambientes más limitantes, aunque su producción y calidad no sean las más altas en ambientes más productivos.

Cuarta. **-**A pesar del ligero aumento del rendimiento a lo largo de estos años, la concentración de minerales presentes en el grano parece haberse estancado. Siendo el ambiente el factor que más influye en la concentración final de los nutrientes obtenidos en el grano de cosecha. No obstante, se identificaron variedades con una mayor concentración de nutrientes, en nuestro panel de estudio.

Quinta.- Las correlaciones obtenidas entre parámetros específicos como el rendimiento y la proteína en grano con la concentración de ciertos minerales, como el Ca, el K, el S y el Fe, podrían ser utilizadas para la mejora del cultivo.

Sexta.- La combinación del fenotipado de alto rendimiento a nivel de dosel vegetal con el fenotipado de los metabolitos presentes de manera específica en los diferentes órganos, ha supuesto el enfoque ideal para estudiar las dinámicas fuente-sumidero e identificar los factores que controlan ciertos caracteres complejos, como el rendimiento y la calidad en grano. Lo cual puede ser implementado en la mejora de cultivos bajo condiciones óptimas o desfavorables de crecimiento. Nuestros estudios muestran que la contribución de los órganos no laminares al llenado del grano, como las espigas, deben ser valorados en los planes de mejora, especialmente bajo condiciones limitantes como las presentes en la Cuenca Mediterránea, debido a su metabolismo constitutivamente adaptado a los diferentes estreses.

Séptima.- El metabolismo primario de los órganos fotosintéticos sugirió que todos tienen funciones importantes en la contribución al llenado temprano y tardío del grano. Aunque en términos absolutos las hojas presentaron la mayor actividad metabólica entre los órganos verdes fotosintéticos, sólo su metabolismo de los carbohidratos se asoció con el rendimiento. En particular, las hojas de manera generalizada mostraron niveles más altos de proteína Rubisco y de actividades enzimáticas claves para la síntesis de asimilados para el grano. Sin embargo, el metabolismo de otros órganos verdes no foliares se correlacionó en mayor medida con el rendimiento y la calidad del grano en ambientes contrastantes de fertilización nitrogenada.

Octava.- El análisis de las actividades enzimáticas relacionadas con los pasos limitantes de la fijación del carbono y la asimilación del nitrógeno, junto al análisis de isótopos estables en materia seca de los distintos órganos de la planta, confirmaron el papel esencial del metabolismo que ejercen los órganos de la espiga (arista, gluma y lema), durante el llenado del grano.

Novena.- Respecto al aporte de nitrógeno realizado, la obtención de un alto rendimiento se asoció con plantas con pedúnculos más cortos y espigas más largas, un fenotipo con una senescencia más tardía (“stay-green”) que presente una biomasa fotosintéticamente activa durante más tiempo, sobre todo en etapas tardías del crecimiento, un mejor estado hídrico y nitrogenado de las hojas, y un metabolismo más activo de las espigas, particularmente en el llenado del grano, con mayores

actividades de enzimas tales como Rubisco, phosphoenolpiruvato carboxilasa, glutamato sintasa y glutamato deshidrogenasa, sin afectar significativamente los parámetros de calidad en grano.

Décima.- El estrés hídrico condujo a una reducción del rendimiento, la biomasa y cambios en la calidad del grano de forma similar en las variedades de trigo duro estudiadas, mientras que diversas técnicas de fenotipado, permitieron predecir y caracterizar los efectos del estrés en las plantas a diferentes niveles, como el análisis de la composición isotópica estable del carbono, el contenido relativo de agua en las hojas, y los índices de vegetación para determinar el verdor del dosel y de las hojas mediante el uso de sensores espectrales.

Undécima.- El perfil metabólico de los diferentes órganos foliares o no foliares fotosintéticos en respuesta a estrés hídrico sugirió que las hojas bandera y los pedúnculos son los órganos más susceptibles al estrés hídrico, observándose efectos negativos en su metabolismo del carbono y del nitrógeno.

Duodécima.- Los órganos de la espiga, como las aristas, las glumas y lemas, mostraron cierto grado de estabilidad en condiciones hídricas contrastantes a diferencia de otros órganos fotosintéticos. Como resultado, estos órganos mostraron un mejor estado hídrico, y de contenido de metabolitos de carbono y nitrógeno, especialmente las glumas y las lemas, lo que puede sugerir que las espigas presentan un metabolismo activo para proveer de nutrientes al grano en desarrollo, particularmente en los estadíos de llenado del grano y cuando las condiciones hídricas son limitantes para el resto de órganos.

Decimotercera.- El contenido de metabolitos de carbono y nitrógeno, se expresaron por órgano de manera novedosa para estudiar el contenido de nutrientes disponibles para el grano. Mostrando que el pedúnculo fue el órgano con mayor cantidad de compuestos disponibles, ya sea para su almacenaje como carbohidratos de reserva para su posterior uso en estadíos tardíos, o bien para su uso directo en estadíos más tempranos. No obstante, el contenido de metabolitos en el pedúnculo se vio significativamente afectado por las condiciones de estrés, lo cual no ha sido observado en las espigas.

9. CHAPTER 9:

Supplementary Material

9.1. Study I

Table S4.1. List of the varieties used in the study, with their corresponding abbreviation (ID), year of release, origin and pedigree.

Variety	ID	Year of release	Country	Pedigree/cross name or origin
Mexa	MEX	1980	Spain	GERARDO-VZ-469/3/JORI(SIB)//ND-61-130/LEEDS
Vitrón	VIT	1983	Spain	TURCHIA-77/3/JORI-69(SIB)/(SIB)ANHINGA//(SIB)FLAMINGO
Simeto	SIM	1988	Italy	CAPEITI-8/VALNOVA[1620][1622][1623][1625][1666]
Regallo	REG	1990	Spain	Diputación General de Aragón CIMMYT
Gallareta	GAL	1994	Spain	RUFF/FLAMINGO//MEXICALI-75/3/SHEARWATER
Claudio	CLA	1998	Italy	SEL.CIMMYT-35/DURANGO//ISEA-1938/GRAZIA
Burgos	BUR	1999	Spain	SUDDEUTSCHE SAATZ
Dorondón	DOR	1999	Spain	Genética y Gestión,S.C.
Amilcar	AMI	2002	Spain	ZEGZAG-1/LUNDE-5//GREENSHANK-32
Avispa	AVI	2003	Italy	Limagrain-CIMMYT
Don Ricardo	DRI	2008	Spain	Agrovegetal-CIMMYT
Kiko Nick	KNI	2009	Spain	SEL.CIMMYT-35/DURANGO//ISEA-1938/GRAZIA
Sculptur	SCU	2011	France	RAGT Semence
Olivadur	OLI	2013	Spain	RAGT 2N SAS seeds

Table S4.2. Mean performance of 14 durum wheat varieties across experiments and mean of experiments considering the panel of durum wheat varieties for grain yield and agronomic traits. Within columns, numbers followed by the same letter indicate non-statistically significant differences at $p<0.05$ as determined by Tukey's HSD tests. The abbreviations of the varieties and the environments are detailed in Supplementary Table S1 and Table 1, respectively. GY, grain yield; DH, days from emergence to heading; GDDH, growing degree days at heading; HI, harvest index, NSP, number of spikes per m^2; NKS, number of kernels per spike; PL, peduncle length; SL, spike length.

	GY		**DH**		**GDDH**		**HI**		**NSP**		**NKS**		**PL**		**SL**	
Varieties																
AMI	6149	ab	154.0	ef	1118	fg	0.41	abc	374.4	bcd	40.0	b	32.3	cd	6.5	de
AVI	6037	abc	153.2	fg	1110	gh	0.43	a	342.1	d	39.1	b	32.2	cd	6.6	cd
BUR	5568	abc	158.8	a	1181	a	0.35	d	423.8	a	26.9	d	33.4	bcd	6.3	e
CLA	5883	abc	157.6	b	1166	bc	0.37	bcd	399.8	ab	31.6	c	35.1	ab	5.9	f
DRI	5594	abc	156.7	cd	1155	c	0.39	abcd	368.3	bcd	33.1	c	35.5	ab	6.7	cd
DOR	5913	abc	154.5	e	1127	ef	0.43	a	363.5	bcd	38.6	b	34.2	abc	6.6	cd
GAL	5858	abc	157.8	b	1168	b	0.39	abc	389.5	abc	38.5	b	34.0	bc	6.4	de
KNI	5507	bc	153.9	ef	1112	gh	0.39	abc	421.8	a	27.0	d	34.8	ab	5.9	f
MEX	5760	abc	152.9	g	1106	h	0.36	cd	383.1	abcd	34.5	c	36.5	a	6.4	de
OLI	6255	a	157.0	bc	1159	bc	0.41	ab	357.4	bcd	43.9	a	27.6	e	7.6	a
REG	6014	abc	157.2	bc	1158	bc	0.37	cd	386.9	abcd	34.1	c	31.9	cd	6.7	c
SCU	6011	abc	156.1	d	1138	de	0.39	abc	384.3	abcd	38.9	b	26.5	e	7.0	b
SIM	5373	c	154.3	e	1124	f	0.37	bcd	352.6	cd	31.7	c	31.4	d	5.9	f
VIT	5802	abc	155.8	d	1141	d	0.37	bcd	391.0	abc	32.5	c	33.7	bcd	6.6	cd
Environments																
1	7750	b	158.9	e	1066	g	0.52	a	379.0	c	39.6	c	33.8	b	6.1	g
2	4182	f	158.8	e	1064	g	0.39	cd	353.8	cd	33.4	de	30.6	c	6.3	ef
3	9622	a	166.0	d	1236	b	0.43	bc	429.8	b	47.5	a	36.9	a	7.8	a
4	7326	bc	166.0	d	1235	b	0.44	b	341.6	cd	46.4	ab	35.7	ab	7.5	b
5	7042	c	150.8	h	1148	d	0.34	ef	478.0	a	37.8	c	36.8	a	6.8	c
6	2943	g	146.6	i	1105	f	0.27	g	489.2	a	24.8	f	27.0	d	6.7	cd
7	5043	e	93.6	j	1024	h	0.41	bc	356.7	cd	43.2	b	35.8	ab	6.6	cd
8	6337	d	182.7	a	1253	a	0.36	de	347.1	cd	36.7	cd	35.2	ab	6.1	g
9	7023	c	171.7	b	1179	c	0.42	bc	349.0	cd	37.5	c	36.5	a	6.5	de
10	5206	e	170.0	c	1158	d	0.50	a	320.1	d	34.1	de	33.9	b	6.1	fg
11	7822	b	154.6	f	1137	e	0.41	bc	445.1	ab	31.2	e	37.1	a	6.2	fg
12	2778	g	152.1	g	1106	f	0.27	g	353.9	cd	19.2	g	23.3	e	6.0	g
13	2814	g	152.5	g	1111	f	0.31	fg	313.8	d	23.9	f	23.8	e	6.0	g

Table S4.3. Mean performance of 14 durum wheat varieties across experiments and mean of experiments considering the panel of durum wheat varieties for quality traits. Within columns, numbers followed by the same letter indicate non-statistically significant differences at $p<0.05$ as determined by Tukey's HSD tests. The abbreviations of the varieties and the environments are detailed in Supplementary Table S1 and Table 1, respectively. PROT, protein content; TW, test weight; TKW, thousand kernel weight; VTR, vitreousness, b*, yellow pigment content; SDSS, SDS sedimentation; WG, wet gluten; GI, gluten index.

	PROT		**TW**		**TKW**		**VTR**		**b***		**SDSS**		**WG**		**GI**	
Varieties																
AMI	13.8	de	80.9	a	48.1	ef	86.3	bcd	15.0	d	39.7	defg	25.9	fg	75.7	bcd
AVI	13.5	e	80.8	a	47.9	ef	83.2	cd	14.9	d	40.7	def	24.9	g	80.0	b
BUR	15.8	a	79.4	bc	51.3	bc	95.0	a	15.2	d	50.0	a	31.6	ab	77.7	bc
CLA	14.7	bcd	81.6	a	48.3	de	83.5	cd	15.2	d	44.2	c	29.2	bcde	79.2	bc
DRI	14.9	bc	80.8	a	53.2	b	92.2	ab	14.3	e	38.6	efg	28.1	def	71.8	def
DOR	13.8	de	81.0	a	46.1	efg	85.7	bcd	14.9	d	40.6	def	25.3	g	75.2	bcde
GAL	14.3	cde	80.9	a	45.4	g	87.6	abc	15.6	c	38.5	fg	28.8	cde	70.0	defg
KNI	15.0	abc	78.9	bcd	52.5	bc	86.4	bcd	16.3	b	45.4	bc	28.6	cde	74.5	bcdef
MEX	14.2	cde	79.6	b	50.5	cd	85.8	bcd	15.7	c	41.3	d	29.4	bcd	69.5	efg
OLI	14.2	cde	78.2	d	41.9	h	80.1	cd	17.0	a	46.9	b	26.9	efg	89.9	a
REG	15.3	ab	81.3	a	47.5	efg	87.8	abc	15.7	c	41.2	de	31.1	abc	73.8	cdef
SCU	13.9	de	79.5	bc	46.0	fg	78.1	d	17.0	a	47.9	ab	28.2	def	64.2	g
SIM	15.9	a	78.7	cd	55.6	a	95.7	a	14.8	d	41.3	d	32.0	a	69.3	fg
VIT	14.3	cde	81.1	a	50.8	c	87.8	abc	14.1	e	37.8	g	28.3	def	52.1	h
Environments																
1	13.1	h	84.2	a	54.2	b	94.6	abcd	14.9	e	43.3	d	26.4	ef	79.2	bc
2	16.5	b	76.8	g	35.0	g	99.5	a	16.4	c	57.6	a	30.8	cd	91.4	a
3	14.2	defg	84.1	a	58.8	a	90.4	bcde	14.6	ef	38.2	e	28.8	de	64.2	fg
4	13.4	gh	81.6	b	50.4	cd	87.2	def	15.5	d	41.5	d	24.8	f	79.8	bc
5	15.2	c	79.9	c	48.5	de	93.8	abcd	15.4	d	42.9	d	32.0	bc	63.6	fg
6	17.4	a	79.2	cde	50.3	cd	97.3	ab	14.8	ef	38.3	e	36.9	a	46.1	h
7	14.7	cd	78.1	f	47.8	e	66.6	g	15.3	d	46.7	c	28.2	e	81.9	b
8	11.6	i	80.0	c	50.7	c	51.0	h	14.2	g	32.2	f	20.3	g	78.5	bc
9	14.3	cdef	81.2	b	54.3	b	88.7	cde	14.4	fg	35.9	e	28.6	de	67.9	ef
10	13.8	efgh	79.9	c	55.1	b	80.3	f	13.4	h	33.2	f	26.9	ef	62.1	g
11	13.5	fgh	79.4	cd	54.5	b	85.1	ef	16.8	bc	36.2	e	25.5	f	71.8	de
12	16.8	ab	78.6	ef	37.0	g	98.2	ab	17.4	a	52.4	b	33.5	b	75.3	cd
13	14.6	cde	79.1	de	39.4	f	95.8	abc	17.1	ab	53.5	b	27.0	ef	87.6	a

Table S4.4. Mean performance of 14 durum wheat varieties across experiments and mean of experiments considering the panel of durum wheat varieties for isotopic composition. Within columns, numbers followed by the same letter indicate non-statistically significant differences at $p<0.05$ as determined by Tukey's HSD tests. The abbreviations of the varieties and the environments are detailed in Supplementary Table S1 and Table 1, respectively. %N, nitrogen percentage; %C, carbon percentage; $\delta^{15}N$, nitrogen isotope; $\delta^{13}C$, carbon isotope; C/N, carbon/nitrogen ratio; GNY, grain nitrogen yield; GCY, grain carbon yield.

	%N		%C		δ 15N		δ 13C		C/N		GNY		GCY	
Varieties														
AMI	2.33	cd	42.9	a	2.4	a	-25.1	ab	19.2	ab	134.9	a	2622	ab
AVI	2.31	cd	42.4	a	2.3	a	-25.1	ab	19.2	ab	133.3	a	2546	abc
BUR	2.59	ab	42.8	a	2.5	a	-25.2	ab	16.9	de	138.4	a	2362	bc
CLA	2.37	bcd	42.2	a	2.4	a	-25.1	ab	18.4	abcd	134.4	a	2468	abc
DRI	2.51	abcd	42.7	a	2.4	a	-24.9	a	17.6	bcde	134.4	a	2373	abc
DOR	2.35	cd	42.5	a	2.4	a	-24.9	a	19.0	abc	130.7	a	2499	abc
GAL	2.45	abcd	42.4	a	2.3	a	-25.2	ab	18.0	abcde	136.8	a	2478	abc
KNI	2.50	abcd	42.5	a	2.4	a	-25.2	ab	17.3	cde	133.9	a	2336	bc
MEX	2.32	cd	42.7	a	2.4	a	-25.1	ab	18.7	abc	130.6	a	2447	abc
OLI	2.31	cd	42.9	a	2.2	a	-25.0	ab	19.3	a	136.2	a	2666	a
REG	2.52	abc	42.6	a	2.5	a	-25.4	b	17.6	bcde	143.0	a	2549	abc
SCU	2.29	d	42.5	a	2.3	a	-25.2	ab	19.4	a	131.2	a	2549	abc
SIM	2.63	a	42.6	a	2.5	a	-25.0	ab	16.5	e	135.7	a	2284	c
VIT	2.34	cd	42.7	a	2.4	a	-25.2	ab	18.6	abc	133.0	a	2468	abc
Environments														
1	2.25	c	42.5	d	3.4	a	-25.9	f	19.0	c	173.9	b	3295	bc
2	2.83	a	42.8	cd	3.4	a	-24.2	c	15.3	e	117.8	d	1789	g
3	2.23	c	42.5	d	2.9	bc	-26.6	g	19.3	c	214.9	a	4088	a
4	2.05	cd	40.6	e	1.5	f	-25.5	ef	20.0	bc	149.9	c	2975	d
5	2.56	b	43.0	cd	2.6	cd	-25.0	d	17.0	d	178.0	b	3025	cd
6	3.02	a	44.4	a	0.9	g	-23.6	b	14.7	e	87.5	e	1305	h
7	2.54	b	42.8	cd	2.5	cd	-25.9	f	17.1	d	125.9	d	2159	f
8	1.86	d	41.1	e	2.0	e	-26.5	g	22.4	a	118.0	d	2602	e
9	2.21	c	41.2	e	3.2	ab	-26.5	g	19.3	bc	153.9	c	2888	de
10	2.13	c	42.2	d	2.5	d	-26.5	g	20.3	bc	110.7	d	2201	f
11	2.12	c	43.4	bc	2.4	d	-25.3	de	20.9	ab	164.8	bc	3398	b
12	3.01	a	43.4	bc	1.2	fg	-22.5	a	14.6	e	83.3	e	1207	h
13	2.59	b	44.1	ab	2.4	d	-22.4	a	17.4	d	72.9	e	1241	h

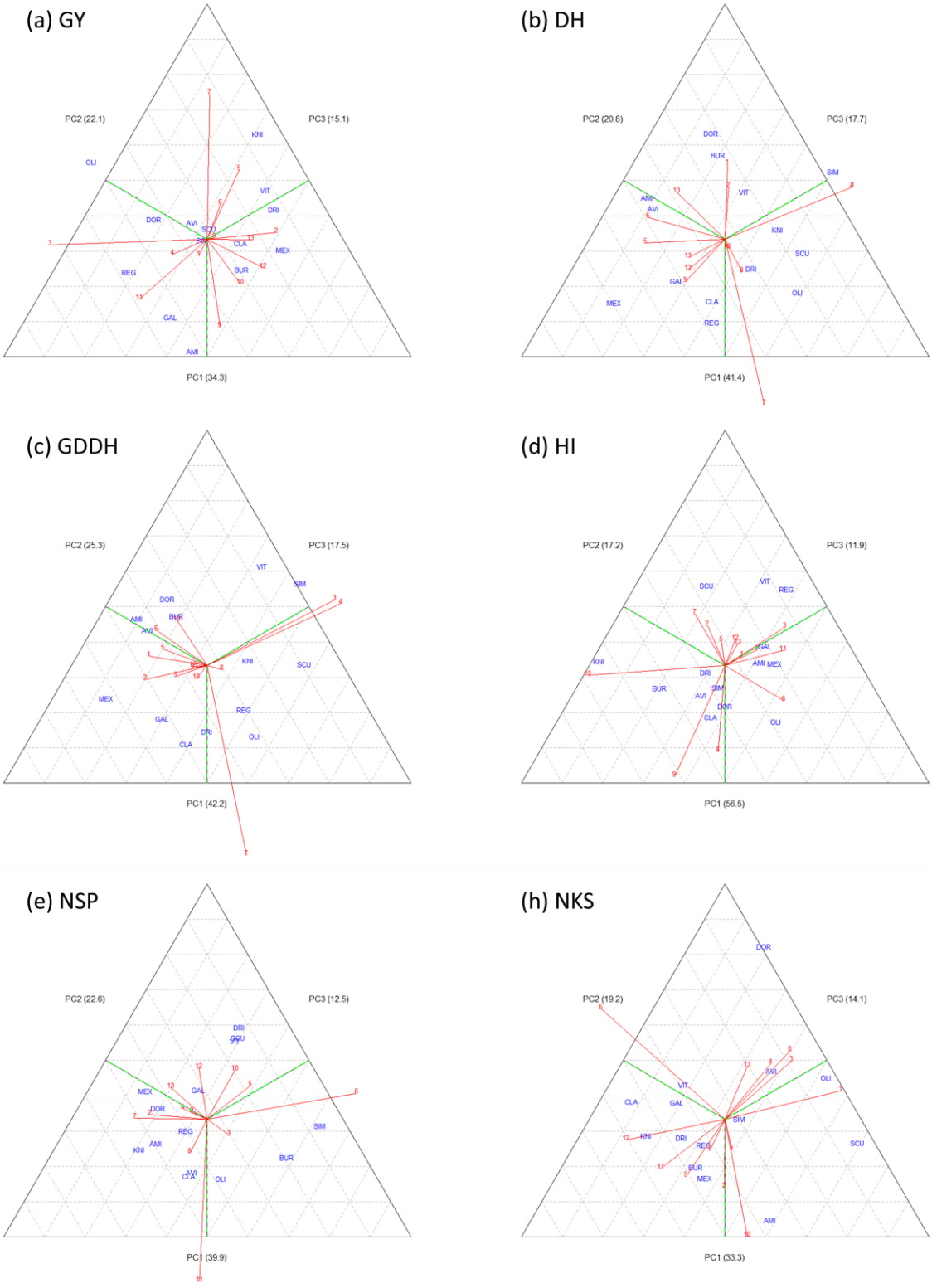


Figure S4.1. Continued.

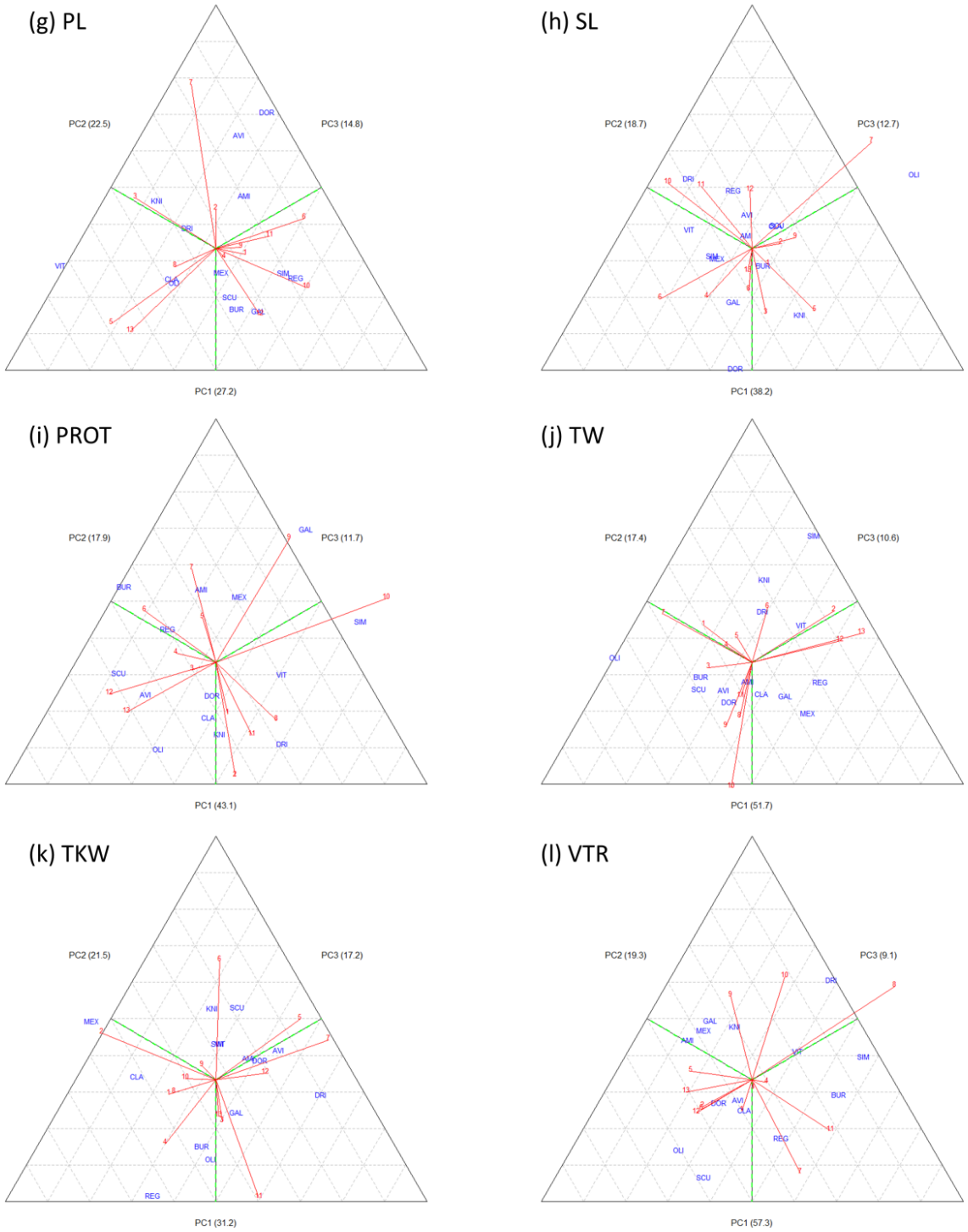


Figure S4.1. Continued.

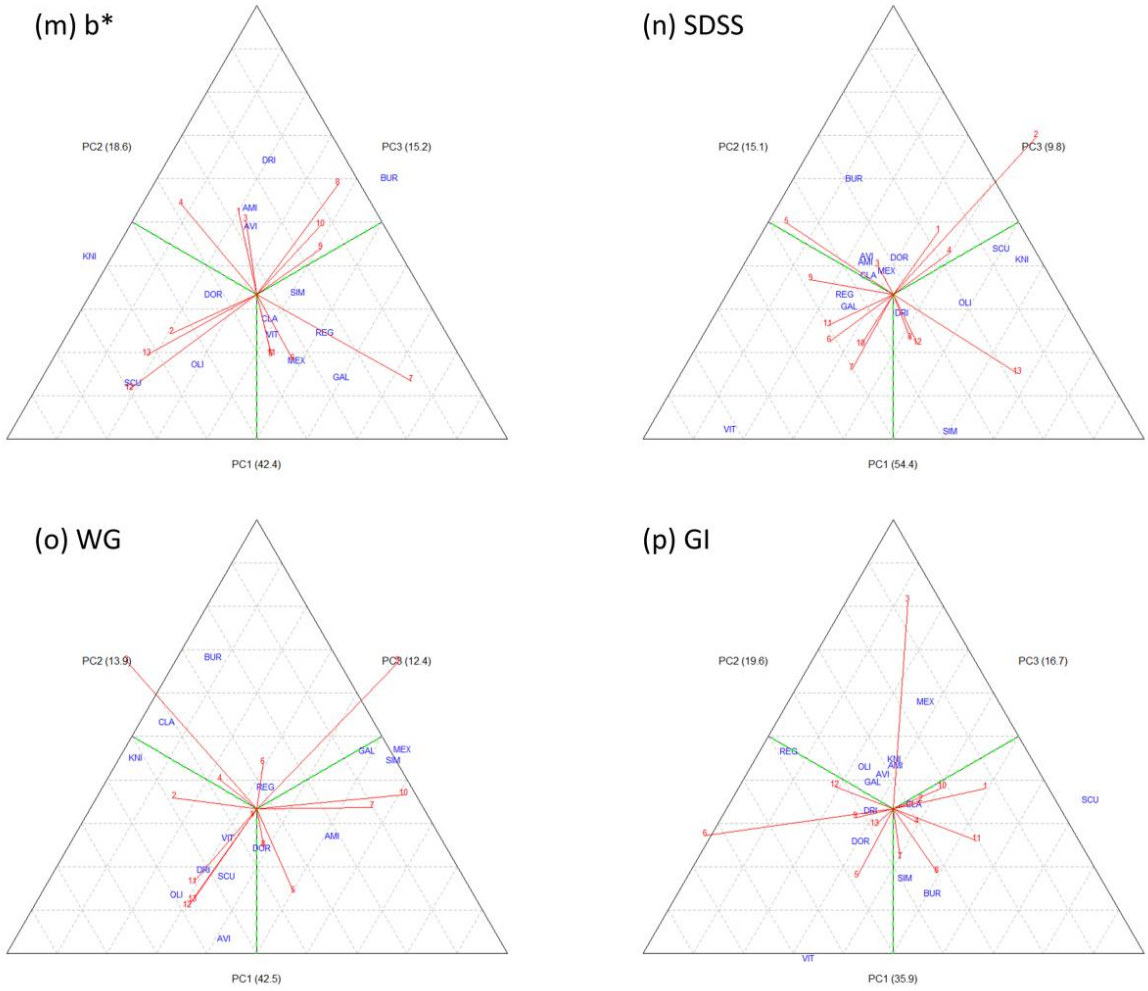


Figure S4.1. Three-dimensional AMMI plots among the 14 durum wheat varieties and the specific genotype-by-environment interactions evaluated across 13 environments during five growing seasons for: (a) GY (grain yield), (b) DH (days from emergence to heading), (c) GDDH (growing degree days at heading), (d) HI (harvest index), (e) NSP (number of spikes per m^2), (f) NKS (number of kernels per spike), (g) PL (peduncle length), (h) SL (spike length), (i) PROT (protein content), (j) TW (test weight), (k) TKW (thousand kernel weight), (l) VTR (vitreousness), (m) b* (yellow pigment content), (n) SDSS (SDS sedimentation), (o) WG (wet gluten) and (p) GI (gluten index). The abbreviations for the varieties are detailed in Table S1 and for the environments in Table 1.

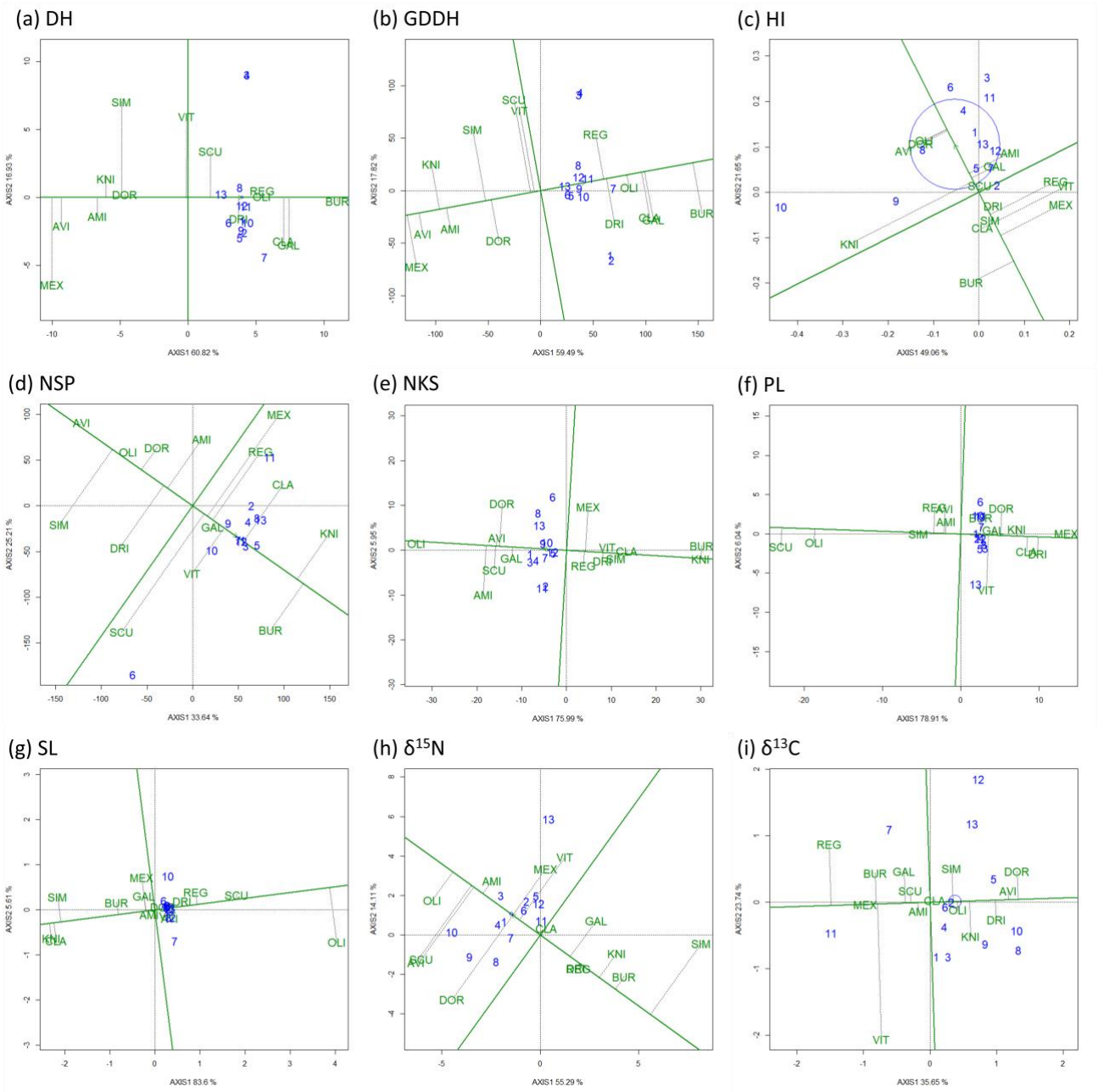


Figure S4.2. GGE biplots of "Mean and Stability" among the 14 durum wheat varieties and the specific genotype × environment interactions were evaluated across 13 environments during five growing seasons for: (a) DH (days from emergence to heading), (b) GDDH (growing degree days at heading), (c) HI (harvest index), (d) NSP (number of spikes per m^2), (e) NKS (number of kernels per spike), (f) PL (peduncle length), (g) SL (spike length), (h) $\delta^{15}N$ (nitrogen isotope composition), and (i) $\delta^{13}C$ (carbon isotope composition). The average-environment coordination (AEC) solid green lines represented the average environment. The dotted lines show the stability of the varieties being the shorter line, the higher the stability. The abbreviations for the varieties are detailed in Table S1 and for the environments in Table 1.

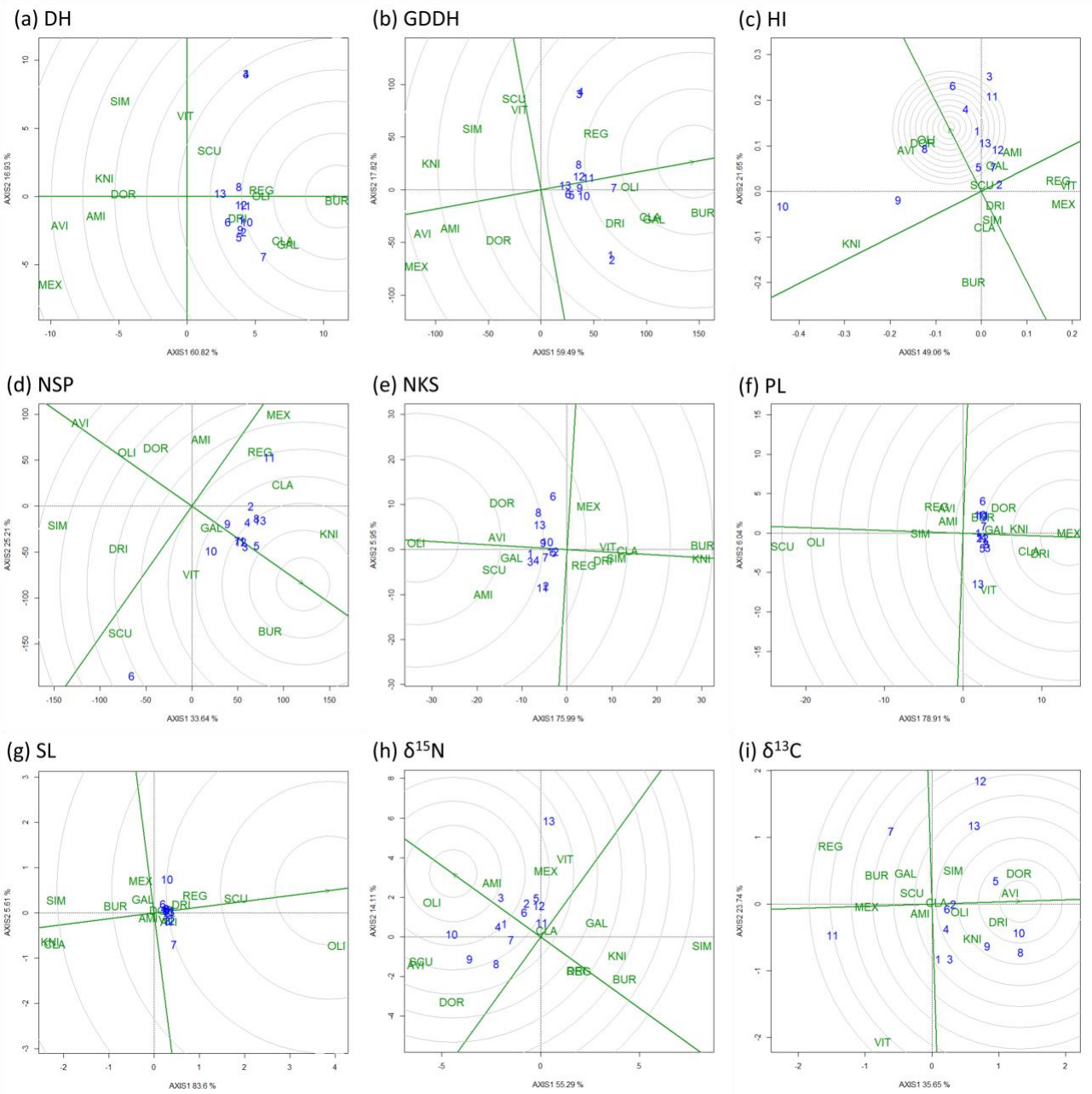


Figure S4.3. GGE biplots of "Ranking Genotype" (ideal genotype) among the 14 durum wheat varieties and the specific genotype × environment interactions evaluated across 13 environments during five growing seasons for: (a) DH (days from emergence to heading), (b) GDDH (growing degree days at heading), (c) HI (harvest index), (d) NSP (number of spikes per m^2), (e) NKS (number of kernels per spike), (f) PL (peduncle length), (g) SL (spike length), (h) $\delta^{15}N$ (nitrogen isotope composition), and (i) $\delta^{13}C$ (carbon isotope composition). The average-environment coordination (AEC) solid green lines represented the average environment. The dotted lines show the stability of the varieties being the shorter line, the higher the stability of each variety. The abbreviations of the varieties and the environments are detailed in Supplementary Table 1 and Table 1, respectively.

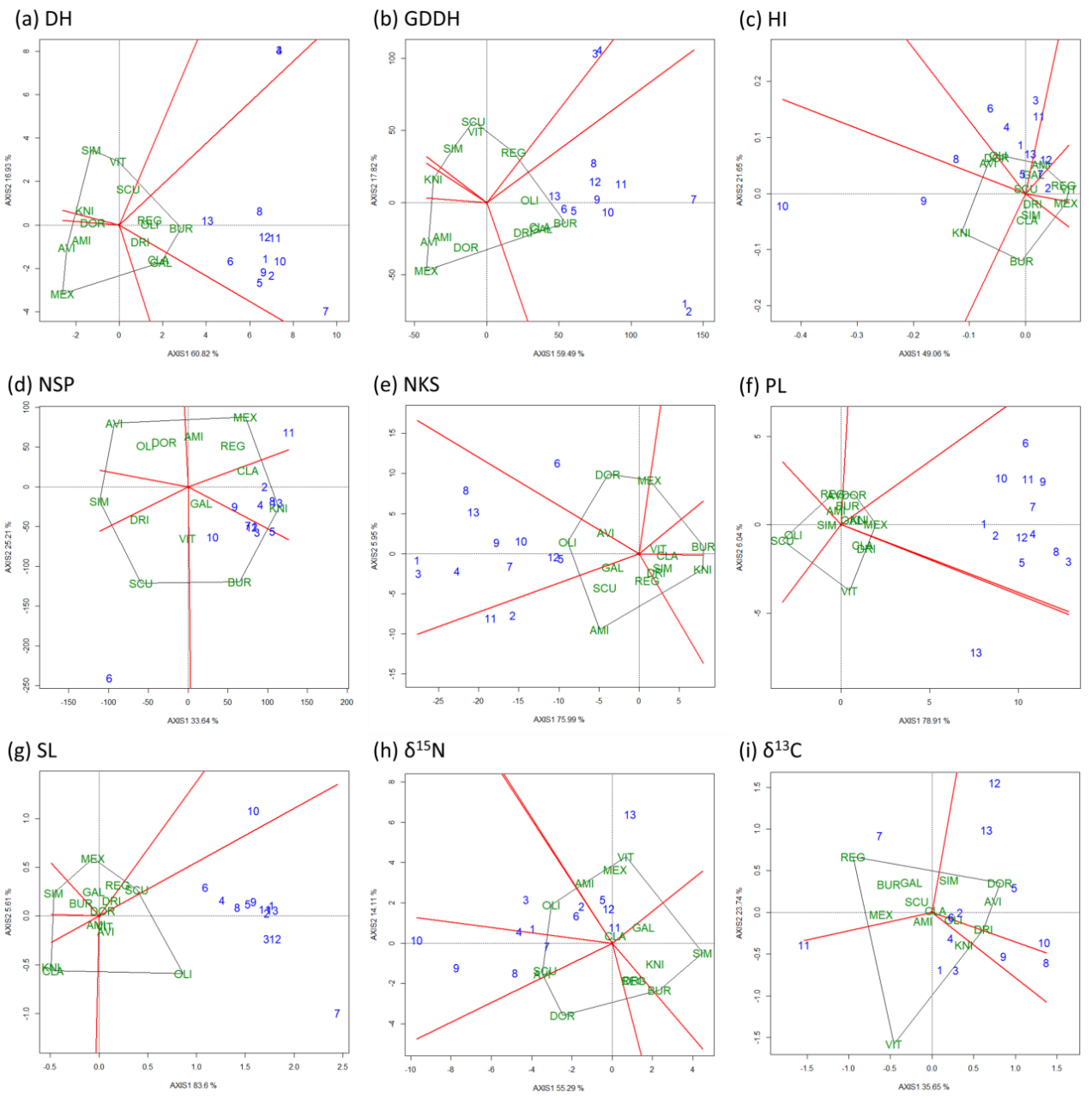


Figure S4.4. GGE biplot of "Which Won Where/What" among the 14 durum wheat varieties and the specific genotype × environment interactions evaluated across 13 environments during five growing seasons for: (a) DH (days from emergence to heading), (b) GDDH (growing degree days at heading), (c) HI (harvest index), (d) NSP (number of spikes per m^2), (e) NKS (number of kernels per spike), (f) PL (peduncle length), (g) SL (spike length), (h) $\delta^{15}N$ (nitrogen isotope composition), and (i) $\delta^{13}C$ (carbon isotope composition). This plot consists of a polygon with perpendicular lines, called equality lines, drawn onto its sides. These lines divide the polygon into various sectors. Varieties located on the polygon's vertices are the best in each mega-environment from a particular sector. The abbreviations for the varieties are detailed in Table S1 and for the environments in Table 1.

9.2. Study III

Table S6.2. List of varieties and traits analysed in this study, including the abbreviations and units used.

Group	Trait	Description	Units
Varieties	HAR	Durum wheat variety Haristide	-
	EUR	Durum wheat variety Euroduro	-
	DRI	Durum wheat variety Don Ricardo	-
	KNI	Durum wheat variety Kiko Nick	-
Agronomic components	GY	Grain yield	kg ha^{-1}
	biomass	Biomass	kg ha^{-1}
	HI	Harvest index	g grain g $biomass^{-1}$
	plants.m2	Number of plants per area	plants m^{-2}
	ears.plant	Number of ears per plant	ears $plant^{-1}$
	grains.ear	Number of grains per ear	grains ear^{-1}
	TGW	Thousand grain weight	g
	ped.length	Length of peduncle (last stem internode)	cm
	ear.length	Length of ear	cm
	height	Plant height	cm
Conventional grain quality traits	prot.grain	Protein content of grain	%
	moisture.grain	Moisture content of grain	%
	SW.grain	Specific weight of grain	g L^{-1}
	vitreo.grain	Vitreousness of grain	%
	sedim.grain	Sodium dodecyl sulphate (SDS) sedimentation volumes of grain, sedimentation index	mL g^{-1}
	b.grain	CIELAB b* coordinate, yellowness index, yellow colour of semolina	(dimensionless)
	WG.grain	Wet gluten of grain	mg
	GI.grain	Gluten index of grain	%
Canopy and leaf phenotyping	GA.15	Green area index at Zadoks 15	(dimensionless)
	GA.25	Green area index at Zadoks 25	(dimensionless)
	GA.35	Green area index at Zadoks 35	(dimensionless)
	GA.55	Green area index at Zadoks 55	(dimensionless)
	GA.65	Green area index at Zadoks 65	(dimensionless)
	GA.75	Green area index at Zadoks 75	(dimensionless)
	GGA.15	Greener area index at Zadoks 15	(dimensionless)
	GGA.25	Greener area index at Zadoks 25	(dimensionless)
	GGA.35	Greener area index at Zadoks 35	(dimensionless)
	GGA.55	Greener area index at Zadoks 55	(dimensionless)
	GGA.65	Greener area index at Zadoks 65	(dimensionless)
	GGA.75	Greener area index at Zadoks 75	(dimensionless)
	CSI.15	Crop senescence index at Zadoks 15	(dimensionless)
	CSI.25	Crop senescence index at Zadoks 25	(dimensionless)
	CSI.35	Crop senescence index at Zadoks 35	(dimensionless)
	CSI.55	Crop senescence index at Zadoks 55	(dimensionless)
	CSI.65	Crop senescence index at Zadoks 65	(dimensionless)
	CSI.75	Crop senescence index at Zadoks 75	(dimensionless)
	NDVI.25	Normalized difference vegetation index at Zadoks 25	(dimensionless)
	NDVI.35	Normalized difference vegetation index at Zadoks 35	(dimensionless)
	NDVI.55	Normalized difference vegetation index at Zadoks 55	(dimensionless)

	NDVI.65	Normalized difference vegetation index at Zadoks 65	(dimensionless)
	NDVI.75	Normalized difference vegetation index at Zadoks 75	(dimensionless)
	NDVI.85	Normalized difference vegetation index at Zadoks 85	(dimensionless)
	chl.65	Relative chlorophyll content at Zadoks 65	(dimensionless)
	chl.75	Relative chlorophyll content at Zadoks 75	(dimensionless)
	flav.65	Relative flavonols content at Zadoks 65	(dimensionless)
	flav.75	Relative flavonols content at Zadoks 75	(dimensionless)
	anth.65	Relative anthocyanins content at Zadoks 65	(dimensionless)
	anth.75	Relative anthocyanins content at Zadoks 75	(dimensionless)
	NBI.65	Nitrogen balance index at Zadoks 65	(dimensionless)
	NBI.75	Nitrogen balance index at Zadoks 75	(dimensionless)
	LRWC.65	Leaf relative water content at Zadoks 65	%
	LRWC.75	Leaf relative water content at Zadoks 75	%
Weight per organ	FW.b.65	Fresh weight of flag leaf blade at Zadoks 65	g
	FW.s.65	Fresh weight of flag leaf sheath at Zadoks 65	g
	FW.p.65	Fresh weight of peduncle at Zadoks 65	g
	FW.ear.65	Fresh weight of ear at Zadoks 65	g
	FW.b.75	Fresh weight of flag leaf blade at Zadoks 75	g
	FW.s.75	Fresh weight of flag leaf sheath at Zadoks 75	g
	FW.p.75	Fresh weight of peduncle at Zadoks 75	g
	FW.ear.75	Fresh weight of ear at Zadoks 75	g

Table S6.1. Continued.

Group	Trait	Description	Units
Weight per organ	DW.b.65	Dry weight of flag leaf blade at Zadoks 65	g
	DW.s.65	Dry weight of flag leaf sheath at Zadoks 65	g
	DW.p.65	Dry weight of peduncle at Zadoks 65	g
	DW.ear.65	Dry weight of ear at Zadoks 65	g
	DW.b.75	Dry weight of flag leaf blade at Zadoks 75	g
	DW.s.75	Dry weight of flag leaf sheath at Zadoks 75	g
	DW.p.75	Dry weight of peduncle at Zadoks 75	g
	DW.ear.75	Dry weight of ear at Zadoks 75	g
	DW.b.85	Dry weight of flag leaf blade at Zadoks 85	g
	DW.s.85	Dry weight of flag leaf sheath at Zadoks 85	g
	DW.p.85	Dry weight of peduncle at Zadoks 85	g
	DW.ear.85	Dry weight of ear at Zadoks 85	g
Carbohydrate content per organ	glc.b.65	Glucose content of flag leaf blade at Zadoks 65	µmol g FW^{-1}
	glc.s.65	Glucose content of flag leaf sheath at Zadoks 65	µmol g FW^{-1}
	glc.p.65	Glucose content of peduncle at Zadoks 65	µmol g FW^{-1}
	glc.a.65	Glucose content of awn at Zadoks 65	µmol g FW^{-1}
	glc.g.65	Glucose content of glume at Zadoks 65	µmol g FW^{-1}
	glc.l.65	Glucose content of lemma at Zadoks 65	µmol g FW^{-1}
	glc.b.75	Glucose content of flag leaf blade at Zadoks 75	µmol g FW^{-1}
	glc.s.75	Glucose content of flag leaf sheath at Zadoks 75	µmol g FW^{-1}
	glc.p.75	Glucose content of peduncle at Zadoks 75	µmol g FW^{-1}
	glc.a.75	Glucose content of awn at Zadoks 75	µmol g FW^{-1}
	glc.g.75	Glucose content of glume at Zadoks 75	µmol g FW^{-1}
	glc.l.75	Glucose content of lemma at Zadoks 75	µmol g FW^{-1}
	fru.b.65	Fructose content of flag leaf blade at Zadoks 65	µmol g FW^{-1}
	fru.s.65	Fructose content of flag leaf sheath at Zadoks 65	µmol g FW^{-1}
	fru.p.65	Fructose content of peduncle at Zadoks 65	µmol g FW^{-1}
	fru.a.65	Fructose content of awn at Zadoks 65	µmol g FW^{-1}
	fru.g.65	Fructose content of glume at Zadoks 65	µmol g FW^{-1}
	fru.l.65	Fructose content of lemma at Zadoks 65	µmol g FW^{-1}
	fru.b.75	Fructose content of flag leaf blade at Zadoks 75	µmol g FW^{-1}
	fru.s.75	Fructose content of flag leaf sheath at Zadoks 75	µmol g FW^{-1}
	fru.p.75	Fructose content of peduncle at Zadoks 75	µmol g FW^{-1}
	fru.a.75	Fructose content of awn at Zadoks 75	µmol g FW^{-1}
	fru.g.75	Fructose content of glume at Zadoks 75	µmol g FW^{-1}
	fru.l.75	Fructose content of lemma at Zadoks 75	µmol g FW^{-1}
	suc.b.65	Sucrose content of flag leaf blade at Zadoks 65	µmol g FW^{-1}
	suc.s.65	Sucrose content of flag leaf sheath at Zadoks 65	µmol g FW^{-1}
	suc.p.65	Sucrose content of peduncle at Zadoks 65	µmol g FW^{-1}
	suc.a.65	Sucrose content of awn at Zadoks 65	µmol g FW^{-1}
	suc.g.65	Sucrose content of glume at Zadoks 65	µmol g FW^{-1}
	suc.l.65	Sucrose content of lemma at Zadoks 65	µmol g FW^{-1}
	suc.b.75	Sucrose content of flag leaf blade at Zadoks 75	µmol g FW^{-1}
	suc.s.75	Sucrose content of flag leaf sheath at Zadoks 75	µmol g FW^{-1}
	suc.p.75	Sucrose content of peduncle at Zadoks 75	µmol g FW^{-1}

	suc.a.75	Sucrose content of awn at Zadoks 75	µmol g FW^{-1}
	suc.g.75	Sucrose content of glume at Zadoks 75	µmol g FW^{-1}
	suc.l.75	Sucrose content of lemma at Zadoks 75	µmol g FW^{-1}
Carbohydrate content per organ	fructan.b.65	Fructan content of flag leaf blade at Zadoks 65	µmol g FW^{-1}
	fructan.s.65	Fructan content of flag leaf sheath at Zadoks 65	µmol g FW^{-1}
	fructan.p.65	Fructan content of peduncle at Zadoks 65	µmol g FW^{-1}
	fructan.a.65	Fructan content of awn at Zadoks 65	µmol g FW^{-1}
	fructan.g.65	Fructan content of glume at Zadoks 65	µmol g FW^{-1}
	fructan.l.65	Fructan content of lemma at Zadoks 65	µmol g FW^{-1}
	fructan.b.75	Fructan content of flag leaf blade at Zadoks 75	µmol g FW^{-1}
	fructan.s.75	Fructan content of flag leaf sheath at Zadoks 75	µmol g FW^{-1}
	fructan.p.75	Fructan content of peduncle at Zadoks 75	µmol g FW^{-1}
	fructan.a.75	Fructan content of awn at Zadoks 75	µmol g FW^{-1}
	fructan.g.75	Fructan content of glume at Zadoks 75	µmol g FW^{-1}
	fructan.l.75	Fructan content of lemma at Zadoks 75	µmol g FW^{-1}
	starch.b.65	Starch content of flag leaf blade at Zadoks 65	µmol g FW^{-1}
	starch.s.65	Starch content of flag leaf sheath at Zadoks 65	µmol g FW^{-1}
	starch.p.65	Starch content of peduncle at Zadoks 65	µmol g FW^{-1}
	starch.a.65	Starch content of awn at Zadoks 65	µmol g FW^{-1}

Table S6.1. Continued.

Group	Trait	Description	Units
Carbohydrate content per organ	starch.g.65	Starch content of glume at Zadoks 65	µmol g FW^{-1}
	starch.l.65	Starch content of lemma at Zadoks 65	µmol g FW^{-1}
	starch.b.75	Starch content of flag leaf blade at Zadoks 75	µmol g FW^{-1}
	starch.s.75	Starch content of flag leaf sheath at Zadoks 75	µmol g FW^{-1}
	starch.p.75	Starch content of peduncle at Zadoks 75	µmol g FW^{-1}
	starch.a.75	Starch content of awn at Zadoks 75	µmol g FW^{-1}
	starch.g.75	Starch content of glume at Zadoks 75	µmol g FW^{-1}
	starch.l.75	Starch content of lemma at Zadoks 75	µmol g FW^{-1}
Rubisco content per organ	rbcL.b.65	Rubisco large subunit content of flag leaf blade at Zadoks 65	mg g FW^{-1}
	rbcL.s.65	Rubisco large subunit content of flag leaf sheath at Zadoks 65	mg g FW^{-1}
	rbcL.p.65	Rubisco large subunit content of peduncle at Zadoks 65	mg g FW^{-1}
	rbcL.a.65	Rubisco large subunit content of awn at Zadoks 65	mg g FW^{-1}
	rbcL.g.65	Rubisco large subunit content of glume at Zadoks 65	mg g FW^{-1}
	rbcL.l.65	Rubisco large subunit content of lemma at Zadoks 65	mg g FW^{-1}
	rbcL.b.75	Rubisco large subunit content of flag leaf blade at Zadoks 75	mg g FW^{-1}
	rbcL.s.75	Rubisco large subunit content of flag leaf sheath at Zadoks 75	mg g FW^{-1}
	rbcL.p.75	Rubisco large subunit content of peduncle at Zadoks 75	mg g FW^{-1}
	rbcL.a.75	Rubisco large subunit content of awn at Zadoks 75	mg g FW^{-1}
	rbcL.g.75	Rubisco large subunit content of glume at Zadoks 75	mg g FW^{-1}
	rbcL.l.75	Rubisco large subunit content of lemma at Zadoks 75	mg g FW^{-1}
Enzyme activities per organ	RCOI.b.65	Initial Rubisco activity of flag leaf blade at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOI.s.65	Initial Rubisco activity of flag leaf sheath at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOI.p.65	Initial Rubisco activity of peduncle at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOI.a.65	Initial Rubisco activity of awn at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOI.g.65	Initial Rubisco activity of glume at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOI.l.65	Initial Rubisco activity of lemma at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOI.b.75	Initial Rubisco activity of flag leaf blade at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOI.s.75	Initial Rubisco activity of flag leaf sheath at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOI.p.75	Initial Rubisco activity of peduncle at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOI.a.75	Initial Rubisco activity of awn at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOI.g.75	Initial Rubisco activity of glume at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOI.l.75	Initial Rubisco activity of lemma at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOT.b.65	Total Rubisco activity of flag leaf blade at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOT.s.65	Total Rubisco activity of flag leaf sheath at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOT.p.65	Total Rubisco activity of peduncle at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOT.a.65	Total Rubisco activity of awn at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOT.g.65	Total Rubisco activity of glume at Zadoks 65	µmol g FW^{-1} min^{-1}
	RCOT.l.65	Total Rubisco activity of lemma at Zadoks 65	µmol g FW^{-1} min^{-1}
Enzyme activities per organ	RCOT.b.75	Total Rubisco activity of flag leaf blade at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOT.s.75	Total Rubisco activity of flag leaf sheath at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOT.p.75	Total Rubisco activity of peduncle at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOT.a.75	Total Rubisco activity of awn at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOT.g.75	Total Rubisco activity of glume at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOT.l.75	Total Rubisco activity of lemma at Zadoks 75	µmol g FW^{-1} min^{-1}
	RCOas.b.65	Rubisco activation state of flag leaf blade at Zadoks 65	%

RCOas.s.65	Rubisco activation state of flag leaf sheath at Zadoks 65	%
RCOas.p.65	Rubisco activation state of peduncle at Zadoks 65	%
RCOas.a.65	Rubisco activation state of awn at Zadoks 65	%
RCOas.g.65	Rubisco activation state of glume at Zadoks 65	%
RCOas.l.65	Rubisco activation state of lemma at Zadoks 65	%
RCOas.b.75	Rubisco activation state of flag leaf blade at Zadoks 75	%
RCOas.s.75	Rubisco activation state of flag leaf sheath at Zadoks 75	%
RCOas.p.75	Rubisco activation state of peduncle at Zadoks 75	%
RCOas.a.75	Rubisco activation state of awn at Zadoks 75	%
RCOas.g.75	Rubisco activation state of glume at Zadoks 75	%
RCOas.l.75	Rubisco activation state of lemma at Zadoks 75	%
PEPC.b.65	Phosphoenolpyruvate carboxylase activity of flag leaf blade at Zadoks 65	nmol g FW^{-1} min^{-1}
PEPC.s.65	Phosphoenolpyruvate carboxylase activity of flag leaf sheath at Zadoks 65	nmol g FW^{-1} min^{-1}
PEPC.p.65	Phosphoenolpyruvate carboxylase activity of peduncle at Zadoks 65	nmol g FW^{-1} min^{-1}
PEPC.a.65	Phosphoenolpyruvate carboxylase activity of awn at Zadoks 65	nmol g FW^{-1} min^{-1}
PEPC.g.65	Phosphoenolpyruvate carboxylase activity of glume at Zadoks 65	nmol g FW^{-1} min^{-1}
PEPC.l.65	Phosphoenolpyruvate carboxylase activity of lemma at Zadoks 65	nmol g FW^{-1} min^{-1}

Table S6.1. Continued.

Group	Trait	Description	Units
Enzyme activities per organ	PEPC.b.75	Phosphoenolpyruvate carboxylase activity of flag leaf blade at Zadoks 75	nmol g FW^{-1} min^{-1}
	PEPC.s.75	Phosphoenolpyruvate carboxylase activity of flag leaf sheath at Zadoks 75	nmol g FW^{-1} min^{-1}
	PEPC.p.75	Phosphoenolpyruvate carboxylase activity of peduncle at Zadoks 75	nmol g FW^{-1} min^{-1}
	PEPC.a.75	Phosphoenolpyruvate carboxylase activity of awn at Zadoks 75	nmol g FW^{-1} min^{-1}
	PEPC.g.75	Phosphoenolpyruvate carboxylase activity of glume at Zadoks 75	nmol g FW^{-1} min^{-1}
	PEPC.l.75	Phosphoenolpyruvate carboxylase activity of lemma at Zadoks 75	nmol g FW^{-1} min^{-1}
	GS.b.65	Glutamine synthetase activity of flag leaf blade at Zadoks 65	nmol g FW^{-1} min^{-1}
	GS.s.65	Glutamine synthetase activity of flag leaf sheath at Zadoks 65	nmol g FW^{-1} min^{-1}
	GS.p.65	Glutamine synthetase activity of peduncle at Zadoks 65	nmol g FW^{-1} min^{-1}
	GS.a.65	Glutamine synthetase activity of awn at Zadoks 65	nmol g FW^{-1} min^{-1}
	GS.g.65	Glutamine synthetase activity of glume at Zadoks 65	nmol g FW^{-1} min^{-1}
	GS.l.65	Glutamine synthetase activity of lemma at Zadoks 65	nmol g FW^{-1} min^{-1}
	GS.b.75	Glutamine synthetase activity of flag leaf blade at Zadoks 75	nmol g FW^{-1} min^{-1}
	GS.s.75	Glutamine synthetase activity of flag leaf sheath at Zadoks 75	nmol g FW^{-1} min^{-1}
	GS.p.75	Glutamine synthetase activity of peduncle at Zadoks 75	nmol g FW^{-1} min^{-1}
	GS.a.75	Glutamine synthetase activity of awn at Zadoks 75	nmol g FW^{-1} min^{-1}
	GS.g.75	Glutamine synthetase activity of glume at Zadoks 75	nmol g FW^{-1} min^{-1}
	GS.l.75	Glutamine synthetase activity of lemma at Zadoks 75	nmol g FW^{-1} min^{-1}
	GOGAT.b.65	Ferredoxin-dependent glutamate synthase activity of flag leaf blade at Zadoks 65	nmol g FW^{-1} min^{-1}
	GOGAT.s.65	Ferredoxin-dependent glutamate synthase activity of flag leaf sheath at Zadoks 65	nmol g FW^{-1} min^{-1}
	GOGAT.p.65	Ferredoxin-dependent glutamate synthase activity of peduncle at Zadoks 65	nmol g FW^{-1} min^{-1}
	GOGAT.a.65	Ferredoxin-dependent glutamate synthase activity of awn at Zadoks 65	nmol g FW^{-1} min^{-1}
	GOGAT.g.65	Ferredoxin-dependent glutamate synthase activity of glume at Zadoks 65	nmol g FW^{-1} min^{-1}
	GOGAT.l.65	Ferredoxin-dependent glutamate synthase activity of lemma at Zadoks 65	nmol g FW^{-1} min^{-1}
	GOGAT.b.75	Ferredoxin-dependent glutamate synthase activity of flag leaf blade at Zadoks 75	nmol g FW^{-1} min^{-1}
	GOGAT.s.75	Ferredoxin-dependent glutamate synthase activity of flag leaf sheath at Zadoks 75	nmol g FW^{-1} min^{-1}
	GOGAT.p.75	Ferredoxin-dependent glutamate synthase activity of peduncle at Zadoks 75	nmol g FW^{-1} min^{-1}
	GOGAT.a.75	Ferredoxin-dependent glutamate synthase activity of awn at Zadoks 75	nmol g FW^{-1} min^{-1}
	GOGAT.g.75	Ferredoxin-dependent glutamate synthase activity of glume at Zadoks 75	nmol g FW^{-1} min^{-1}
	GOGAT.l.75	Ferredoxin-dependent glutamate synthase activity of lemma at Zadoks 75	nmol g FW^{-1} min^{-1}
	GDH.b.65	NADH-dependent glutamate dehydrogenase activity of flag leaf blade at Zadoks 65	nmol g FW^{-1} min^{-1}
	GDH.s.65	NADH-dependent glutamate dehydrogenase activity of flag leaf sheath at Zadoks 65	nmol g FW^{-1} min^{-1}
	GDH.p.65	NADH-dependent glutamate dehydrogenase activity of peduncle at Zadoks 65	nmol g FW^{-1} min^{-1}
	GDH.a.65	NADH-dependent glutamate dehydrogenase activity of awn at Zadoks 65	nmol g FW^{-1} min^{-1}
	GDH.g.65	NADH-dependent glutamate dehydrogenase activity of glume at Zadoks 65	nmol g FW^{-1} min^{-1}
	GDH.l.65	NADH-dependent glutamate dehydrogenase activity of lemma at Zadoks 65	nmol g FW^{-1} min^{-1}
	GDH.b.75	NADH-dependent glutamate dehydrogenase activity of flag leaf blade at Zadoks 75	nmol g FW^{-1} min^{-1}
	GDH.s.75	NADH-dependent glutamate dehydrogenase activity of flag leaf sheath at Zadoks 75	nmol g FW^{-1} min^{-1}
	GDH.p.75	NADH-dependent glutamate dehydrogenase activity of peduncle at Zadoks 75	nmol g FW^{-1} min^{-1}
	GDH.a.75	NADH-dependent glutamate dehydrogenase activity of awn at Zadoks 75	nmol g FW^{-1} min^{-1}
	GDH.g.75	NADH-dependent glutamate dehydrogenase activity of glume at Zadoks 75	nmol g FW^{-1} min^{-1}
	GDH.l.75	NADH-dependent glutamate dehydrogenase activity of lemma at Zadoks 75	nmol g FW^{-1} min^{-1}
Nutrient composition per organ	C.b.65	Carbon content of flag leaf blade at Zadoks 65	g kg DW^{-1}
	C.s.65	Carbon content of flag leaf sheath at Zadoks 65	g kg DW^{-1}
	C.p.65	Carbon content of peduncle at Zadoks 65	g kg DW^{-1}
	C.a.65	Carbon content of awn at Zadoks 65	g kg DW^{-1}

C.g.65	Carbon content of glume at Zadoks 65	g kg DW^{-1}
C.l.65	Carbon content of lemma at Zadoks 65	g kg DW^{-1}
C.b.75	Carbon content of flag leaf blade at Zadoks 75	g kg DW^{-1}
C.s.75	Carbon content of flag leaf sheath at Zadoks 75	g kg DW^{-1}
C.p.75	Carbon content of peduncle at Zadoks 75	g kg DW^{-1}
C.a.75	Carbon content of awn at Zadoks 75	g kg DW^{-1}
C.g.75	Carbon content of glume at Zadoks 75	g kg DW^{-1}
C.l.75	Carbon content of lemma at Zadoks 75	g kg DW^{-1}
N.b.65	Nitrogen content of flag leaf blade at Zadoks 65	g kg DW^{-1}
N.s.65	Nitrogen content of flag leaf sheath at Zadoks 65	g kg DW^{-1}
N.p.65	Nitrogen content of peduncle at Zadoks 65	g kg DW^{-1}
N.a.65	Nitrogen content of awn at Zadoks 65	g kg DW^{-1}
N.g.65	Nitrogen content of glume at Zadoks 65	g kg DW^{-1}
N.l.65	Nitrogen content of lemma at Zadoks 65	g kg DW^{-1}
N.b.75	Nitrogen content of flag leaf blade at Zadoks 75	g kg DW^{-1}
N.s.75	Nitrogen content of flag leaf sheath at Zadoks 75	g kg DW^{-1}
N.p.75	Nitrogen content of peduncle at Zadoks 75	g kg DW^{-1}
N.a.75	Nitrogen content of awn at Zadoks 75	g kg DW^{-1}
N.g.75	Nitrogen content of glume at Zadoks 75	g kg DW^{-1}
N.l.75	Nitrogen content of lemma at Zadoks 75	g kg DW^{-1}

Table S6.1. Continued.

Group	Trait	Description	Units
Nutrient composition per organ	CN.b.65	Carbon-to-nitrogen ratio of flag leaf blade at Zadoks 65	g C g N^{-1}
	CN.s.65	Carbon-to-nitrogen ratio of flag leaf sheath at Zadoks 65	g C g N^{-1}
	CN.p.65	Carbon-to-nitrogen ratio of peduncle at Zadoks 65	g C g N^{-1}
	CN.a.65	Carbon-to-nitrogen ratio of awn at Zadoks 65	g C g N^{-1}
	CN.g.65	Carbon-to-nitrogen ratio of glume at Zadoks 65	g C g N^{-1}
	CN.l.65	Carbon-to-nitrogen ratio of lemma at Zadoks 65	g C g N^{-1}
	CN.b.75	Carbon-to-nitrogen ratio of flag leaf blade at Zadoks 75	g C g N^{-1}
	CN.s.75	Carbon-to-nitrogen ratio of flag leaf sheath at Zadoks 75	g C g N^{-1}
	CN.p.75	Carbon-to-nitrogen ratio of peduncle at Zadoks 75	g C g N^{-1}
	CN.a.75	Carbon-to-nitrogen ratio of awn at Zadoks 75	g C g N^{-1}
	CN.g.75	Carbon-to-nitrogen ratio of glume at Zadoks 75	g C g N^{-1}
	CN.l.75	Carbon-to-nitrogen ratio of lemma at Zadoks 75	g C g N^{-1}
	K.b.65	Potassium content of flag leaf blade at Zadoks 65	g kg DW^{-1}
	K.s.65	Potassium content of flag leaf sheath at Zadoks 65	g kg DW^{-1}
	K.p.65	Potassium content of peduncle at Zadoks 65	g kg DW^{-1}
	K.a.65	Potassium content of awn at Zadoks 65	g kg DW^{-1}
	K.g.65	Potassium content of glume at Zadoks 65	g kg DW^{-1}
	K.l.65	Potassium content of lemma at Zadoks 65	g kg DW^{-1}
Nutrient composition per organ	K.b.75	Potassium content of flag leaf blade at Zadoks 75	g kg DW^{-1}
	K.s.75	Potassium content of flag leaf sheath at Zadoks 75	g kg DW^{-1}
	K.p.75	Potassium content of peduncle at Zadoks 75	g kg DW^{-1}
	K.a.75	Potassium content of awn at Zadoks 75	g kg DW^{-1}
	K.g.75	Potassium content of glume at Zadoks 75	g kg DW^{-1}
	K.l.75	Potassium content of lemma at Zadoks 75	g kg DW^{-1}
	Ca.b.65	Calcium content of flag leaf blade at Zadoks 65	g kg DW^{-1}
	Ca.s.65	Calcium content of flag leaf sheath at Zadoks 65	g kg DW^{-1}
	Ca.p.65	Calcium content of peduncle at Zadoks 65	g kg DW^{-1}
	Ca.a.65	Calcium content of awn at Zadoks 65	g kg DW^{-1}
	Ca.g.65	Calcium content of glume at Zadoks 65	g kg DW^{-1}
	Ca.l.65	Calcium content of lemma at Zadoks 65	g kg DW^{-1}
	Ca.b.75	Calcium content of flag leaf blade at Zadoks 75	g kg DW^{-1}
	Ca.s.75	Calcium content of flag leaf sheath at Zadoks 75	g kg DW^{-1}
	Ca.p.75	Calcium content of peduncle at Zadoks 75	g kg DW^{-1}
	Ca.a.75	Calcium content of awn at Zadoks 75	g kg DW^{-1}
	Ca.g.75	Calcium content of glume at Zadoks 75	g kg DW^{-1}
	Ca.l.75	Calcium content of lemma at Zadoks 75	g kg DW^{-1}
	P.b.65	Phosphorus content of flag leaf blade at Zadoks 65	g kg DW^{-1}
	P.s.65	Phosphorus content of flag leaf sheath at Zadoks 65	g kg DW^{-1}
	P.p.65	Phosphorus content of peduncle at Zadoks 65	g kg DW^{-1}
	P.a.65	Phosphorus content of awn at Zadoks 65	g kg DW^{-1}
	P.g.65	Phosphorus content of glume at Zadoks 65	g kg DW^{-1}
	P.l.65	Phosphorus content of lemma at Zadoks 65	g kg DW^{-1}
	P.b.75	Phosphorus content of flag leaf blade at Zadoks 75	g kg DW^{-1}
	P.s.75	Phosphorus content of flag leaf sheath at Zadoks 75	g kg DW^{-1}
	P.p.75	Phosphorus content of peduncle at Zadoks 75	g kg DW^{-1}

P.a.75	Phosphorus content of awn at Zadoks 75	g kg DW^{-1}
P.g.75	Phosphorus content of glume at Zadoks 75	g kg DW^{-1}
P.l.75	Phosphorus content of lemma at Zadoks 75	g kg DW^{-1}
Mg.b.65	Magnesium content of flag leaf blade at Zadoks 65	g kg DW^{-1}
Mg.s.65	Magnesium content of flag leaf sheath at Zadoks 65	g kg DW^{-1}
Mg.p.65	Magnesium content of peduncle at Zadoks 65	g kg DW^{-1}
Mg.a.65	Magnesium content of awn at Zadoks 65	g kg DW^{-1}
Mg.g.65	Magnesium content of glume at Zadoks 65	g kg DW^{-1}
Mg.l.65	Magnesium content of lemma at Zadoks 65	g kg DW^{-1}
Mg.b.75	Magnesium content of flag leaf blade at Zadoks 75	g kg DW^{-1}
Mg.s.75	Magnesium content of flag leaf sheath at Zadoks 75	g kg DW^{-1}
Mg.p.75	Magnesium content of peduncle at Zadoks 75	g kg DW^{-1}
Mg.a.75	Magnesium content of awn at Zadoks 75	g kg DW^{-1}
Mg.g.75	Magnesium content of glume at Zadoks 75	g kg DW^{-1}
Mg.l.75	Magnesium content of lemma at Zadoks 75	g kg DW^{-1}
Fe.b.65	Iron content of flag leaf blade at Zadoks 65	mg kg DW^{-1}
Fe.s.65	Iron content of flag leaf sheath at Zadoks 65	mg kg DW^{-1}
Fe.p.65	Iron content of peduncle at Zadoks 65	mg kg DW^{-1}
Fe.a.65	Iron content of awn at Zadoks 65	mg kg DW^{-1}
Fe.g.65	Iron content of glume at Zadoks 65	mg kg DW^{-1}
Fe.l.65	Iron content of lemma at Zadoks 65	mg kg DW^{-1}

Table S6.1. Continued.

Group	Trait	Description	Units
Nutrient composition per organ	Fe.b.75	Iron content of flag leaf blade at Zadoks 75	mg kg DW^{-1}
	Fe.s.75	Iron content of flag leaf sheath at Zadoks 75	mg kg DW^{-1}
	Fe.p.75	Iron content of peduncle at Zadoks 75	mg kg DW^{-1}
	Fe.a.75	Iron content of awn at Zadoks 75	mg kg DW^{-1}
	Fe.g.75	Iron content of glume at Zadoks 75	mg kg DW^{-1}
	Fe.l.75	Iron content of lemma at Zadoks 75	mg kg DW^{-1}
Nutrient composition per organ	Mn.b.65	Manganese content of flag leaf blade at Zadoks 65	mg kg DW^{-1}
	Mn.s.65	Manganese content of flag leaf sheath at Zadoks 65	mg kg DW^{-1}
	Mn.p.65	Manganese content of peduncle at Zadoks 65	mg kg DW^{-1}
	Mn.a.65	Manganese content of awn at Zadoks 65	mg kg DW^{-1}
	Mn.g.65	Manganese content of glume at Zadoks 65	mg kg DW^{-1}
	Mn.l.65	Manganese content of lemma at Zadoks 65	mg kg DW^{-1}
	Mn.b.75	Manganese content of flag leaf blade at Zadoks 75	mg kg DW^{-1}
	Mn.s.75	Manganese content of flag leaf sheath at Zadoks 75	mg kg DW^{-1}
	Mn.p.75	Manganese content of peduncle at Zadoks 75	mg kg DW^{-1}
	Mn.a.75	Manganese content of awn at Zadoks 75	mg kg DW^{-1}
	Mn.g.75	Manganese content of glume at Zadoks 75	mg kg DW^{-1}
	Mn.l.75	Manganese content of lemma at Zadoks 75	mg kg DW^{-1}
	Cu.b.65	Cupper content of flag leaf blade at Zadoks 65	mg kg DW^{-1}
	Cu.s.65	Cupper content of flag leaf sheath at Zadoks 65	mg kg DW^{-1}
	Cu.p.65	Cupper content of peduncle at Zadoks 65	mg kg DW^{-1}
	Cu.a.65	Cupper content of awn at Zadoks 65	mg kg DW^{-1}
	Cu.g.65	Cupper content of glume at Zadoks 65	mg kg DW^{-1}
	Cu.l.65	Cupper content of lemma at Zadoks 65	mg kg DW^{-1}
	Cu.b.75	Cupper content of flag leaf blade at Zadoks 75	mg kg DW^{-1}
	Cu.s.75	Cupper content of flag leaf sheath at Zadoks 75	mg kg DW^{-1}
	Cu.p.75	Cupper content of peduncle at Zadoks 75	mg kg DW^{-1}
	Cu.a.75	Cupper content of awn at Zadoks 75	mg kg DW^{-1}
	Cu.g.75	Cupper content of glume at Zadoks 75	mg kg DW^{-1}
	Cu.l.75	Cupper content of lemma at Zadoks 75	mg kg DW^{-1}
	C.grain.75	Carbon content of grain at Zadoks 75	g kg DW^{-1}
	C.grain.85	Carbon content of grain at Zadoks 85	g kg DW^{-1}
	C.grain.92	Carbon content of grain at Zadoks 92	g kg DW^{-1}
	N.grain.75	Nitrogen content of grain at Zadoks 75	g kg DW^{-1}
	N.grain.85	Nitrogen content of grain at Zadoks 85	g kg DW^{-1}
	N.grain.92	Nitrogen content of grain at Zadoks 92	g kg DW^{-1}
	CN.grain.75	Carbon-to-nitrogen ratio of grain at Zadoks 75	g C g N^{-1}
	CN.grain.85	Carbon-to-nitrogen ratio of grain at Zadoks 85	g C g N^{-1}
	CN.grain.92	Carbon-to-nitrogen ratio of grain at Zadoks 92	g C g N^{-1}
	K.grain.92	Potassium content of grain at Zadoks 92	g kg DW^{-1}
	P.grain.92	Phosphorus content of grain at Zadoks 92	g kg DW^{-1}
	S.grain.92	Sulphur content of grain at Zadoks 92	g kg DW^{-1}
	Mg.grain.92	Magnesium content of grain at Zadoks 92	g kg DW^{-1}
	Ca.grain.92	Calcium content of grain at Zadoks 92	g kg DW^{-1}
	Mn.grain.92	Manganese content of grain at Zadoks 92	mg kg DW^{-1}

	Fe.grain.92	Iron content of grain at Zadoks 92	mg kg DW^{-1}
	Na.grain.92	Sodium content of grain at Zadoks 92	mg kg DW^{-1}
	Zn.grain.92	Zinc content of grain at Zadoks 92	mg kg DW^{-1}
	Cu.grain.92	Cupper content of grain at Zadoks 92	mg kg DW^{-1}
	Mo.grain.92	Molybdenum content of grain at Zadoks 92	mg kg DW^{-1}
Nutrient and protein yield	GCY	Grain carbon yield	kg ha^{-1}
	GNY	Grain nitrogen yield	kg ha^{-1}
	GKY	Grain potassium yield	kg ha^{-1}
	GPY	Grain phosphorus yield	kg ha^{-1}
	GSY	Grain sulphur yield	kg ha^{-1}
	GMgY	Grain magnesium yield	kg ha^{-1}
	GCaY	Grain calcium yield	kg ha^{-1}
	GMnY	Grain manganese yield	kg ha^{-1}
	GFeY	Grain iron yield	kg ha^{-1}
	GNaY	Grain sodium yield	kg ha^{-1}
	GZnY	Grain zinc yield	kg ha^{-1}
	GCuY	Grain cupper yield	g ha^{-1}
	GMoY	Grain molybdenum yield	g ha^{-1}
	GProtY	Grain protein yield	kg ha^{-1}

Table S6.1. Continued.

Group	Trait	Description	Units
Carbon and nitrogen isotope composition per organ	d13C.b.65	Carbon isotope composition ($\delta^{13}C$) of flag leaf blade at Zadoks 65	‰
	d13C.s.65	Carbon isotope composition ($\delta^{13}C$) of flag leaf sheath at Zadoks 65	‰
	d13C.p.65	Carbon isotope composition ($\delta^{13}C$) of peduncle at Zadoks 65	‰
	d13C.a.65	Carbon isotope composition ($\delta^{13}C$) of awn at Zadoks 65	‰
	d13C.g.65	Carbon isotope composition ($\delta^{13}C$) of glume at Zadoks 65	‰
	d13C.l.65	Carbon isotope composition ($\delta^{13}C$) of lemma at Zadoks 65	‰
	d13C.b.75	Carbon isotope composition ($\delta^{13}C$) of flag leaf blade at Zadoks 75	‰
	d13C.s.75	Carbon isotope composition ($\delta^{13}C$) of flag leaf sheath at Zadoks 75	‰
	d13C.p.75	Carbon isotope composition ($\delta^{13}C$) of peduncle at Zadoks 75	‰
	d13C.a.75	Carbon isotope composition ($\delta^{13}C$) of awn at Zadoks 75	‰
	d13C.g.75	Carbon isotope composition ($\delta^{13}C$) of glume at Zadoks 75	‰
	d13C.l.75	Carbon isotope composition ($\delta^{13}C$) of lemma at Zadoks 75	‰
	d13C.grain.75	Carbon isotope composition ($\delta^{13}C$) of grain at Zadoks 75	‰
	d13C.grain.85	Carbon isotope composition ($\delta^{13}C$) of grain at Zadoks 85	‰
	d13C.grain.92	Carbon isotope composition ($\delta^{13}C$) of grain at Zadoks 92	‰
	d15N.b.65	Nitrogen isotope composition ($\delta^{15}N$) of flag leaf blade at Zadoks 65	‰
	d15N.s.65	Nitrogen isotope composition ($\delta^{15}N$) of flag leaf sheath at Zadoks 65	‰
	d15N.p.65	Nitrogen isotope composition ($\delta^{15}N$) of peduncle at Zadoks 65	‰
	d15N.a.65	Nitrogen isotope composition ($\delta^{15}N$) of awn at Zadoks 65	‰
	d15N.g.65	Nitrogen isotope composition ($\delta^{15}N$) of glume at Zadoks 65	‰
	d15N.l.65	Nitrogen isotope composition ($\delta^{15}N$) of lemma at Zadoks 65	‰
	d15N.b.75	Nitrogen isotope composition ($\delta^{15}N$) of flag leaf blade at Zadoks 75	‰
	d15N.s.75	Nitrogen isotope composition ($\delta^{15}N$) of flag leaf sheath at Zadoks 75	‰
	d15N.p.75	Nitrogen isotope composition ($\delta^{15}N$) of peduncle at Zadoks 75	‰
	d15N.a.75	Nitrogen isotope composition ($\delta^{15}N$) of awn at Zadoks 75	‰
	d15N.b.75	Nitrogen isotope composition ($\delta^{15}N$) of glume at Zadoks 75	‰
	d15N.l.75	Nitrogen isotope composition ($\delta^{15}N$) of lemma at Zadoks 75	‰
	d15N.grain75	Nitrogen isotope composition ($\delta^{15}N$) of grain at Zadoks 75	‰
	d15N.grain.85	Nitrogen isotope composition ($\delta^{15}N$) of grain at Zadoks 85	‰
	d15N.grain.92	Nitrogen isotope composition ($\delta^{15}N$) of grain at Zadoks 92	‰

Table S6.2. Effects of N supply and genotypic variability on agronomic, physiological and metabolic traits analysed in field-grown durum wheat. Values are means ± SEM (n = 3) per N (control vs. low N) and variety (Kiko Nick, KNI; Don Ricardo, DRI; Euroduro, EUR; Haristide, HAR) combination. The means in each row with different letters differ statistically ($P < 0.05$; two-way ANOVA, TUKEY test). The colour scale indicates the minimum (darkest red) and maximum (darkest blue) values per trait. The abbreviations are described in Supplementary Table 1.

Trait	Control				Low N				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
GY	6061 ± 215^{ad}	6465 ± 773^{abc}	7130 ± 411^{ab}	7571 ± 50^{a}	4227 ± 437^{d}	5063 ± 282^{cd}	5394 ± 461^{bd}	6479 ± 289^{abc}	**<0.001**	**0.003**	0.804
biomass	14453 ± 694^{a}	12973 ± 827^{ab}	13053 ± 488^{ab}	15493 ± 653^{a}	8080 ± 711^{c}	12467 ± 1266^{ab}	9907 ± 1144^{bc}	11680 ± 1058^{ac}	**<0.001**	0.062	**0.035**
HI	0.420 ± 0.008^{b}	0.498 ± 0.046^{ab}	0.545 ± 0.011^{a}	0.490 ± 0.019^{ab}	0.523 ± 0.026^{ab}	0.410 ± 0.018^{b}	0.548 ± 0.018^{a}	0.559 ± 0.025^{a}	0.222	**0.004**	**0.006**
plants.m2	211 ± 20^{a}	189 ± 15^{ab}	161 ± 15^{ac}	215 ± 13^{a}	161 ± 21^{ac}	115 ± 7^{c}	130.7 ± 9^{bc}	147 ± 5^{ac}	**<0.001**	**0.026**	0.427
ears.plant	1.91 ± 0.23^{b}	1.82 ± 0.14^{b}	2.20 ± 0.24^{b}	1.80 ± 0.06^{b}	1.99 ± 0.17^{b}	3.05 ± 0.17^{a}	2.49 ± 0.19^{ab}	1.90 ± 0.11^{b}	**0.003**	**0.009**	**0.013**
grains.ear	28.3 ± 3.2^{ab}	31.7 ± 0.7^{ab}	34.0 ± 2.1^{ab}	36.1 ± 2.4^{a}	25.5 ± 1.0^{b}	31.9 ± 2.5^{ab}	30.1 ± 2.1^{ab}	38.1 ± 0.8^{a}	0.450	**0.001**	0.472
TGW	47.3 ± 2.1^{ab}	48.9 ± 1.1^{ab}	45.7 ± 1.3^{ab}	43.6 ± 0.2^{b}	48.7 ± 0.2^{ab}	50.6 ± 1.9^{ab}	49.9 ± 1.9^{ab}	52.5 ± 1.7^{a}	**0.001**	0.530	0.074
ped.length	38.5 ± 1.4^{ab}	38.9 ± 1.3^{a}	36.8 ± 0.1^{ab}	37.2 ± 0.1^{ab}	34.3 ± 1.5^{ab}	35.2 ± 0.8^{ab}	34.9 ± 1.1^{ab}	34.0 ± 0.2^{b}	**<0.001**	0.476	0.665
ear.length	5.97 ± 0.32^{b}	6.53 ± 0.03^{b}	5.82 ± 0.24^{b}	9.59 ± 0.12^{a}	5.07 ± 0.08^{b}	6.57 ± 0.53^{b}	6.31 ± 0.76^{b}	8.83 ± 0.22^{a}	0.296	**<0.001**	0.226
height	83.8 ± 0.3^{a}	84.8 ± 1.9^{a}	81.5 ± 1.9^{ab}	82.0 ± 1.8^{ab}	72.0 ± 2.1^{b}	72.7 ± 3.8^{b}	75.0 ± 0.6^{ab}	73.0 ± 2.1^{b}	**<0.001**	0.940	0.495
prot.grain	14.6 ± 1.7	14.3 ± 1.3	13.8 ± 1.6	13.5 ± 0.9	14.1 ± 1.5	14.2 ± 0.6	13.7 ± 1.1	13.4 ± 0.2	0.827	0.873	0.997
moisture.grain	11.3 ± 0.1^{ab}	11.0 ± 0.1^{ab}	11.2 ± 0.2^{ab}	10.9 ± 0.1^{b}	11.6 ± 0.1^{a}	11.3 ± 0.1^{ab}	11.4 ± 0.1^{ab}	11.6 ± 0.1^{a}	**0.001**	0.172	0.299
SW.grain	78.5 ± 0.6^{ab}	81.1 ± 0.9^{ab}	82.4 ± 0.5^{a}	80.5 ± 0.1^{ab}	78.0 ± 1.8^{b}	79.8 ± 0.2^{ab}	81.4 ± 0.8^{ab}	78.7 ± 0.5^{ab}	0.065	**0.004**	0.883
vitreo.grain	92.2 ± 5.1	97.3 ± 1.5	93.0 ± 4.0	77.2 ± 16.9	79.0 ± 9.1	95.8 ± 1.0	78.2 ± 6.3	69.2 ± 1.2	0.097	0.050	0.813
sedim.grain	34.3 ± 1.4^{ab}	30.3 ± 1.3^{b}	39.3 ± 1.2^{a}	37.7 ± 1.9^{a}	14.0 ± 0.3^{c}	12.9 ± 0.3^{c}	13.9 ± 0.3^{c}	14.2 ± 0.1^{c}	**<0.001**	**0.001**	**0.009**
b.grain	15.2 ± 0.1^{c}	13.9 ± 0.3^{c}	15.1 ± 0.3^{c}	16.6 ± 0.1^{c}	34.7 ± 0.7^{a}	29.7 ± 0.9^{b}	37.7 ± 1.8^{a}	38.7 ± 0.9^{a}	**<0.001**	**<0.001**	**0.003**
WG.grain	27.9 ± 3.8	26.6 ± 3.7	25.0 ± 5.9	26.9 ± 2.9	25.5 ± 3.9	25.9 ± 1.8	27.3 ± 2.9	26.2 ± 0.58	0.883	0.999	0.924
GI.grain	68.5 ± 2.5^{ac}	70.9 ± 5.0^{ab}	86.0 ± 4.4^{a}	40.4 ± 8.8^{c}	65.2 ± 10.9^{ac}	68.5 ± 5.0^{ac}	77.6 ± 2.5^{a}	44.3 ± 1.4^{bc}	0.552	**<0.001**	0.781
GA.15	0.094 ± 0.013	0.066 ± 0.004	0.085 ± 0.015	0.066 ± 0.004	0.063 ± 0.024	0.070 ± 0.014	0.055 ± 0.014	0.046 ± 0.012	0.070	0.462	0.564
GA.25	0.425 ± 0.050	0.306 ± 0.027	0.360 ± 0.059	0.373 ± 0.024	0.319 ± 0.087	0.268 ± 0.063	0.261 ± 0.062	0.273 ± 0.029	**0.040**	0.480	0.912
GA.35	0.709 ± 0.049	0.693 ± 0.056	0.728 ± 0.055	0.679 ± 0.063	0.489 ± 0.076	0.540 ± 0.066	0.508 ± 0.029	0.489 ± 0.026	**<0.001**	0.911	0.913
GA.55	0.801 ± 0.042	0.801 ± 0.062	0.793 ± 0.047	0.802 ± 0.047	0.615 ± 0.042	0.645 ± 0.068	0.687 ± 0.040	0.645 ± 0.019	**<0.001**	0.925	0.864
GA.65	0.776 ± 0.020	0.761 ± 0.048	0.811 ± 0.047	0.841 ± 0.031	0.657 ± 0.062	0.717 ± 0.054	0.779 ± 0.028	0.781 ± 0.024	**0.046**	0.115	0.737
GA.75	0.619 ± 0.064^{ac}	0.620 ± 0.071^{ac}	0.702 ± 0.057^{ab}	0.882 ± 0.017^{a}	0.353 ± 0.075^{c}	0.475 ± 0.114^{bc}	0.472 ± 0.061^{bc}	0.668 ± 0.025^{ac}	**<0.001**	**0.003**	0.829
GGA.15	0.052 ± 0.008	0.036 ± 0.001	0.046 ± 0.009	0.029 ± 0.003	0.037 ± 0.016	0.045 ± 0.011	0.031 ± 0.008	0.022 ± 0.009	0.289	0.245	0.518
GGA.25	0.161 ± 0.035	0.115 ± 0.014	0.142 ± 0.035	0.152 ± 0.014	0.116 ± 0.043	0.094 ± 0.037	0.091 ± 0.029	0.097 ± 0.021	0.059	0.712	0.939
GGA.35	0.491 ± 0.052^{ab}	0.495 ± 0.078^{ab}	0.539 ± 0.053^{a}	0.472 ± 0.076^{ab}	0.257 ± 0.050^{b}	0.329 ± 0.065^{ab}	0.302 ± 0.015^{ab}	0.276 ± 0.021^{ab}	**<0.001**	0.758	0.905
GGA.55	0.667 ± 0.056	0.670 ± 0.091	0.660 ± 0.062	0.675 ± 0.057	0.418 ± 0.035	0.483 ± 0.080	0.530 ± 0.053	0.476 ± 0.032	**<0.001**	0.857	0.811
GGA.65	0.577 ± 0.053	0.608 ± 0.056	0.609 ± 0.070	0.649 ± 0.040	0.541 ± 0.057	0.616 ± 0.052	0.657 ± 0.033	0.680 ± 0.019	0.717	0.234	0.849
GGA.75	0.239 ± 0.061^{ab}	0.234 ± 0.067^{ab}	0.256 ± 0.047^{ab}	0.467 ± 0.029^{a}	0.116 ± 0.056^{b}	0.168 ± 0.063^{b}	0.137 ± 0.038^{b}	0.305 ± 0.033^{ab}	**0.005**	**0.003**	0.825
CSI.15	45.0 ± 1.2	45.5 ± 1.8	45.8 ± 2.1	56.4 ± 2.9	47.5 ± 7.0	37.6 ± 3.6	44.5 ± 1.0	53.6 ± 9.2	0.468	0.052	0.726
CSI.25	62.7 ± 3.7	62.6 ± 1.4	61.9 ± 3.8	59.3 ± 1.2	66.4 ± 5.4	67.3 ± 5.3	67.0 ± 4.0	65.3 ± 3.6	0.091	0.900	0.992
CSI.35	31.2 ± 2.5^{ab}	29.4 ± 5.2^{b}	26.3 ± 1.7^{b}	31.3 ± 4.5^{ab}	48.0 ± 2.6^{a}	40.3 ± 5.9^{ab}	40.5 ± 0.9^{ab}	43.6 ± 2.3^{ab}	**<0.001**	0.350	0.856
CSI.55	17.0 ± 2.9	17.2 ± 4.7	17.1 ± 3.1	16.1 ± 2.3	32.2 ± 1.3	26.1 ± 5.0	23.3 ± 3.3	26.4 ± 3.1	**0.001**	0.613	0.608
CSI.65	25.8 ± 5.2	20.4 ± 2.6	25.4 ± 4.6	23.0 ± 2.4	17.6 ± 3.4	14.1 ± 2.0	15.7 ± 1.6	12.9 ± 0.5	**0.001**	0.451	0.925
CSI.75	62.5 ± 6.0	63.8 ± 8.1	63.9 ± 5.0	47.1 ± 2.6	70.8 ± 9.0	67.0 ± 6.2	72.0 ± 4.0	54.5 ± 4.1	0.127	**0.036**	0.969

Table S6.2. Continued.

Trait	Control				Low N				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
NDVI.25	0.540 ± 0.045	0.530 ± 0.031	0.490 ± 0.031	0.500 ± 0.050	0.420 ± 0.068	0.400 ± 0.031	0.423 ± 0.037	0.423 ± 0.034	**0.005**	0.952	0.846
NDVI.35	0.633 ± 0.030	0.633 ± 0.039	0.587 ± 0.032	0.607 ± 0.042	0.497 ± 0.075	0.497 ± 0.049	0.510 ± 0.015	0.470 ± 0.010	**0.001**	0.892	0.850
NDVI.55	0.733 ± 0.032^{ab}	0.723 ± 0.038^{ab}	0.693 ± 0.012^{ab}	0.767 ± 0.027^{a}	0.510 ± 0.047^{b}	0.573 ± 0.087^{ab}	0.583 ± 0.069^{ab}	0.527 ± 0.035^{b}	**<0.001**	0.944	0.518
NDVI.65	0.667 ± 0.018^{ab}	0.637 ± 0.023^{ab}	0.643 ± 0.024^{ab}	0.713 ± 0.009^{a}	0.533 ± 0.026^{b}	0.560 ± 0.067^{ab}	0.620 ± 0.023^{ab}	0.633 ± 0.029^{ab}	**0.003**	0.099	0.414
NDVI.75	0.583 ± 0.032^{ab}	0.557 ± 0.037^{ab}	0.590 ± 0.025^{ab}	0.690 ± 0.006^{a}	0.450 ± 0.046^{b}	0.473 ± 0.033^{b}	0.577 ± 0.033^{ab}	0.590 ± 0.021^{ab}	**0.002**	**0.003**	0.306
NDVI.85	0.237 ± 0.027^{ab}	0.200 ± 0.021^{b}	0.213 ± 0.032^{ab}	0.353 ± 0.049^{a}	0.233 ± 0.038^{ab}	0.220 ± 0.021^{ab}	0.223 ± 0.034^{ab}	0.350 ± 0.010^{ab}	0.794	**0.001**	0.977
chl.65	37.8 ± 4.9	43.4 ± 1.2	46.1 ± 2.7	43.8 ± 4.1	42.2 ± 2.6	42.7 ± 1.7	43.2 ± 2.2	40.7 ± 2.4	0.793	0.491	0.568
chl.75	43.2 ± 2.3	43.3 ± 1.7	47.8 ± 3.0	46.9 ± 3.6	43.9 ± 3.7	47.0 ± 1.4	49.6 ± 4.3	45.94 ± 2.38	0.545	0.380	0.876
flav.65	1.64 ± 0.08^{ac}	1.60 ± 0.07^{ac}	1.47 ± 0.04^{bc}	1.40 ± 0.05^{c}	1.73 ± 0.04^{a}	1.68 ± 0.03^{ab}	1.56 ± 0.05^{ac}	1.47 ± 0.02^{bc}	**0.040**	**0.001**	0.998
flav.75	1.63 ± 0.03^{ab}	1.64 ± 0.09^{ab}	1.52 ± 0.10^{ab}	1.42 ± 0.08^{b}	1.76 ± 0.03^{a}	1.67 ± 0.02^{ab}	1.62 ± 0.03^{ab}	1.42 ± 0.05^{b}	0.143	**0.002**	0.692
anth.65	0.113 ± 0.006	0.118 ± 0.004	0.121 ± 0.009	0.113 ± 0.008	0.123 ± 0.007	0.117 ± 0.005	0.111 ± 0.007	0.127 ± 0.002	0.456	0.949	0.269
anth.75	0.106 ± 0.002	0.114 ± 0.006	0.082 ± 0.013	0.103 ± 0.010	0.107 ± 0.015	0.095 ± 0.009	0.086 ± 0.006	0.109 ± 0.006	0.751	0.067	0.519
NBI.65	27.4 ± 1.2	27.4 ± 2.0	31.6 ± 2.6	31.5 ± 2.2	24.6 ± 2.1	25.8 ± 1.4	28.0 ± 2.3	28.0 ± 1.7	0.057	0.146	0.956
NBI.75	26.9 ± 1.1	27.1 ± 2.8	33.8 ± 2.7	35.9 ± 4.5	25.0 ± 2.0	28.3 ± 1.1	30.9 ± 3.2	32.9 ± 2.7	0.406	**0.022**	0.851
LRWC.65	68.6 ± 2.7^{bcd}	65.4 ± 1.5^{cd}	82.5 ± 0.1^{a}	79.5 ± 1.5^{a}	64.6 ± 2.8^{cd}	62.1 ± 2.9^{d}	76.7 ± 2.3^{ab}	74.4 ± 1.7^{ac}	**0.008**	**<0.001**	0.937
LRWC.75	68.9 ± 5.7	71.3 ± 4.2	68.9 ± 1.2	66.3 ± 1.3	60.0 ± 2.3	57.0 ± 2.6	68.0 ± 1.6	60.8 ± 1.7	**0.003**	0.366	0.184
FW.b.65	1.67 ± 0.14^{ab}	1.54 ± 0.09^{ab}	1.41 ± 0.15^{ab}	2.01 ± 0.18^{a}	1.20 ± 0.06^{b}	1.59 ± 0.14^{ab}	1.28 ± 0.13^{b}	1.62 ± 0.10^{ab}	**0.020**	**0.011**	0.206
FW.s.65	2.07 ± 0.19	2.11 ± 0.23	1.45 ± 0.11	1.90 ± 0.24	1.86 ± 0.11	2.03 ± 0.07	1.45 ± 0.10	1.91 ± 0.11	0.540	**0.006**	0.881
FW.p.65	5.65 ± 0.44	5.14 ± 0.36	4.55 ± 0.35	5.51 ± 0.63	4.92 ± 0.54	5.08 ± 0.38	4.45 ± 0.33	5.16 ± 0.29	0.316	0.227	0.856
FW.ear.65	6.95 ± 0.68	6.53 ± 0.43	5.92 ± 0.29	6.18 ± 0.97	6.28 ± 0.56	7.47 ± 0.21	6.13 ± 0.45	7.18 ± 0.37	0.348	0.370	0.407
FW.b.75	2.09 ± 0.23^{ab}	2.10 ± 0.19^{ab}	1.95 ± 0.15^{b}	2.77 ± 0.16^{a}	1.55 ± 0.07^{b}	1.85 ± 0.13^{b}	1.56 ± 0.08^{b}	2.03 ± 0.14^{ab}	**<0.001**	**0.003**	0.448
FW.s.75	2.23 ± 0.28	2.20 ± 0.12	1.91 ± 0.12	2.58 ± 0.15	2.04 ± 0.08	2.33 ± 0.23	1.91 ± 0.16	2.30 ± 0.16	0.500	**0.046**	0.651
FW.p.75	5.48 ± 0.82	5.49 ± 0.48	5.42 ± 0.41	6.87 ± 0.28	5.29 ± 0.77	5.88 ± 0.40	5.07 ± 0.16	6.10 ± 0.39	0.532	0.111	0.737
FW.ear.75	19.4 ± 3.0	21.4 ± 2.5	19.1 ± 1.8	20.1 ± 1.3	19.4 ± 1.9	24.2 ± 2.3	20.9 ± 1.9	22.4 ± 1.8	0.262	0.413	0.918
DW.b.65	0.529 ± 0.048	0.580 ± 0.035	0.497 ± 0.039	0.676 ± 0.061	0.445 ± 0.027	0.634 ± 0.048	0.560 ± 0.126	0.562 ± 0.026	0.639	0.121	0.345
DW.s.65	0.703 ± 0.106	0.910 ± 0.083	0.633 ± 0.052	0.777 ± 0.069	0.826 ± 0.051	0.921 ± 0.044	0.649 ± 0.035	0.821 ± 0.058	0.312	**0.007**	0.818
DW.p.65	1.81 ± 0.18	1.48 ± 0.07	1.37 ± 0.16	1.60 ± 0.06	1.79 ± 0.14	1.56 ± 0.03	1.68 ± 0.07	1.54 ± 0.03	0.327	**0.048**	0.325
DW.ear.65	2.57 ± 0.28	2.50 ± 0.15	2.31 ± 0.10	2.30 ± 0.34	2.41 ± 0.22	2.82 ± 0.16	2.33 ± 0.14	2.72 ± 0.16	0.333	0.463	0.520
DW.b.75	0.674 ± 0.083^{ab}	0.755 ± 0.062^{ab}	0.654 ± 0.051^{ab}	0.851 ± 0.044^{a}	0.605 ± 0.020^{ab}	0.768 ± 0.062^{ab}	0.585 ± 0.030^{b}	0.782 ± 0.053^{ab}	0.221	**0.005**	0.833
DW.s.75	0.963 ± 0.136	0.984 ± 0.056	0.798 ± 0.087	1.035 ± 0.038	0.915 ± 0.017	1.082 ± 0.102	0.847 ± 0.068	1.015 ± 0.061	0.727	0.056	0.785
DW.p.75	2.38 ± 0.36	2.32 ± 0.19	2.25 ± 0.22	2.93 ± 0.10	2.26 ± 0.34	2.54 ± 0.19	2.21 ± 0.10	2.89 ± 0.13	0.966	**0.035**	0.883
DW.ear.75	8.74 ± 1.47	9.43 ± 0.98	8.46 ± 0.84	8.25 ± 0.60	9.01 ± 1.01	11.36 ± 1.21	10.01 ± 0.87	9.90 ± 0.90	0.078	0.460	0.851
DW.b.85	0.537 ± 0.028	0.746 ± 0.119	0.881 ± 0.228	0.884 ± 0.097	0.441 ± 0.007	0.637 ± 0.0870	0.561 ± 0.0316	0.649 ± 0.149	**0.034**	0.123	0.736
DW.s.85	1.47 ± 0.15^{ab}	1.41 ± 0.13^{ab}	1.38 ± 0.18^{ab}	1.86 ± 0.16^{a}	0.88 ± 0.01^{b}	1.13 ± 0.05^{b}	0.97 ± 0.13^{b}	0.88 ± 0.13^{b}	**<0.001**	0.411	0.080
DW.p.85	4.33 ± 0.30^{a}	3.63 ± 0.18^{ab}	3.76 ± 0.07^{a}	4.81 ± 0.31^{a}	2.11 ± 0.20^{c}	2.13 ± 0.10^{c}	2.15 ± 0.25^{c}	2.35 ± 0.51^{bc}	**<0.001**	0.080	0.267
DW.ear.85	13.6 ± 1.2^{b}	16.2 ± 1.1^{ab}	14.3 ± 0.8^{ab}	18.2 ± 1.4^{a}	12.1 ± 0.7^{b}	18.5 ± 0.6^{a}	15.3 ± 0.2^{ab}	14.9 ± 0.8^{ab}	0.560	**0.001**	**0.034**
glc.b.65	2.86 ± 1.0	3.11 ± 0.29	2.68 ± 0.02	3.69 ± 0.38	2.92 ± 0.35	3.29 ± 0.81	2.37 ± 0.25	3.03 ± 0.29	0.625	0.400	0.838
glc.s.65	19.7 ± 4.0	25.3 ± 18.4	7.5 ± 0.2	23.4 ± 5.4	20.8 ± 8.0	20.4 ± 10.9	6.7 ± 1.5	10.8 ± 0.9	0.484	0.297	0.857
glc.p.65	83.8 ± 15.2^{ab}	105.5 ± 5.1^{a}	27.8 ± 8.3^{b}	59.9 ± 24.8^{ab}	81.6 ± 20.5^{ab}	76.2 ± 15.5^{ab}	20.0 ± 13.4^{b}	70.1 ± 7.3^{ab}	0.507	**0.002**	0.628
glc.a.65	30.8 ± 5.8	17.9 ± 2.7	20.9 ± 1.8	18.9 ± 1.1	26.5 ± 2.7	20.3 ± 4.5	20.5 ± 5.1	16.9 ± 1.6	0.673	**0.035**	0.819
glc.g.65	21.9 ± 6.5	20.2 ± 5.5	27.2 ± 3.4	22.1 ± 1.3	18.6 ± 3.9	22.0 ± 5.1	23.9 ± 3.8	17.2 ± 3.4	0.446	0.539	0.880
glc.l.65	30.6 ± 5.5	27.9 ± 3.6	21.6 ± 1.2	29.8 ± 3.1	26.1 ± 4.8	26.2 ± 3.8	20.1 ± 3.5	24.4 ± 2.7	0.228	0.220	0.936

Table S6.2. Continued.

Trait	Control				Low N				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
glc.b.75	5.26 ± 1.14^{b}	4.71 ± 0.87^{b}	3.93 ± 0.16^{b}	18.55 ± 1.29^{a}	3.99 ± 0.35^{b}	2.71 ± 0.65^{b}	1.70 ± 0.50^{b}	20.00 ± 1.30^{a}	0.122	**<0.001**	0.182
glc.s.75	6.99 ± 1.12^{b}	5.22 ± 1.48^{b}	5.54 ± 0.34^{b}	15.95 ± 3.35^{a}	7.12 ± 0.10^{b}	5.55 ± 0.20^{b}	5.45 ± 1.59^{b}	16.47 ± 2.41^{a}	0.856	**<0.001**	0.998
glc.p.75	2.48 ± 0.28	4.12 ± 0.72	5.42 ± 1.66	6.12 ± 0.60	3.30 ± 0.54	5.51 ± 1.73	8.69 ± 3.22	8.93 ± 0.07	0.064	**0.022**	0.820
glc.a.75	15.6 ± 1.4	11.8 ± 2.4	18.7 ± 1.7	13.5 ± 1.2	8.2 ± 2.3	8.9 ± 3.7	14.1 ± 3.7	15.3 ± 1.1	0.069	0.096	0.313
glc.g.75	13.6 ± 2.7	19.4 ± 5.6	24.7 ± 2.7	11.7 ± 1.1	9.3 ± 1.4	16.8 ± 5.7	16.7 ± 3.3	10.8 ± 1.8	0.125	**0.032**	0.763
glc.l.75	13.8 ± 1.7	14.4 ± 4.0	18.3 ± 1.2	16.2 ± 1.5	8.8 ± 2.6	12.8 ± 4.1	13.6 ± 3.1	14.8 ± 1.0	0.113	0.314	0.849
fru.b.65	5.24 ± 1.06	4.62 ± 1.15	7.29 ± 1.43	5.88 ± 1.30	3.39 ± 0.53	3.32 ± 0.87	6.64 ± 1.55	6.65 ± 0.84	0.359	**0.047**	0.691
fru.s.65	13.7 ± 3.3	22.2 ± 17.2	8.0 ± 1.0	20.7 ± 4.3	13.5 ± 5.1	17.6 ± 9.2	7.4 ± 0.7	14.5 ± 2.0	0.590	0.404	0.971
fru.p.65	50.7 ± 9.8^{ab}	79.1 ± 6.8^{a}	19.4 ± 6.1^{b}	37.8 ± 15.1^{ab}	47.2 ± 11.5^{ab}	48.6 ± 16.0^{ab}	10.1 ± 5.0^{b}	43.9 ± 5.9^{ab}	0.222	**0.002**	0.367
fru.a.65	6.43 ± 0.87^{ab}	6.22 ± 0.58^{ab}	10.67 ± 0.23^{ab}	8.37 ± 0.60^{ab}	5.47 ± 0.44^{b}	6.19 ± 0.62^{ab}	10.86 ± 1.79^{ab}	11.87 ± 2.51^{a}	0.440	**0.001**	0.307
fru.g.65	8.3 ± 1.8^{bc}	7.6 ± 1.0^{c}	25.1 ± 1.2^{a}	15.1 ± 0.6^{b}	7.6 ± 1.0^{c}	7.5 ± 0.4^{c}	24.0 ± 1.8^{a}	13.1 ± 2.9^{bc}	0.396	**<0.001**	0.936
fru.l.65	17.1 ± 1.8^{ab}	18.3 ± 1.6^{ab}	23.5 ± 1.2^{a}	21.7 ± 1.9^{ab}	14.8 ± 2.2^{b}	16.5 ± 2.0^{ab}	20.7 ± 1.9^{ab}	20.6 ± 0.2^{ab}	0.117	**0.007**	0.962
fru.b.75	6.20 ± 0.64^{b}	4.95 ± 1.00^{b}	4.49 ± 0.36^{b}	16.45 ± 1.15^{a}	6.30 ± 1.60^{b}	5.33 ± 0.74^{b}	2.64 ± 0.33^{b}	19.74 ± 0.99^{a}	0.480	**<0.001**	0.091
fru.s.75	8.94 ± 0.28^{b}	6.01 ± 1.01^{b}	6.21 ± 0.86^{b}	40.22 ± 5.32^{a}	13.25 ± 1.15^{b}	8.88 ± 0.25^{b}	7.67 ± 0.38^{b}	44.77 ± 1.83^{a}	0.041	<0.001	0.872
fru.p.75	2.23 ± 0.42^{b}	3.27 ± 0.13^{b}	4.24 ± 0.74^{b}	11.75 ± 1.08^{ab}	8.86 ± 4.19^{b}	6.13 ± 1.19^{b}	6.54 ± 1.30^{b}	21.9 ± 3.68^{a}	**0.002**	**<0.001**	0.256
fru.a.75	7.28 ± 0.83^{cd}	5.13 ± 0.62^{d}	9.46 ± 1.27^{bcd}	12.32 ± 0.79^{ab}	6.76 ± 0.32^{d}	5.04 ± 0.90^{d}	11.88 ± 1.21^{ac}	15.16 ± 1.49^{a}	0.118	**<0.001**	0.261
fru.g.75	11.7 ± 1.5	12.8 ± 1.2	18.7 ± 2.9	11.0 ± 0.4	11.2 ± 1.1	12.9 ± 1.2	15.5 ± 1.3	11.9 ± 1.8	0.566	**0.008**	0.601
fru.l.75	14.1 ± 1.0^{ac}	13.8 ± 1.1^{ac}	18.4 ± 1.2^{a}	12.9 ± 0.4^{bc}	9.8 ± 1.1^{c}	13.1 ± 1.5^{bc}	15.8 ± 0.9^{ab}	12.2 ± 1.1^{bc}	**0.015**	**0.001**	0.314
suc.b.65	118 ± 11^{bc}	122 ± 7^{ab}	82 ± 7^{cd}	72 ± 8^{d}	158 ± 3^{a}	127 ± 4^{ab}	117 ± 13^{bc}	91 ± 2^{bd}	**<0.001**	**<0.001**	0.162
suc.s.65	135 ± 28^{ab}	190 ± 30^{a}	131 ± 8^{ac}	50 ± 11^{c}	182 ± 11^{a}	183 ± 18^{a}	140 ± 3^{a}	55 ± 3^{bc}	0.280	**<0.001**	0.453
suc.p.65	197 ± 67^{ab}	41 ± 5^{c}	109 ± 46^{bc}	40 ± 11^{c}	283 ± 18^{a}	62 ± 13^{bc}	55 ± 4^{bc}	40 ± 1^{c}	0.532	**<0.001**	0.178
suc.a.65	91.2 ± 4.2^{a}	108.1 ± 4.0^{a}	65.9 ± 5.2^{b}	46.3 ± 0.2^{b}	100.1 ± 7.3^{a}	97.9 ± 3.9^{a}	65.9 ± 3.4^{b}	49.5 ± 1.2^{b}	0.878	**<0.001**	0.184
suc.g.65	56.5 ± 8.8^{ac}	80.7 ± 3.6^{a}	43.8 ± 3.3^{c}	43.1 ± 4.9^{c}	68.6 ± 6.1^{ac}	74.7 ± 6.1^{ab}	49.8 ± 0.3^{bc}	48.0 ± 4.9^{c}	0.270	**<0.001**	0.412
suc.l.65	84.3 ± 1.1^{ab}	97.0 ± 3.9^{a}	57.6 ± 4.3^{cd}	45.0 ± 5.2^{d}	90.7 ± 2.1^{a}	91.0 ± 5.2^{a}	69.6 ± 2.3^{bc}	43.2 ± 1.2^{d}	0.306	**<0.001**	0.088
suc.b.75	114 ± 12^{abc}	122 ± 1^{abc}	110 ± 3^{bd}	133 ± 5^{ab}	106 ± 13^{bd}	85 ± 6^{cd}	74 ± 11^{d}	152 ± 6^{a}	**0.013**	**<0.001**	**0.009**
suc.s.75	152 ± 1^{a}	159 ± 14^{a}	147 ± 7^{a}	92 ± 4^{b}	133 ± 14^{ab}	132 ± 5^{ab}	125 ± 11^{ab}	90 ± 9^{b}	**0.017**	**<0.001**	0.590
suc.p.75	190 ± 5^{a}	92 ± 4^{bc}	85 ± 8^{bc}	109 ± 8^{bc}	149 ± 36^{ab}	101 ± 5^{bc}	79 ± 4^{c}	102 ± 1^{bc}	0.267	**<0.001**	0.356
suc.a.75	78.4 ± 6.1^{a}	80.3 ± 4.4^{a}	82.8 ± 3.5^{a}	75.4 ± 4.1^{a}	47.1 ± 3.5^{b}	46.9 ± 1.7^{b}	47.1 ± 6.1^{b}	79.7 ± 0.6^{a}	**<0.001**	**0.008**	**0.001**
suc.g.75	53.7 ± 5.5^{bd}	57.9 ± 1.4^{bc}	56.3 ± 0.9^{bc}	70.1 ± 2.0^{ab}	45.0 ± 0.9^{cd}	41.4 ± 1.2^{cd}	36.8 ± 0.1^{d}	85.3 ± 9.1^{a}	**0.016**	**<0.001**	**0.002**
suc.l.75	78.0 ± 4.0^{b}	77.1 ± 0.6^{bc}	77.6 ± 2.5^{bc}	86.9 ± 3.9^{ab}	61.7 ± 1.1^{d}	61.1 ± 1.1^{d}	64.7 ± 3.0^{cd}	98.7 ± 3.1^{a}	**0.001**	**<0.001**	**<0.001**
fructan.b.65	124 ± 21^{ab}	175 ± 29^{a}	57 ± 18^{ab}	27 ± 8^{b}	157 ± 32^{ab}	175 ± 39^{a}	112 ± 43^{ab}	51 ± 12^{ab}	0.170	**0.001**	0.798
fructan.s.65	320 ± 48	261 ± 44	331 ± 60	254 ± 69	164 ± 33	290 ± 55	330 ± 79	397 ± 62	0.929	0.389	0.121
fructan.p.65	66 ± 5^{b}	61 ± 9^{b}	215 ± 76^{ab}	76 ± 30^{b}	205 ± 56^{ab}	129 ± 32^{ab}	299 ± 55^{a}	132 ± 33^{ab}	**0.012**	**0.007**	0.780
fructan.a.65	77.4 ± 15.5	104.1 ± 25.0	70.2 ± 17.0	21.6 ± 1.2	68.6 ± 18.6	82.6 ± 23.5	91.2 ± 27.4	37.9 ± 13.7	0.898	**0.025**	0.655
fructan.g.65	148.7 ± 10.8	145.2 ± 38.6	88.8 ± 20.9	60.1 ± 9.4	146.1 ± 19.9	120.4 ± 35.8	93.0 ± 23.6	89.2 ± 38.0	0.938	0.050	0.798
fructan.l.65	86.9 ± 15.8	101.7 ± 22.7	63.9 ± 8.8	22.4 ± 5.1	78.2 ± 18.3	78.6 ± 25.0	55.4 ± 14.0	39.3 ± 9.8	0.618	**0.010**	0.675
fructan.b.75	66 ± 24	143 ± 65	69 ± 4	47 ± 3	180 ± 28	156 ± 75	106 ± 40	68 ± 3	0.122	0.149	0.578
fructan.s.75	252 ± 36	338 ± 27	300 ± 27	346 ± 40	260 ± 24	338 ± 49	336 ± 35	367 ± 12	0.497	**0.039**	0.947
fructan.p.75	576 ± 77^{ab}	638 ± 93^{ab}	475 ± 39^{b}	771 ± 27^{ab}	570 ± 28^{ab}	617 ± 59^{ab}	550 ± 94^{ab}	798 ± 36^{a}	0.676	**0.003**	0.873
fructan.a.75	55.8 ± 5.9^{ac}	69.6 ± 18.5^{a}	67.6 ± 8.3^{ab}	13.4 ± 0.97^{c}	33.7 ± 5.3^{ac}	62.9 ± 10.0^{ac}	64.5 ± 15.5^{ab}	18.8 ± 3.8^{bc}	0.369	**<0.001**	0.602
fructan.g.75	38.3 ± 5.9	55.8 ± 18	56.8 ± 5.6	26.9 ± 3.9	36.0 ± 6.2	38.7 ± 8.4	42.7 ± 13.9	43.2 ± 7.1	0.537	0.370	0.337
fructan.l.75	38.9 ± 3.2	55.7 ± 19.9	52.0 ± 3.4	18.5 ± 3.1	28.8 ± 5.6	44.0 ± 10.9	42.6 ± 10.1	24.9 ± 6.3	0.370	**0.032**	0.757

Table S6.2. Continued.

Trait	Control				Low N				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
starch.b.65	15.12 ± 3.60^{bc}	16.28 ± 1.22^{bc}	2.96 ± 0.82^{d}	3.53 ± 0.26^{d}	32.41 ± 2.01^{a}	23.85 ± 1.57^{ab}	8.20 ± 2.11^{cd}	7.24 ± 1.10^{cd}	**<0.001**	**<0.001**	**0.009**
starch.s.65	4.59 ± 0.33^{ac}	6.30 ± 0.89^{ab}	2.05 ± 0.36^{c}	1.99 ± 0.35^{c}	5.62 ± 1.30^{ac}	7.00 ± 0.78^{a}	4.32 ± 1.36^{ac}	2.89 ± 0.32^{bc}	0.051	**<0.001**	0.771
starch.p.65	3.47 ± 0.82^{ab}	2.29 ± 0.34^{ab}	1.47 ± 0.12^{b}	2.01 ± 0.36^{ab}	4.13 ± 0.32^{a}	3.66 ± 0.41^{ab}	2.00 ± 0.53^{ab}	3.17 ± 0.49^{ab}	**0.012**	**0.004**	0.772
starch.a.65	12.50 ± 0.96^{a}	17.75 ± 2.06^{a}	3.26 ± 0.74^{b}	6.35 ± 0.16^{b}	14.44 ± 1.63^{a}	16.62 ± 1.55^{a}	4.44 ± 0.88^{b}	6.11 ± 0.90^{b}	0.626	**<0.001**	0.614
starch.g.65	7.77 ± 0.20^{a}	8.33 ± 1.31^{a}	2.95 ± 0.51^{b}	5.15 ± 0.32^{ab}	8.28 ± 0.76^{a}	7.96 ± 0.82^{a}	3.80 ± 0.47^{b}	5.54 ± 0.13^{ab}	0.476	**<0.001**	0.831
starch.l.65	11.7 ± 1.0^{bc}	18.4 ± 1.0^{a}	8.3 ± 0.6^{c}	12.0 ± 1.7^{bc}	13.6 ± 0.3^{ac}	17.3 ± 2.2^{ab}	10.0 ± 0.6^{c}	10.5 ± 0.9^{c}	0.782	**<0.001**	0.354
starch.b.75	8.57 ± 1.65^{b}	11.61 ± 2.36^{b}	5.60 ± 0.30^{b}	12.59 ± 2.57^{ab}	10.15 ± 1.96^{b}	6.68 ± 1.35^{b}	5.37 ± 1.26^{b}	20.49 ± 1.83^{a}	0.405	**<0.001**	**0.019**
starch.s.75	2.31 ± 0.47^{ab}	3.52 ± 0.89^{a}	2.17 ± 0.18^{ab}	1.42 ± 0.09^{ab}	0.94 ± 0.06^{b}	2.49 ± 0.58^{ab}	2.11 ± 0.47^{ab}	2.22 ± 0.12^{ab}	0.211	**0.036**	0.109
starch.p.75	1.13 ± 0.53	1.01 ± 0.07	0.84 ± 0.09	0.80 ± 0.11	1.35 ± 0.18	1.10 ± 0.59	0.84 ± 0.30	0.62 ± 0.10	0.891	0.368	0.934
starch.a.75	4.15 ± 0.46^{bd}	7.81 ± 0.59^{a}	4.94 ± 0.60^{bc}	4.66 ± 0.50^{bc}	2.28 ± 0.31^{d}	4.02 ± 0.49^{bd}	2.74 ± 0.45^{cd}	6.06 ± 0.34^{ab}	**<0.001**	**<0.001**	**0.001**
starch.g.75	2.04 ± 0.27^{bd}	2.72 ± 0.21^{b}	2.65 ± 0.31^{bc}	2.84 ± 0.06^{b}	1.38 ± 0.34^{d}	1.40 ± 0.16^{d}	1.49 ± 0.11^{cd}	4.34 ± 0.37^{a}	**0.035**	**<0.001**	**<0.001**
starch.l.75	7.85 ± 2.66^{a}	5.68 ± 0.46^{ab}	7.04 ± 1.48^{ab}	3.22 ± 0.21^{ab}	2.28 ± 0.24^{b}	3.72 ± 0.33^{ab}	3.69 ± 0.23^{ab}	3.66 ± 0.36^{ab}	**0.004**	0.355	0.091
rbcL.b.65	10.45 ± 0.70^{a}	5.95 ± 1.08^{bc}	6.65 ± 0.89^{ac}	9.86 ± 0.59^{ab}	8.70 ± 1.05^{ab}	4.37 ± 0.71^{c}	4.15 ± 0.07^{c}	6.15 ± 0.84^{bc}	**0.001**	**<0.001**	0.547
rbcL.s.65	2.97 ± 0.49	1.67 ± 0.16	2.23 ± 0.31	3.15 ± 0.72	2.40 ± 0.05	1.61 ± 0.10	2.48 ± 0.18	2.87 ± 0.62	0.568	**0.022**	0.779
rbcL.p.65	1.63 ± 0.24^{ac}	0.76 ± 0.18^{c}	0.64 ± 0.26^{c}	2.52 ± 0.58^{ab}	2.64 ± 0.09^{ab}	1.78 ± 0.43^{ac}	3.00 ± 0.48^{a}	1.18 ± 0.09^{bc}	**0.006**	0.123	**0.001**
rbcL.a.65	6.54 ± 0.15^{a}	4.19 ± 0.34^{ab}	3.50 ± 0.23^{b}	5.47 ± 0.29^{ab}	5.95 ± 0.59^{ab}	5.48 ± 0.64^{ab}	3.54 ± 0.49^{b}	3.82 ± 0.86^{b}	0.528	**0.001**	0.061
rbcL.g.65	1.82 ± 0.07^{ab}	1.76 ± 0.18^{ac}	1.01 ± 0.12^{c}	1.39 ± 0.16^{ac}	1.18 ± 0.05^{bc}	2.12 ± 0.25^{a}	1.54 ± 0.21^{ac}	1.22 ± 0.10^{bc}	0.846	**0.002**	**0.008**
rbcL.l.65	2.56 ± 0.07^{b}	2.91 ± 0.18^{ab}	2.22 ± 0.37^{b}	2.42 ± 0.27^{b}	2.37 ± 0.30^{b}	3.13 ± 0.03^{ab}	3.03 ± 0.03^{ab}	4.22 ± 0.61^{a}	**0.006**	**0.043**	**0.022**
rbcL.b.75	13.64 ± 0.66^{a}	3.66 ± 0.21^{e}	9.42 ± 0.30^{b}	7.03 ± 1.36^{bd}	6.92 ± 0.31^{bd}	4.04 ± 0.73^{de}	5.40 ± 0.25^{cde}	7.94 ± 0.15^{bc}	**<0.001**	**<0.001**	**<0.001**
rbcL.s.75	6.93 ± 1.17^{a}	1.59 ± 0.45^{b}	2.78 ± 0.19^{b}	4.01 ± 0.34^{b}	3.55 ± 0.46^{b}	2.93 ± 0.16^{b}	1.79 ± 0.51^{b}	2.17 ± 0.58^{b}	**0.008**	**<0.001**	**0.006**
rbcL.p.75	1.35 ± 0.07^{ac}	0.67 ± 0.07^{c}	1.87 ± 0.33^{a}	1.02 ± 0.25^{ac}	1.63 ± 0.02^{ab}	0.54 ± 0.08^{c}	0.58 ± 0.18^{c}	0.98 ± 0.19^{bc}	**0.034**	**0.001**	**0.003**
rbcL.a.75	3.82 ± 0.16	3.33 ± 0.40	3.59 ± 0.38	3.23 ± 0.77	2.83 ± 0.46	3.62 ± 0.92	3.69 ± 0.88	2.69 ± 0.06	0.501	0.693	0.686
rbcL.g.75	1.70 ± 0.08^{ac}	1.02 ± 0.13^{ac}	0.78 ± 0.23^{bc}	2.17 ± 0.57^{a}	1.28 ± 0.09^{ac}	0.88 ± 0.02^{bc}	0.66 ± 0.24^{c}	1.94 ± 0.27^{ab}	0.230	**<0.001**	0.939
rbcL.l.75	1.35 ± 0.11	1.01 ± 0.16	1.24 ± 0.35	1.22 ± 0.32	1.21 ± 0.11	1.44 ± 0.08	1.39 ± 0.13	1.52 ± 0.16	0.213	0.901	0.538
RCOI.b.65	20.5 ± 8.0^{ab}	19.9 ± 1.1^{ab}	22.2 ± 1.4^{ab}	14.1 ± 0.7^{b}	23.3 ± 0.7^{a}	22.2 ± 2.1^{ab}	23.2 ± 2.9^{a}	20.0 ± 2.5^{ab}	**0.026**	**0.023**	0.554
RCOI.s.65	5.50 ± 0.63^{ab}	8.85 ± 0.50^{a}	6.95 ± 1.07^{ab}	6.03 ± 0.31^{ab}	4.55 ± 0.23^{b}	4.42 ± 0.79^{b}	7.41 ± 1.21^{ab}	4.28 ± 0.33^{b}	**0.005**	**0.019**	**0.025**
RCOI.p.65	1.81 ± 0.39^{c}	1.68 ± 0.25^{c}	3.45 ± 0.37^{bc}	3.46 ± 0.21^{bc}	4.96 ± 0.69^{ab}	3.19 ± 0.52^{bc}	6.01 ± 0.95^{a}	1.92 ± 0.17^{c}	**0.001**	**0.001**	**0.001**
RCOI.a.65	11.9 ± 7.2	11.3 ± 0.8	8.79 ± 0.63	10.9 ± 0.7	11.1± 1.3	12.6 ± 0.9	9.8 ± 0.9	9.5 ± 0.8	0.997	**0.027**	0.334
RCOI.g.65	3.08 ± 0.19^{ab}	2.50 ± 0.26^{ab}	2.10 ± 0.33^{ab}	3.19 ± 0.30^{a}	2.65 ± 0.08^{ab}	2.78 ± 0.48^{ab}	1.75 ± 0.34^{b}	2.50 ± 0.09^{ab}	0.163	**0.015**	0.404
RCOI.l.65	2.65 ± 0.22	2.56 ± 0.17	1.89 ± 0.22	2.65 ± 0.48	2.36 ± 0.27	2.38 ± 0.14	2.30 ± 0.34	3.12 ± 0.29	0.629	0.091	0.437
RCOI.b.75	21.5 ± 1.2^{ab}	22.9 ± 04^{a}	22.9 ± 2.7^{a}	13.6 ± 1.5^{b}	13.8 ± 1.6^{b}	16.5 ± 2.5^{ab}	20.0 ± 1.5^{ab}	14.3 ± 0.6^{b}	**0.004**	**0.003**	0.096
RCOI.s.75	9.38 ± 0.77^{a}	6.17 ± 0.68^{ac}	7.84 ± 0.56^{ac}	8.06 ± 0.51^{ab}	6.43 ± 1.07^{ac}	6.33 ± 0.32^{ac}	4.48 ± 0.16^{c}	5.77 ± 1.17^{bc}	**0.001**	0.104	0.114
RCOI.p.75	4.72 ± 0.24^{a}	3.41 ± 0.12^{ab}	3.40 ± 0.38^{ab}	2.49 ± 0.41^{bc}	3.59 ± 0.41^{ab}	2.56 ± 0.27^{bc}	3.95 ± 0.57^{ab}	1.55 ± 0.20^{c}	**0.030**	**<0.001**	0.108
RCOI.a.75	9.36 ± 0.27^{ab}	9.77 ± 1.14^{a}	9.06 ± 1.44^{ab}	7.01 ± 0.31^{ab}	4.87 ± 0.72^{b}	7.23 ± 1.06^{ab}	7.59 ± 1.43^{ab}	7.63 ± 0.34^{ab}	**0.010**	0.393	0.099
RCOI.g.75	3.66 ± 0.34^{ac}	3.13 ± 0.06^{ac}	2.77 ± 0.37^{bc}	4.99 ± 0.89^{a}	1.76 ± 0.14^{c}	2.74 ± 0.19^{bc}	2.13 ± 0.27^{bc}	3.99 ± 0.66^{ab}	**0.007**	**0.001**	0.388
RCOI.l.75	2.57 ± 0.19^{bc}	2.42 ± 0.27^{bd}	2.74 ± 0.11^{ab}	3.69 ± 0.15^{a}	1.72 ± 0.30^{cd}	2.32 ± 0.08^{bd}	1.51 ± 0.29^{d}	2.84 ± 0.14^{ab}	**<0.001**	**<0.001**	0.084
RCOT.b.65	24.3 ± 0.8^{ab}	23.9 ± 1.3^{ab}	25.7 ± 3.0^{ab}	16.7 ± 0.6^{b}	28.2 ± 2.4^{a}	24.4 ± 1.6^{ab}	25.0 ± 3.0^{ab}	21.3 ± 2.6^{ab}	0.189	**0.015**	0.542
RCOT.s.65	6.13 ± 0.45^{b}	10.99 ± 0.36^{a}	8.55 ± 1.52^{ab}	6.72 ± 0.10^{b}	5.55 ± 0.26^{b}	5.19 ± 0.81^{b}	8.60 ± 1.61^{ab}	4.93 ± 0.30^{b}	**0.005**	**0.008**	**0.017**
RCOT.p.65	2.69 ± 0.12^{c}	2.40 ± 0.37^{c}	4.72 ± 0.46^{ac}	3.93 ± 0.13^{bc}	6.48 ± 0.45^{ab}	3.84 ± 0.64^{bc}	7.37 ± 1.24^{a}	2.32 ± 0.11^{c}	**0.001**	**<0.001**	**0.001**
RCOT.a.65	14.8 ± 0.4^{a}	13.4 ± 0.7^{ab}	11.3 ± 1.1^{ab}	13.8 ± 0.5^{ab}	12.7 ± 1.1^{ab}	13.8 ± 0.8^{ab}	10.5 ± 0.9^{b}	11.4 ± 0.6^{ab}	0.053	**0.010**	0.318
RCOT.g.65	3.40 ± 0.16	3.26 ± 0.33	2.59 ± 0.41	3.57 ± 0.33	3.08 ± 0.05	3.23 ± 0.05	2.09 ± 0.39	3.00 ± 0.09	0.133	**0.025**	0.837
RCOT.l.65	3.37 ± 0.18	3.53 ± 0.12	2.64 ± 0.21	3.23 ± 0.51	3.15 ± 0.27	3.06 ± 0.17	3.05 ± 0.37	3.35 ± 0.20	0.851	0.344	0.449

Table S6.2. Continued.

Trait	Control				Low N				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
RCOT.b.75	26.3 ± 1.5	25.6 ± 0.2	26.7 ± 4.1	17.0 ± 2.2	18.2 ± 2.5	20.2 ± 3.8	23.9 ± 3.1	17.8 ± 0.6	0.052	0.051	0.401
RCOT.s.75	10.81 ± 0.96^{a}	7.10 ± 0.97^{ab}	9.56 ± 0.35^{ab}	9.48 ± 0.70^{ab}	8.02 ± 1.07^{ab}	7.79 ± 0.53^{ab}	5.71 ± 0.14^{b}	7.09 ± 1.3^{ab}	**0.003**	0.121	0.080
RCOT.p.75	5.65 ± 0.44^{a}	4.13 ± 0.18^{ab}	4.46 ± 0.46^{ab}	2.72 ± 0.31^{bc}	5.15 ± 0.28^{a}	3.48 ± 0.41^{ac}	5.41 ± 0.87^{a}	1.58 ± 0.28^{c}	0.307	**<0.001**	0.155
RCOT.a.75	12.14 ± 0.37^{a}	9.85 ± 1.37^{ab}	11.02 ± 1.07^{a}	9.23 ± 0.64^{ab}	5.98 ± 0.67^{b}	8.33 ± 0.83^{ab}	8.14 ± 1.27^{ab}	8.71 ± 0.23^{ab}	**<0.001**	0.902	**0.031**
RCOT.g.75	3.82 ± 0.35^{ac}	3.61 ± 0.22^{ac}	3.17 ± 0.52^{bc}	5.58 ± 0.83^{a}	1.87 ± 0.09^{c}	3.29 ± 0.14^{bc}	2.58 ± 0.20^{bc}	4.21 ± 0.39^{ab}	**0.002**	**<0.001**	0.215
RCOT.l.75	3.23 ± 0.12^{ac}	2.96 ± 0.43^{ac}	3.71 ± 0.38^{ab}	4.08 ± 0.02^{a}	2.13 ± 0.30^{c}	2.60 ± 0.10^{bc}	2.00 ± 0.38^{c}	3.12 ± 0.14^{ac}	**<0.001**	**0.017**	0.154
RCOas.b.65	84.4 ± 2.8	83.5 ± 4.2	87.8 ± 5.1	84.7 ± 5.8	83.3 ± 5.3	91.0 ± 2.6	93.0 ± 2.2	94.0 ± 1.2	0.079	0.392	0.590
RCOas.s.65	89.3 ± 3.6	80.5 ± 2.7	82.3 ± 3.2	89.6 ± 3.5	81.9 ± 0.8	85.1 ± 5.7	87.2 ± 3.4	86.7 ± 1.5	0.940	0.469	0.226
RCOas.p.65	67.5 ± 4.5^{b}	70.3 ± 1.1^{ab}	72.9 ± 3.4^{ab}	88.2 ± 5.9^{a}	75.9 ± 5.9^{ab}	83.1 ± 0.7^{ab}	81.9 ± 2.1^{ab}	82.3 ± 3.4^{ab}	**0.043**	**0.023**	0.122
RCOas.a.65	80.7 ± 3.5	84.2 ± 1.2	78.5 ± 7.5	79.5 ± 5.4	86.7 ± 2.7	90.6 ± 1.4	92.9 ± 0.9	82.7 ± 3.7	**0.015**	0.432	0.540
RCOas.g.65	90.7 ± 4.2^{a}	76.8 ± 1.4^{b}	80.7 ± 0.7^{ab}	89.6 ± 4.0^{a}	86.0 ± 2.0^{ab}	85.8 ± 2.1^{ab}	83.7 ± 0.6^{ab}	83.3 ± 0.6^{ab}	0.882	**0.026**	**0.018**
RCOas.l.65	78.6 ± 2.5^{b}	72.6 ± 2.9^{b}	71.3 ± 3.1^{b}	81.4 ± 2.4^{ab}	74.6 ± 2.3^{b}	77.8 ± 0.9^{b}	75.0 ± 4.5^{b}	92.7 ± 3.0^{a}	0.060	**0.001**	0.103
RCOas.b.75	81.7 ± 0.2	89.4 ± 2.2	87.1 ± 4.6	80.3 ± 3.7	76.6 ± 3.1	83.4 ± 6.3	84.6 ± 4.3	80.8 ± 1.8	0.233	0.164	0.824
RCOas.s.75	86.9 ± 0.8	87.5 ± 4.2	82.1 ± 5.1	85.2 ± 1.3	79.5 ± 3.9	81.4 ± 1.5	78.5 ± 2.1	80.7 ± 3.1	**0.026**	0.597	0.931
RCOas.p.75	84.0 ± 2.2^{ac}	82.6 ± 0.9^{ac}	76.2 ± 1.7^{bc}	90.1 ± 5.0^{ab}	69.7 ± 6.5^{c}	73.6 ± 0.9^{bc}	73.4 ± 1.5^{bc}	99.7 ± 4.7^{a}	0.121	**<0.001**	**0.022**
RCOas.a.75	77.2 ± 2.3^{b}	99.9 ± 4.6^{a}	81.5 ± 6.0^{b}	76.2 ± 2.1^{b}	80.7 ± 3.1^{b}	86.1 ± 4.3^{ab}	92.4 ± 3.3^{ab}	87.5 ± 1.7^{ab}	0.259	**0.008**	**0.011**
RCOas.g.75	95.7 ± 1.1	87.3 ± 3.8	88.2 ± 2.9	88.7 ± 4.1	94.4 ± 6.3	83.1 ± 5.0	81.9 ± 4.6	93.6 ± 7.2	0.619	0.138	0.667
RCOas.l.75	79.3 ± 3.3^{ab}	82.4 ± 3.3^{ab}	75.0 ± 5.3^{b}	90.3 ± 3.2^{ab}	79.7 ± 3.1^{ab}	89.2 ± 1.6^{ab}	75.3 ± 0.7^{ab}	91.0 ± 3.7^{a}	0.391	**0.001**	0.707
PEPC.b.65	1624 ± 133	1319 ± 73	1690 ± 110	1599 ± 157	1695 ± 95	1347 ± 159	1802 ± 11	1805 ± 17	0.194	**0.006**	0.863
PEPC.s.65	753 ± 42	777 ± 100	840 ± 121	880 ± 47	760 ± 84	626 ± 62	800 ± 78	844 ± 48	0.330	0.214	0.772
PEPC.p.65	694 ± 46^{ab}	502 ± 17^{b}	705 ± 33^{ab}	629 ± 67^{ab}	618 ± 17^{ab}	652 ± 24^{ab}	775 ± 97^{a}	563 ± 46^{ab}	0.594	**0.022**	0.109
PEPC.a.65	1104 ± 153	956 ± 31	1057 ± 64	1337 ± 179	880 ± 60	993 ± 35	1054 ± 94	949 ± 104	0.065	0.380	0.177
PEPC.g.65	818 ± 37^{ab}	678 ± 34^{b}	848 ± 12^{ab}	958 ± 104^{a}	728 ± 89^{ab}	682 ± 18^{ab}	758 ± 14^{ab}	712 ± 60^{ab}	**0.018**	0.076	0.215
PEPC.l.65	759 ± 74	769 ± 21	830 ± 93	917 ± 152	694 ± 14	775 ± 27	990 ± 43	974 ± 114	0.502	**0.045**	0.579
PEPC.b.75	1924 ± 97	1377 ± 35	1732 ± 263	1623 ± 180	1730 ± 291	1630 ± 123	1514 ± 88	1803 ± 145	0.965	0.325	0.417
PEPC.s.75	1048 ± 80^{a}	937 ± 93^{ab}	807 ± 100^{ab}	890 ± 102^{ab}	813 ± 24^{ab}	739 ± 71^{ab}	564 ± 53^{b}	905 ± 108^{ab}	**0.012**	**0.043**	0.390
PEPC.p.75	1028 ± 84^{a}	783 ± 104^{ab}	734 ± 46^{ab}	676 ± 72^{b}	706 ± 29^{b}	570 ± 41^{b}	732 ± 71^{ab}	522 ± 14^{b}	**0.002**	**0.005**	0.132
PEPC.a.75	1467 ± 165^{a}	1246 ± 62^{ab}	1294 ± 119^{ab}	1241 ± 113^{ab}	818 ± 41^{b}	1339 ± 146^{ab}	1026 ± 122^{ab}	1550 ± 73^{a}	0.125	0.123	**0.003**
PEPC.g.75	764 ± 25^{ab}	742 ± 23^{ab}	719 ± 58^{ab}	819 ± 99^{a}	484 ± 55^{b}	575 ± 25^{ab}	789 ± 111^{ab}	719 ± 81^{ab}	**0.024**	0.128	0.111
PEPC.l.75	1101 ± 57	881 ± 51	877 ± 27	895 ± 107	775 ± 132	948 ± 29	765 ± 60	871 ± 42	0.071	0.419	0.079
GS.b.65	3205 ± 203^{ab}	3918 ± 156^{a}	3592 ± 353^{a}	2245 ± 29^{b}	3777 ± 220^{a}	3621 ± 375^{a}	3516 ± 293^{ab}	2856 ± 314^{ab}	0.297	**0.002**	0.256
GS.s.65	782 ± 52^{bcd}	1455 ± 37^{a}	1224 ± 101^{ab}	895 ± 114^{bcd}	790 ± 44^{bcd}	756 ± 19^{cd}	1058 ± 187^{ac}	594 ± 85^{d}	**0.001**	**0.001**	**0.012**
GS.p.65	468 ± 33^{cd}	386 ± 46^{cd}	629 ± 46^{bc}	568 ± 42^{bc}	773 ± 25^{ab}	577 ± 83^{bc}	1019 ± 82^{a}	223 ± 18^{d}	**0.002**	**<0.001**	**<0.001**
GS.a.65	1870 ± 49^{ab}	2045 ± 105^{a}	1563 ± 138^{ac}	1169 ± 59^{c}	1931 ± 117^{ab}	2048 ± 135^{a}	1425 ± 65^{bc}	1210 ± 168^{c}	0.921	**<0.001**	0.811
GS.g.65	506 ± 10^{b}	527 ± 65^{b}	395 ± 52^{b}	512 ± 56^{b}	492 ± 28^{b}	508 ± 62^{b}	299 ± 46^{b}	805 ± 21^{a}	0.235	**<0.001**	**0.004**
GS.l.65	516 ± 77^{ab}	457 ± 36^{ab}	346 ± 37^{b}	626 ± 34^{a}	447 ± 77^{ab}	452 ± 54^{ab}	420 ± 23^{ab}	522 ± 39^{ab}	0.482	**0.014**	0.348
GS.b.75	3390 ± 294^{ab}	3935 ± 85^{a}	4011 ± 179^{a}	2556 ± 121^{bc}	3099 ± 161^{ab}	3673 ± 374^{a}	3716 ± 210^{a}	1989 ± 207^{c}	**0.038**	**<0.001**	0.889
GS.s.75	1342 ± 155^{a}	959 ± 73^{ac}	1116 ± 36.9^{ab}	984 ± 65^{ac}	767 ± 92^{bc}	993 ± 52^{ac}	749 ± 79^{bc}	623 ± 24^{c}	**<0.001**	**0.046**	**0.013**
GS.p.75	677 ± 30^{a}	619 ± 8^{a}	572 ± 58^{a}	321 ± 13^{b}	644 ± 24^{a}	576 ± 17^{a}	736 ± 118^{a}	175 ± 21^{b}	0.678	**<0.001**	**0.043**
GS.a.75	1688 ± 75^{a}	1768 ± 185^{a}	1885 ± 107^{a}	534 ± 57^{b}	754 ± 147^{b}	1488 ± 109^{a}	1538 ± 108^{a}	581 ± 102^{b}	**<0.001**	**<0.001**	**0.006**
GS.g.75	695 ± 42^{ab}	729 ± 55^{ab}	507 ± 74^{b}	711 ± 86^{ab}	489 ± 39^{bc}	850 ± 36^{a}	235 ± 46^{c}	602 ± 29^{ab}	**0.008**	**<0.001**	**0.012**
GS.l.75	481 ± 29^{ab}	485 ± 77^{ab}	520 ± 39^{ab}	388 ± 90^{b}	426 ± 11^{ab}	401 ± 90^{b}	365 ± 44^{b}	710 ± 30^{a}	0.872	0.241	**0.004**

Table S6.2. Continued.

Trait	Control				Low N				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
GOGAT.b.65	2351 ± 141^{b}	3761 ± 180^{a}	2775 ± 90^{b}	2207 ± 87^{b}	2821 ± 199^{b}	3877 ± 240^{a}	2941 ± 232^{b}	2212 ± 75^{b}	0.129	**<0.001**	0.563
GOGAT.s.65	907 ± 66^{b}	1412 ± 101^{a}	926 ± 174^{ab}	804 ± 85^{b}	654 ± 63^{b}	463 ± 79^{b}	932 ± 119^{ab}	611 ± 89^{b}	**<0.001**	0.102	**0.002**
GOGAT.p.65	474 ± 41^{bd}	285 ± 69^{d}	593 ± 25^{abc}	362 ± 4^{cd}	660 ± 72^{ab}	809 ± 73^{a}	572 ± 46^{abc}	312 ± 47^{d}	**0.001**	**0.001**	**<0.001**
GOGAT.a.65	1550 ± 68^{a}	1389 ± 30^{ab}	1462 ± 48^{ab}	1480 ± 171^{ab}	1484 ± 122^{ab}	1447 ± 71^{ab}	1071 ± 89^{b}	1212 ± 36^{ab}	**0.020**	0.077	0.102
GOGAT.g.65	301 ± 66^{b}	394 ± 74^{b}	326 ± 23^{b}	506 ± 60^{b}	387 ± 27^{b}	355 ± 20^{b}	387 ± 10^{b}	1097 ± 83^{a}	**<0.001**	**<0.001**	**<0.001**
GOGAT.l.65	407 ± 17^{ab}	543 ± 51^{a}	413 ± 23^{ab}	434 ± 23^{ab}	364 ± 34^{ab}	546 ± 55^{a}	510 ± 38^{a}	284 ± 59^{b}	0.428	**0.001**	0.050
GOGAT.b.75	2794 ± 226	3931 ± 276	2970 ± 92	2290 ± 268	2352 ± 389	4049 ± 822	2418 ± 186	2742 ± 67	0.687	**0.003**	0.493
GOGAT.s.75	1194 ± 151^{a}	1084 ± 76^{ab}	925 ± 84^{ab}	1161 ± 121^{a}	1159 ± 106^{a}	976 ± 42^{ab}	682 ± 44^{b}	916 ± 89^{ab}	**0.033**	**0.011**	0.632
GOGAT.p.75	541 ± 51^{a}	566 ± 80^{a}	462 ± 71^{ab}	230 ± 49^{bc}	595 ± 43^{a}	452 ± 9^{ab}	558 ± 67^{a}	147 ± 26^{c}	0.764	**<0.001**	0.192
GOGAT.a.75	1205 ± 116^{ab}	1226 ± 149^{ab}	1345 ± 130^{ab}	1575 ± 151^{a}	736 ± 138^{b}	1442 ± 141^{a}	1055 ± 158^{ab}	1630 ± 24^{a}	0.211	**0.002**	0.073
GOGAT.g.75	490 ± 54^{b}	380 ± 26^{bc}	452 ± 58^{b}	751 ± 62^{a}	194 ± 34^{c}	324 ± 87^{bc}	373 ± 21^{bc}	468 ± 29^{b}	**<0.001**	**<0.001**	0.051
GOGAT.l.75	554 ± 11^{ab}	578 ± 82^{a}	560 ± 76^{ab}	550 ± 16^{ab}	440 ± 26^{ab}	576 ± 2^{a}	378 ± 30^{ab}	356 ± 19^{b}	**0.001**	**0.049**	0.141
GDH.b.65	481 ± 47^{ab}	593 ± 51^{ab}	476 ± 74^{ab}	353 ± 10^{b}	500 ± 79^{ab}	723 ± 62^{a}	670 ± 77^{a}	497 ± 63^{ab}	**0.013**	**0.010**	0.561
GDH.s.65	331 ± 2^{b}	381 ± 47^{ab}	315 ± 23^{b}	481 ± 28^{a}	308 ± 18^{b}	351 ± 17^{b}	329 ± 15^{b}	287 ± 33^{b}	**0.006**	0.057	**0.005**
GDH.p.65	239 ± 10^{ce}	187 ± 6^{e}	274 ± 3^{bcd}	357 ± 22^{a}	220 ± 12de	301 ± 13^{ac}	258 ± 25^{cd}	343 ± 10^{ab}	0.120	**<0.001**	**0.001**
GDH.a.65	295 ± 33^{ab}	315 ± 10^{ab}	288 ± 31^{ab}	370 ± 15^{a}	243 ± 2^{b}	288 ± 14^{ab}	350 ± 36^{ab}	366 ± 13^{a}	0.767	**0.004**	0.112
GDH.g.65	290 ± 10^{c}	404 ± 38^{bc}	295 ± 30^{c}	674 ± 39^{a}	266 ± 30^{c}	382 ± 4^{bc}	294 ± 33^{c}	471 ± 24^{b}	**0.007**	**<0.001**	**0.010**
GDH.l.65	401 ± 41^{cd}	544 ± 17^{ad}	441 ± 31^{bcd}	618 ± 34^{ab}	370 ± 17^{d}	493 ± 56^{ad}	576 ± 34^{ac}	650 ± 67^{a}	0.475	**<0.001**	0.135
GDH.b.75	493 ± 36^{b}	492 ± 22^{b}	488 ± 18^{b}	596 ± 53^{b}	495 ± 51^{b}	1016 ± 152^{a}	650 ± 71^{b}	1000 ± 72^{a}	**<0.001**	**0.001**	**0.009**
GDH.s.75	334 ± 0^{ac}	461 ± 51^{ab}	287 ± 8^{c}	424 ± 30^{ac}	342 ± 60^{ac}	300 ± 30^{bc}	355 ± 36^{ac}	483 ± 19^{a}	0.793	**0.007**	**0.015**
GDH.p.75	325 ± 32	334 ± 6	313 ± 4	395 ± 15	408 ± 19	374 ± 93	328 ± 14	445 ± 23	0.093	0.096	0.836
GDH.a.75	303 ± 23^{bc}	282 ± 44^{bc}	439 ± 26^{bc}	928 ± 21^{a}	254 ± 60^{c}	492 ± 62^{b}	417 ± 47^{bc}	810 ± 70^{a}	0.881	**<0.001**	**0.018**
GDH.g.75	186 ± 34^{b}	562 ± 11^{a}	477 ± 5^{a}	532 ± 53^{a}	193 ± 13^{b}	503 ± 48^{a}	437 ± 33^{a}	469 ± 43^{a}	0.129	**<0.001**	0.727
GDH.l.75	407 ± 69^{e}	694 ± 10^{cd}	595 ± 34^{ce}	969 ± 53^{a}	472 ± 58de	740 ± 39^{bc}	647 ± 50^{cd}	933 ± 32^{ab}	0.350	**<0.001**	0.697
C.b.65	459 ± 7	454 ± 6	454 ± 4	439 ± 3	441 ± 4	451 ± 9	457 ± 2	438 ± 4	0.245	**0.032**	0.302
C.s.65	457 ± 3^{a}	448 ± 3^{ac}	451 ± 3^{ac}	441 ± 1^{bc}	448 ± 2^{ac}	452 ± 4^{ab}	449 ± 2^{ac}	439 ± 0^{c}	0.265	**<0.001**	0.123
C.p.65	462 ± 1^{a}	461 ± 1^{a}	456 ± 3^{a}	443 ± 2^{b}	453 ± 2^{a}	461 ± 1^{a}	460 ± 3^{a}	442 ± 1^{b}	0.423	**<0.001**	**0.044**
C.a.65	446 ± 3	449 ± 3	434 ± 10	428 ± 0	430 ± 4	446 ± 7	437 ± 7	434 ± 7	0.555	0.072	0.288
C.g.65	461 ± 2	456 ± 5	457 ± 4	453 ± 1	457 ± 1	456 ± 5	455 ± 2	449 ± 2	0.249	0.161	0.849
C.l.65	430 ± 4^{ab}	444 ± 1^{a}	426 ± 8^{ab}	419 ± 3^{b}	421 ± 3^{b}	447 ± 9^{a}	417 ± 3^{b}	413 ± 2^{b}	0.144	**<0.001**	0.554
C.b.75	432 ± 10	449 ± 7	445 ± 7	437 ± 8	424 ± 9	418 ± 12	424 ± 6	426 ± 7	**0.009**	0.876	0.550
C.s.75	453 ± 2^{a}	451 ± 2^{ab}	449 ± 1^{ab}	454 ± 4^{a}	444 ± 1^{ab}	438 ± 5^{b}	443 ± 3^{ab}	446 ± 1^{ab}	**<0.001**	0.226	0.712
C.p.75	460 ± 3	454 ± 3	457 ± 1	455 ± 0	457 ± 2	451 ± 1	455 ± 3	459 ± 3	0.572	0.151	0.548
C.a.75	407 ± 4^{ab}	415 ± 5^{a}	401 ± 3^{ab}	395 ± 9^{ab}	402 ± 11^{ab}	401 ± 10^{ab}	385 ± 3^{ab}	381 ± 2^{b}	**0.021**	**0.027**	0.835
C.g.75	438 ± 2^{ab}	444 ± 6^{a}	430 ± 6^{ab}	444 ± 3^{a}	432 ± 8^{ab}	424 ± 13^{ab}	411 ± 1^{b}	438 ± 3^{ab}	**0.009**	**0.034**	0.552
C.l.75	388 ± 4^{ab}	416 ± 5^{a}	392 ± 2^{ab}	392 ± 5^{ab}	381 ± 11^{ab}	388 ± 15^{ab}	371 ± 3^{b}	382 ± 5^{ab}	**0.006**	0.063	0.495
N.b.65	46.1 ± 4.6	40.2 ± 5.1	32.0 ± 7.8	41.8 ± 3.6	33.9 ± 5.5	40.0 ± 5.0	42.8 ± 3.9	36.8 ± 1.7	0.644	0.944	0.166
N.s.65	15.0 ± 2.7	11.7 ± 1.0	13.6 ± 2.3	12.1 ± 1.7	10.8 ± 2.1	13.6 ± 2.1	13.0 ± 2.1	11.1 ± 1.0	0.497	0.854	0.505
N.p.65	15.0 ± 1.9	15.8 ± 1.6	14.2 ± 1.2	14.4 ± 0.5	12.2 ± 2.4	16.0 ± 2.1	15.5 ± 1.5	10.4 ± 0.4	0.282	0.192	0.345
N.a.65	20.0 ± 1.2	19.4 ± 1.7	20.3 ± 3.0	20.5 ± 0.7	16.3 ± 1.5	18.7 ± 2.0	18.1 ± 1.5	18.9 ± 0.5	0.105	0.820	0.846
N.g.65	13.8 ± 0.9	13.5 ± 1.2	14.5 ± 1.5	13.3 ± 0.7	11.7 ± 0.9	13.3 ± 1.2	13.5 ± 1.2	12.3 ± 0.2	0.166	0.599	0.828
N.l.65	14.0 ± 1.2	13.7 ± 1.1	14.1 ± 1.4	13.5 ± 0.4	12.3 ± 0.8	14.0 ± 1.2	12.9 ± 0.9	12.6 ± 0.4	0.255	0.857	0.780

Table S6.2. Continued.

Trait	Control				Low N				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
N.b.75	27.5 ± 4.1	32.8 ± 0.8	32.3 ± 4.9	35.8 ± 1.7	25.2 ± 2.9	28.3 ± 4.5	26.3 ± 3.0	30.0 ± 1.9	0.060	0.279	0.935
N.s.75	9.4 ± 1.1	10.3 ± 0.5	10.4 ± 1.7	11.4 ± 0.3	9.6 ± 1.2	9.1 ± 1.3	7.8 ± 1.0	11.0 ± 1.2	0.235	0.298	0.642
N.p.75	7.9 ± 0.9	9.9 ± 0.6	8.0 ± 0.7	8.1 ± 0.2	9.3 ± 1.6	8.8 ± 1.3	7.1 ± 0.8	7.0 ± 0.4	0.489	0.178	0.495
N.a.75	10.9 ± 1.0^{ac}	13.2 ± 1.7^{ab}	9.7 ± 0.8^{bc}	16.0 ± 1.1^{a}	11.3 ± 1.5^{ac}	11.9 ± 0.9^{ac}	7.8 ± 0.4^{c}	13.2 ± 0.7^{ab}	0.098	**0.001**	0.520
N.g.75	10.5 ± 1.8^{ab}	11.7 ± 1.1^{ab}	10.3 ± 1.3^{ab}	14.1 ± 0.7^{a}	10.4 ± 1.7^{ab}	10.0 ± 0.7^{ab}	7.7 ± 0.1^{b}	11.6 ± 0.9^{ab}	0.052	**0.034**	0.680
N.l.75	9.05 ± 1.1^{ab}	10.3 ± 0.7^{ab}	7.7 ± 0.7^{bc}	11.6 ± 0.8^{a}	7.3 ± 0.7^{bc}	8.4 ± 0.3^{ac}	5.6 ± 0.1^{c}	10.3 ± 0.8^{ab}	**0.002**	**<0.001**	0.967
CN.b.65	10.1 ± 0.8	11.7 ± 1.5	11.4 ± 0.4	10.6 ± 0.8	13.6 ± 1.8	11.6 ± 1.4	10.9 ± 1.0	11.9 ± 0.5	0.210	0.909	0.305
CN.s.65	32.7 ± 6.2	38.9 ± 3.7	35.1 ± 5.6	38.0 ± 6.0	44.4 ± 7.6	35.0 ± 5.9	36.1 ± 4.8	39.6 ± 3.3	0.516	0.931	0.565
CN.p.65	32.0 ± 4.5	29.9 ± 3.2	32.7 ± 2.8	30.9 ± 1.1	40.1 ± 7.6	29.9 ± 4.4	30.1 ± 2.4	42.5 ± 1.6	0.144	0.261	0.272
CN.a.65	22.4 ± 1.2	23.5 ± 2.1	22.2 ± 2.5	20.9 ± 0.7	26.7 ± 2.0	24.4 ± 2.6	24.4 ± 1.6	22.9 ± 0.3	0.078	0.499	0.824
CN.g.65	33.6 ± 1.8	34.3 ± 3.5	32.0 ± 2.9	34.3 ± 1.7	39.6 ± 2.8	34.3 ± 3.6	34.1 ± 2.7	36.4 ± 0.3	0.185	0.588	0.706
CN.l.65	31.2 ± 2.3	33.4 ± 2.0	30.8 ± 2.6	31.0 ± 0.9	34.3 ± 2.0	32.2 ± 2.1	32.6 ± 1.9	32.8 ± 1.0	0.323	0.912	0.719
CN.b.75	16.3 ± 2.0	13.7 ± 0.3	14.3 ± 1.9	12.2 ± 0.4	17.2 ± 1.9	15.4 ± 2.3	16.5 ± 1.7	14.3 ± 0.8	0.139	0.202	0.978
CN.s.75	49.9 ± 6.2	43.9 ± 2.1	46.2 ± 8.8	39.8 ± 1.0	47.8 ± 5.9	49.9 ± 6.6	58.7 ± 7.5	41.8 ± 5.1	0.293	0.294	0.660
CN.p.75	59.3 ± 6.0	46.1 ± 3.4	57.6 ± 4.6	55.2 ± 2.0	51.9 ± 7.4	53.9 ± 9.1	65.4 ± 6.6	66.1 ± 3.5	0.261	0.210	0.414
CN.a.75	37.9 ± 3.1^{ac}	32.6 ± 4.2^{bc}	42.0 ± 2.9^{ab}	24.9 ± 1.3^{c}	36.6 ± 4.2^{ac}	33.9 ± 1.8^{bc}	49.6 ± 2.6^{a}	29.1 ± 1.6^{bc}	0.171	**<0.001**	0.471
CN.g.75	44 ± 7.9	38.6 ± 3.1	42.8 ± 4.5	31.7 ± 1.4	44.0 ± 8.0	42.7 ± 2.1	53.7 ± 0.4	38.1 ± 2.7	0.125	0.061	0.673
CN.l.75	44.2 ± 5.4^{bc}	40.8 ± 2.3^{bc}	51.9 ± 3.9^{ab}	34.0 ± 2.0^{c}	53.3 ± 5.4^{ab}	46.2± 0.1^{bc}	66.1 ± 0.5^{a}	37.5 ± 2.3^{bc}	**0.004**	**<0.001**	0.424
K.b.65	17.0 ± 1.0^{ab}	13.7 ± 1.5^{ab}	18.1 ± 1.2^{a}	14.7 ± 1.9^{ab}	12.8 ± 1.3^{ab}	10.1 ± 1.4^{b}	17.7 ± 0.9^{a}	16.0 ± 2.2^{ab}	0.126	**0.009**	0.251
K.s.65	13.1 ± 1.5	9.2 ± 1.0	13.4 ± 1.5	12.6 ± 1.0	10.6 ± 0.4	8.7 ± 0.2	11.2 ± 0.9	9.4 ± 1.0	**0.010**	**0.023**	0.598
K.p.65	13.4 ± 0.6^{bc}	13.1 ± 0.8^{bc}	13.3 ± 1.4^{bc}	17.9 ± 0.8^{a}	10.3 ± 0.9^{c}	11.4 ± 1.1^{bc}	11.5 ± 0.7^{bc}	14.7 ± 0.5^{ab}	**0.001**	**<0.001**	0.742
K.a.65	8.26 ± 0.62^{ab}	8.40 ± 1.16^{ab}	5.37 ± 0.72^{ac}	6.70 ± 1.07^{ac}	6.93 ± 0.54^{ac}	9.02 ± 1.02^{a}	3.79 ± 0.56^{c}	4.60 ± 0.42^{bc}	0.073	**<0.001**	0.384
K.g.65	7.80 ± 0.29	5.70 ± 1.06	7.71 ± 0.30	5.63 ± 0.36	6.83 ± 0.53	7.33 ± 0.19	6.11 ± 0.07	5.68 ± 0.15	0.510	**0.018**	**0.018**
K.l.65	6.17 ± 0.35	5.92 ± 0.06	7.04 ± 1.05	7.06 ± 0.64	5.92 ± 0.35	5.92 ± 0.21	6.51 ± 0.48	6.41 ± 0.10	0.329	0.232	0.922
K.b.75	15.9 ± 2.0	12.9 ± 0.8	18.0 ± 3.0	13.8 ± 1.6	13.1 ± 1.3	12.6 ± 0.8	15.9 ± 0.4	12.1 ± 1.2	0.151	0.063	0.880
K.s.75	10.9 ± 1.4^{ab}	11.7 ± 2.2^{ab}	14.4 ± 1.9^{a}	9.3 ± 0.5^{ab}	10.6 ± 0.4^{ab}	10.7 ± 1.0^{ab}	11.5 ± 0.8^{ab}	7.2 ± 0.6^{b}	0.100	**0.014**	0.742
K.p.75	5.45 ± 0.63^{b}	9.33 ± 0.81^{a}	8.35 ± 0.76^{ab}	8.31 ± 0.54^{ab}	6.18 ± 0.61^{ab}	9.10 ± 0.67^{a}	7.46 ± 0.96^{ab}	4.86 ± 0.75^{b}	0.081	**0.001**	0.061
K.a.75	4.99 ± 0.72	5.40 ± 0.93	5.47 ± 0.27	8.32 ± 0.90	6.93 ± 1.39	7.16 ± 0.28	5.61 ± 0.76	7.47 ± 0.95	0.229	0.064	0.327
K.g.75	12.3 ± 0.3	9.4 ± 1.3	13.5 ± 0.2	13.0 ± 0.9	11.6 ± 0.7	12.0 ± 1.4	13.6 ± 1.2	11.1 ± 0.3	0.930	**0.044**	0.111
K.l.75	7.93 ± 0.39	6.85 ± 0.72	7.33 ± 0.36	8.72 ± 0.03	7.99 ± 0.38	7.89 ± 1.33	7.32 ± 0.69	7.18 ± 0.29	0.809	0.625	0.282
Ca.b.65	5.97 ± 0.15	4.43 ± 0.56	3.82 ± 0.26	5.29 ± 0.41	4.62 ± 0.65	5.59 ± 1.10	4.80 ± 0.82	6.05 ± 0.30	0.386	0.193	0.183
Ca.s.65	2.11 ± 0.31	1.31 ± 0.18	1.29 ± 0.16	2.62 ± 0.55	1.53 ± 0.18	1.96 ± 0.26	1.82 ± 0.30	2.17 ± 0.16	0.856	**0.043**	0.100
Ca.p.65	0.79 ± 0.13^{ab}	0.65 ± 0.03^{b}	0.72 ± 0.06^{b}	1.08 ± 0.17^{ab}	0.86 ± 0.10^{ab}	0.76 ± 0.10^{b}	0.89 ± 0.08^{ab}	1.27 ± 0.08^{a}	0.077	**0.001**	0.925
Ca.a.65	2.53 ± 0.22^{ac}	1.74 ± 0.35^{c}	2.13 ± 0.17^{ac}	3.35 ± 0.37^{ab}	2.12 ± 0.32^{ac}	1.98 ± 0.39^{bc}	2.07 ± 0.32^{bc}	3.58 ± 0.20^{a}	0.996	**<0.001**	0.679
Ca.g.65	1.21 ± 0.16	0.93 ± 0.12	0.99 ± 0.06	0.96 ± 0.11	1.02 ± 0.14	0.89 ± 0.18	1.03 ± 0.18	1.44 ± 0.16	0.485	0.239	0.158
Ca.l.65	1.81 ± 0.36^{ac}	0.68 ± 0.08^{c}	1.24 ± 0.20^{bc}	2.12 ± 0.36^{ab}	1.19 ± 0.15^{bc}	1.15 ± 0.10^{bc}	1.32 ± 0.21^{bc}	2.71 ± 0.40^{a}	0.495	**<0.001**	0.125
Ca.b.75	10.0 ± 1.5	8.1 ± 0.6	6.8 ± 0.4	9.0 ± 0.6	11.0 ± 2.3	11.3 ± 0.2	7.8 ± 0.3	11.7 ± 0.3	**0.017**	**0.024**	0.613
Ca.s.75	2.65 ± 0.01^{ab}	1.95 ± 0.12^{b}	2.22 ± 0.25^{ab}	3.05 ± 0.12^{ab}	2.32 ± 0.46^{ab}	2.41 ± 0.05^{ab}	1.86 ± 0.11^{b}	3.19 ± 0.40^{a}	0.902	**0.002**	0.309
Ca.p.75	1.46 ± 0.11^{ac}	1.28 ± 0.21^{ac}	0.94 ± 0.07^{c}	1.05 ± 0.11^{bc}	1.69 ± 0.26^{ab}	1.83 ± 0.16^{a}	1.12 ± 0.12^{ac}	1.27 ± 0.06^{ac}	**0.015**	**0.004**	0.597
Ca.a.75	2.27 ± 0.17^{bc}	1.74 ± 0.18^{c}	2.15 ± 0.16^{bc}	3.37 ± 0.34^{ab}	2.51 ± 0.53^{ac}	2.58 ± 0.37^{ac}	2.15 ± 0.13^{bc}	3.84 ± 0.35^{a}	0.091	**<0.001**	0.573
Ca.g.75	0.84 ± 0.12^{ab}	0.54 ± 0.05^{b}	0.77 ± 0.06^{ab}	0.99 ± 0.10^{ab}	0.94 ± 0.19^{ab}	0.81 ± 0.14^{ab}	0.65 ± 0.02^{b}	1.24 ± 0.08^{a}	0.123	**0.003**	0.278
Ca.l.75	0.95 ± 0.07^{ac}	0.54 ± 0.07^{c}	0.68 ± 0.10^{ac}	1.03 ± 0.04^{ab}	1.04 ± 0.18^{ab}	0.79 ± 0.14^{ac}	0.57 ± 0.06^{bc}	1.12 ± 0.03^{a}	0.261	**<0.001**	0.377

Table S6.2. Continued.

Trait	Control				Low N				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
P.b.65	2.60 ± 0.22	2.76 ± 0.33	3.99 ± 0.67	2.56 ± 0.30	2.61 ± 0.10	2.58 ± 0.18	4.03 ± 0.23	2.61 ± 0.39	0.936	**0.002**	0.986
P.s.65	1.09 ± 0.07^{bc}	0.96 ± 0.11^{c}	1.61 ± 0.04^{a}	1.16 ± 0.06^{bc}	1.27 ± 0.03^{ac}	1.07 ± 0.05^{bc}	1.40 ± 0.08^{ab}	1.39 ± 0.15^{ab}	0.174	**<0.001**	0.066
P.p.65	2.79 ± 0.10^{a}	3.43 ± 0.15^{a}	2.81 ± 0.29^{a}	3.18 ± 0.20^{a}	1.93 ± 0.09^{b}	3.12 ± 0.06^{a}	3.00 ± 0.18^{a}	2.83 ± 0.20^{a}	**0.016**	**0.001**	0.057
P.a.65	1.77 ± 0.23^{ab}	2.11 ± 0.35^{a}	1.82 ± 0.13^{ab}	1.41 ± 0.17^{ab}	1.67 ± 0.12^{ab}	1.94 ± 0.17^{ab}	1.16 ± 0.10^{b}	1.36 ± 0.13^{ab}	0.091	**0.019**	0.380
P.g.65	2.00 ± 0.31	1.85 ± 0.30	2.34 ± 0.12	1.60 ± 0.17	1.97 ± 0.07	2.35 ± 0.01	1.63 ± 0.07	1.66 ± 0.12	0.716	0.082	**0.027**
P.l.65	1.82 ± 0.15	1.92 ± 0.05	2.17 ± 0.28	1.94 ± 0.23	1.70 ± 0.13	1.82 ± 0.06	1.59 ± 0.09	2.58 ± 0.54	0.821	0.224	0.135
P.b.75	1.62 ± 0.10^{b}	2.00 ± 0.36^{b}	2.80 ± 0.21^{ab}	2.14 ± 0.27^{b}	2.04 ± 0.30^{b}	1.98 ± 0.05^{b}	3.78 ± 0.34^{a}	1.77 ± 0.14^{b}	0.168	**<0.001**	0.076
P.s.75	0.71 ± 0.05^{c}	0.72 ± 0.09^{c}	1.05 ± 0.05^{ac}	1.28 ± 0.22^{ab}	1.33 ± 0.06^{ab}	0.80 ± 0.11^{bc}	1.55 ± 0.11^{a}	0.84 ± 0.07^{bc}	**0.023**	**0.002**	**0.001**
P.p.75	0.68 ± 0.09^{c}	1.68 ± 0.14^{ab}	1.52 ± 0.26^{ab}	1.54 ± 0.20^{ab}	0.87 ± 0.14^{bc}	1.28 ± 0.23^{ac}	1.87 ± 0.10^{a}	1.11 ± 0.15^{ac}	0.575	**<0.001**	0.081
P.a.75	0.71 ± 0.04^{c}	1.05 ± 0.23^{ac}	0.80 ± 0.04^{bc}	1.39 ± 0.05^{ab}	1.48 ± 0.21^{a}	0.97 ± 0.13^{ac}	0.89 ± 0.09^{ac}	1.00 ± 0.08^{ac}	0.318	0.093	**0.003**
P.g.75	0.97 ± 0.02	1.33 ± 0.27	1.24 ± 0.07	1.52 ± 0.14	1.40 ± 0.22	1.01 ± 0.15	1.18 ± 0.04	1.26 ± 0.06	0.632	0.435	0.080
P.l.75	0.94 ± 0.05^{b}	1.07 ± 0.15^{b}	0.91 ± 0.05^{b}	2.30 ± 0.43^{a}	1.06 ± 0.06^{b}	1.08 ± 0.13^{b}	0.80 ± 0.06^{b}	1.06 ± 0.10^{b}	**0.027**	**0.001**	**0.005**
Mg.b.65	1.48 ± 0.20^{ad}	1.65 ± 0.08^{ac}	1.26 ± 0.11^{cd}	1.92 ± 0.11^{a}	1.30 ± 0.08^{bcd}	1.69 ± 0.08^{ac}	1.08 ± 0.02^{d}	1.77 ± 0.04^{ab}	0.114	**<0.001**	0.660
Mg.s.65	0.510 ± 0.041^{b}	0.476 ± 0.029^{b}	0.534 ± 0.027^{ab}	0.730 ± 0.077^{a}	0.405 ± 0.024^{b}	0.598 ± 0.056^{ab}	0.526 ± 0.051^{ab}	0.603 ± 0.016^{ab}	0.365	**0.002**	0.052
Mg.p.65	0.739 ± 0.011^{a}	0.732 ± 0.025^{a}	0.652 ± 0.020^{ab}	0.737 ± 0.019^{a}	0.686 ± 0.024^{ab}	0.763 ± 0.055^{a}	0.647 ± 0.027^{ab}	0.565 ± 0.012^{b}	**0.019**	**0.005**	**0.010**
Mg.a.65	1.12 ± 0.06^{ab}	1.17 ± 0.03^{ab}	0.86 ± 0.09^{bc}	1.36 ± 0.04^{a}	1.09 ± 0.03^{ac}	1.21 ± 0.15^{ab}	0.74 ± 0.06^{c}	1.31 ± 0.05^{a}	0.470	**<0.001**	0.784
Mg.g.65	0.900 ± 0.067^{a}	0.836 ± 0.028^{ac}	0.663 ± 0.065^{bc}	0.830 ± 0.024^{ac}	0.906 ± 0.019^{a}	0.854 ± 0.045^{ab}	0.644 ± 0.019^{c}	0.821 ± 0.020^{ac}	0.976	**<0.001**	0.971
Mg.l.65	0.997 ± 0.073^{bc}	0.804 ± 0.031^{cd}	0.606 ± 0.027^{de}	1.249 ± 0.019^{ab}	0.923 ± 0.032^{c}	0.864 ± 0.042^{cd}	0.524 ± 0.040^{e}	1.267± 0.106^{a}	0.618	**<0.001**	0.486
Mg.b.75	1.98 ± 0.14^{ab}	1.99 ± 0.07^{ab}	1.31 ± 0.05^{c}	2.09 ± 0.13^{ab}	1.90 ± 0.07^{ac}	1.85 ± 0.11^{ac}	1.49 ± 0.14^{bc}	2.13 ± 0.21^{a}	0.995	**<0.001**	0.601
Mg.s.75	0.779 ± 0.069^{ab}	0.409 ± 0.016^{b}	0.692 ± 0.029^{ab}	0.954 ± 0.044^{a}	0.780 ± 0.056^{ab}	0.717 ± 0.105^{ab}	0.728 ± 0.060^{ab}	1.020 ± 0.147^{a}	0.076	**<0.001**	0.215
Mg.p.75	0.341 ± 0.052	0.402 ± 0.010	0.389 ± 0.044	0.327 ± 0.014	0.314 ± 0.059	0.366 ± 0.062	0.301 ± 0.020	0.256 ± 0.034	0.078	0.205	0.873
Mg.a.75	1.06 ± 0.11^{ac}	1.06 ± 0.03^{ac}	0.83 ± 0.05^{c}	1.40 ± 0.12^{a}	1.15 ± 0.03^{ac}	1.26 ± 0.13^{ab}	0.93 ± 0.09^{bc}	1.30 ± 0.02^{ab}	0.224	**<0.001**	0.337
Mg.g.75	0.532 ± 0.049^{ab}	0.498 ± 0.033^{ab}	0.398 ± 0.040^{b}	0.651 ± 0.0489^{a}	0.639 ± 0.068^{a}	0.526 ± 0.027^{ab}	0.409 ± 0.034^{b}	0.682 ± 0.028^{a}	0.164	**<0.001**	0.689
Mg.l.75	0.556 ± 0.021^{a}	0.444 ± 0.038^{ab}	0.278 ± 0.028^{c}	0.480 ± 0.034^{a}	0.301 ± 0.046^{bc}	0.488 ± 0.023^{a}	0.259 ± 0.007^{c}	0.479 ± 0.047^{a}	**0.025**	**<0.001**	**0.001**
Fe.b.65	159 ± 8	113 ± 19	124 ± 12	160 ± 33	118 ± 10	119 ± 8	152 ± 8	153 ± 10	0.752	0.126	0.214
Fe.s.65	46.1 ± 10.4	43.6 ± 6.6	52.8 ± 6.0	82.4 ± 16.4	33.0 ± 5.4	57.2 ± 6.9	69.9 ± 15.3	76.2 ± 11.5	0.710	**0.011**	0.428
Fe.p.65	21.6 ± 3.3^{c}	28.1 ± 3.7^{bc}	29.7 ± 1.0^{bc}	53.0 ± 7.3^{a}	30.4 ± 4.8^{bc}	29.5 ± 3.7^{bc}	37.6 ± 4.7^{ac}	44.4 ± 5.7^{ab}	0.474	**0.001**	0.245
Fe.a.65	44.9 ± 2.3^{b}	55.8 ± 8.6^{ab}	64.2 ± 12.7^{ab}	110.0 ± 23.9^{ab}	49.1 ± 5.1^{b}	66.4 ± 6.0^{ab}	109.1 ± 2.1^{ab}	125.0 ± 28.1^{a}	0.086	**0.001**	0.524
Fe.g.65	76.4 ± 9.9^{c}	72.3 ± 3.9^{c}	344.9 ± 15.9^{b}	266.5 ± 23.2^{bc}	81.4 ± 15.3^{c}	87.9 ± 15.3^{c}	707.2 ± 128.5^{a}	171.3 ± 11.6^{bc}	**0.048**	**<0.001**	**0.001**
Fe.l.65	51.7 ± 8.2^{c}	81.8 ± 9.6^{bc}	166.0 ± 31.7^{ac}	196.7 ± 24.0^{ab}	60.7 ± 7.4^{c}	56.2 ± 6.3^{c}	253.1 ± 47.0^{a}	173.1 ± 33.3^{ac}	0.522	**<0.001**	0.134
Fe.b.75	88 ± 6	133 ± 19	119 ± 11	113 ± 13	143 ± 15	128 ± 22	146 ± 6	133 ± 17	**0.033**	0.652	0.277
Fe.s.75	51.6 ± 2.9	62.1 ± 10.6	74.8 ± 12.4	61.5 ± 5.2	52.0 ± 14.4	73.2 ± 6.0	63.3 ± 5.7	75.8 ± 3.3	0.566	0.173	0.453
Fe.p.75	22.2 ± 2.4	33.7 ± 7.4	28.0 ± 8.4	20.5 ± 2.0	21.1 ± 2.2	21.9 ± 4.8	24.4 ± 3.5	22.6 ± 2.6	0.300	0.471	0.525
Fe.a.75	49.4 ± 2.5	71.4 ± 7.3	50.5 ± 7.3	43.7 ± 1.3	62.4 ± 10.4	65.7 ± 8.3	62.9 ± 5.3	53.7 ± 2.1	0.119	**0.047**	0.434
Fe.g.75	218 ± 11^{bc}	124 ± 7^{c}	425 ± 43^{b}	126 ± 17^{c}	262 ± 37^{bc}	208 ± 18^{c}	776 ± 99^{a}	239 ± 39^{bc}	**<0.001**	**<0.001**	**0.012**
Fe.l.75	117 ± 13^{bc}	73 ± 2^{c}	177 ± 9^{ab}	83 ± 11^{c}	172 ± 36^{ab}	93 ± 7^{bc}	229 ± 13^{a}	126 ± 22^{bc}	**0.003**	**<0.001**	0.734
Mn.b.65	63.9 ± 5.2^{a}	35.1 ± 7.0^{b}	36.0 ± 2.4^{b}	55.6 ± 4.6^{ab}	38.1 ± 6.0^{ab}	45.7 ± 4.2^{ab}	51.0 ± 9.4^{ab}	49.9 ± 1.6^{ab}	0.715	0.120	**0.008**
Mn.s.65	26.7 ± 2.7^{ab}	17.9 ± 1.3^{b}	27.8 ± 5.3^{ab}	35.2 ± 4.8^{a}	21.9 ± 3.2^{ab}	24.5 ± 2.1^{ab}	26.6 ± 4.3^{ab}	27.9 ± 1.8^{ab}	0.497	**0.049**	0.247
Mn.p.65	41.0 ± 1.4	36.8 ± 2.1	38.5 ± 5.5	40.8 ± 2.8	32.8 ± 4.1	37.8 ± 4.9	35.8 ± 4.0	35.2 ± 1.3	0.145	0.992	0.623
Mn.a.65	13.4 ± 1.9^{b}	10.0 ± 2.1^{b}	16.8 ± 3.4^{b}	36.0 ± 5.4^{a}	12.6 ± 1.9^{b}	13.4 ± 1.0^{b}	12.5 ± 2.4^{b}	31.6 ± 0.9^{a}	0.436	**<0.001**	0.459
Mn.g.65	12.3 ± 1.1^{b}	12.0 ± 2.5^{b}	15.1 ± 2.1^{ab}	23.4 ± 2.4^{a}	12.6 ± 2.3^{b}	13.1 ± 1.0^{b}	18.2 ± 3.1^{ab}	20.7 ± 0.5^{ab}	0.757	**0.001**	0.573
Mn.l.65	26.9 ± 4.5^{ab}	10.3 ± 1.7^{c}	16.3 ± 2.1^{bc}	36.0 ± 2.9^{a}	22.0 ± 2.4^{bc}	17.7 ± 1.4^{bc}	14.3 ± 2.5^{c}	35.7 ± 0.7^{a}	0.975	**<0.001**	0.124

Table S6.2. Continued.

Trait	Control				Low N				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
Mn.b.75	117.3 ± 12.8^{ab}	62.3 ± 10.7^{b}	91.6 ± 9.1^{ab}	83.5 ± 2.7^{ab}	142.3 ± 19.8^{a}	102.3 ± 10.1^{ab}	113.3 ± 14.9^{ab}	92.1 ± 9.4^{ab}	**0.013**	**0.006**	0.646
Mn.s.75	37.5 ± 3.2^{ab}	31.7 ± 4.6^{b}	39.9 ± 4.6^{ab}	39.3 ± 2.5^{ab}	35.9 ± 2.3^{ab}	39.1 ± 0.6^{ab}	34.1 ± 4.2^{ab}	49.5 ± 4.2^{a}	0.320	0.081	0.124
Mn.p.75	9.9 ± 0.7	14.4 ± 2.0	10.8 ± 2.2	14.0 ± 1.6	11.1 ± 1.5	11.9 ± 2.1	9.7 ± 0.7	11.1 ± 0.2	0.231	0.193	0.543
Mn.a.75	33.4 ± 3.6^{ab}	22.1 ± 5.1^{b}	36.2 ± 4.6^{ab}	48.2 ± 10.1^{ab}	23.8 ± 4.1^{ab}	31.1 ± 5.1^{ab}	31.5 ± 5.5^{ab}	51.2 ± 4.8^{a}	0.883	**0.004**	0.403
Mn.g.75	16.0 ± 2.0^{ab}	9.1 ± 1.3^{b}	20.3 ± 4.6^{a}	19.1 ± 1.1^{ab}	12.9 ± 2.0^{ab}	11.9 ± 0.9^{ab}	15.1 ± 1.5^{ab}	20.7 ± 2.5^{a}	0.551	**0.004**	0.282
Mn.l.75	26.8 ± 2.7^{abc}	12.9 ± 1.4^{d}	19.1 ± 2.9^{bd}	28.0 ± 2.0^{ab}	18.0 ± 2.5^{bd}	20.2 ± 1.9^{bd}	16.3 ± 0.1^{cd}	34.5 ± 2.7^{a}	0.733	**<0.001**	**0.005**
Cu.b.65	7.59 ± 0.23	6.26 ± 0.65	7.73 ± 0.48	5.91 ± 0.69	6.29 ± 1.19	6.66 ± 0.93	6.34 ± 0.66	5.43 ± 0.80	0.212	0.288	0.616
Cu.s.65	3.72 ± 0.53	2.31 ± 0.26	3.65 ± 0.45	2.31 ± 0.30	3.73 ± 0.37	3.09 ± 0.60	2.61 ± 0.43	1.72 ± 0.19	0.481	**0.006**	0.181
Cu.p.65	4.71 ± 0.36^{ab}	4.48 ± 0.15^{ab}	4.74 ± 0.39^{ab}	5.30 ± 0.20^{a}	4.42 ± 0.43^{ab}	4.52 ± 0.66^{ab}	3.53 ± 0.41^{ab}	3.31 ± 0.45^{b}	**0.009**	0.719	0.090
Cu.a.65	4.62 ± 0.61	3.88 ± 0.33	4.52 ± 0.70	4.54 ± 0.95	4.78 ± 0.55	4.63 ± 0.92	2.73 ± 0.06	3.16 ± 0.39	0.225	0.368	0.179
Cu.g.65	4.74 ± 0.59	3.63 ± 0.26	4.97 ± 0.11	2.80 ± 0.22	4.67 ± 0.94	4.41 ± 0.86	3.81 ± 0.84	2.33 ± 0.26	0.598	**0.012**	0.461
Cu.l.65	4.83 ± 0.49^{a}	3.74 ± 0.48^{ab}	4.36 ± 0.46^{ab}	2.62 ± 0.48^{b}	4.51 ± 0.11^{ab}	4.00 ± 0.56^{ab}	2.52 ± 0.20^{b}	2.87 ± 0.45^{ab}	0.197	**0.003**	0.085
Cu.b.75	3.97 ± 0.16	5.79 ± 0.32	3.95 ± 0.48	4.95 ± 0.73	4.76 ± 0.36	4.49 ± 0.68	5.16 ± 0.63	4.55 ± 0.27	0.830	0.454	0.081
Cu.s.75	1.28 ± 0.17^{c}	2.70 ± 0.11^{ab}	1.29 ± 0.23^{c}	1.15 ± 0.26^{c}	1.43 ± 0.17^{c}	1.63 ± 0.21^{bc}	2.96 ± 0.45^{a}	1.16 ± 0.12^{c}	0.273	**0.001**	**<0.001**
Cu.p.75	1.24 ± 0.25^{b}	3.05 ± 0.25^{a}	1.97 ± 0.36^{ab}	1.54 ± 0.19^{b}	1.89 ± 0.28^{ab}	2.11 ± 0.26^{ab}	2.62 ± 0.44^{ab}	1.25 ± 0.18^{b}	0.932	**0.002**	**0.037**
Cu.a.75	1.23 ± 0.13^{b}	3.47 ± 0.06^{a}	1.12 ± 0.15^{b}	2.88 ± 0.23^{a}	2.01 ± 0.34^{ab}	2.56 ± 0.10^{ab}	3.10 ± 0.64^{a}	2.28 ± 0.36^{ab}	0.171	**0.002**	**0.001**
Cu.g.75	2.15 ± 0.42^{bc}	3.47 ± 0.14^{ab}	1.29 ± 0.29^{c}	1.61 ± 0.30^{c}	2.59 ± 0.32^{ac}	2.18 ± 0.11^{bc}	4.32 ± 0.70^{a}	1.93 ± 0.30^{bc}	**0.027**	**0.035**	**<0.001**
Cu.l.75	0.24 ± 0.01^{c}	3.30 ± 0.30^{a}	1.35 ± 0.28^{bc}	2.28 ± 0.21^{ab}	1.86 ± 0.21^{b}	1.86 ± 0.26^{b}	2.31 ± 0.16^{ab}	2.05 ± 0.25^{b}	0.177	**<0.001**	**<0.001**
C.grain.75	441 ± 2	439 ± 3	435 ± 2	438 ± 4	437 ± 2	435 ± 0	434 ± 3	437 ± 0	0.156	0.334	0.854
C.grain.85	436 ± 2	435 ± 1	435 ± 3	438 ± 0	434 ± 1	432 ± 0	433 ± 1	436 ± 0	0.063	0.122	0.940
C.grain.92	435 ± 2	437 ± 0	436 ± 5	439 ± 1	435 ± 2	434 ± 0	433 ± 2	437 ± 0	0.185	0.390	0.784
N.grain.75	24.9 ± 1.4^{ab}	25.0 ± 0.4^{ab}	23.8 ± 0.5^{ac}	20.4 ± 1.2^{bc}	23.2 ± 1.7^{ac}	25.7 ± 0.7^{a}	21.3 ± 0.2^{ac}	19.7 ± 0.6^{c}	0.163	**<0.001**	0.425
N.grain.85	27.1 ± 2.8	24.2 ± 1.2	24.1 ± 3.6	21.3 ± 2.4	26.7 ± 2.9	24.0 ± 1.6	21.9 ± 3.5	21.0 ± 1.5	0.695	0.209	0.974
N.grain.92	26.1 ± 2.7	23.7 ± 0.6	26.3 ± 1.9	22.4 ± 1.0	26.0 ± 2.8	23.3 ± 1.5	19.0 ± 1.2	20.9 ± 1.2	0.080	0.113	0.189
CN.grain.75	17.0 ± 1.3^{b}	17.6 ± 0.4^{ab}	18.3 ± 0.5^{ab}	21.6 ± 1.2^{a}	19.0 ± 1.3^{ab}	16.7 ± 0.5^{b}	20.4 ± 0.3^{ab}	21.3 ± 0.2^{a}	0.247	**0.001**	0.202
CN.grain.85	16.5 ± 1.9	17.9 ± 1.1	18.9 ± 2.9	20.3 ± 1.9	16.7 ± 1.9	18.0 ± 1.1	20.7 ± 2.8	21.0 ± 1.5	0.638	0.213	0.971
CN.grain.92	17.0 ± 1.9	18.0 ± 1.0	16.7 ± 1.0	19.2 ± 0.4	17.2 ± 2.0	18.4 ± 0.9	22.9 ± 1.3	21.1 ± 1.3	**0.033**	0.108	0.119
K.grain.92	4.25 ± 0.21	4.00 ± 0.07	4.36 ± 0.12	4.42 ± 0.21	4.05 ± 0.14	3.86 ± 0.12	4.17 ± 0.13	4.19 ± 0.25	0.133	0.153	0.994
P.grain.92	4.00 ± 0.07	4.19 ± 0.05	4.15 ± 0.06	3.82 ± 0.04	3.96 ± 0.08	3.98 ± 0.15	4.13 ± 0.12	3.77 ± 0.13	0.250	**0.012**	0.729
S.grain.92	1.53 ± 0.11	1.31 ± 0.04	1.39 ± 0.10	1.59 ± 0.11	1.37 ± 0.07	1.25 ± 0.04	1.35 ± 0.11	1.46 ± 0.02	0.108	0.050	0.900
Mg.grain.92	1.21 ± 0.05	1.20 ± 0.04	1.15 ± 0.03	1.13 ± 0.04	1.24 ± 0.04	1.20 ± 0.03	1.17 ± 0.05	1.12 ± 0.04	0.687	0.124	0.964
Ca.grain.92	0.279 ± 0.007^{b}	0.229 ± 0.010^{c}	0.273 ± 0.008^{bc}	0.375 ± 0.011^{a}	0.284 ± 0.011^{b}	0.247 ± 0.008^{bc}	0.288 ± 0.011^{b}	0.384 ± 0.009^{a}	0.104	**<0.001**	0.913
Mn.grain.92	32.69 ± 2.68	32.71 ± 2.13	32.47 ± 1.04	34.39 ± 1.77	30.62 ± 1.39	31.51 ± 0.97	30.03 ± 2.45	31.63 ± 1.60	0.125	0.802	0.977
Fe.grain.92	33.60 ± 0.9	41.4 ± 4.3	35.6 ± 2.5	31.1 ± 0.6	34.0 ± 2.3	38.2 ± 2.5	33.0 ± 3.0	30.5 ± 2.4	0.416	**0.020**	0.884
Na.grain.92	17.8 ± 4.6	15.9 ± 3.1	19.9 ± 4.5	21.8 ± 3.8	15.7 ± 2.1	21.9 ± 2.9	19.7 ± 0.8	29.9 ± 2.3	0.215	0.070	0.369
Zn.grain.92	21.0 ± 1.2^{ab}	18.8 ± 2.2^{ab}	21.0 ± 1.6^{ab}	15.6 ± 0.2^{b}	22.5 ± 2.1^{ab}	22.9 ± 1.4^{ab}	24.1 ± 2.2^{a}	18.1 ± 1.8^{ab}	**0.033**	**0.018**	0.895
Cu.grain.92	5.01 ± 0.47	4.49 ± 0.16	4.37 ± 0.28	4.73 ± 0.30	4.26 ± 0.37	3.80 ± 0.17	3.85 ± 0.21	4.20 ± 0.07	**0.006**	0.220	0.966
Mo.grain.92	0.552 ± 0.101	0.709 ± 0.074	0.567 ± 0.099	0.754 ± 0.033	0.622 ± 0.049	0.493 ± 0.078	0.582 ± 0.075	0.670 ± 0.024	0.304	0.242	0.243
GCY	2638 ± 105^{ad}	2827 ± 337^{abc}	3110 ± 203^{ab}	3323 ± 17^{a}	1842 ± 195^{d}	2196 ± 121^{cd}	2337 ± 208^{bd}	2831 ± 127^{abc}	**<0.001**	**0.003**	0.836
GNY	158 ± 17^{ab}	153 ± 18^{ab}	188 ± 17^{a}	169 ± 8^{ab}	112 ± 22^{b}	118 ± 11^{ab}	104 ± 15^{b}	135 ± 3^{ab}	**<0.001**	0.629	0.340
GKY	25.8 ± 1.6^{ad}	25.8 ± 2.9^{ad}	31.0 ± 1.3^{ab}	33.4 ± 1.6^{a}	17.2 ± 2.2^{d}	19.5 ± 0.8^{cd}	22.5 ± 2.1^{bcd}	27.2 ± 2.6^{ac}	**<0.001**	**0.001**	0.882
GPY	24.2 ± 0.5^{ab}	27.1 ± 3.2^{a}	29.6 ± 2.1^{a}	29.0 ± 0.3^{a}	16.8 ± 1.8^{b}	20.2 ± 1.4^{ab}	22.4 ± 2.5^{ab}	24.5 ± 1.9^{ab}	**<0.001**	**0.022**	0.859
GSY	9.22 ± 0.52^{ab}	8.49 ± 1.09^{ab}	10.01 ± 1.21^{ab}	12.01 ± 0.86^{a}	5.80 ± 0.72^{b}	6.33 ± 0.52^{b}	7.35 ± 1.21^{b}	9.47 ± 0.54^{ab}	**0.001**	**0.005**	0.908
GMgY	7.31 ± 0.11^{ab}	7.76 ± 1.00^{ab}	8.19 ± 0.34^{a}	8.54 ± 0.31^{a}	5.27 ± 0.67^{b}	6.09 ± 0.32^{ab}	6.38 ± 0.83^{ab}	7.27 ± 0.53^{ab}	**0.001**	0.083	0.927

Table S6.2. Continued.

Trait	Control				Low N				*P*-value		
	KNI	DRI	EUR	HAR	KNI	DRI	EUR	HAR	N	G	G×N
GCaY	1.69 ± 0.10^{c}	1.49 ± 0.23^{c}	1.96 ± 0.17^{bc}	2.84 ± 0.07^{a}	1.21 ± 0.16^{c}	1.25 ± 0.09^{c}	1.56 ± 0.19^{c}	2.49 ± 0.17^{ab}	**0.004**	**<0.001**	0.886
GMnY	0.197 ± 0.011^{ac}	0.211 ± 0.027^{ac}	0.232 ± 0.019^{ab}	0.260 ± 0.012^{a}	0.130 ± 0.017^{c}	0.160 ± 0.014^{bc}	0.164 ± 0.027^{bc}	0.205 ± 0.017^{ac}	**<0.001**	**0.015**	0.957
GFeY	0.204 ± 0.011	0.270 ± 0.049	0.256 ± 0.032	0.236 ± 0.006	0.146 ± 0.023	0.193 ± 0.011	0.181 ± 0.029	0.197 ± 0.017	**0.004**	0.188	0.868
GNaY	0.108 ± 0.028^{ab}	0.102 ± 0.0204^{ab}	0.140 ± 0.025^{ab}	0.165 ± 0.029^{ab}	0.068 ± 0.015^{b}	0.110 ± 0.011^{ab}	0.107 ± 0.013^{ab}	0.195 ± 0.023^{a}	0.573	**0.004**	0.351
GZnY	0.128 ± 0.009	0.119 ± 0.010	0.149 ± 0.012	0.118 ± 0.002	0.096 ± 0.015	0.115 ± 0.002	0.132 ± 0.022	0.116 ± 0.007	0.119	0.104	0.569
GCuY	30.2 ± 2.1^{ac}	29.0 ± 3.7^{ac}	31.2 ± 2.9^{ab}	35.8 ± 2.3^{a}	18.2 ± 3.1^{c}	19.2 ± 9.3^{bc}	21.0 ± 2.8^{bc}	27.2 ± 1.6^{ac}	**<0.001**	**0.035**	0.931
GMoY	3.32 ± 0.52^{bc}	4.48 ± 0.17^{ab}	4.04 ± 0.72^{ac}	5.70 ± 0.22^{a}	2.66 ± 0.44^{bc}	2.46 ± 0.29^{c}	3.10 ± 0.35^{bc}	4.33 ± 0.12^{ac}	**<0.001**	**0.001**	0.372
GProtY	882 ± 90	917 ± 115	994 ± 148	1022 ± 72	597 ± 97	721 ± 52	751 ± 120	868 ± 42	**0.006**	0.233	0.916
d13C.b.65	-29.2 ± 0.1	-29.0 ± 0.1	-28.9 ± 0.6	-29.9 ± 0.3	-29.5 ± 0.2	-28.7 ± 0.3	-29.4 ± 0.3	-29.8 ± 0.2	0.649	**0.030**	0.590
d13C.s.65	-27.8 ± 0.2^{ab}	-27.4 ± 0.2^{a}	-28.6 ± 0.2^{ac}	-29.0 ± 0.4^{c}	-28.2 ± 0.2^{ac}	-27.5 ± 0.3^{a}	-28.8 ± 0.07^{bc}	-29.0 ± 0.1^{c}	0.365	**<0.001**	0.853
d13C.p.65	-25.0 ± 0.2^{a}	-25.7 ± 0.1^{ab}	-26.1 ± 0.06^{bc}	-27.4 ± 0.5^{d}	-25.9 ± 0.1^{abc}	-25.6 ± 0.2^{ab}	-26.5 ± 0.3^{bd}	-27.0 ± 0.2^{cd}	0.290	**<0.001**	0.060
d13C.a.65	-27.1 ± 0.5	-27.0 ± 0.0	-27.9 ± 0.4	-28.2 ± 0.5	-27.2 ± 0.1	-26.9 ± 0.2	-27.9 ± 0.1	-27.9 ± 0.5	0.689	**0.016**	0.928
d13C.g.65	-26.9 ± 0.4	-26.7 ± 0.0	-27.2 ± 0.6	-28.1 ± 0.6	-27.1 ± 0.3	-26.8 ± 0.4	-27.9 ± 0.2	-28.0 ± 0.3	0.425	**0.012**	0.761
d13C.l.65	-27.1 ± 0.3	-26.8 ± 0.2	-26.9 ± 0.6	-27.8 ± 0.4	-27.1 ± 0.2	-26.9 ± 0.3	-27.7 ± 0.1	-27.8 ± 0.0	0.312	**0.031**	0.547
d13C.b.75	-29.5 ± 0.0^{ab}	-29.0 ± 0.2^{a}	-29.3 ± 0.3^{ab}	-29.9 ± 0.2^{b}	-29.3 ± 0.1^{ab}	-29.3 ± 0.2^{ab}	-29.3 ± 0.1^{ab}	-29.9 ± 0.1^{b}	0.970	**0.003**	0.415
d13C.s.75	-28.6 ± 0.3^{ab}	-27.9 ± 0.1^{a}	-28.3 ± 0.1^{ab}	-28.9 ± 0.3^{ab}	-28.4 ± 0.1^{ab}	-28.0 ± 0.4^{a}	-28.3 ± 0.1^{ab}	-29.1 ± 0.1^{b}	0.906	**0.001**	0.884
d13C.p.75	-26.6 ± 0.2^{ab}	-26.2 ± 0.4^{a}	-26.3 ± 0.2^{a}	-27.9 ± 0.3^{b}	-26.3 ± 0.2^{a}	-26.3 ± 0.3^{a}	-26.4 ± 0.1^{a}	-27.3 ± 0.4^{ab}	0.406	**0.001**	0.693
d13C.a.75	-27.4 ± 0.1^{ab}	-26.7 ± 0.4^{ab}	-26.5 ± 0.2^{a}	-28.1 ± 0.4^{b}	-27.3 ± 0.4^{ab}	-26.6 ± 0.3^{a}	-26.9 ± 0.3^{ab}	-27.9 ± 0.2^{ab}	0.939	**0.001**	0.701
d13C.g.75	-27.9 ± 0.2	-27.2 ± 0.4	-26.9 ± 0.3	-28.4 ± 0.5	-28.0 ± 0.4	-27.4 ± 0.2	-27.8 ± 0.2	-28.5 ± 0.3	0.177	**0.008**	0.548
d13C.l.75	-27.5 ± 0.1	-27.0 ± 0.4	-26.6 ± 0.3	-28.0 ± 0.5	-27.5 ± 0.3	-27.2 ± 0.3	-27.2 ± 0.1	-27.8 ± 0.3	0.542	**0.022**	0.619
d13C.grain.75	-26.7 ± 0.2	-26.2 ± 0.4	-26.6 ± 0.1	-27.1 ± 0.4	-26.0 ± 0.3	-26.2 ± 0.2	-26.6 ± 0.2	-26.8 ± 0.3	0.279	0.122	0.619
d13C.grain.85	-26.0 ± 0.1^{ac}	-25.7 ± 0.4^{ab}	-26.7 ± 0.3^{bc}	-26.7 ± 0.1^{bc}	-26.3 ± 0.2^{ac}	-25.4 ± 0.1^{a}	-26.2 ± 0.2^{ac}	-27.0 ± 0.2^{c}	0.852	**<0.001**	0.273
d13C.grain.92	-25.8 ± 0.1^{ac}	-25.6 ± 0.4^{ab}	-26.5 ± 0.3^{bc}	-26.6 ± 0.1^{bc}	-26.1 ± 0.1^{ac}	-25.3 ± 0.1^{a}	-26.1 ± 0.2^{ac}	-26.8 ± 0.2^{c}	0.726	**<0.001**	0.324
d15N.b.65	1.38 ± 0.30	2.10 ± 0.24	1.98 ± 0.71	2.49 ± 0.14	1.87 ± 0.30	1.42 ± 0.33	2.21 ± 0.30	2.43 ± 0.37	0.991	0.150	0.457
d15N.s.65	0.58 ± 0.41	1.46 ± 0.09	1.93 ± 0.65	1.57 ± 0.18	0.86 ± 0.33	0.54 ± 0.08	0.88 ± 0.34	0.93 ± 0.24	**0.029**	0.247	0.240
d15N.p.65	1.64 ± 0.23	2.07 ± 0.33	2.07 ± 0.62	2.20 ± 0.44	1.02 ± 0.45	1.33 ± 0.30	1.47 ± 0.36	1.19 ± 0.42	**0.021**	0.708	0.954
d15N.a.65	2.20 ± 0.21	3.03 ± 0.38	2.77 ± 0.46	2.41 ± 0.16	2.01 ± 0.30	1.70 ± 0.45	2.01 ± 0.56	2.01 ± 0.60	**0.037**	0.890	0.554
d15N.g.65	1.71 ± 0.19	2.23 ± 0.56	1.87 ± 0.56	1.37 ± 0.10	1.62 ± 0.37	1.29 ± 0.40	1.34 ± 0.59	1.41 ± 0.40	0.230	0.849	0.662
d15N.l.65	1.99 ± 0.18	2.45 ± 0.31	2.41 ± 0.41	1.54 ± 0.20	1.75 ± 0.31	1.19 ± 0.49	1.43 ± 0.47	1.59 ± 0.66	0.051	0.824	0.364
d15N.b.75	1.74 ± 0.36	2.03 ± 0.24	2.23 ± 0.21	2.53 ± 0.24	1.39 ± 0.08	1.35 ± 0.44	1.60 ± 0.60	2.32 ± 0.09	0.063	0.082	0.869
d15N.s.75	0.80 ± 0.31^{ab}	0.99 ± 0.09^{ab}	0.93 ± 0.07^{ab}	1.51 ± 0.06^{a}	0.18 ± 0.13^{b}	-0.01 ± 0.46^{b}	0.33 ± 0.47^{ab}	1.04 ± 0.14^{ab}	**0.003**	**0.029**	0.782
d15N.p.75	1.30 ± 0.31	1.16 ± 0.22	1.28 ± 0.08	1.33 ± 0.28	0.40 ± 0.09	0.42 ± 0.37	0.71 ± 0.39	0.61 ± 0.34	**0.002**	0.861	0.954
d15N.a.75	2.35 ± 0.51	1.95 ± 0.12	2.13 ± 0.05	2.18 ± 0.34	1.47 ± 0.19	1.02 ± 0.27	1.76 ± 0.57	1.66 ± 0.08	**0.009**	0.445	0.784
d15N.b.75	1.65 ± 0.61	1.40 ± 0.09	1.30 ± 0.10	1.63 ± 0.22	0.95 ± 0.43	0.34 ± 0.36	0.76 ± 0.49	1.04 ± 0.03	**0.010**	0.505	0.874
d15N.l.75	1.88 ± 0.51^{ab}	2.07 ± 0.23^{a}	1.84 ± 0.16^{ab}	2.18 ± 0.22^{a}	0.38 ± 0.37^{b}	0.23 ± 0.50^{b}	1.23 ± 0.39^{ab}	1.45 ± 0.02^{ab}	**<0.001**	0.164	0.231
d15N.grain75	4.32 ± 0.68^{a}	3.68 ± 0.13^{ab}	3.45 ± 0.19^{ab}	3.49 ± 0.26^{ab}	1.63 ± 0.59^{b}	2.54 ± 0.43^{ab}	2.32 ± 0.58^{ab}	2.50 ± 0.21^{ab}	**<0.001**	0.962	0.197
d15N.grain.85	3.33 ± 0.37	2.87 ± 0.09	3.10 ± 0.78	2.38 ± 0.57	1.83 ± 0.46	1.57 ± 0.34	2.15 ± 0.76	2.38 ± 0.23	**0.018**	0.840	0.478
d15N.grain.92	3.43 ± 0.38	3.01 ± 0.06	3.35 ± 0.80	2.61 ± 0.67	1.93 ± 0.47	1.71 ± 0.32	2.23 ± 0.79	2.65 ± 0.25	**0.020**	0.868	0.479

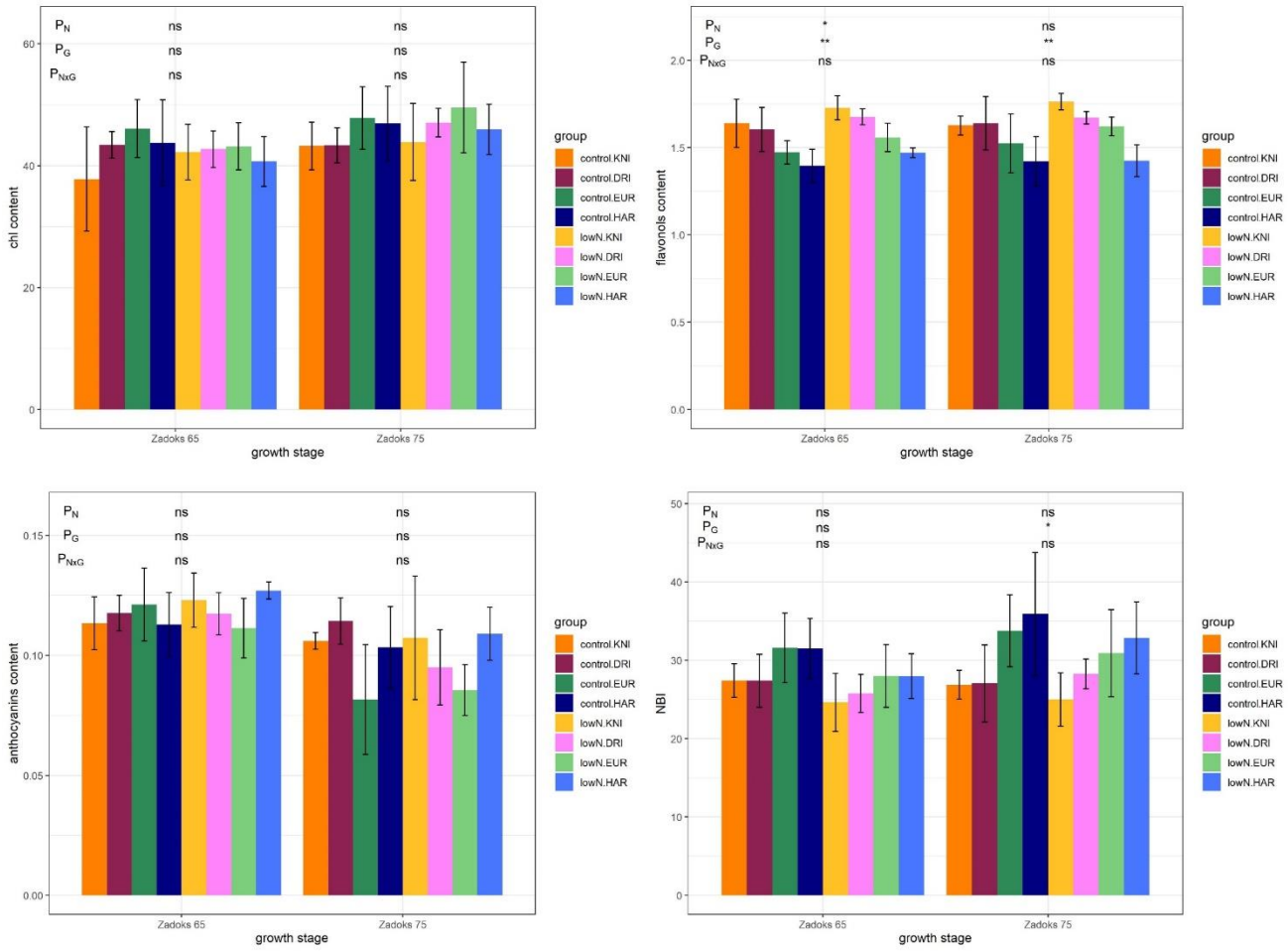


Figure S6.1. Leaf chlorophyll, flavonols and anthocyanins contents and N balanced index (NBI) in four varieties of field-grown durum wheat (Kiko Nick, Don Ricardo, Euroduro and Haristide) at two N levels (control vs. low N). Asterisks indicate a significant difference between varieties and N levels (*, P<0.05; **, P<0.01; ***, P<0.001) according to two-way ANOVA.

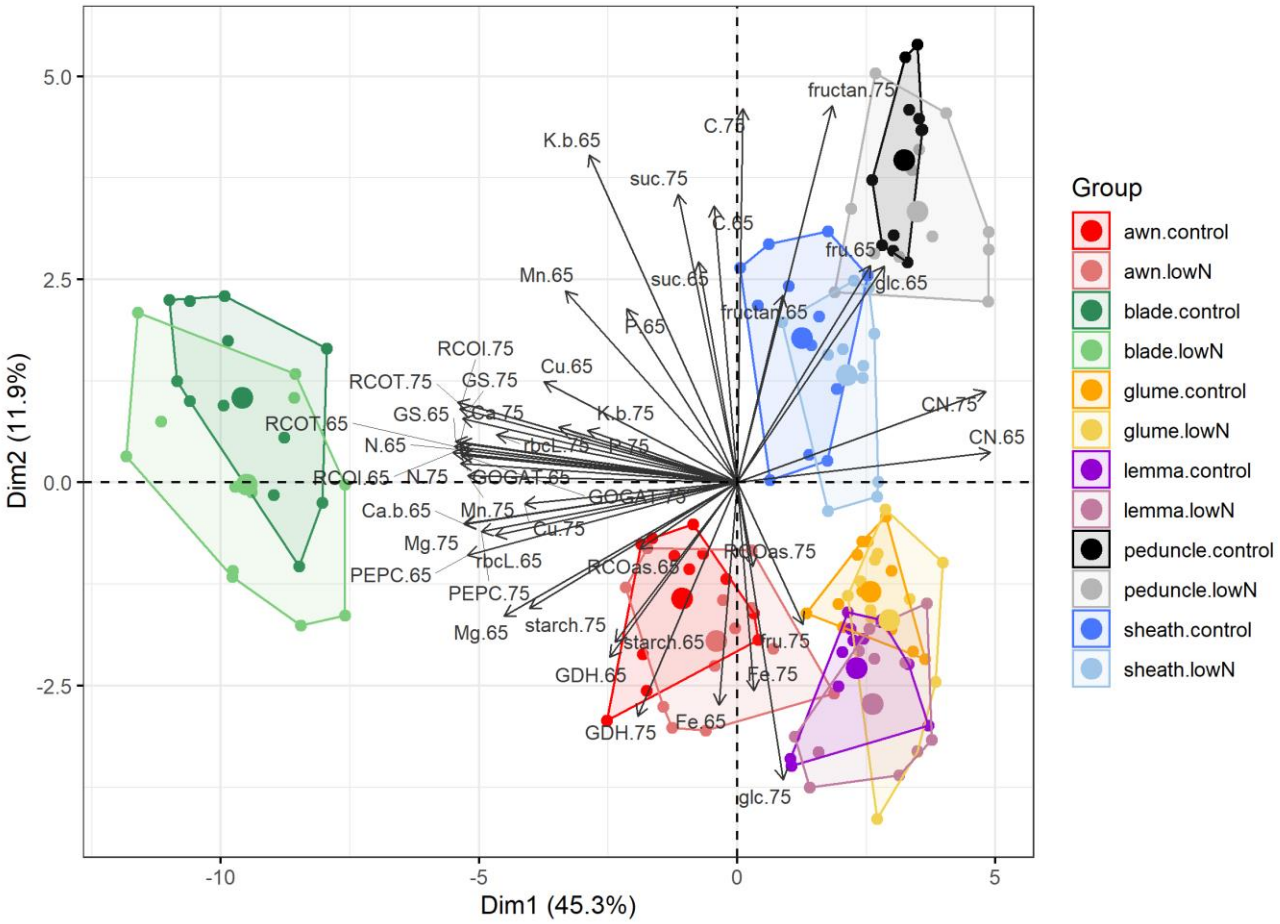


Figure S6.2. Principal component analysis of metabolites, enzyme activities and nutrient composition in field-grown durum wheat per organ (blade, sheath, peduncle, awn, glume and lemma) at two N levels (control vs. low N). The abbreviations are described in Supplementary Table 1.

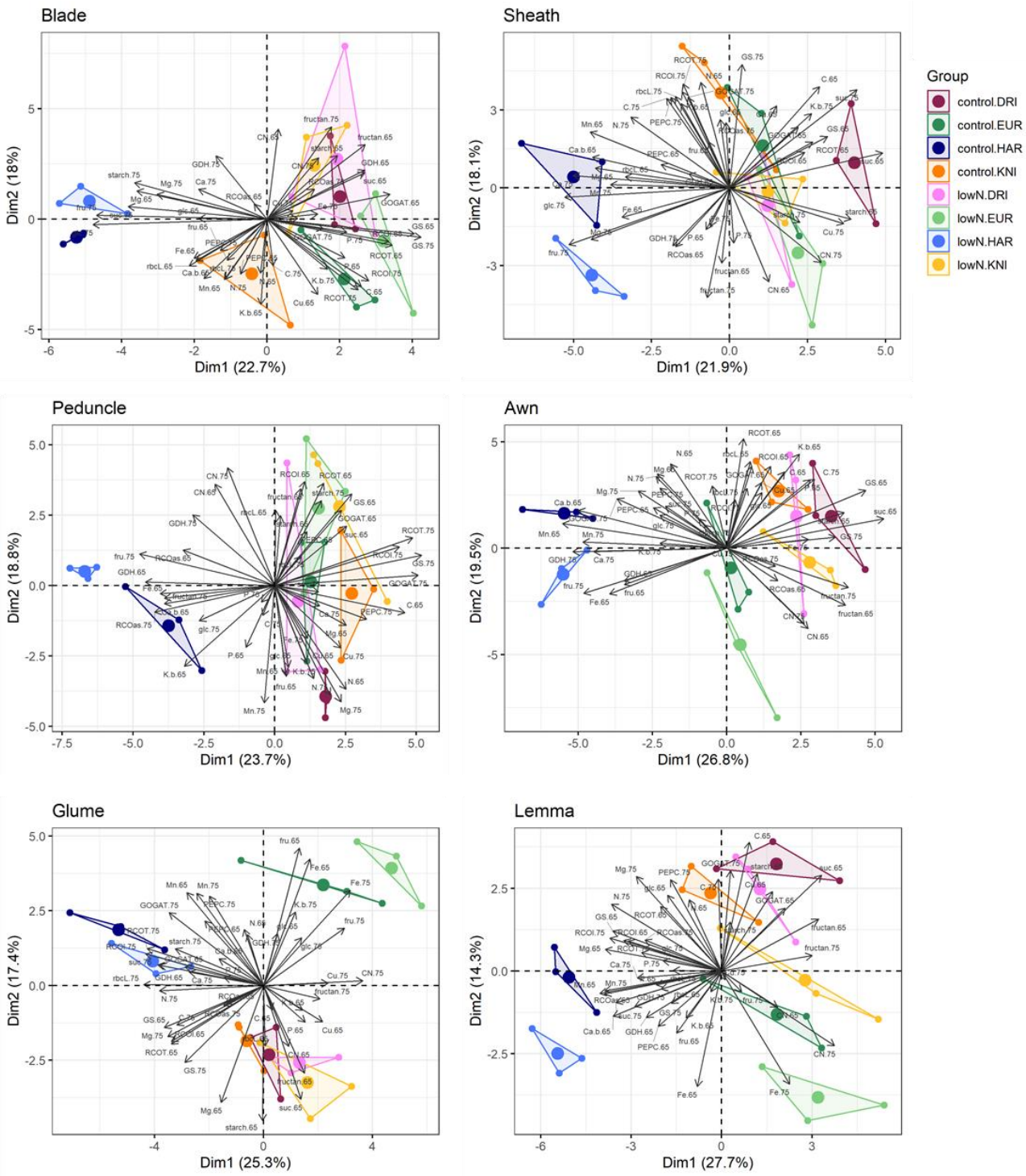


Figure S6.3. Principal component analysis of metabolites, enzyme activities and nutrient composition in four varieties of field-grown durum wheat (Kiko Nick, Don Ricardo, Euroduro and Haristide) at two N levels (control vs. low N) per organ (blade, sheath, peduncle, awn, glume and lemma). The abbreviations are described in Supplementary Table 1.

9.3. Study IV

Table S7.1. Effect of genotypic variability (G), water regime (W) and their interaction (G×W) on agronomic traits, carbon and nitrogen isotope composition, grain quality traits, grain mineral content, spectral vegetation indices, leaf relative water content, and organ-specific fresh and dry weights, water content, and carbon (glucose, glucose-6-phosphate, fructose, sucrose, starch, and malate) and nitrogen (glutamate, total amino acids, proteins, chlorophylls a, b and total) metabolites. The numbers in the traits represent the Zadoks scale when they were measured. The organ-specific traits were expressed as concentration in dry weight (DW) and as total organ content. The means in each row with different letters are statistically different (p<0.05; two-way ANOVA, TUKEY test; yellow colour indicates the significance of a factor). The colour scale in the means shows the minimum (red) and maximum (blue) values per trait. The rest of the abbreviations are described throughout the text. MEX, Mexa; EUR, Euroduro; DRI, Don Ricardo; KNI, Kiko Nick; HAR, Haristide; b, blade; s, sheath; p, peduncle; a, awn; g, glume; l, lemma; e, the whole ear.

Traits	Units	Irrigated					Rainfed					*p*-value		
		MEX	EUR	DRI	KNI	HAR	MEX	EUR	DRI	KNI	HAR	G	W	G×W
GY	kg ha^{-1}	7220 ± 291^{a}	7270 ± 852^{a}	7050 ± 979^{a}	7550 ± 1050^{a}	7070 ± 818^{a}	2520 ± 222^{b}	2390 ± 291^{b}	3380 ± 241^{b}	2410 ± 279^{b}	2180 ± 105^{b}	0.909	0.000	0.784
GNY	kg ha^{-1}	169 ± 31.8^{ab}	197 ± 13.2^{a}	154 ± 18.4^{abc}	165 ± 25^{ab}	159 ± 19.1^{ab}	72.5 ± 6.71^{cd}	75.2 ± 10.6^{cd}	108 ± 5.6^{bd}	75.2 ± 8.36^{cd}	66.8 ± 2.16^{d}	0.645	0.000	0.284
GCY	kg ha^{-1}	3120 ± 141^{a}	3190 ± 401^{a}	3070 ± 418^{a}	3320 ± 474^{a}	3080 ± 368^{a}	1110 ± 103^{b}	1050 ± 121^{b}	1480 ± 106^{b}	1050 ± 118^{b}	954 ± 45.9^{b}	0.916	0.000	0.780
biomass	kg ha^{-1}	14100 ± 536^{a}	14000 ± 473^{a}	14400 ± 2700^{a}	15100 ± 1270^{a}	15300 ± 1740^{a}	6950 ± 803^{b}	6540 ± 116^{b}	9190 ± 1320^{ab}	6440 ± 955^{b}	5900 ± 528^{b}	0.780	0.000	0.553
HI	g grain g biomass^{-1}	0.418 ± 0.017^{a}	0.362 ± 0.017^{abc}	0.373 ± 0.014^{ab}	0.35 ± 0.022^{abc}	0.399 ± 0.008^{ab}	0.276 ± 0.031^{cde}	0.218 ± 0.022^{e}	0.314 ± 0.009^{bd}	0.247 ± 0.014^{de}	0.247 ± 0.017^{de}	0.016	0.000	0.094
plant.m2	plants m^{-2}	180 ± 22	211 ± 25	201 ± 34	221 ± 22	243 ± 17	196 ± 6	175 ± 1	187 ± 28	212 ± 10	224 ± 12	0.163	0.340	0.784
ears.m2	ears m^{-2}	439 ± 22^{ab}	535 ± 22^{a}	339 ± 99^{ab}	521 ± 31.2^{a}	433 ± 58.7^{ab}	368 ± 26^{ab}	360 ± 21.2^{ab}	371 ± 49.7^{ab}	404 ± 20^{ab}	281 ± 21.8^{b}	0.072	0.003	0.187
ears.plant	ears plant^{-1}	2.49 ± 0.23^{a}	2.6 ± 0.28^{a}	1.65 ± 0.26^{ab}	2.37 ± 0.10^{a}	1.83 ± 0.35^{ab}	2.04 ± 0.26^{ab}	2.06 ± 0.14^{ab}	2.00 ± 0.10^{ab}	1.91 ± 0.09^{ab}	1.26 ± 0.10^{b}	0.006	0.020	0.181
grains.ear	grains ear^{-1}	27.3 ± 3.7^{ac}	24.9 ± 5.1^{ac}	28.3 ± 4.3^{ab}	22.9 ± 2.3^{ac}	30.4 ± 2.0^{a}	19.2 ± 3.5^{ac}	13.6 ± 0.5^{bc}	21.5 ± 2.8^{ac}	12.1 ± 2.1^{c}	17.3 ± 2.2^{ac}	0.119	0.000	0.850
d15N.grain	‰	2.19 ± 0.14^{ac}	1.69 ± 0.10^{ad}	2.44 ± 0.26^{ab}	2.79 ± 0.06^{a}	2.78 ± 0.32^{a}	1.42 ± 0.23^{bcd}	0.98 ± 0.17^{cd}	0.95 ± 0.53^{cd}	0.74 ± 0.24^{d}	0.83 ± 0.18^{d}	0.336	0.000	0.040
d13C.grain	‰	-24.5 ± 0.8^{be}	-24.9 ± 0.7^{cde}	-25.6 ± 0.7^{e}	-26.1 ± 0.3^{e}	-25.2 ± 0.3^{de}	-22.4 ± 0.4^{abc}	-22.2 ± 0.7^{ab}	-22.3 ± 0.4^{ab}	-22.8 ± 0.2^{abd}	-21.8 ± 0.9^{a}	0.299	0.000	0.628
prot.grain	%	13.0 ± 0.5^{c}	14.5 ± 1.1^{bc}	15.4 ± 0.6^{ac}	14.8 ± 0.5^{ac}	12.5 ± 0.5^{c}	15.5 ± 0.8^{ac}	17.7 ± 0.7^{a}	16.3 ± 0.3^{ab}	16.5 ± 0.5^{ab}	17.2 ± 0.2^{ab}	0.040	0.000	0.057
TW.grain	g L^{-1}	78.9 ± 1.35^{ab}	80.5 ± 0.8^{a}	78.8 ± 0.6^{ab}	76.7 ± 0.2^{b}	79.5 ± 0.4^{ab}	78.3 ± 0.5^{ab}	79.0 ± 0.5^{ab}	80.3 ± 0.2^{a}	77.7 ± 0.2^{ab}	79.0 ± 0.1^{ab}	0.004	0.945	0.116
TKW.grain	g	52.8 ± 1.5^{bc}	55.5 ± 2.0^{ab}	61.2 ± 1.2^{a}	56.3 ± 3.5^{ab}	51.1 ± 1.8^{bc}	37.2 ± 0.1^{d}	39.6 ± 0.9^{d}	44.9 ± 0.2^{cd}	39.6 ± 1.3^{d}	38.0 ± 0.7^{d}	0.000	0.000	0.819
vit.grain	%	81.2 ± 9.3	92.2 ± 4.2	93.0 ± 2.8	80.2 ± 8.0	86.3 ± 10.7	97.8 ± 0.3	98.0 ± 0.3	98.2 ± 0.6	97.5 ± 0.5	97.8 ± 0.2	0.628	0.004	0.688
b.grain		17.8 ± 0.3^{ad}	16.6 ± 0.4^{cde}	15.6 ± 0.5^{e}	17.0 ± 0.9^{bde}	18.6 ± 0.2^{abc}	17.8 ± 0.0^{ad}	16.4 ± 0.3^{de}	15.9 ± 0.2^{de}	19.4 ± 0.5^{a}	19.0 ± 0.3^{ab}	0.000	0.041	0.033
SDSS.grain	mL g^{-1}	33.0 ± 1.5^{c}	37.7 ± 0.3^{bc}	33.0 ± 1.2^{c}	38.7 ± 5.5^{bc}	37.0 ± 4.7^{bc}	47.0 ± 3.2^{ab}	56.0 ± 1.5^{a}	49.0 ± 0.6^{ab}	59.0 ± 2.7^{a}	58.3 ± 0.9^{a}	0.011	0.000	0.668
WG.grain	mg	24.7 ± 1.1^{cd}	28.8 ± 2.1^{ad}	30.3 ± 2.2^{ad}	26.9 ± 1.5^{bcd}	23.6 ± 0.8^{d}	31.5 ± 3.3^{ad}	35.0 ± 1.3^{ab}	31.3 ± 1.0^{ad}	33.7 ± 1.7^{ac}	36.3 ± 2.2^{a}	0.383	0.000	0.072
GI.grain	%	74 ± 3.3^{a}	71.7 ± 1.8^{a}	54.4 ± 5.4^{b}	71.6 ± 0.9^{a}	50.4 ± 2.5^{b}	77.1 ± 4.1^{a}	85.6 ± 0.4^{a}	80.9 ± 0.8^{a}	82.1 ± 5.6^{a}	49.8 ± 1.0^{b}	0.000	0.000	0.003
N.grain	%	2.31 ± 0.34	2.75 ± 0.16	2.27 ± 0.35	2.19 ± 0.16	2.25 ± 0.02	2.89 ± 0.12	3.14 ± 0.10	3.22 ± 0.21	3.16 ± 0.32	3.07 ± 0.12	0.574	0.000	0.623
C.grain	%	43.2 ± 0.2	43.7 ± 0.4	43.6 ± 0.2	43.9 ± 0.3	43.6 ± 0.3	44.2 ± 0.5	43.8 ± 0.2	43.6 ± 0.1	43.4 ± 0.2	43.8 ± 0.1	0.986	0.368	0.159
Ca.grain	g kg DW^{-1}	0.409 ± 0.012^{ab}	0.387 ± 0.023^{b}	0.338 ± 0.023^{b}	0.333 ± 0.023^{b}	0.42 ± 0.025^{ab}	0.411 ± 0.020^{ab}	0.363 ± 0.01^{b}	0.324 ± 0.019^{b}	0.384 ± 0.021^{b}	0.506 ± 0.020^{a}	0.000	0.127	0.058
K.grain	g kg DW^{-1}	5.00 ± 0.17	4.73 ± 0.26	4.59 ± 0.06	4.69 ± 0.10	4.68 ± 0.11	4.71 ± 0.29	4.74 ± 0.25	4.03 ± 0.11	4.52 ± 0.34	4.93 ± 0.32	0.144	0.295	0.471
Mg.grain	g kg DW^{-1}	1.32 ± 0.05^{bcd}	1.42 ± 0.10^{ab}	1.64 ± 0.09^{a}	1.42 ± 0.04^{ac}	1.19 ± 0.02^{bcd}	1.13 ± 0.02^{d}	1.15 ± 0.05^{cd}	1.09 ± 0.01^{d}	1.15 ± 0.03^{bcd}	1.15 ± 0.05^{cd}	0.019	0.000	0.003
P.grain	g kg DW^{-1}	3.32 ± 0.15^{bc}	3.79 ± 0.34^{ab}	4.45 ± 0.27^{a}	3.66 ± 0.19^{ac}	2.76 ± 0.06^{bc}	2.84 ± 0.25^{bc}	3.07 ± 0.16^{bc}	2.67 ± 0.02^{c}	2.87 ± 0.11^{bc}	2.94 ± 0.30^{bc}	0.023	0.000	0.003
S.grain	g kg DW^{-1}	1.69 ± 0.14^{c}	1.77 ± 0.07^{c}	1.83 ± 0.06^{c}	1.88 ± 0.15^{c}	1.89 ± 0.03^{c}	1.99 ± 0.08^{ac}	2.17 ± 0.13^{ac}	1.9 ± 0.04^{bc}	2.39 ± 0.08^{ab}	2.41 ± 0.11^{a}	0.010	0.000	0.164
Cu.grain	mg kg DW^{-1}	3.76 ± 0.21	4.46 ± 0.32	5.54 ± 0.44	4.59 ± 0.76	3.87 ± 0.13	3.68 ± 0.17	4.04 ± 0.38	3.84 ± 0.35	4.06 ± 0.23	3.77 ± 0.34	0.102	0.027	0.215
Fe.grain	mg kg DW^{-1}	33.7 ± 3.7^{b}	44.7 ± 3.9^{ab}	58.8 ± 8.6^{a}	43.8 ± 2.9^{ab}	34.4 ± 3.3^{b}	35.1 ± 3.0^{b}	39.4 ± 2.2^{ab}	38.5 ± 1.8^{b}	42.0 ± 2.23^{ab}	37.6 ± 3.6^{b}	0.013	0.086	0.055
Mn.grain	mg kg DW^{-1}	32.5 ± 2.1^{bd}	36.8 ± 1.1^{bc}	46.1 ± 3.7^{a}	38.9 ± 1.6^{ab}	23.4 ± 0.7^{ef}	25.6 ± 0.8^{df}	30.3 ± 1.4^{cde}	27.0 ± 1.5^{df}	25.6 ± 1.3^{df}	20.6 ± 0.5^{f}	0.000	0.000	0.001
Zn.grain	mg kg DW^{-1}	14.4 ± 1.2	19.6 ± 0.7	22.9 ± 2.0	16.2 ± 2.7	15.8 ± 2.0	20.0 ± 3.0	19.1 ± 1.4	14.5 ± 1.4	16.9 ± 1.4	22.9 ± 1.8	0.472	0.447	0.005
NDVI_25		0.437 ± 0.041^{ab}	0.410 ± 0.045^{ab}	0.390 ± 0.025^{b}	0.460 ± 0.023^{ab}	0.400 ± 0.027^{ab}	0.483 ± 0.018^{ab}	0.523 ± 0.009^{a}	0.470 ± 0.020^{ab}	0.520 ± 0.015^{ab}	0.467 ± 0.019^{ab}	0.170	0.000	0.760
NDVI_35		0.660 ± 0.025^{ab}	0.650 ± 0.017^{ab}	0.670 ± 0.021^{ab}	0.703 ± 0.022^{a}	0.617 ± 0.035^{ab}	0.630 ± 0.030^{ab}	0.670 ± 0.015^{ab}	0.663 ± 0.009^{ab}	0.667 ± 0.032^{ab}	0.577 ± 0.017^{b}	0.015	0.226	0.688
NDVI_40		0.783 ± 0.009^{ab}	0.767 ± 0.018^{abc}	0.790 ± 0.012^{a}	0.793 ± 0.009^{a}	0.757 ± 0.017^{abd}	0.643 ± 0.037^{de}	0.670 ± 0.023^{be}	0.720 ± 0.000^{abd}	0.660 ± 0.040^{cde}	0.563 ± 0.032^{e}	0.010	0.000	0.128
NDVI_45		0.763 ± 0.015^{ac}	0.710 ± 0.047^{ad}	0.783 ± 0.023^{a}	0.773 ± 0.007^{ab}	0.743 ± 0.015^{ac}	0.633 ± 0.037^{cd}	0.633 ± 0.020^{cd}	0.713 ± 0.003^{ad}	0.643 ± 0.026^{bcd}	0.587 ± 0.038^{d}	0.040	0.000	0.440
NDVI_55		0.747 ± 0.024^{a}	0.693 ± 0.061^{ab}	0.760 ± 0.027^{a}	0.743 ± 0.017^{a}	0.743 ± 0.012^{a}	0.577 ± 0.052^{bc}	0.560 ± 0.017^{bc}	0.680 ± 0.010^{ab}	0.543 ± 0.017^{bc}	0.490 ± 0.029^{c}	0.027	0.000	0.103
NDVI_65		0.697 ± 0.015^{a}	0.683 ± 0.041^{a}	0.677 ± 0.054^{a}	0.687 ± 0.044^{a}	0.680 ± 0.015^{a}	0.290 ± 0.023^{b}	0.320 ± 0.020^{b}	0.320 ± 0.021^{b}	0.270 ± 0.027^{b}	0.283 ± 0.018^{b}	0.920	0.000	0.818

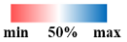


Table S7.1. Continued.

Traits	Units	Irrigated					Rainfed					*p*-value		
		MEX	EUR	DRI	KNI	HAR	MEX	EUR	DRI	KNI	HAR	G	W	G×W
Chl_65		40.1 ± 2.0^{ab}	48.0 ± 1.9^{a}	49.3 ± 1.6^{a}	43.9 ± 1.5^{ab}	37.4 ± 2.2^{ab}	32.8 ± 2.3^{b}	37.2 ± 5.0^{ab}	38.2 ± 3.2^{ab}	38.3 ± 4.4^{ab}	37.1 ± 2.4^{ab}	0.070	0.001	0.353
Chl_75		37.5 ± 0.6^{ab}	36.3 ± 3.1^{ab}	45.8 ± 2.4^{a}	37.0 ± 3.0^{ab}	42.2 ± 2.6^{a}	24.5 ± 3.8^{bc}	32.1 ± 3.8^{ac}	30.1 ± 4.3^{ac}	18.1 ± 2.1^{c}	35.1 ± 6.2^{ac}	0.022	0.000	0.235
Flav_65		1.42 ± 0.03	1.49 ± 0.04	1.51 ± 0.01	1.49 ± 0.01	1.39 ± 0.04	1.46 ± 0.01	1.51 ± 0.03	1.54 ± 0.05	1.55 ± 0.03	1.48 ± 0.05	0.029	0.041	0.845
Flav_75		1.48 ± 0.05^{ab}	1.53 ± 0.04^{ab}	1.45 ± 0.03^{ab}	1.38 ± 0.06^{b}	1.36 ± 0.04^{b}	1.57 ± 0.05^{ab}	1.56 ± 0.07^{ab}	1.6 ± 0.05^{a}	1.65 ± 0.02^{a}	1.55 ± 0.04^{ab}	0.337	0.000	0.099
Anth_65		0.100 ± 0.004	0.109 ± 0.007	0.110 ± 0.009	0.108 ± 0.012	0.144 ± 0.005	0.134 ± 0.013	0.118 ± 0.012	0.113 ± 0.013	0.114 ± 0.008	0.133 ± 0.017	0.093	0.239	0.370
Anth_75		0.098 ± 0.018^{bc}	0.086 ± 0.004^{bc}	0.071 ± 0.007^{c}	0.098 ± 0.004^{bc}	0.118 ± 0.015^{bc}	0.199 ± 0.026^{ab}	0.171 ± 0.012^{ac}	0.190 ± 0.030^{ac}	0.289 ± 0.037^{a}	0.186 ± 0.043^{ac}	0.080	0.000	0.126
NBI_65		28.4 ± 1.1	32.4 ± 2.0	32.5 ± 1.3	29.6 ± 1.6	27 ± 2.5	22.3 ± 1.7	24.3 ± 3.2	24.6 ± 1.5	24.4 ± 2.7	25.4 ± 2.5	0.512	0.000	0.543
NBI_75		25.5 ± 1.2^{ab}	23.6 ± 1.9^{ab}	32.0 ± 0.8^{a}	27.0 ± 2.8^{ab}	31.7 ± 1.3^{a}	15.5 ± 2.7^{bc}	20.1 ± 2.1^{ac}	19.1 ± 3.2^{bc}	10.7 ± 1.4^{c}	22.9 ± 4.3^{ab}	0.011	0.000	0.135
RWC_55	%	85.1 ± 0.3^{ac}	88.9 ± 1.7^{ac}	92.1 ± 1.4^{a}	85.6 ± 2.9^{ac}	80.0 ± 1.3^{cd}	88.4 ± 1.7^{ac}	80.3 ± 1.7^{cd}	81.8 ± 2.5^{bcd}	90.6 ± 0.8^{ab}	74.4 ± 2.5^{d}	0.000	0.012	0.001
RWC_65	%	82.3 ± 2.1^{ac}	87.0 ± 2.6^{ab}	89.8 ± 1.2^{a}	87.8 ± 1.7^{ab}	82.6 ± 1.2^{ac}	72.2 ± 4.9^{c}	81.3 ± 1.9^{ac}	83.3 ± 3.7^{ac}	72.5 ± 1.5^{c}	76.5 ± 2.6^{bc}	0.012	0.000	0.323
RWC_75	%	80.9 ± 3.4^{ab}	83.2 ± 4.8^{a}	85.6 ± 1.8^{a}	77.9 ± 6.4^{ab}	80.9 ± 2.1^{ab}	62.7 ± 4.4^{b}	71.7 ± 2.0^{ab}	73.5 ± 1.5^{ab}	62.3 ± 5.3^{b}	75.8 ± 3.8^{ab}	0.088	0.000	0.539
FW.b.65	g	0.323 ± 0.020^{a}	0.275 ± 0.035^{ac}	0.280 ± 0.026^{ab}	0.255 ± 0.010^{ad}	0.38 ± 0.026^{a}	0.186 ± 0.047^{bcd}	0.152 ± 0.008^{cd}	0.192 ± 0.011^{bcd}	0.137 ± 0.027^{d}	0.196 ± 0.010^{bcd}	0.014	0.000	0.438
FW.s.65	g	0.461 ± 0.030^{a}	0.374 ± 0.037^{ad}	0.403 ± 0.029^{abc}	0.409 ± 0.018^{abc}	0.437 ± 0.021^{ab}	0.342 ± 0.027^{ad}	0.276 ± 0.001^{d}	0.330 ± 0.023^{bd}	0.292 ± 0.032^{cd}	0.340 ± 0.016^{ad}	0.046	0.000	0.884
FW.p.65	g	0.968 ± 0.260^{ac}	1.00 ± 0.115^{ac}	1.100 ± 0.028^{ab}	1.210 ± 0.048^{a}	0.766 ± 0.004^{ac}	0.637 ± 0.165^{bc}	0.466 ± 0.015^{c}	0.603 ± 0.099^{bc}	0.594 ± 0.090^{bc}	0.507 ± 0.013^{c}	0.202	0.000	0.516
FW.a.65	g	0.409 ± 0.066^{a}	0.275 ± 0.025^{ac}	0.251 ± 0.028^{bc}	0.268 ± 0.042^{ac}	0.368 ± 0.036^{ab}	0.255 ± 0.008^{ac}	0.258 ± 0.024^{ac}	0.239 ± 0.013^{bc}	0.158 ± 0.010^{c}	0.258 ± 0.004^{ac}	0.007	0.001	0.132
FW.g.65	g	0.244 ± 0.030^{a}	0.161 ± 0.011^{bc}	0.252 ± 0.013^{a}	0.206 ± 0.022^{ac}	0.246 ± 0.013^{a}	0.159 ± 0.003^{bc}	0.157 ± 0.006^{bc}	0.222 ± 0.008^{ab}	0.138 ± 0.006^{c}	0.221 ± 0.005^{ab}	0.000	0.000	0.057
FW.l.65	g	0.369 ± 0.052^{a}	0.235 ± 0.014^{bd}	0.310 ± 0.014^{abc}	0.290 ± 0.0378^{abc}	0.338 ± 0.029^{ab}	0.23 ± 0.009^{bd}	0.208 ± 0.004^{cd}	0.284 ± 0.010^{ad}	0.164 ± 0.012^{d}	0.226 ± 0.010^{bd}	0.006	0.000	0.064
FW.e.65	g	1.440 ± 0.083^{ab}	1.270 ± 0.120^{bc}	1.340 ± 0.086^{ab}	1.210 ± 0.082^{bc}	1.680 ± 0.075^{a}	1.240 ± 0.053^{bc}	1.100 ± 0.042^{bc}	1.080 ± 0.063^{bc}	0.879 ± 0.104^{c}	1.220 ± 0.037^{bc}	0.001	0.000	0.361
FW.b.75	g	0.239 ± 0.020^{ac}	0.271 ± 0.001^{ac}	0.333 ± 0.011^{ab}	0.261 ± 0.038^{ac}	0.375 ± 0.070^{a}	0.119 ± 0.016^{c}	0.149 ± 0.012^{bc}	0.191 ± 0.020^{ac}	0.116 ± 0.035^{c}	0.206 ± 0.089^{ac}	0.052	0.000	0.975
FW.s.75	g	0.322 ± 0.011^{ab}	0.280 ± 0.008^{ab}	0.400 ± 0.029^{ab}	0.314 ± 0.042^{ab}	0.425 ± 0.033^{a}	0.258 ± 0.011^{ab}	0.273 ± 0.008^{ab}	0.348 ± 0.033^{ab}	0.246 ± 0.037^{b}	0.362 ± 0.077^{ab}	0.005	0.033	0.907
FW.p.75	g	0.876 ± 0.120^{ac}	0.781 ± 0.053^{ac}	1.070 ± 0.027^{ab}	0.936 ± 0.148^{ac}	1.140 ± 0.139^{a}	0.439 ± 0.056^{c}	0.435 ± 0.022^{c}	0.591 ± 0.056^{bc}	0.489 ± 0.096^{c}	0.678 ± 0.161^{ac}	0.040	0.000	0.969
FW.a.75	g	0.288 ± 0.009^{ac}	0.279 ± 0.048^{ac}	0.210 ± 0.010^{c}	0.214 ± 0.032^{bc}	0.452 ± 0.075^{ab}	0.271 ± 0.036^{ac}	0.255 ± 0.014^{ac}	0.235 ± 0.022^{ac}	0.168 ± 0.010^{c}	0.465 ± 0.109^{a}	0.000	0.756	0.946
FW.g.75	g	0.172 ± 0.003^{bc}	0.138 ± 0.015^{c}	0.208 ± 0.012^{ac}	0.159 ± 0.028^{c}	0.264 ± 0.023^{ab}	0.172 ± 0.012^{bc}	0.153 ± 0.004^{c}	0.211 ± 0.017^{ac}	0.148 ± 0.007^{c}	0.280 ± 0.038^{a}	0.000	0.689	0.950
FW.l.75	g	0.234 ± 0.006	0.179 ± 0.031	0.239 ± 0.018	0.182 ± 0.032	0.287 ± 0.035	0.196 ± 0.010	0.196 ± 0.003	0.235 ± 0.019	0.167 ± 0.014	0.282 ± 0.049	0.003	0.581	0.876
FW.e.75	g	2.40 ± 0.37^{ab}	2.02 ± 0.25^{ab}	2.87 ± 0.44^{ab}	1.76 ± 0.18^{b}	3.88 ± 0.47^{a}	1.71 ± 0.26^{b}	1.63 ± 0.05^{b}	2.24 ± 0.15^{ab}	1.54 ± 0.25^{b}	3.87 ± 0.79^{a}	0.000	0.120	0.886
DW.b.65	g	0.094 ± 0.004^{ab}	0.089 ± 0.011^{ac}	0.096 ± 0.007^{ab}	0.076 ± 0.003^{bc}	0.122 ± 0.008^{a}	0.069 ± 0.013^{bc}	0.063 ± 0.003^{bc}	0.071 ± 0.010^{bc}	0.054 ± 0.010^{c}	0.075 ± 0.003^{bc}	0.007	0.000	0.520
DW.s.65	g	0.160 ± 0.008	0.140 ± 0.014	0.161 ± 0.011	0.143 ± 0.009	0.163 ± 0.007	0.148 ± 0.010	0.126 ± 0.001	0.151 ± 0.009	0.127 ± 0.014	0.146 ± 0.006	0.047	0.034	0.994
DW.p.65	g	0.257 ± 0.004^{ac}	0.272 ± 0.014^{ab}	0.291 ± 0.018^{a}	0.238 ± 0.002^{ad}	0.194 ± 0.012^{bcd}	0.207 ± 0.028^{bcd}	0.185 ± 0.007^{cd}	0.182 ± 0.020^{cd}	0.199 ± 0.027^{bcd}	0.168 ± 0.006^{d}	0.019	0.000	0.100
DW.a.65	g	0.152 ± 0.009^{ab}	0.140 ± 0.014^{ac}	0.114 ± 0.010^{bcd}	0.114 ± 0.007^{bcd}	0.169 ± 0.009^{a}	0.137 ± 0.004^{ac}	0.107 ± 0.004^{cd}	0.103 ± 0.008^{cd}	0.080 ± 0.012^{d}	0.124 ± 0.007^{bcd}	0.000	0.000	0.299
DW.g.65	g	0.066 ± 0.003^{bcd}	0.063 ± 0.007^{cd}	0.084 ± 0.006^{ab}	0.063 ± 0.004^{cd}	0.092 ± 0.001^{a}	0.068 ± 0.004^{bcd}	0.058 ± 0.003^{d}	0.081 ± 0.003^{ac}	0.059 ± 0.004^{d}	0.083 ± 0.003^{ab}	0.000	0.169	0.777
DW.l.65	g	0.085 ± 0.0041^{ab}	0.080 ± 0.012^{ab}	0.101 ± 0.007^{a}	0.077 ± 0.005^{ab}	0.099 ± 0.002^{a}	0.080 ± 0.005^{ab}	0.067 ± 0.002^{b}	0.087 ± 0.002^{ab}	0.065 ± 0.008^{b}	0.068 ± 0.006^{b}	0.008	0.001	0.303
DW.e.65	g	0.463 ± 0.021^{ac}	0.445 ± 0.043^{ac}	0.481 ± 0.039^{ac}	0.387 ± 0.023^{bc}	0.548 ± 0.020^{a}	0.491 ± 0.024^{ab}	0.437 ± 0.014^{ac}	0.445 ± 0.017^{ac}	0.346 ± 0.041^{c}	0.478 ± 0.013^{ac}	0.001	0.167	0.476
DW.b.75	g	0.084 ± 0.001^{ac}	0.100 ± 0.003^{ac}	0.130 ± 0.007^{ab}	0.085 ± 0.008^{ac}	0.139 ± 0.019^{a}	0.055 ± 0.005^{c}	0.067 ± 0.006^{bc}	0.089 ± 0.010^{ac}	0.047 ± 0.011^{c}	0.097 ± 0.033^{ac}	0.003	0.000	0.983
DW.s.75	g	0.133 ± 0.010	0.123 ± 0.006	0.180 ± 0.013	0.130 ± 0.016	0.193 ± 0.014	0.126 ± 0.006	0.133 ± 0.005	0.172 ± 0.017	0.115 ± 0.015	0.171 ± 0.034	0.001	0.395	0.878
DW.p.75	g	0.373 ± 0.058^{ac}	0.331 ± 0.023^{ac}	0.456 ± 0.014^{ab}	0.395 ± 0.050^{ac}	0.522 ± 0.064^{a}	0.207 ± 0.029^{c}	0.201 ± 0.011^{c}	0.278 ± 0.028^{bc}	0.226 ± 0.043^{c}	0.306 ± 0.066^{bc}	0.017	0.000	0.902
DW.a.75	g	0.163 ± 0.021^{ab}	0.150 ± 0.014^{ab}	0.156 ± 0.013^{ab}	0.120 ± 0.013^{ab}	0.237 ± 0.024^{a}	0.140 ± 0.009^{ab}	0.132 ± 0.003^{ab}	0.132 ± 0.015^{ab}	0.078 ± 0.015^{b}	0.237 ± 0.060^{a}	0.000	0.169	0.935
DW.g.75	g	0.076 ± 0.008^{bc}	0.060 ± 0.007^{c}	0.110 ± 0.008^{ab}	0.066 ± 0.006^{c}	0.114 ± 0.009^{a}	0.067 ± 0.004^{c}	0.060 ± 0.003^{c}	0.107 ± 0.006^{ab}	0.057 ± 0.006^{c}	0.116 ± 0.011^{a}	0.000	0.374	0.912
DW.l.75	g	0.091 ± 0.009^{ac}	0.087 ± 0.011^{ac}	0.129 ± 0.011^{a}	0.082 ± 0.012^{ac}	0.119 ± 0.005^{ab}	0.077 ± 0.007^{ac}	0.069 ± 0.004^{bc}	0.116 ± 0.006^{ab}	0.058 ± 0.011^{c}	0.114 ± 0.020^{ab}	0.000	0.039	0.928
DW.e.75	g	0.910 ± 0.154^{bc}	0.758 ± 0.097^{c}	1.100 ± 0.172^{ac}	0.632 ± 0.060^{c}	1.570 ± 0.211^{ab}	0.679 ± 0.091^{c}	0.631 ± 0.017^{c}	0.895 ± 0.056^{bc}	0.580 ± 0.092^{c}	1.680 ± 0.287^{a}	0.000	0.286	0.770
WC.b.65	%	70.7 ± 0.8^{a}	67.7 ± 0.8^{ab}	65.7 ± 1.0^{ab}	70.1 ± 0.3^{a}	67.8 ± 0.2^{ab}	61.6 ± 2.3^{ab}	58.7 ± 0.2^{b}	63.2 ± 4.9^{ab}	60.3 ± 1.2^{b}	61.6 ± 0.5^{ab}	0.603	0.000	0.276
WC.s.65	%	65.3 ± 0.6^{a}	62.4 ± 0.7^{b}	60.1 ± 0.8^{b}	65.2 ± 0.8^{a}	62.6 ± 0.36^{ab}	56.7 ± 0.6^{c}	54.3 ± 0.1^{c}	54.2 ± 0.4^{c}	56.6 ± 0.4^{c}	56.9 ± 0.3^{c}	0.000	0.000	0.022
WC.p.65	%	67.1 ± 11.8	72.5 ± 1.9	73.4 ± 2.3	80.2 ± 0.7	74.7 ± 1.6	75.0 ± 6.7	60.3 ± 0.2	69.2 ± 1.7	66.3 ± 0.8	66.8 ± 0.5	0.641	0.045	0.153
WC.a.65	%	61.2 ± 4.8	48.4 ± 6.9	53.7 ± 5.9	56 ± 4.3	53.5 ± 2.4	46.1 ± 3.4	58.2 ± 3.1	57.1 ± 1.4	50.1 ± 5.1	52.0 ± 3.4	0.975	0.507	0.090
WC.g.65	%	72.5 ± 2.6^{a}	60.8 ± 5.1^{ab}	66.6 ± 3.1^{ab}	69.2 ± 1.9^{ab}	62.6 ± 1.6^{ab}	57.5 ± 2.1^{b}	62.9 ± 2.7^{ab}	63.7 ± 0.5^{ab}	57.2 ± 2.0^{b}	62.4 ± 0.7^{ab}	0.620	0.002	0.011
WC.l.65	%	76.1 ± 2.9^{a}	65.7 ± 5.9^{ab}	67.3 ± 1.9^{ab}	72.8 ± 2.4^{ab}	70.2 ± 2.3^{ab}	65.2 ± 1.0^{ab}	67.7 ± 1.3^{ab}	69.3 ± 1.2^{ab}	60.7 ± 3.2^{b}	70 ± 1.9^{ab}	0.494	0.040	0.032
WC.e.65	%	67.8 ± 0.5^{a}	64.9 ± 0.6^{ab}	64.2 ± 0.8^{b}	67.9 ± 0.4^{a}	67.3 ± 1.0^{ab}	60.5 ± 0.9^{c}	60.3 ± 0.2^{c}	58.6 ± 0.9^{c}	60.6 ± 0.6^{c}	60.6 ± 0.3^{c}	0.001	0.000	0.244

Table S7.1. Continued.

Traits	Units	Irrigated					Rainfed					*p*-value		
		MEX	EUR	DRI	KNI	HAR	MEX	EUR	DRI	KNI	HAR	G	W	G×W
WC.b.75	%	64.5 ± 2.8^{ab}	63.2 ± 0.9^{ab}	61.1 ± 1.3^{ab}	66.7 ± 2.2^{a}	62.1 ± 2.5^{ab}	52.8 ± 2.6^{ab}	54.9 ± 0.4^{ab}	53.7 ± 0.5^{ab}	56.9 ± 4.6^{ab}	44.6 ± 10.8^{b}	0.370	0.000	0.746
WC.s.75	%	58.8 ± 1.7^{a}	56.2 ± 0.7^{ab}	54.9 ± 0.8^{ac}	58.2 ± 2.0^{a}	54.5 ± 0.3^{ac}	51.1 ± 0.3^{bc}	51.3 ± 0.3^{bc}	50.5 ± 0.3^{c}	53.0 ± 1.2^{bc}	52.6 ± 0.7^{bc}	0.074	0.000	0.132
WC.p.75	%	57.6 ± 0.7^{a}	57.6 ± 0.2^{a}	57.3 ± 1.0^{a}	57.3 ± 1.7^{a}	54.2 ± 0.6^{ab}	53.2 ± 0.8^{b}	53.7 ± 0.1^{ab}	53.0 ± 0.3^{b}	53.7 ± 0.3^{ab}	54.4 ± 1.1^{ab}	0.528	0.000	0.051
WC.a.75	%	42.9 ± 8.7	42.4 ± 12.1	26.3 ± 3.2	43.2 ± 3.6	46.3 ± 4.4	47.6 ± 3.4	47.8 ± 3.8	43.4 ± 5.5	53.1 ± 9.5	45.8 ± 14.9	0.510	0.160	0.844
WC.g.75	%	55.9 ± 4.9	55.5 ± 5.4	47.1 ± 0.5	57.5 ± 3.4	56.6 ± 0.4	61.1 ± 2.3	60.8 ± 2.7	49.1 ± 2.1	61.4 ± 5.2	57.0 ± 7.7	0.073	0.210	0.968
WC.l.75	%	60.8 ± 4.9	50 ± 6.1	45.9 ± 2.2	54 ± 3.6	57.7 ± 3.0	60.6 ± 3.5	64.6 ± 1.5	50.5 ± 1.9	64.6 ± 8.2	56.9 ± 11.3	0.217	0.111	0.568
WC.e.75	%	62.4 ± 0.7^{a}	62.5 ± 0.3^{a}	61.5 ± 0.1^{a}	64.1 ± 1.1^{a}	59.7 ± 2.0^{ab}	60.0 ± 0.7^{ab}	61.2 ± 0.2^{a}	60.1 ± 0.2^{ab}	62.2 ± 0.4^{a}	55.9 ± 1.8^{b}	0.000	0.002	0.700
glc.b.65	*µmol g DW*$^{-1}$	17.3 ± 1.1^{bc}	10.8 ± 2.9^{bc}	14.0 ± 5.8^{bc}	7.5 ± 0.4^{c}	43.1 ± 14.3^{ab}	57.0 ± 9.2^{a}	12.7 ± 2.4^{bc}	32.7 ± 6.6^{ac}	59.3 ± 6.3^{a}	18.5 ± 4.7^{bc}	0.009	0.000	0.000
glc.s.65	*µmol g DW*$^{-1}$	57.4 ± 4.2^{abc}	32.5 ± 7.6^{bd}	22.6 ± 9.2^{cd}	44.2 ± 3.5^{ad}	61.1 ± 8.0^{ab}	73.5 ± 15.1^{a}	16.0 ± 3.24^{d}	42.0 ± 5.9^{ad}	60.0 ± 3.5^{ab}	55.6 ± 3.5^{abc}	0.000	0.219	0.086
glc.p.65	*µmol g DW*$^{-1}$	227 ± 70^{ab}	243 ± 40^{ab}	222 ± 38^{ab}	338 ± 28^{a}	318 ± 40^{a}	273 ± 41^{a}	71 ± 10^{b}	305 ± 22^{a}	299 ± 38^{a}	169 ± 15^{ab}	0.007	0.068	0.009
glc.a.65	*µmol g DW*$^{-1}$	85.1 ± 19.4^{ac}	36.8 ± 5.5^{bc}	37.5 ± 3.5^{bc}	75.7 ± 9.3^{ac}	85.4 ± 3.4^{ac}	113.0 ± 27^{a}	28.3 ± 5.2^{c}	67.5 ± 16.8^{ac}	102.0 ± 7.8^{ab}	50.0 ± 16.5^{ac}	0.001	0.357	0.100
glc.g.65	*µmol g DW*$^{-1}$	123.0 ± 20.0^{a}	69.0 ± 9.8^{bc}	71.6 ± 0.8^{ac}	121.0 ± 9.0^{ab}	79.2 ± 0.9^{ac}	94.4 ± 4.9^{ac}	48.6 ± 8.9^{c}	73.6 ± 2.8^{ac}	95.9 ± 7.6^{ac}	52.2 ± 17.7^{c}	0.000	0.007	0.576
glc.l.65	*µmol g DW*$^{-1}$	199.0 ± 23.4^{a}	89.1 ± 11.5^{ce}	87.1 ± 13.4^{de}	172.0 ± 17.9^{ab}	167.0 ± 19.8^{ac}	141.0 ± 6.75^{acd}	42.7 ± 1.79^{e}	99.9 ± 15.1^{bce}	129.0 ± 13.1^{acd}	105.0 ± 22.1^{bce}	0.000	0.001	0.166
glc.b.75	*µmol g DW*$^{-1}$	44.9 ± 17.1^{ab}	19.7 ± 4.7^{b}	24.0 ± 9.32^{b}	32.2 ± 17.1^{b}	85.6 ± 9.3^{a}	48.6 ± 10.0^{ab}	19.2 ± 2.6^{b}	23.1 ± 3.7^{b}	29.2 ± 4.3^{b}	14.6 ± 2.3^{b}	0.014	0.028	0.004
glc.s.75	*µmol g DW*$^{-1}$	28.3 ± 5.7^{ab}	14.7 ± 3.9^{b}	12.6 ± 3.7^{b}	19.8 ± 5.0^{b}	41.7 ± 3.4^{a}	20.9 ± 2.6^{b}	14.7 ± 1.0^{b}	13.2 ± 0.8^{b}	17.8 ± 2.6^{b}	24.2 ± 4.3^{ab}	0.000	0.034	0.114
glc.p.75	*µmol g DW*$^{-1}$	37.1 ± 16.4	31.7 ± 15.0	31.4 ± 11.5	22.0 ± 7.24	40.6 ± 9.9	26.5 ± 2.8	19.2 ± 5.8	17.5 ± 3.5	18.6 ± 4.8	35.9 ± 14.9	0.471	0.185	0.980
glc.a.75	*µmol g DW*$^{-1}$	30.0 ± 9.1^{ab}	25.1 ± 14.4^{ab}	16.4 ± 5.1^{b}	20.0 ± 6.6^{b}	61.7 ± 7.3^{a}	24.8 ± 3.2^{ab}	20.0 ± 6.7^{b}	11.9 ± 1.8^{b}	18.8 ± 2.8^{b}	34.1 ± 11.9^{ab}	0.004	0.095	0.471
glc.g.75	*µmol g DW*$^{-1}$	41.6 ± 11.9	44.1 ± 15.4	40.4 ± 10.4	37.2 ± 7.7	44.8 ± 2.3	33.7 ± 5.7	28.4 ± 5.4	21.9 ± 5.8	15.4 ± 2.4	22.4 ± 6.8	0.685	0.004	0.908
glc.l.75	*µmol g DW*$^{-1}$	52.9 ± 14.5^{ab}	35.0 ± 14.3^{ab}	29.6 ± 4.9^{ab}	29.4 ± 8.2^{ab}	63.5 ± 4.9^{a}	28.2 ± 3.0^{ab}	21.2 ± 2.6^{b}	19.6 ± 5.4^{b}	25.2 ± 4.2^{ab}	33.4 ± 10.9^{ab}	0.045	0.006	0.537
glc6P.b.65	*nmol g DW*$^{-1}$	1950 ± 140^{a}	2020 ± 132^{a}	1950 ± 113^{a}	1780 ± 136^{a}	1790 ± 193^{a}	1050 ± 53^{b}	986 ± 23^{b}	1110 ± 106^{b}	1110 ± 92^{b}	901 ± 32^{b}	0.541	0.000	0.613
glc6P.s.65	*nmol g DW*$^{-1}$	1480 ± 91^{a}	1280 ± 63^{a}	1300 ± 66^{a}	1450 ± 107^{a}	1200 ± 165^{a}	707 ± 38^{b}	573 ± 8^{b}	593 ± 18^{b}	750 ± 37^{b}	658 ± 26^{b}	0.062	0.000	0.674
glc6P.p.65	*nmol g DW*$^{-1}$	1710 ± 490^{ab}	1920 ± 215^{ab}	1740 ± 86^{ab}	2380 ± 273^{a}	1950 ± 261^{ab}	1400 ± 187^{ab}	938 ± 8^{b}	1510 ± 103^{ab}	1450 ± 99^{ab}	1280 ± 70^{ab}	0.317	0.000	0.342
glc6P.a.65	*nmol g DW*$^{-1}$	2090 ± 304	1520 ± 190	1740 ± 258	1920 ± 133	1710 ± 81	1500 ± 60	1530 ± 69	1660 ± 81	1470 ± 206	1530 ± 53	0.592	0.025	0.365
glc6P.g.65	*nmol g DW*$^{-1}$	2490 ± 311^{a}	1740 ± 275^{ac}	2040 ± 278^{ac}	2340 ± 124^{ab}	1830 ± 201^{ac}	1530 ± 82^{bc}	1490 ± 58^{bc}	1700 ± 63^{ac}	1600 ± 56^{ac}	1180 ± 85^{c}	0.045	0.000	0.309
glc6P.l.65	*nmol g DW*$^{-1}$	3450 ± 338^{a}	2360 ± 444^{bc}	2170 ± 124^{bc}	2850 ± 267^{ab}	2510 ± 19^{ac}	1820 ± 58^{bc}	1800 ± 80^{bc}	2040 ± 121^{bc}	1780 ± 41^{c}	2020 ± 136^{bc}	0.099	0.000	0.017
glc6P.b.75	*nmol g DW*$^{-1}$	1450 ± 74^{ab}	1460 ± 162^{ab}	1440 ± 101^{ab}	1720 ± 218^{a}	1440 ± 148^{ab}	940 ± 33^{b}	1120 ± 89^{ab}	929 ± 44^{b}	1140 ± 74^{ab}	1060 ± 212^{ab}	0.372	0.000	0.890
glc6P.s.75	*nmol g DW*$^{-1}$	735 ± 55^{ab}	704 ± 33^{ab}	743 ± 84^{ab}	736 ± 80^{ab}	630 ± 30^{ab}	590 ± 22^{b}	561 ± 16^{b}	586 ± 33^{b}	660 ± 43^{ab}	830 ± 18^{a}	0.324	0.045	0.005
glc6P.p.75	*nmol g DW*$^{-1}$	556 ± 24^{bc}	577 ± 41^{bc}	569 ± 79^{bc}	527 ± 11^{c}	561 ± 55^{bc}	751 ± 41^{b}	619 ± 21^{bc}	607 ± 30^{bc}	682 ± 62^{bc}	981 ± 35^{a}	0.003	0.000	0.002
glc6P.a.75	*nmol g DW*$^{-1}$	1200 ± 129	1140 ± 198	832 ± 59	1200 ± 188	1290 ± 196	1100 ± 84	1080 ± 109	888 ± 112	1300 ± 301	1470 ± 358	0.142	0.795	0.948
glc6P.g.75	*nmol g DW*$^{-1}$	1440 ± 184	1270 ± 141	1200 ± 63	1530 ± 166	1000 ± 77	1640 ± 140	1390 ± 135	1190 ± 112	1900 ± 344	1500 ± 300	0.063	0.059	0.684
glc6P.l.75	*nmol g DW*$^{-1}$	1530 ± 212^{ab}	1100 ± 104^{ab}	968 ± 42^{b}	1290 ± 150^{ab}	1110 ± 116^{ab}	1450 ± 102^{ab}	1720 ± 127^{ab}	1130 ± 79^{ab}	2090 ± 513^{a}	1630 ± 304^{ab}	0.100	0.009	0.296
fru.b.65	*µmol g DW*$^{-1}$	21.4 ± 1.6^{ac}	10.5 ± 3.3^{c}	13.4 ± 4.9^{bc}	15.9 ± 0.6^{bc}	36.0 ± 11.9^{ac}	45.6 ± 7.3^{a}	10.6 ± 2.2^{bc}	21.6 ± 4.8^{ac}	39.5 ± 8.1^{ab}	14.7 ± 2.7^{bc}	0.007	0.072	0.004
fru.s.65	*µmol g DW*$^{-1}$	29.5 ± 1.7^{ac}	23.8 ± 5.0^{ac}	17.9 ± 7.6^{bc}	24.5 ± 1.6^{ac}	42.9 ± 7.0^{a}	33.4 ± 5.4^{ab}	10.1 ± 1.6^{c}	22.4 ± 4.2^{ac}	27.4 ± 3.1^{ac}	23.6 ± 3.9^{ac}	0.008	0.152	0.041
fru.p.65	*µmol g DW*$^{-1}$	120.0 ± 36.7^{ab}	132.0 ± 16.9^{ab}	125.0 ± 16.2^{ab}	179.0 ± 19.5^{a}	168.0 ± 16.3^{a}	133.0 ± 22.1^{ab}	46.3 ± 3.5^{b}	157.0 ± 10.9^{a}	128.0 ± 10.9^{ab}	93.9 ± 6.1^{ab}	0.025	0.009	0.012
fru.a.65	*µmol g DW*$^{-1}$	13.7 ± 2.4	9.9 ± 3.9	7.8 ± 0.6	11.4 ± 1.7	14.1 ± 2.7	18.2 ± 5.3	10.2 ± 1.5	13.9 ± 4.9	17.1 ± 1.8	11.6 ± 3.4	0.343	0.171	0.596
fru.g.65	*µmol g DW*$^{-1}$	28.8 ± 3.7	28.9 ± 7.7	20.9 ± 0.9	27.3 ± 1.5	25.2 ± 3.3	31.0 ± 2.6	30.4 ± 3.9	24.0 ± 0.3	35.4 ± 3.3	30.0 ± 11.1	0.433	0.219	0.964
fru.l.65	*µmol g DW*$^{-1}$	77.3 ± 10.3^{ab}	52.8 ± 3.4^{ac}	38.9 ± 4.6^{c}	62.7 ± 7.4^{ac}	78.9 ± 12.6^{a}	55.9 ± 3.7^{ac}	35.1 ± 2.4^{c}	43.3 ± 5.2^{bc}	54.7 ± 3.5^{ac}	58.8 ± 8.1^{ac}	0.001	0.009	0.331
fru.b.75	*µmol g DW*$^{-1}$	49.9 ± 17.2^{ac}	27.8 ± 7.5^{bc}	29.0 ± 8.0^{bc}	28.8 ± 13.3^{bc}	98.7 ± 6.8^{a}	89.6 ± 23.0^{ab}	30.0 ± 5.8^{bc}	47.7 ± 21.1^{ac}	74.8 ± 5.2^{ac}	15.9 ± 0.6^{c}	0.041	0.566	0.000
fru.s.75	*µmol g DW*$^{-1}$	44.4 ± 11.1^{ab}	40.9 ± 18.7^{ab}	31.9 ± 15.3^{b}	27.0 ± 6.8^{b}	93.2 ± 15.1^{a}	38.3 ± 4.4^{b}	29.0 ± 3.13^{b}	25.5 ± 7.9^{b}	24.7 ± 3.8^{b}	48.1 ± 9.9^{ab}	0.004	0.050	0.305
fru.p.75	*µmol g DW*$^{-1}$	50.2 ± 13.0	35.1 ± 8.6	29.9 ± 9.1	30.2 ± 13.4	66.1 ± 17.9	32.0 ± 8.7	22.9 ± 6.6	20.7 ± 3.6	23.8 ± 2.3	56.7 ± 11.9	0.013	0.111	0.984
fru.a.75	*µmol g DW*$^{-1}$	15.5 ± 4.5^{b}	12.3 ± 6.4^{b}	7.4 ± 1.1^{b}	8.3 ± 2.6^{b}	43.1 ± 3.3^{a}	14.9 ± 2.7^{b}	12.3 ± 3.1^{b}	12.8 ± 2.2^{b}	14.6 ± 3.8^{b}	29.7 ± 9.7^{ab}	0.000	0.878	0.249
fru.g.75	*µmol g DW*$^{-1}$	21.9 ± 7.7^{ab}	28.6 ± 9.2^{ab}	21.5 ± 5.2^{ab}	20.4 ± 3.8^{b}	51.3 ± 5.7^{a}	23 ± 3.5^{ab}	20.6 ± 3.4^{b}	17.6 ± 5.0^{b}	13.6 ± 1.9^{b}	37.7 ± 9.9^{ab}	0.002	0.118	0.807
fru.l.75	*µmol g DW*$^{-1}$	33.8 ± 10.1^{ab}	28.9 ± 8.7^{ab}	18.7 ± 2.4^{b}	18.5 ± 3.3^{b}	57.8 ± 5.4^{a}	30.3 ± 2.0^{ab}	26.6 ± 3.6^{ab}	20.6 ± 5.0^{b}	27.5 ± 6.5^{ab}	42.1 ± 12.1^{ab}	0.002	0.630	0.487
suc.b.65	*µmol g DW*$^{-1}$	115 ± 13^{d}	171 ± 29^{ad}	193 ± 21^{ad}	137 ± 12^{cd}	231 ± 24^{ac}	163 ± 18^{bcd}	265 ± 5^{a}	226 ± 37^{ac}	208 ± 6^{ad}	254 ± 5^{ab}	0.000	0.000	0.408
suc.s.65	*µmol g DW*$^{-1}$	139 ± 8^{e}	180 ± 19^{bce}	167 ± 3^{ce}	142 ± 10de	225 ± 7^{ab}	183 ± 18^{bce}	219 ± 3^{ac}	193 ± 11^{bcd}	208 ± 3^{ac}	245 ± 2^{a}	0.000	0.000	0.241
suc.p.65	*µmol g DW*$^{-1}$	90 ± 27^{c}	175 ± 44^{ac}	196 ± 20^{ab}	115 ± 22^{bc}	199 ± 7^{ab}	140 ± 9^{bc}	265 ± 6^{a}	166 ± 24^{ac}	176 ± 13^{ac}	263 ± 1^{a}	0.000	0.002	0.093
suc.a.65	*µmol g DW*$^{-1}$	84 ± 10^{d}	134 ± 20^{bcd}	139 ± 21^{bcd}	86 ± 10^{d}	127 ± 2^{cd}	116 ± 10^{cd}	233 ± 14^{a}	188 ± 17^{ac}	130 ± 24^{bcd}	212 ± 25^{ab}	0.000	0.000	0.245
suc.g.65	*µmol g DW*$^{-1}$	102 ± 5de	130 ± 38^{ce}	169 ± 16^{ace}	90 ± 6^{e}	185 ± 8^{acd}	153 ± 11^{bce}	238 ± 24^{ab}	204 ± 9^{ac}	152 ± 13^{ce}	244 ± 14^{a}	0.000	0.000	0.321
suc.l.65	*µmol g DW*$^{-1}$	132 ± 6^{cd}	158 ± 50^{cd}	148 ± 10^{cd}	112 ± 12^{d}	173 ± 5^{bcd}	173 ± 16^{bcd}	286 ± 18^{ab}	237 ± 26^{ac}	165 ± 17^{bcd}	316 ± 42^{a}	0.002	0.000	0.205

Table S7.1. Continued.

Traits	Units	Irrigated					Rainfed					*p*-value		
		MEX	EUR	DRI	KNI	HAR	MEX	EUR	DRI	KNI	HAR	G	W	G×W
suc.b.75	*µmol g DW*$^{-1}$	149 ± 29	172 ± 41	175 ± 20	148 ± 12	153 ± 21	162 ± 6	239 ± 9	200 ± 16	231 ± 29	193 ± 34	0.341	0.008	0.583
suc.s.75	*µmol g DW*$^{-1}$	193 ± 23	189 ± 29	178 ± 15	200 ± 4	170 ± 5	192 ± 10	204 ± 8	197 ± 12	195 ± 13	195 ± 7	0.821	0.277	0.804
suc.p.75	*µmol g DW*$^{-1}$	253 ± 13	239 ± 25	253 ± 13	274 ± 17	226 ± 19	254 ± 3	287 ± 10	298 ± 13	272 ± 5	261 ± 18	0.213	0.014	0.297
suc.a.75	*µmol g DW*$^{-1}$	89.1 ± 9.3^{ab}	93.1 ± 24.8^{ab}	56.2 ± 3.9^{b}	68.2 ± 7.7^{ab}	97.1 ± 5.1^{ab}	144.0 ± 13.0^{ab}	155.0 ± 10.7^{ab}	149.0 ± 18.6^{ab}	186.0 ± 49.5^{a}	154.0 ± 47.9^{ab}	0.857	0.000	0.663
suc.g.75	*µmol g DW*$^{-1}$	104 ± 10^{bc}	121 ± 28^{ac}	84 ± 6^{c}	106 ± 17^{bc}	153 ± 17^{ac}	180 ± 16^{ab}	209 ± 7^{a}	151 ± 20^{ac}	186 ± 26^{ab}	171 ± 27^{ac}	0.128	0.000	0.396
suc.l.75	*µmol g DW*$^{-1}$	133 ± 11^{ac}	121 ± 22^{ac}	78 ± 3^{c}	88 ± 17^{bc}	140 ± 12^{ac}	196 ± 35^{ac}	232 ± 15^{ab}	153 ± 10^{ac}	263 ± 66^{a}	178 ± 40^{ac}	0.255	0.000	0.200
starch.b.65	*µmol g DW*$^{-1}$	12.8 ± 2.0^{b}	16.1 ± 3.8^{b}	25.7 ± 4.2^{ab}	19.2 ± 1.6^{b}	28.5 ± 16.0^{ab}	7.2 ± 3.4^{b}	54.0 ± 4.3^{a}	23.0 ± 5.2^{ab}	7.8 ± 2.7^{b}	22.3 ± 5.3^{b}	0.005	0.556	0.005
starch.s.65	*µmol g DW*$^{-1}$	4.08 ± 0.10^{c}	5.48 ± 2.17^{c}	7.25 ± 1.73^{c}	4.42 ± 0.47^{c}	8.35 ± 3.81^{bc}	3.64 ± 1.21^{c}	17.70 ± 2.61^{ab}	6.43 ± 0.84^{c}	5.34 ± 1.43^{c}	19.20 ± 3.17^{a}	0.000	0.003	0.007
starch.p.65	*µmol g DW*$^{-1}$	6.07 ± 1.32^{c}	9.50 ± 2.69^{bc}	8.49 ± 1.18^{bc}	10.20 ± 2.81^{bc}	9.05 ± 1.67^{bc}	5.32 ± 0.52^{c}	17.30 ± 0.53^{ab}	9.38 ± 0.79^{bc}	7.62 ± 2.83^{bc}	25.50 ± 2.93^{a}	0.000	0.002	0.001
starch.a.65	*µmol g DW*$^{-1}$	13.1 ± 2.4^{c}	22.3 ± 6.7^{bc}	21.5 ± 4.4^{bc}	13.4 ± 2.0^{c}	20.5 ± 6.0^{bc}	15.8 ± 2.8^{bc}	47.1 ± 3.8^{a}	29.9 ± 0.6^{ac}	18.0 ± 3.8^{bc}	38.8 ± 9.8^{ab}	0.002	0.001	0.157
starch.g.65	*µmol g DW*$^{-1}$	11.3 ± 0.5^{ab}	12 ± 5.2^{ab}	10.3 ± 1.5^{b}	10.1 ± 1.3^{b}	13.4 ± 3.5^{ab}	12.5 ± 2.0^{ab}	18.8 ± 3.0^{ab}	16.4 ± 2.2^{ab}	13.0 ± 2.7^{ab}	24.5 ± 2.3^{a}	0.077	0.004	0.433
starch.l.65	*µmol g DW*$^{-1}$	35.3 ± 4.5^{b}	28.9 ± 11.4^{b}	30.6 ± 3.3^{b}	22.7 ± 2.4^{b}	38.3 ± 3.3^{b}	29.7 ± 3.4^{b}	52.5 ± 10.6^{ab}	44.1 ± 5.1^{b}	25.3 ± 5.6^{b}	80.2 ± 11.6^{a}	0.001	0.003	0.026
starch.b.75	*µmol g DW*$^{-1}$	11.9 ± 7.7	14.4 ± 7.2	13.2 ± 6.5	15.3 ± 8.2	4.6 ± 1.6	4.96 ± 1.3	14.1 ± 1.1	10.2 ± 2.6	8.41 ± 1.7	23.2 ± 2.1	0.777	0.923	0.091
starch.s.75	*µmol g DW*$^{-1}$	2.35 ± 1.3^{ab}	4.15 ± 2.4^{ab}	3.14 ± 0.8^{ab}	2.76 ± 1.1^{ab}	1.38 ± 0.3^{b}	3.38 ± 0.79^{ab}	5.42 ± 0.52^{ab}	4.47 ± 1.12^{ab}	5.40 ± 0.43^{ab}	7.56 ± 0.80^{a}	0.481	0.002	0.144
starch.p.75	*µmol g DW*$^{-1}$	1.08 ± 0.42	1.32 ± 0.62	0.978 ± 0.37	1.59 ± 0.24	1.02 ± 0.24	1.82 ± 0.09	2.43 ± 0.39	2.84 ± 0.56	2.26 ± 0.44	2.36 ± 0.76	0.805	0.001	0.682
starch.a.75	*µmol g DW*$^{-1}$	10.5 ± 3.4^{ab}	10.5 ± 6.0^{ab}	7.3 ± 1.7^{b}	6.8 ± 0.8^{b}	6.7 ± 0.9^{b}	20.2 ± 3.7^{ab}	21.2 ± 3.0^{ab}	21.1 ± 3.1^{ab}	27.7 ± 7.3^{a}	13.4 ± 3.4^{ab}	0.422	0.000	0.434
starch.g.75	*µmol g DW*$^{-1}$	6.6 ± 1.6^{bc}	9.0 ± 3.8^{ac}	4.2 ± 1.3^{c}	7.9 ± 1.7^{ac}	7.9 ± 1.3^{ac}	16.1 ± 1.8^{ab}	17.9 ± 1.9^{a}	12.1 ± 2.4^{ac}	17.3 ± 2.5^{a}	14.0 ± 1.2^{ac}	0.157	0.000	0.919
starch.l.75	*µmol g DW*$^{-1}$	14.7 ± 4.2^{bc}	17.9 ± 6.0^{bc}	8.8 ± 1.4^{c}	8.4 ± 0.8^{c}	9.2 ± 1.3^{bc}	26.0 ± 4.0^{ac}	42.9 ± 8.2^{a}	19.0 ± 1.0^{ac}	33.5 ± 9.6^{ab}	18.9 ± 3.2^{ac}	0.020	0.000	0.292
malate.b.65	*µmol g DW*$^{-1}$	14.7 ± 1.0^{a}	6.6 ± 1.2^{c}	10.6 ± 1.0^{ac}	12.3 ± 0.1^{ab}	7.9 ± 1.0^{bc}	12.3 ± 0.5^{ab}	6.8 ± 0.7^{c}	9.2 ± 0.6^{bc}	11.2 ± 2.4^{ac}	7.2 ± 0.3^{bc}	0.000	0.120	0.795
malate.s.65	*µmol g DW*$^{-1}$	12.5 ± 1.3^{a}	8.7 ± 0.4^{bd}	8.9 ± 0.4^{bc}	10.8 ± 0.7^{ab}	8.4 ± 0.2^{bd}	9.3 ± 0.3^{bc}	5.9 ± 0.6^{d}	7.0 ± 0.4^{cd}	9.5 ± 0.0^{bc}	5.9 ± 0.6^{d}	0.000	0.000	0.526
malate.p.65	*µmol g DW*$^{-1}$	5.77 ± 1.66^{ab}	5.12 ± 1.05^{ab}	4.91 ± 0.55^{ab}	8.33 ± 1.31^{a}	7.85 ± 1.59^{a}	4.93 ± 1.20^{ab}	1.29 ± 0.41^{b}	4.94 ± 0.79^{ab}	4.35 ± 0.36^{ab}	3.38 ± 0.25^{ab}	0.071	0.001	0.140
malate.a.65	*µmol g DW*$^{-1}$	3.09 ± 0.69^{a}	1.01 ± 0.25^{b}	1.27 ± 0.27^{b}	3.33 ± 0.12^{a}	2.61 ± 0.35^{ab}	2.02 ± 0.35^{ab}	1.24 ± 0.08^{b}	1.98 ± 0.21^{ab}	1.85 ± 0.30^{ab}	1.47 ± 0.17^{b}	0.001	0.013	0.009
malate.g.65	*µmol g DW*$^{-1}$	1.28 ± 0.43	0.62 ± 0.11	0.76 ± 0.13	1.65 ± 0.21	1.55 ± 0.30	2.49 ± 1.11	1.96 ± 0.15	1.47 ± 0.23	2.31 ± 0.48	1.35 ± 0.25	0.257	0.015	0.460
malate.l.65	*µmol g DW*$^{-1}$	4.87 ± 0.52^{a}	1.95 ± 0.49^{b}	1.90 ± 0.13^{b}	4.83 ± 0.54^{a}	4.31 ± 0.46^{ab}	3.35 ± 0.96^{ab}	2.01 ± 0.04^{b}	2.56 ± 0.58^{ab}	3.70 ± 0.67^{ab}	3.10 ± 0.47^{ab}	0.001	0.081	0.239
malate.b.75	*µmol g DW*$^{-1}$	12.00 ± 0.81^{a}	6.18 ± 0.39^{b}	9.23 ± 0.77^{ab}	12.00 ± 1.94^{a}	10.20 ± 0.79^{ab}	8.97 ± 1.35^{ab}	6.07 ± 0.31^{b}	7.63 ± 1.13^{ab}	11.00 ± 1.38^{ab}	7.81 ± 1.01^{ab}	0.001	0.030	0.709
malate.s.75	*µmol g DW*$^{-1}$	9.17 ± 0.56^{ab}	7.85 ± 0.77^{ac}	7.46 ± 0.36^{ac}	9.29 ± 0.40^{a}	6.55 ± 0.34^{bc}	6.46 ± 0.58^{c}	5.87 ± 0.56^{c}	6.01 ± 0.77^{c}	6.75 ± 0.23^{ac}	6.07 ± 0.51^{c}	0.020	0.000	0.263
malate.p.75	*µmol g DW*$^{-1}$	0.955 ± 0.092^{ac}	0.674 ± 0.143^{ac}	1.010 ± 0.25^{ab}	0.566 ± 0.053^{ac}	0.477 ± 0.116^{bc}	0.926 ± 0.042^{ac}	0.429 ± 0.009^{c}	0.972 ± 0.140^{ac}	0.564 ± 0.054^{ac}	1.050 ± 0.014^{a}	0.002	0.482	0.023
malate.a.75	*µmol g DW*$^{-1}$	2.10 ± 0.22	1.77 ± 0.28	1.24 ± 0.06	2.82 ± 0.26	2.88 ± 0.23	0.72 ± 0.10	0.94 ± 0.12	0.62 ± 0.09	1.56 ± 0.50	2.35 ± 1.23	0.009	0.004	0.842
malate.g.75	*µmol g DW*$^{-1}$	1.12 ± 0.23^{ac}	0.90 ± 0.28^{bc}	0.77 ± 0.10^{c}	2.01 ± 0.25^{a}	1.14 ± 0.07^{ac}	1.33 ± 0.17^{ac}	1.43 ± 0.07^{ac}	0.94 ± 0.08^{bc}	1.73 ± 0.18^{ab}	0.91 ± 0.21^{bc}	0.000	0.491	0.176
malate.l.75	*µmol g DW*$^{-1}$	1.86 ± 0.30	0.83 ± 0.22	0.96 ± 0.04	2.18 ± 0.22	2.18 ± 0.12	1.29 ± 0.14	1.46 ± 0.05	1.24 ± 0.02	2.37 ± 0.47	1.92 ± 0.85	0.007	0.801	0.459
Glu.b.65	*µmol g DW*$^{-1}$	23.7 ± 3.5^{ac}	26.2 ± 2.5^{ab}	31.0 ± 3.5^{a}	28.2 ± 0.3^{ab}	23.1 ± 1.0^{ac}	18.1 ± 3.1^{bc}	18.1 ± 0.5^{bc}	21.2 ± 1.8^{ac}	18.9 ± 2.6^{bc}	14.0 ± 1.6^{c}	0.044	0.000	0.902
Glu.s.65	*µmol g DW*$^{-1}$	13.6 ± 1.5^{ab}	13.9 ± 0.2^{ab}	16.2 ± 0.4^{a}	13.4 ± 0.6^{ab}	12.8 ± 0.6^{ac}	11.3 ± 0.1^{bc}	9.5 ± 0.9^{c}	10.6 ± 0.6^{bc}	12.5 ± 0.8^{bc}	9.8 ± 0.4^{c}	0.049	0.000	0.034
Glu.p.65	*µmol g DW*$^{-1}$	8.6 ± 2.7	13.8 ± 0.8	13.8 ± 2.0	13.1 ± 1.9	12.3 ± 0.9	10.2 ± 1.1	12.9 ± 1.0	13.0 ± 0.4	12.1 ± 0.3	12.3 ± 0.5	0.050	0.772	0.875
Glu.a.65	*µmol g DW*$^{-1}$	8.72 ± 1.64	8.46 ± 0.50	8.94 ± 0.96	10.40 ± 1.19	8.14 ± 0.45	6.35 ± 0.39	9.22 ± 0.95	10.60 ± 1.02	7.60 ± 1.42	6.35 ± 0.64	0.100	0.172	0.135
Glu.g.65	*µmol g DW*$^{-1}$	9.5 ± 1.0	11.2 ± 2.9	11.7 ± 1.7	12.1 ± 0.6	13.2 ± 0.6	11.3 ± 0.9	15.3 ± 1.2	11.4 ± 0.5	12.9 ± 1.6	13.2 ± 1.0	0.234	0.158	0.529
Glu.l.65	*µmol g DW*$^{-1}$	15.0 ± 1.8	14.0 ± 3.7	13.4 ± 1.3	14.8 ± 1.4	15.5 ± 0.8	11.6 ± 0.5	17.2 ± 1.5	13.6 ± 2.7	14.2 ± 2.2	14.8 ± 1.0	0.711	0.844	0.569
Glu.b.75	*µmol g DW*$^{-1}$	19.7 ± 1.8^{ab}	20.5 ± 1.2^{ab}	24.1 ± 0.9^{a}	22.1 ± 1.1^{ab}	16.3 ± 1.6^{bc}	11.8 ± 0.7^{c}	11.6 ± 0.7^{c}	10.1 ± 0.6^{c}	12.2 ± 1.9^{c}	12.0 ± 1.5^{c}	0.158	0.000	0.020
Glu.s.75	*µmol g DW*$^{-1}$	9.71 ± 0.81	9.44 ± 2.36	9.63 ± 0.51	10.9 ± 0.64	8.02 ± 1.41	7.42 ± 0.35	6.76 ± 0.22	6.35 ± 0.13	7.84 ± 0.28	7.73 ± 0.16	0.543	0.001	0.556
Glu.p.75	*µmol g DW*$^{-1}$	7.78 ± 0.59	10.40 ± 2.11	7.79 ± 0.95	9.32 ± 0.38	5.59 ± 0.40	9.89 ± 0.73	9.91 ± 0.67	9.56 ± 1.33	8.46 ± 0.79	6.52 ± 0.12	0.007	0.274	0.462
Glu.a.75	*µmol g DW*$^{-1}$	6.68 ± 0.71	8.50 ± 2.63	5.50 ± 0.04	9.20 ± 0.58	5.89 ± 1.15	5.27 ± 0.44	7.53 ± 0.58	6.31 ± 0.88	10.10 ± 2.33	7.03 ± 2.18	0.074	0.923	0.846
Glu.g.75	*µmol g DW*$^{-1}$	9.4 ± 0.6^{b}	9.0 ± 1.6^{b}	8.2 ± 0.5^{b}	12.8 ± 1.1^{ab}	10.3 ± 0.7^{ab}	13.2 ± 1.1^{ab}	14.6 ± 0.1^{a}	9.2 ± 0.3^{b}	16.7 ± 3.1^{a}	10.9 ± 1.9^{ab}	0.006	0.003	0.345
Glu.l.75	*µmol g DW*$^{-1}$	11.5 ± 1.2^{ab}	8.96 ± 2.0^{ab}	7.7 ± 0.9^{b}	11.0 ± 0.4^{ab}	10.4 ± 1.9^{ab}	12.2 ± 0.9^{ab}	15.6 ± 1.2^{ab}	9.1 ± 0.7^{ab}	18.4 ± 4.6^{a}	11.2 ± 3.0^{ab}	0.078	0.018	0.297
aa.b.65	*µmol g DW*$^{-1}$	62.0 ± 7.6	67.6 ± 6.3	82.4 ± 4.1	76.4 ± 1.0	69.2 ± 8.2	71.2 ± 7.2	60.6 ± 5.4	69.6 ± 4.2	64.8 ± 5.3	67.1 ± 1.1	0.298	0.183	0.316
aa.s.65	*µmol g DW*$^{-1}$	43.8 ± 0.4	38.4 ± 4.9	48.6 ± 2.3	40.5 ± 4.9	41.7 ± 2.5	46.6 ± 4.7	44.0 ± 5.2	39.3 ± 3.4	56.5 ± 4.4	51.2 ± 4.9	0.465	0.067	0.058
aa.p.65	*µmol g DW*$^{-1}$	26.9 ± 8.0^{d}	40.9 ± 6.4^{cd}	47.8 ± 7.9^{bcd}	36.9 ± 5.7^{cd}	57 ± 7.7^{ac}	38.4 ± 1.0^{cd}	76.5 ± 5.4^{ab}	52.1 ± 0.4^{ad}	53.1 ± 1.6^{ad}	77.5 ± 6.7^{a}	0.000	0.000	0.128
aa.a.65	*µmol g DW*$^{-1}$	35.8 ± 6.4	29.5 ± 2.6	33.0 ± 6.6	34.6 ± 1.8	33.6 ± 4.6	28.8 ± 5.0	49.2 ± 6.1	32.3 ± 2.4	38.5 ± 7.0	34.8 ± 5.5	0.618	0.303	0.151
aa.g.65	*µmol g DW*$^{-1}$	32.8 ± 3.4^{d}	53.7 ± 12.5^{cd}	47.9 ± 6.4^{cd}	48.6 ± 5.1^{cd}	76.3 ± 9.1^{ac}	59.1 ± 8.9^{bcd}	97.4 ± 9.9^{ab}	56.7 ± 0.9^{cd}	83.1 ± 11.2^{ac}	109.0 ± 2.8^{a}	0.000	0.000	0.289
aa.l.65	*µmol g DW*$^{-1}$	47.6 ± 7.3^{c}	44.0 ± 15.3^{c}	43.2 ± 3.0^{c}	45.3 ± 7.6^{c}	73.2 ± 8.3^{ac}	63.3 ± 8.3^{bc}	110.0 ± 6.6^{a}	48.1 ± 5.9^{c}	80.4 ± 7.6^{ac}	92.1 ± 9.3^{ab}	0.002	0.000	0.016

Table S7.1. Continued.

Traits	Units	Irrigated					Rainfed					*p*-value		
		MEX	EUR	DRI	KNI	HAR	MEX	EUR	DRI	KNI	HAR	G	W	G×W
aa.b.75	*µmol g DW^{-1}*	48.6 ± 4.3^{ab}	51.1 ± 4.1^{ab}	51.6 ± 1.5^{ab}	62.7 ± 0.8^{a}	51.9 ± 6.1^{ab}	59.7 ± 1.7^{ab}	43.7 ± 6.2^{ab}	55.5 ± 2.8^{ab}	50.2 ± 5.78^{ab}	41.2 ± 4.9^{b}	0.120	0.262	0.051
aa.s.75	*µmol g DW^{-1}*	32.0 ± 3.0^{b}	27.0 ± 3.1^{bc}	28.4 ± 2.1^{bc}	31.3 ± 1.1^{bc}	21.2 ± 1.5^{c}	45.5 ± 2.0^{a}	28.6 ± 1.2^{bc}	30.9 ± 0.8^{bc}	45.4 ± 3.1^{a}	26.8 ± 1.2^{bc}	0.000	0.000	0.014
aa.p.75	*µmol g DW^{-1}*	29.7 ± 1.0^{bc}	46.1 ± 2.1^{a}	35.0 ± 3.3^{ac}	43.7 ± 1.8^{ab}	27.8 ± 3.2^{c}	49.9 ± 3.3^{a}	46.7 ± 4.9^{a}	44.1 ± 4.3^{ab}	41.8 ± 3.6^{ac}	28.5 ± 2.3^{bc}	0.000	0.010	0.014
aa.a.75	*µmol g DW^{-1}*	35.1 ± 4.5	37.6 ± 9.6	22.1 ± 2.7	38.2 ± 4.4	27.4 ± 0.8	29.9 ± 1.1	38.3 ± 6.4	23.0 ± 3.8	44.8 ± 12.4	28.8 ± 7.4	0.053	0.827	0.926
aa.g.75	*µmol g DW^{-1}*	40.8 ± 4.6^{c}	34.6 ± 4.0^{c}	31.4 ± 4.8^{c}	81.5 ± 4.7^{a}	30.1 ± 2.2^{c}	73.7 ± 5.9^{ab}	88.0 ± 6.3^{a}	30.5 ± 0.91^{c}	85.8 ± 13.9^{a}	43.7 ± 7.5^{bc}	0.000	0.000	0.002
aa.l.75	*µmol g DW^{-1}*	54.7 ± 1.1^{b}	38.9 ± 7.7^{b}	30.3 ± 4.2^{b}	66.5 ± 7.3^{b}	42.5 ± 7.5^{b}	82.2 ± 13.9^{ab}	138.0 ± 14.5^{a}	37.7 ± 0.3^{b}	137.0 ± 23.1^{a}	63.4 ± 19.8^{b}	0.000	0.000	0.007
prot.b.65	*mg g DW^{-1}*	56.3 ± 5.7	66.0 ± 7.4	76.4 ± 7.0	64.6 ± 3.6	70.8 ± 3.9	62.4 ± 7.3	55.0 ± 2.8	66.7 ± 6.2	59.1 ± 4.6	59.3 ± 6.1	0.239	0.093	0.518
prot.s.65	*mg g DW^{-1}*	34.8 ± 4.9	32.0 ± 2.0	34.7 ± 1.0	34.1 ± 1.3	32.4 ± 1.2	33.3 ± 2.6	31.6 ± 1.1	30.7 ± 1.5	34.4 ± 1.4	33.3 ± 0.3	0.771	0.494	0.775
prot.p.65	*mg g DW^{-1}*	32.7 ± 8.3	34.8 ± 1.3	37.0 ± 2.1	45.6 ± 3.4	33.8 ± 1.7	30.6 ± 2.5	30.6 ± 0.4	36.9 ± 0.7	32.6 ± 0.9	32.2 ± 0.5	0.124	0.048	0.290
prot.a.65	*mg g DW^{-1}*	35.6 ± 4.9	29.3 ± 3.0	34 ± 6.6	35.4 ± 3.9	30.3 ± 1.7	30.6 ± 1.8	41 ± 2.1	39.7 ± 1.8	34.8 ± 4.5	31.5 ± 3.6	0.570	0.278	0.248
prot.g.65	*mg g DW^{-1}*	37.6 ± 3.6	27.5 ± 4.0	28.3 ± 3.0	31.9 ± 2.1	31.3 ± 1.3	30.1 ± 2.3	36 ± 2.3	29.9 ± 1.7	30.5 ± 1.5	31.7 ± 1.7	0.485	0.843	0.061
prot.l.65	*mg g DW^{-1}*	40 ± 4.3	30 ± 7.4	27 ± 1.4	33.9 ± 3.4	35.4 ± 1.7	29.9 ± 2.5	35.7 ± 1.5	31 ± 2.9	31.2 ± 2.0	38.7 ± 2.1	0.218	0.985	0.152
prot.b.75	*mg g DW^{-1}*	60.0 ± 3.4^{ab}	55.3 ± 4.3^{ac}	65.1 ± 0.9^{a}	60.3 ± 2.9^{a}	49.1 ± 3.3^{ac}	40.3 ± 2.0^{c}	43.7 ± 0.4^{bc}	41.1 ± 4.1^{c}	42.2 ± 3.7^{c}	40.2 ± 4.8^{c}	0.154	0.000	0.179
prot.s.75	*mg g DW^{-1}*	30.3 ± 2.6	27.9 ± 2.1	33.0 ± 1.2	30.4 ± 1.0	24.8 ± 2.8	29.0 ± 1.4	27.4 ± 0.3	29.9 ± 1.3	29.5 ± 1.4	30.9 ± 0.6	0.164	0.967	0.099
prot.p.75	*mg g DW^{-1}*	22.9 ± 1.2^{ab}	24.8 ± 0.9^{ab}	24.0 ± 1.1^{ab}	24.0 ± 0.1^{ab}	20.6 ± 1.4^{b}	24.5 ± 2.2^{ab}	27.6 ± 0.8^{a}	28.0 ± 0.6^{a}	25.3 ± 1.4^{ab}	23.5 ± 1.3^{ab}	0.015	0.004	0.815
prot.a.75	*mg g DW^{-1}*	22.0 ± 1.5	29.3 ± 7.8	18.8 ± 1.6	28.7 ± 2.1	24.3 ± 2.2	25.4 ± 0.72	33.0 ± 2.0	28.7 ± 3.9	39.7 ± 10.7	30.3 ± 9.6	0.267	0.064	0.933
prot.g.75	*mg g DW^{-1}*	24.7 ± 2.5	27.3 ± 5.3	18.8 ± 0.9	28.0 ± 3.3	24.5 ± 1.4	30.8 ± 1.8	35.2 ± 2.0	23.4 ± 1.9	35.0 ± 6.1	29.3 ± 5.5	0.052	0.014	0.989
prot.l.75	*mg g DW^{-1}*	25.8 ± 2.6	19.9 ± 2.2	17.9 ± 1.6	20.6 ± 1.2	23.2 ± 2.6	27.0 ± 4.6	33.6 ± 1.0	20.3 ± 0.8	35.8 ± 9.6	26.7 ± 4.5	0.220	0.009	0.263
chla.b.65	*mg g DW^{-1}*	9.13 ± 1.08^{ab}	10.3 ± 0.43^{ab}	9.28 ± 0.65^{ab}	10.80 ± 0.96^{a}	7.88 ± 0.45^{ab}	7.35 ± 0.78^{ab}	8.67 ± 0.40^{ab}	10.20 ± 1.47^{ab}	7.86 ± 0.44^{ab}	6.55 ± 0.67^{b}	0.026	0.015	0.223
chla.s.65	*mg g DW^{-1}*	5.57 ± 0.12^{b}	6.79 ± 0.60^{ab}	6.98 ± 0.49^{ab}	5.86 ± 0.54^{ab}	5.98 ± 0.75^{ab}	5.35 ± 0.33^{b}	9.05 ± 1.38^{a}	7.07 ± 0.67^{ab}	7.54 ± 0.27^{ab}	8.75 ± 0.90^{ab}	0.024	0.007	0.163
chla.p.65	*mg g DW^{-1}*	3.39 ± 0.74^{b}	3.53 ± 0.38^{ab}	4.17 ± 0.61^{ab}	4.49 ± 0.07^{ab}	2.92 ± 0.03^{b}	3.62 ± 0.26^{ab}	6.08 ± 0.95^{a}	4.02 ± 0.33^{ab}	3.61 ± 0.33^{ab}	5.13 ± 0.79^{ab}	0.242	0.031	0.016
chla.a.65	*mg g DW^{-1}*	5.51 ± 0.87	4.74 ± 0.58	5.45 ± 1.21	5.71 ± 0.64	3.95 ± 0.21	3.33 ± 0.15	5.85 ± 0.43	4.96 ± 0.46	4.16 ± 0.61	3.52 ± 0.75	0.148	0.104	0.167
chla.g.65	*mg g DW^{-1}*	3.96 ± 0.43	3.09 ± 0.37	3.71 ± 0.39	3.85 ± 0.13	3.2 ± 0.21	2.81 ± 0.18	3.67 ± 0.40	3.32 ± 0.18	2.69 ± 0.21	3.54 ± 0.29	0.952	0.075	0.021
chla.l.65	*mg g DW^{-1}*	4.85 ± 0.62	3.95 ± 1.04	3.79 ± 0.44	4.58 ± 0.27	3.82 ± 0.36	3.22 ± 0.18	4.66 ± 0.47	4.28 ± 0.45	3.08 ± 0.39	4.44 ± 0.70	0.935	0.456	0.084
chla.b.75	*mg g DW^{-1}*	7.41 ± 0.55	7.35 ± 0.57	9.11 ± 0.64	9.62 ± 1.09	8.72 ± 3.51	6.25 ± 1.44	7.85 ± 1.59	5.44 ± 1.21	4.33 ± 0.11	3.67 ± 0.59	0.896	0.004	0.234
chla.s.75	*mg g DW^{-1}*	5.82 ± 0.63	6.18 ± 0.69	6.1 ± 0.16	5.53 ± 0.12	7.57 ± 2.11	7.21 ± 1.34	7.09 ± 0.88	7.30 ± 2.37	7.53 ± 1.03	6.05 ± 1.21	0.999	0.333	0.687
chla.p.75	*mg g DW^{-1}*	5.08 ± 0.83	6.35 ± 1.16	5.11 ± 0.91	5.26 ± 0.59	5.48 ± 1.00	4.45 ± 0.55	6.22 ± 1.88	5.05 ± 1.13	5.77 ± 1.04	5.05 ± 0.53	0.654	0.820	0.985
chla.a.75	*mg g DW^{-1}*	3.01 ± 0.38	3.93 ± 0.89	3.08 ± 0.07	4.48 ± 0.64	2.70 ± 0.74	3.13 ± 0.17	3.61 ± 0.51	3.56 ± 0.78	3.37 ± 0.56	3.15 ± 1.17	0.511	0.861	0.743
chla.g.75	*mg g DW^{-1}*	2.52 ± 0.30	2.65 ± 0.45	2.05 ± 0.15	2.71 ± 0.10	2.41 ± 0.15	2.77 ± 0.29	3.53 ± 0.32	2.32 ± 0.35	2.42 ± 0.06	2.74 ± 0.51	0.097	0.146	0.471
chla.l.75	*mg g DW^{-1}*	2.74 ± 0.33	2.21 ± 0.28	2.07 ± 0.17	2.38 ± 0.19	2.52 ± 0.31	2.71 ± 0.34	3.61 ± 0.25	2.21 ± 0.31	2.98 ± 0.31	3.01 ± 1.14	0.500	0.082	0.561
chlb.b.65	*mg g DW^{-1}*	3.72 ± 0.35^{b}	3.38 ± 0.20^{b}	3.68 ± 0.27^{b}	3.70 ± 0.16^{b}	3.23 ± 0.18^{b}	3.36 ± 0.11^{b}	6.16 ± 0.46^{a}	6.27 ± 1.15^{a}	5.04 ± 0.27^{ab}	3.26 ± 0.31^{b}	0.003	0.000	0.005
chlb.s.65	*mg g DW^{-1}*	3.10 ± 0.17^{b}	4.53 ± 0.41^{b}	4.39 ± 0.18^{b}	3.11 ± 0.18^{b}	4.73 ± 1.41^{b}	4.39 ± 0.62^{b}	10.40 ± 1.67^{a}	6.99 ± 1.31^{ab}	7.97 ± 0.87^{ab}	10.20 ± 1.54^{a}	0.007	0.000	0.145
chlb.p.65	*mg g DW^{-1}*	2.54 ± 0.65^{b}	2.77 ± 0.23^{b}	3.15 ± 0.35^{b}	3.34 ± 0.12^{b}	3.11 ± 0.20^{b}	3.21 ± 0.63^{b}	6.89 ± 1.22^{a}	2.90 ± 0.25^{b}	3.84 ± 0.49^{ab}	6.87 ± 1.17^{a}	0.008	0.000	0.007
chlb.a.65	*mg g DW^{-1}*	2.12 ± 0.19^{b}	1.80 ± 0.21^{b}	1.95 ± 0.31^{b}	1.91 ± 0.18^{b}	1.84 ± 0.06^{b}	1.55 ± 0.09^{b}	3.73 ± 0.25^{a}	2.44 ± 0.32^{b}	1.88 ± 0.35^{b}	2.33 ± 0.32^{b}	0.009	0.008	0.001
chlb.g.65	*mg g DW^{-1}*	2.71 ± 0.26^{ab}	2.11 ± 0.29^{ab}	2.12 ± 0.20^{ab}	2.23 ± 0.10^{ab}	2.03 ± 0.19^{ab}	2.07 ± 0.17^{ab}	3.16 ± 0.42^{a}	2.35 ± 0.12^{ab}	1.95 ± 0.16^{b}	3.17 ± 0.27^{a}	0.145	0.061	0.003
chlb.l.65	*mg g DW^{-1}*	3.24 ± 0.43^{ab}	2.70 ± 0.64^{ab}	2.44 ± 0.20^{b}	2.66 ± 0.21^{b}	2.56 ± 0.29^{b}	2.57 ± 0.26^{b}	4.77 ± 0.56^{a}	3.66 ± 0.54^{ab}	2.17 ± 0.22^{b}	4.24 ± 0.52^{ab}	0.051	0.010	0.009
chlb.b.75	*mg g DW^{-1}*	3.52 ± 0.55	4.16 ± 0.83	3.96 ± 0.37	3.84 ± 0.21	8.96 ± 6.34	6.18 ± 3.20	8.29 ± 3.18	4.92 ± 1.84	3.43 ± 0.76	2.29 ± 0.19	0.867	0.934	0.294
chlb.s.75	*mg g DW^{-1}*	5.11 ± 1.40	6.05 ± 1.26	4.11 ± 0.56	3.92 ± 0.27	8.89 ± 3.31	8.97 ± 3.24	10.30 ± 1.56	8.44 ± 3.73	9.73 ± 1.65	6.59 ± 1.91	0.918	0.033	0.416
chlb.p.75	*mg g DW^{-1}*	5.98 ± 1.60	7.08 ± 1.90	4.81 ± 1.33	5.24 ± 0.79	6.43 ± 1.03	5.02 ± 1.34	7.50 ± 2.89	5.68 ± 1.55	7.25 ± 1.37	7.01 ± 0.92	0.684	0.563	0.920
chlb.a.75	*mg g DW^{-1}*	1.45 ± 0.17	1.81 ± 0.29	1.21 ± 0.03	1.73 ± 0.15	1.67 ± 0.42	1.77 ± 0.14	2.51 ± 0.42	2.44 ± 0.77	2.23 ± 0.17	2.26 ± 1.02	0.810	0.034	0.892
chlb.g.75	*mg g DW^{-1}*	1.79 ± 0.25^{ab}	1.98 ± 0.30^{ab}	1.27 ± 0.03^{b}	1.82 ± 0.15^{ab}	1.84 ± 0.12^{ab}	2.08 ± 0.12^{ab}	2.89 ± 0.40^{a}	1.85 ± 0.40^{ab}	2.35 ± 0.13^{ab}	2.17 ± 0.43^{ab}	0.058	0.005	0.787
chlb.l.75	*mg g DW^{-1}*	1.96 ± 0.21	1.7 ± 0.19	1.30 ± 0.05	1.55 ± 0.06	2.14 ± 0.35	2.29 ± 0.40	3.71 ± 0.47	2.08 ± 0.57	2.92 ± 0.29	2.85 ± 1.28	0.368	0.004	0.525
chltot.b.65	*mg g DW^{-1}*	12.9 ± 1.4^{ab}	13.7 ± 0.5^{ab}	13.0 ± 0.6^{ab}	14.5 ± 1.1^{ab}	11.1 ± 0.6^{ab}	10.7 ± 0.8^{b}	14.8 ± 0.8^{ab}	16.5 ± 2.5^{a}	12.9 ± 0.5^{ab}	9.8 ± 0.9^{b}	0.005	0.911	0.104
chltot.s.65	*mg g DW^{-1}*	8.7 ± 0.1^{c}	11.3 ± 1.0^{ac}	11.4 ± 0.5^{ac}	9.0 ± 0.7^{c}	10.7 ± 2.2^{bc}	9.7 ± 0.9^{c}	19.5 ± 3.0^{a}	14.1 ± 2.0^{ac}	15.5 ± 1.1^{ac}	19.0 ± 2.4^{ab}	0.010	0.000	0.139
chltot.p.65	*mg g DW^{-1}*	5.92 ± 1.39^{c}	6.30 ± 0.61^{bc}	7.31 ± 0.94^{ac}	7.84 ± 0.18^{ac}	6.03 ± 0.18^{c}	6.83 ± 0.88^{bc}	13.00 ± 2.18^{a}	6.91 ± 0.56^{bc}	7.45 ± 0.81^{ac}	12.00 ± 1.96^{ab}	0.062	0.002	0.010
chltot.a.65	*mg g DW^{-1}*	7.64 ± 1.05^{ab}	6.54 ± 0.79^{ab}	7.39 ± 1.52^{ab}	7.61 ± 0.80^{ab}	5.80 ± 0.27^{ab}	4.87 ± 0.23^{b}	9.58 ± 0.67^{a}	7.39 ± 0.77^{ab}	6.03 ± 0.94^{ab}	5.85 ± 1.06^{ab}	0.132	0.660	0.042
chltot.g.65	*mg g DW^{-1}*	6.67 ± 0.69	5.20 ± 0.66	5.83 ± 0.59	6.08 ± 0.19	5.22 ± 0.41	4.88 ± 0.33	6.83 ± 0.82	5.66 ± 0.30	4.64 ± 0.33	6.70 ± 0.55	0.747	0.863	0.008
chltot.l.65	*mg g DW^{-1}*	8.09 ± 1.05	6.65 ± 1.68	6.24 ± 0.64	7.25 ± 0.44	6.38 ± 0.63	5.80 ± 0.43	9.43 ± 1.03	7.94 ± 0.99	5.25 ± 0.61	8.68 ± 1.21	0.432	0.416	0.031

Table S7.1. Continued.

Traits	Units	Irrigated					Rainfed					*p*-value		
		MEX	EUR	DRI	KNI	HAR	MEX	EUR	DRI	KNI	HAR	G	W	G×W
chltot.b.75	*mg g DW*$^{-1}$	10.9 ± 1.1	11.5 ± 1.4	13.1 ± 0.9	13.5 ± 1.3	17.7 ± 9.8	12.4 ± 4.6	16.1 ± 4.8	10.4 ± 3.0	7.8 ± 0.9	6.0 ± 0.4	0.949	0.276	0.305
chltot.s.75	*mg g DW*$^{-1}$	10.9 ± 2.0	12.2 ± 2.0	10.2 ± 0.7	9.5 ± 0.3	16.5 ± 5.4	16.2 ± 4.6	17.3 ± 2.4	15.7 ± 6.1	17.3 ± 2.7	12.6 ± 3.1	0.980	0.083	0.509
chltot.p.75	*mg g DW*$^{-1}$	11.1 ± 2.4	13.4 ± 3.0	9.9 ± 2.2	10.5 ± 1.4	11.9 ± 2.0	9.5 ± 1.9	13.7 ± 4.8	10.7 ± 2.7	13.0 ± 2.3	12.1 ± 1.4	0.689	0.791	0.954
chltot.a.75	*mg g DW*$^{-1}$	4.45 ± 0.54	5.74 ± 1.17	4.29 ± 0.07	6.21 ± 0.79	4.37 ± 1.14	4.90 ± 0.31	6.13 ± 0.92	6.00 ± 1.54	5.60 ± 0.67	5.40 ± 2.18	0.697	0.404	0.873
chltot.g.75	*mg g DW*$^{-1}$	4.30 ± 0.55^{ab}	4.63 ± 0.71^{ab}	3.32 ± 0.18^{b}	4.52 ± 0.24^{ab}	4.25 ± 0.26^{ab}	4.85 ± 0.39^{ab}	6.42 ± 0.71^{a}	4.16 ± 0.75^{ab}	4.77 ± 0.19^{ab}	4.91 ± 0.91^{ab}	0.066	0.029	0.693
chltot.l.75	*mg g DW*$^{-1}$	4.70 ± 0.54	3.91 ± 0.43	3.36 ± 0.21	3.93 ± 0.24	4.66 ± 0.62	5.00 ± 0.73	7.32 ± 0.72	4.29 ± 0.88	5.90 ± 0.51	5.86 ± 2.42	0.426	0.016	0.537
glc.b.65	*µmol organ*$^{-1}$	1.64 ± 0.16^{ab}	0.96 ± 0.32^{b}	1.38 ± 0.63^{b}	0.57 ± 0.05^{b}	5.31 ± 1.94^{a}	3.75 ± 0.32^{ab}	0.78 ± 0.14^{b}	2.28 ± 0.53^{ab}	3.32 ± 0.87^{ab}	1.39 ± 0.35^{b}	0.037	0.487	0.002
glc.s.65	*µmol organ*$^{-1}$	9.11 ± 0.28^{ac}	4.78 ± 1.40^{bcd}	3.82 ± 1.77^{cd}	6.24 ± 0.09^{ad}	9.95 ± 1.30^{ab}	10.60 ± 1.59^{a}	2.02 ± 0.42^{d}	6.25 ± 0.47^{ad}	7.70 ± 1.23^{ac}	8.12 ± 0.47^{ac}	0.000	0.815	0.096
glc.p.65	*µmol organ*$^{-1}$	58.8 ± 18.7^{ab}	67.1 ± 13.8^{ab}	63.6 ± 9.1^{ab}	80.5 ± 6.3^{a}	60.8 ± 4.4^{ab}	58.9 ± 17.2^{ab}	13.0 ± 1.4^{b}	56.5 ± 10.3^{ab}	61.5 ± 14.5^{ab}	28.4 ± 2.1^{ab}	0.082	0.005	0.171
glc.a.65	*µmol organ*$^{-1}$	13.30 ± 3.57^{ab}	5.29 ± 1.23^{bc}	4.34 ± 0.80^{bc}	8.75 ± 1.42^{ac}	14.50 ± 1.27^{ab}	15.50 ± 3.66^{a}	3.02 ± 0.60^{c}	6.65 ± 1.31^{ac}	8.14 ± 1.31^{ac}	6.40 ± 2.37^{ac}	0.000	0.326	0.105
glc.g.65	*µmol organ*$^{-1}$	8.14 ± 1.54^{a}	4.43 ± 0.97^{ab}	6.00 ± 0.53^{ab}	7.57 ± 0.79^{a}	7.26 ± 0.15^{ab}	6.36 ± 0.33^{ab}	2.85 ± 0.64^{b}	5.91 ± 0.04^{ab}	5.73 ± 0.79^{ab}	4.44 ± 1.58^{ab}	0.007	0.009	0.659
glc.l.65	*µmol organ*$^{-1}$	17.1 ± 2.7^{a}	7.2 ± 1.7^{bc}	9.0 ± 2.0^{ac}	13.5 ± 2.1^{ab}	16.5 ± 1.6^{a}	11.3 ± 0.8^{ac}	2.9 ± 0.2^{c}	8.6 ± 1.1^{ac}	8.5 ± 1.7^{ac}	7.3 ± 2.0^{bc}	0.001	0.000	0.203
glc.b.75	*µmol organ*$^{-1}$	3.74 ± 1.40^{b}	1.97 ± 0.51^{b}	3.17 ± 1.34^{b}	2.53 ± 1.23^{b}	11.9 ± 1.74^{a}	2.69 ± 0.56^{b}	1.26 ± 0.10^{b}	2.08 ± 0.51^{b}	1.45 ± 0.48^{b}	1.51 ± 0.57^{b}	0.000	0.000	0.000
glc.s.75	*µmol organ*$^{-1}$	3.84 ± 0.97^{b}	1.85 ± 0.58^{b}	2.34 ± 0.84^{b}	2.48 ± 0.57^{b}	8.00 ± 0.54^{a}	2.61 ± 0.25^{b}	1.96 ± 0.16^{b}	2.28 ± 0.35^{b}	2.08 ± 0.46^{b}	3.95 ± 0.69^{b}	0.000	0.007	0.012
glc.p.75	*µmol organ*$^{-1}$	15.6 ± 9.1	11.1 ± 6.0	14.2 ± 5.3	8.1 ± 1.7	21.1 ± 5.8	5.3 ± 0.5	3.9 ± 1.2	4.9 ± 1.0	4.5 ± 1.6	12.4 ± 7.4	0.284	0.021	0.964
glc.a.75	*µmol organ*$^{-1}$	5.10 ± 2.06^{b}	4.04 ± 2.61^{b}	2.66 ± 1.03^{b}	2.37 ± 0.79^{b}	14.90 ± 3.10^{a}	3.43 ± 0.31^{b}	2.63 ± 0.85^{b}	1.61 ± 0.41^{b}	1.41 ± 0.25^{b}	7.90 ± 3.69^{ab}	0.000	0.061	0.483
glc.g.75	*µmol organ*$^{-1}$	3.22 ± 1.11^{ab}	2.83 ± 1.28^{ab}	4.51 ± 1.37^{ab}	2.42 ± 0.45^{ab}	5.12 ± 0.50^{a}	2.20 ± 0.29^{ab}	1.73 ± 0.42^{ab}	2.40 ± 0.76^{ab}	0.87 ± 0.17^{b}	2.57 ± 0.87^{ab}	0.094	0.005	0.861
glc.l.75	*µmol organ*$^{-1}$	5.04 ± 1.87^{ab}	3.34 ± 1.75^{ab}	3.94 ± 1.01^{ab}	2.26 ± 0.45^{b}	7.63 ± 0.91^{a}	2.15 ± 0.23^{b}	1.49 ± 0.27^{b}	2.32 ± 0.76^{ab}	1.39 ± 0.19^{b}	3.66 ± 1.38^{ab}	0.018	0.003	0.634
G6P.b.65	*nmol organ*$^{-1}$	184 ± 17^{ab}	176 ± 13^{ab}	188 ± 24^{ab}	135 ± 9^{bc}	217 ± 20^{a}	74 ± 16^{cd}	62 ± 2^{d}	76 ± 7^{cd}	60 ± 13^{d}	68 ± 4^{cd}	0.046	0.000	0.175
G6P.s.65	*nmol organ*$^{-1}$	235 ± 2^{a}	179 ± 13^{a}	208 ± 18^{a}	207 ± 21^{a}	195 ± 22^{a}	104 ± 1^{b}	72 ± 1^{b}	89 ± 3^{b}	94 ± 8^{b}	97 ± 7^{b}	0.036	0.000	0.737
G6P.p.65	*nmol organ*$^{-1}$	442 ± 127^{ac}	527 ± 83^{ab}	502 ± 16^{ab}	566 ± 64^{a}	372 ± 27^{ac}	301 ± 83^{ac}	173 ± 7^{c}	279 ± 51^{ac}	291 ± 52^{ac}	215 ± 18^{bc}	0.326	0.000	0.459
G6P.a.65	*nmol organ*$^{-1}$	323 ± 62^{a}	210 ± 27^{ac}	196 ± 29^{ac}	221 ± 28^{ac}	291 ± 27^{ab}	206 ± 7^{ac}	164 ± 10^{bc}	169 ± 9^{bc}	113 ± 5^{c}	189 ± 8^{ac}	0.007	0.000	0.366
G6P.g.65	*nmol organ*$^{-1}$	164 ± 25^{ab}	106 ± 10^{ac}	170 ± 23^{a}	146 ± 8^{ac}	168 ± 20^{a}	103 ± 1^{ac}	86 ± 4^{c}	137 ± 8^{ac}	94 ± 6^{c}	99 ± 10^{bc}	0.007	0.000	0.383
G6P.l.65	*nmol organ*$^{-1}$	296 ± 38^{a}	178 ± 18^{bcd}	221 ± 26^{ac}	221 ± 28^{ac}	250 ± 7^{ab}	145 ± 7^{cd}	120 ± 7^{d}	177 ± 9^{bcd}	115 ± 12^{d}	135 ± 8^{cd}	0.012	0.000	0.067
G6P.b.75	*nmol organ*$^{-1}$	121 ± 5^{ac}	147 ± 21^{ac}	185 ± 2^{ab}	147 ± 23^{ac}	200 ± 30^{a}	52 ± 3^{c}	75 ± 7^{bc}	82 ± 6^{bc}	55 ± 16^{c}	116 ± 53^{ac}	0.036	0.000	0.938
G6P.s.75	*nmol organ*$^{-1}$	98 ± 13	86 ± 3	134 ± 18	96 ± 17	121 ± 5	75 ± 6	74 ± 1	101 ± 12	76 ± 11	143 ± 31	0.006	0.151	0.399
G6P.p.75	*nmol organ*$^{-1}$	206 ± 28	193 ± 27	261 ± 42	209 ± 31	300 ± 64	153 ± 15	124 ± 3	169 ± 20	149 ± 19	301 ± 70	0.009	0.033	0.795
G6P.a.75	*nmol organ*$^{-1}$	191 ± 8^{ac}	170 ± 30^{ac}	128 ± 2^{c}	141 ± 18^{bc}	311 ± 64^{ab}	154 ± 17^{ac}	142 ± 12^{bc}	114 ± 5^{c}	93 ± 7^{c}	325 ± 85^{a}	0.000	0.335	0.923
G6P.g.75	*nmol organ*$^{-1}$	107 ± 7^{ab}	75 ± 7^{b}	132 ± 4^{ab}	102 ± 19^{ab}	114 ± 9^{ab}	110 ± 12^{ab}	83 ± 5^{b}	127 ± 11^{ab}	104 ± 7^{ab}	171 ± 35^{a}	0.003	0.176	0.235
G6P.l.75	*nmol organ*$^{-1}$	136 ± 7	97 ± 18	124 ± 6	105 ± 14	133 ± 20	110 ± 2	118 ± 2	130 ± 9	111 ± 11	180 ± 41	0.050	0.323	0.331
fru.b.65	*µmol organ*$^{-1}$	2.03 ± 0.23^{ab}	0.97 ± 0.38^{b}	1.32 ± 0.55^{ab}	1.22 ± 0.08^{b}	4.35 ± 1.49^{a}	3.07 ± 0.50^{ab}	0.65 ± 0.13^{b}	1.50 ± 0.38^{ab}	2.28 ± 0.78^{ab}	1.10 ± 0.20^{b}	0.029	0.519	0.013
fru.s.65	*µmol organ*$^{-1}$	4.73 ± 0.44^{ab}	3.48 ± 0.96^{ab}	3.03 ± 1.45^{b}	3.46 ± 0.17^{ab}	6.96 ± 1.07^{a}	4.88 ± 0.59^{ab}	1.27 ± 0.21^{b}	3.33 ± 0.42^{ab}	3.54 ± 0.71^{ab}	3.43 ± 0.51^{ab}	0.007	0.041	0.065
fru.p.65	*µmol organ*$^{-1}$	30.9 ± 9.8^{ab}	36.2 ± 6.2^{a}	35.8 ± 3.5^{ab}	42.6 ± 4.4^{a}	32.3 ± 1.02^{ab}	28.7 ± 8.7^{ab}	8.5 ± 0.3^{b}	29.0 ± 5.27^{ab}	26.0 ± 5.3^{ab}	15.8 ± 1.0^{ab}	0.169	0.001	0.205
fru.a.65	*µmol organ*$^{-1}$	2.14 ± 0.48	1.47 ± 0.67	0.90 ± 0.15	1.33 ± 0.26	2.36 ± 0.42	2.49 ± 0.71	1.08 ± 0.16	1.36 ± 0.41	1.35 ± 0.20	1.48 ± 0.52	0.069	0.764	0.550
fru.g.65	*µmol organ*$^{-1}$	1.90 ± 0.28	1.84 ± 0.60	1.76 ± 0.19	1.72 ± 0.20	2.30 ± 0.27	2.10 ± 0.22	1.78 ± 0.30	1.93 ± 0.06	2.12 ± 0.32	2.56 ± 0.97	0.603	0.484	0.988
fru.l.65	*µmol organ*$^{-1}$	6.67 ± 1.17^{ab}	4.17 ± 0.60^{ac}	4.01 ± 0.76^{ac}	4.92 ± 0.85^{ac}	7.78 ± 1.11^{a}	4.48 ± 0.42^{ac}	2.35 ± 0.20^{c}	3.74 ± 0.36^{bc}	3.59 ± 0.63^{bc}	4.05 ± 0.83^{ac}	0.009	0.001	0.269
fru.b.75	*µmol organ*$^{-1}$	4.16 ± 1.41^{b}	2.81 ± 0.83^{b}	3.83 ± 1.19^{b}	2.30 ± 0.93^{b}	13.90 ± 2.75^{a}	4.94 ± 1.20^{b}	1.98 ± 0.29^{b}	4.61 ± 2.49^{b}	3.46 ± 0.85^{b}	1.56 ± 0.57^{b}	0.013	0.034	0.001
fru.s.75	*µmol organ*$^{-1}$	6.06 ± 1.81^{b}	5.24 ± 2.63^{b}	6.09 ± 3.29^{b}	3.39 ± 0.78^{b}	18.40 ± 4.15^{a}	4.78 ± 0.29^{b}	3.86 ± 0.46^{b}	4.65 ± 1.90^{b}	2.91 ± 0.68^{b}	8.50 ± 3.13^{ab}	0.002	0.061	0.256
fru.p.75	*µmol organ*$^{-1}$	19.0 ± 5.4^{ab}	12.0 ± 3.8^{b}	13.7 ± 4.3^{ab}	10.7 ± 3.6^{b}	33.4 ± 7.4^{a}	6.2 ± 0.9^{b}	4.7 ± 1.5^{b}	5.8 ± 1.1^{b}	5.5 ± 1.3^{b}	17.4 ± 5.6^{ab}	0.002	0.001	0.674
fru.a.75	*µmol organ*$^{-1}$	2.63 ± 1.04^{b}	1.99 ± 1.18^{b}	1.18 ± 0.28^{b}	0.99 ± 0.31^{b}	10.40 ± 1.87^{a}	2.05 ± 0.28^{b}	1.62 ± 0.40^{b}	1.74 ± 0.47^{b}	1.04 ± 0.18^{b}	6.61 ± 2.51^{ab}	0.000	0.268	0.376
fru.g.75	*µmol organ*$^{-1}$	1.69 ± 0.69^{bc}	1.84 ± 0.80^{bc}	2.40 ± 0.69^{bc}	1.35 ± 0.28^{bc}	5.92 ± 0.96^{a}	1.51 ± 0.16^{bc}	1.26 ± 0.28^{bc}	1.93 ± 0.66^{bc}	0.76 ± 0.09^{c}	4.32 ± 1.26^{ab}	0.000	0.131	0.871
fru.l.75	*µmol organ*$^{-1}$	3.23 ± 1.27^{ab}	2.69 ± 1.15^{b}	2.47 ± 0.54^{b}	1.46 ± 0.12^{b}	6.94 ± 0.89^{a}	2.32 ± 0.18^{b}	1.88 ± 0.37^{b}	2.44 ± 0.71^{b}	1.46 ± 0.14^{b}	4.54 ± 1.32^{ab}	0.000	0.119	0.582
suc.b.65	*µmol organ*$^{-1}$	10.9 ± 1.6^{b}	15.0 ± 2.8^{b}	18.5 ± 2.7^{ab}	10.5 ± 1.0^{b}	28.4 ± 3.9^{a}	11.7 ± 3.4^{b}	16.5 ± 0.6^{b}	15.2 ± 0.5^{b}	11.2 ± 1.9^{b}	19.1 ± 1.1^{ab}	0.000	0.195	0.129
suc.s.65	*µmol organ*$^{-1}$	22.2 ± 1.3^{c}	24.9 ± 2.1^{bc}	27.0 ± 2.3^{ac}	20.4 ± 2.5^{c}	36.8 ± 2.6^{a}	27.2 ± 3.6^{ac}	27.6 ± 0.5^{ac}	29.1 ± 1.7^{ac}	26.4 ± 3.1^{ac}	35.9 ± 1.7^{ab}	0.000	0.057	0.607
suc.p.65	*µmol organ*$^{-1}$	23.1 ± 6.8^{b}	46.5 ± 10.1^{ab}	57.5 ± 8.4^{a}	27.3 ± 5.0^{b}	38.5 ± 1.1^{ab}	28.6 ± 2.0^{b}	49.0 ± 3.0^{ab}	29.4 ± 1.9^{b}	34.4 ± 3.4^{ab}	44.2 ± 1.3^{ab}	0.002	0.661	0.014
suc.a.65	*µmol organ*$^{-1}$	12.9 ± 2.2^{de}	18.1 ± 0.7^{bcd}	15.6 ± 2.1^{ce}	10.0 ± 1.7^{e}	21.5 ± 1.5^{ac}	15.8 ± 1.0^{ce}	24.8 ± 1.8^{ab}	19.1 ± 1.1^{acd}	9.8 ± 0.8^{e}	25.9 ± 1.8^{a}	0.000	0.002	0.307
suc.g.65	*µmol organ*$^{-1}$	6.6 ± 0.3^{de}	7.7 ± 1.3^{de}	14.1 ± 1.4^{bc}	5.7 ± 0.7^{e}	17.0 ± 0.9^{ab}	10.3 ± 0.9^{cd}	13.7 ± 1.0^{bc}	16.4 ± 0.2^{ab}	8.9 ± 0.6^{de}	20.2 ± 0.8^{a}	0.000	0.000	0.335
suc.l.65	*µmol organ*$^{-1}$	11.3 ± 1.1^{bc}	11.3 ± 1.5^{bc}	15.2 ± 2.1^{ac}	8.8 ± 1.4^{c}	17.2 ± 0.6^{ab}	13.9 ± 1.7^{ac}	19.1 ± 1.0^{a}	20.5 ± 1.8^{a}	10.4 ± 0.7^{bc}	21.0 ± 1.6^{a}	0.000	0.000	0.255

Table S7.1. Continued.

Traits	Units	Irrigated					Rainfed					*p*-value		
		MEX	EUR	DRI	KNI	HAR	MEX	EUR	DRI	KNI	HAR	G	W	G×W
suc.b.75	*µmol organ*$^{-1}$	12.5 ± 2.4	17.0 ± 3.8	22.5 ± 1.8	12.8 ± 2.3	21.8 ± 5.2	9.1 ± 1.2	16.1 ± 1.4	17.4 ± 1.0	11.4 ± 3.7	20.7 ± 8.8	0.051	0.343	0.978
suc.s.75	*µmol organ*$^{-1}$	25.3 ± 1.1	23.0 ± 2.7	31.7 ± 0.4	26.2 ± 3.4	33.0 ± 3.1	24.4 ± 2.4	27.1 ± 1.3	33.5 ± 1.2	22.6 ± 3.7	33.4 ± 7.2	0.018	0.857	0.810
suc.p.75	*µmol organ*$^{-1}$	93.6 ± 11.5^{ac}	77.9 ± 3.2^{ac}	115.0 ± 3.3^{a}	110.0 ± 20.6^{ab}	118.0 ± 17.0^{a}	52.4 ± 7.4^{c}	57.8 ± 4.4^{bc}	82.5 ± 7.0^{ac}	61.8 ± 12.6^{ac}	77.8 ± 12.1^{ac}	0.040	0.000	0.779
suc.a.75	*µmol organ*$^{-1}$	14.2 ± 0.8^{bc}	13.4 ± 2.4^{bc}	8.8 ± 1.2^{bc}	8.2 ± 1.1^{c}	23.0 ± 2.3^{ab}	20.3 ± 3.0^{ac}	20.5 ± 1.0^{ac}	19.5 ± 2.2^{ac}	13.1 ± 1.5^{bc}	32.5 ± 7.3^{a}	0.000	0.000	0.853
suc.g.75	*µmol organ*$^{-1}$	7.7 ± 0.6^{c}	7.0 ± 0.9^{c}	9.4 ± 1.2^{c}	7.0 ± 1.1^{c}	17.2 ± 0.7^{ab}	11.9 ± 0.5^{bc}	12.5 ± 0.3^{bc}	16.0 ± 2.1^{ab}	10.2 ± 0.4^{c}	19.3 ± 2.0^{a}	0.000	0.000	0.324
suc.l.75	*µmol organ*$^{-1}$	12.0 ± 1.0^{bd}	10.1 ± 1.1^{cd}	10.1 ± 0.6^{cd}	7.0 ± 1.0^{d}	16.7 ± 1.2^{ab}	14.6 ± 1.3^{abc}	16.0 ± 0.3^{abc}	17.8 ± 1.9^{ab}	13.8 ± 1.1^{abc}	18.9 ± 2.3^{a}	0.000	0.000	0.157
starch.b.65	*µmol organ*$^{-1}$	1.22 ± 0.22	1.35 ± 0.14	2.41 ± 0.20	1.47 ± 0.19	3.59 ± 2.11	0.56 ± 0.33	3.40 ± 0.44	1.53 ± 0.16	0.37 ± 0.05	1.68 ± 0.43	0.067	0.284	0.104
starch.s.65	*µmol organ*$^{-1}$	0.65 ± 0.04^{bc}	0.71 ± 0.20^{bc}	1.13 ± 0.22^{bc}	0.64 ± 0.10^{bc}	1.41 ± 0.69^{ac}	0.55 ± 0.19^{c}	2.24 ± 0.34^{ab}	0.99 ± 0.19^{bc}	0.64 ± 0.09^{bc}	2.84 ± 0.55^{a}	0.001	0.017	0.028
starch.p.65	*µmol organ*$^{-1}$	1.56 ± 0.34^{bc}	2.51 ± 0.56^{ac}	2.50 ± 0.48^{ac}	2.43 ± 0.66^{ac}	1.72 ± 0.20^{bc}	1.09 ± 0.13^{c}	3.20 ± 0.14^{ab}	1.72 ± 0.25^{bc}	1.36 ± 0.29^{bc}	4.28 ± 0.49^{a}	0.002	0.461	0.001
starch.a.65	*µmol organ*$^{-1}$	2.04 ± 0.47^{bc}	2.93 ± 0.54^{ac}	2.40 ± 0.44^{ac}	1.56 ± 0.31^{c}	3.51 ± 1.10^{ac}	2.17 ± 0.37^{ac}	5.02 ± 0.40^{a}	3.07 ± 0.26^{ac}	1.36 ± 0.15^{c}	4.67 ± 1.01^{ab}	0.001	0.051	0.348
starch.g.65	*µmol organ*$^{-1}$	0.74 ± 0.04^{b}	0.68 ± 0.23^{b}	0.85 ± 0.11^{b}	0.64 ± 0.11^{b}	1.23 ± 0.33^{ab}	0.86 ± 0.18^{b}	1.07 ± 0.14^{b}	1.31 ± 0.14^{ab}	0.75 ± 0.11^{b}	2.03 ± 0.15^{a}	0.000	0.002	0.274
starch.l.65	*µmol organ*$^{-1}$	3.03 ± 0.44^{bc}	2.03 ± 0.46^{bc}	3.11 ± 0.42^{bc}	1.77 ± 0.26^{bc}	3.81 ± 0.36^{ab}	2.39 ± 0.38^{bc}	3.48 ± 0.64^{ac}	3.82 ± 0.38^{ab}	1.56 ± 0.23^{c}	5.33 ± 0.47^{a}	0.000	0.043	0.061
starch.b.75	*µmol organ*$^{-1}$	1.000 ± 0.638	1.410 ± 0.723	1.620 ± 0.733	1.440 ± 0.891	0.657 ± 0.235	0.282 ± 0.090	0.939 ± 0.056	0.868 ± 0.185	0.418 ± 0.158	2.380 ± 0.994	0.630	0.509	0.157
starch.s.75	*µmol organ*$^{-1}$	0.289 ± 0.141^{b}	0.489 ± 0.276^{b}	0.545 ± 0.117^{ab}	0.392 ± 0.200^{b}	0.258 ± 0.042^{b}	0.435 ± 0.109^{b}	0.719 ± 0.064^{ab}	0.749 ± 0.151^{ab}	0.608 ± 0.045^{ab}	1.250 ± 0.186^{a}	0.137	0.001	0.053
starch.p.75	*µmol organ*$^{-1}$	0.364 ± 0.099	0.419 ± 0.192	0.436 ± 0.150	0.626 ± 0.120	0.555 ± 0.168	0.379 ± 0.065	0.498 ± 0.108	0.772 ± 0.112	0.480 ± 0.074	0.637 ± 0.170	0.366	0.390	0.503
starch.a.75	*µmol organ*$^{-1}$	1.59 ± 0.40	1.42 ± 0.72	1.11 ± 0.24	0.84 ± 0.18	1.61 ± 0.32	2.89 ± 0.69	2.81 ± 0.39	2.76 ± 0.39	1.97 ± 0.29	2.84 ± 0.38	0.315	0.000	0.980
starch.g.75	*µmol organ*$^{-1}$	0.49 ± 0.11^{c}	0.49 ± 0.19^{c}	0.47 ± 0.15^{c}	0.54 ± 0.16^{bc}	0.88 ± 0.09^{ac}	1.07 ± 0.13^{ac}	1.07 ± 0.09^{ac}	1.32 ± 0.33^{ab}	0.95 ± 0.05^{ac}	1.61 ± 0.14^{a}	0.031	0.000	0.718
starch.l.75	*µmol organ*$^{-1}$	1.26 ± 0.25^{b}	1.42 ± 0.39^{b}	1.11 ± 0.13^{b}	0.71 ± 0.15^{b}	1.11 ± 0.17^{b}	1.93 ± 0.13^{ab}	3.04 ± 0.77^{a}	2.19 ± 0.18^{ab}	1.74 ± 0.15^{ab}	2.03 ± 0.13^{ab}	0.055	0.000	0.646
malate.b.65	*µmol organ*$^{-1}$	1.39 ± 0.13^{a}	0.56 ± 0.03^{b}	1.02 ± 0.13^{ab}	0.94 ± 0.05^{ab}	0.97 ± 0.14^{ab}	0.86 ± 0.20^{ab}	0.42 ± 0.04^{b}	0.64 ± 0.06^{b}	0.65 ± 0.22^{b}	0.54 ± 0.04^{b}	0.001	0.000	0.590
malate.s.65	*µmol organ*$^{-1}$	1.97 ± 0.10^{a}	1.22 ± 0.15^{bd}	1.44 ± 0.16^{ab}	1.53 ± 0.07^{ab}	1.38 ± 0.02^{bc}	1.37 ± 0.05^{bc}	0.75 ± 0.07^{d}	1.07 ± 0.12^{bd}	1.20 ± 0.13^{bd}	0.87 ± 0.13^{cd}	0.000	0.000	0.717
malate.p.65	*µmol organ*$^{-1}$	1.49 ± 0.45^{ab}	1.42 ± 0.34^{ab}	1.41 ± 0.08^{ab}	1.99 ± 0.31^{a}	1.49 ± 0.20^{ab}	1.09 ± 0.42^{ab}	0.23 ± 0.07^{b}	0.93 ± 0.25^{ab}	0.88 ± 0.18^{ab}	0.57 ± 0.02^{b}	0.235	0.000	0.497
malate.a.65	*µmol organ*$^{-1}$	0.483 ± 0.126^{a}	0.141 ± 0.038^{c}	0.139 ± 0.019^{c}	0.382 ± 0.032^{ac}	0.447 ± 0.079^{ab}	0.276 ± 0.047^{ac}	0.132 ± 0.006^{c}	0.200 ± 0.008^{bc}	0.144 ± 0.026^{c}	0.183 ± 0.026^{bc}	0.001	0.001	0.019
malate.g.65	*µmol organ*$^{-1}$	0.085 ± 0.029	0.038 ± 0.003	0.062 ± 0.007	0.103 ± 0.016	0.142 ± 0.029	0.162 ± 0.065	0.114 ± 0.014	0.117 ± 0.016	0.135 ± 0.026	0.113 ± 0.024	0.287	0.028	0.331
malate.l.65	*µmol organ*$^{-1}$	0.416 ± 0.048^{ab}	0.153 ± 0.046^{cd}	0.191 ± 0.008^{bcd}	0.376 ± 0.058^{ac}	0.427 ± 0.041^{a}	0.260 ± 0.058^{ad}	0.134 ± 0.004^{d}	0.22 ± 0.045^{ad}	0.243 ± 0.062^{ad}	0.213 ± 0.046^{ad}	0.001	0.003	0.082
malate.b.75	*µmol organ*$^{-1}$	1.00 ± 0.06^{ac}	0.62 ± 0.04^{bc}	1.21 ± 0.16^{ab}	1.00 ± 0.11^{ac}	1.40 ± 0.15^{a}	0.51 ± 0.13^{bc}	0.41 ± 0.02^{c}	0.69 ± 0.16^{ac}	0.54 ± 0.17^{bc}	0.80 ± 0.30^{ac}	0.010	0.000	0.744
malate.s.75	*µmol organ*$^{-1}$	1.22 ± 0.11	0.97 ± 0.14	1.35 ± 0.16	1.21 ± 0.16	1.26 ± 0.09	0.82 ± 0.10	0.78 ± 0.07	1.06 ± 0.24	0.77 ± 0.09	1.01 ± 0.14	0.176	0.002	0.894
malate.p.75	*µmol organ*$^{-1}$	0.353 ± 0.054^{ab}	0.230 ± 0.066^{ab}	0.466 ± 0.123^{a}	0.228 ± 0.048^{ab}	0.263 ± 0.096^{ab}	0.190 ± 0.023^{ab}	0.086 ± 0.005^{b}	0.275 ± 0.058^{ab}	0.124 ± 0.018^{b}	0.323 ± 0.070^{ab}	0.023	0.016	0.361
malate.a.75	*µmol organ*$^{-1}$	0.333 ± 0.017^{bc}	0.270 ± 0.062^{bd}	0.192 ± 0.013^{cd}	0.334 ± 0.025^{bc}	0.673 ± 0.020^{a}	0.099 ± 0.008^{d}	0.124 ± 0.014^{cd}	0.080 ± 0.007^{d}	0.108 ± 0.010^{d}	0.432 ± 0.117^{b}	0.000	0.000	0.487
malate.g.75	*µmol organ*$^{-1}$	0.0824 ± 0.015^{ab}	0.057 ± 0.024^{b}	0.086 ± 0.016^{ab}	0.131 ± 0.013^{ab}	0.132 ± 0.019^{a}	0.087 ± 0.007^{ab}	0.089 ± 0.006^{ab}	0.101 ± 0.013^{ab}	0.096 ± 0.001^{ab}	0.102 ± 0.019^{ab}	0.028	0.727	0.171
malate.l.75	*µmol organ*$^{-1}$	0.165 ± 0.015^{ac}	0.078 ± 0.031^{c}	0.125 ± 0.017^{bc}	0.175 ± 0.007^{ac}	0.261 ± 0.025^{a}	0.098 ± 0.003^{bc}	0.102 ± 0.005^{bc}	0.144 ± 0.010^{bc}	0.127 ± 0.004^{bc}	0.188 ± 0.049^{ab}	0.000	0.048	0.092
glutamate.b.65	*µmol organ*$^{-1}$	2.26 ± 0.39^{ac}	2.31 ± 0.34^{ac}	2.98 ± 0.45^{a}	2.15 ± 0.07^{ac}	2.81 ± 0.07^{ab}	1.33 ± 0.48^{c}	1.13 ± 0.04^{c}	1.46 ± 0.09^{bc}	1.07 ± 0.31^{c}	1.06 ± 0.15^{c}	0.294	0.000	0.621
glutamate.s.65	*µmol organ*$^{-1}$	2.15 ± 0.12^{ab}	1.94 ± 0.16^{abc}	2.60 ± 0.17^{a}	1.90 ± 0.03^{bc}	2.09 ± 0.01^{abc}	1.68 ± 0.13^{bd}	1.20 ± 0.12^{d}	1.60 ± 0.11^{bd}	1.59 ± 0.25^{bd}	1.43 ± 0.12^{cd}	0.013	0.000	0.150
glutamate.p.65	*µmol organ*$^{-1}$	2.22 ± 0.69	3.73 ± 0.21	4.06 ± 0.72	3.13 ± 0.43	2.38 ± 0.10	2.16 ± 0.52	2.37 ± 0.10	2.37 ± 0.28	2.42 ± 0.37	2.07 ± 0.12	0.072	0.005	0.281
glutamate.a.65	*µmol organ*$^{-1}$	1.36 ± 0.32^{a}	1.17 ± 0.10^{ab}	1.01 ± 0.09^{ab}	1.20 ± 0.20^{ab}	1.39 ± 0.14^{a}	0.87 ± 0.04^{ab}	0.98 ± 0.11^{ab}	1.07 ± 0.03^{ab}	0.58 ± 0.10^{b}	0.78 ± 0.04^{ab}	0.567	0.001	0.105
glutamate.g.65	*µmol organ*$^{-1}$	0.62 ± 0.08^{c}	0.66 ± 0.09^{c}	0.98 ± 0.14^{ac}	0.76 ± 0.05^{bc}	1.21 ± 0.07^{a}	0.76 ± 0.07^{bc}	0.88 ± 0.03^{ac}	0.91 ± 0.02^{ac}	0.75 ± 0.05^{bc}	1.11 ± 0.11^{ab}	0.000	0.474	0.252
glutamate.l.65	*µmol organ*$^{-1}$	1.29 ± 0.20	1.02 ± 0.08	1.37 ± 0.22	1.15 ± 0.17	1.54 ± 0.05	0.93 ± 0.06	1.15 ± 0.08	1.17 ± 0.21	0.90 ± 0.15	1.00 ± 0.09	0.345	0.014	0.261
glutamate.b.75	*µmol organ*$^{-1}$	1.65 ± 0.14^{be}	2.04 ± 0.08^{abc}	3.12 ± 0.21^{a}	1.88 ± 0.19^{bd}	2.27 ± 0.35^{ab}	0.66 ± 0.11^{e}	0.79 ± 0.11^{de}	0.89 ± 0.06^{cde}	0.61 ± 0.21^{e}	1.26 ± 0.54^{be}	0.011	0.000	0.106
glutamate.s.75	*µmol organ*$^{-1}$	1.30 ± 0.18	1.19 ± 0.35	1.75 ± 0.22	1.42 ± 0.19	1.52 ± 0.19	0.94 ± 0.08	0.90 ± 0.04	1.09 ± 0.09	0.90 ± 0.11	1.33 ± 0.29	0.206	0.004	0.764
glutamate.p.75	*µmol organ*$^{-1}$	2.85 ± 0.30	3.53 ± 0.94	3.57 ± 0.50	3.65 ± 0.39	2.95 ± 0.50	2.03 ± 0.29	1.99 ± 0.11	2.63 ± 0.34	1.94 ± 0.48	2.00 ± 0.42	0.633	0.001	0.832
glutamate.a.75	*µmol organ*$^{-1}$	1.07 ± 0.12	1.29 ± 0.45	0.857 ± 0.074	1.09 ± 0.07	1.42 ± 0.36	0.74 ± 0.11	1.00 ± 0.07	0.82 ± 0.11	0.72 ± 0.03	1.49 ± 0.35	0.071	0.193	0.825
glutamate.g.75	*µmol organ*$^{-1}$	0.70 ± 0.06^{ab}	0.55 ± 0.12^{b}	0.91 ± 0.11^{ab}	0.85 ± 0.14^{ab}	1.19 ± 0.18^{a}	0.87 ± 0.05^{ab}	0.88 ± 0.05^{ab}	0.99 ± 0.07^{ab}	0.92 ± 0.09^{ab}	1.24 ± 0.18^{a}	0.004	0.075	0.719
glutamate.l.75	*µmol organ*$^{-1}$	1.04 ± 0.10	0.81 ± 0.25	1.02 ± 0.19	0.91 ± 0.14	1.26 ± 0.29	0.93 ± 0.08	1.09 ± 0.14	1.04 ± 0.03	0.97 ± 0.03	1.17 ± 0.19	0.468	0.749	0.774
aa.b.65	*µmol organ*$^{-1}$	5.90 ± 0.93^{ac}	5.87 ± 0.33^{ac}	7.93 ± 0.89^{ab}	5.83 ± 0.27^{ac}	8.57 ± 1.47^{a}	5.11 ± 1.50^{ac}	3.78 ± 0.31^{bc}	4.83 ± 0.42^{ac}	3.60 ± 0.87^{c}	5.04 ± 0.26^{ac}	0.089	0.000	0.565
aa.s.65	*µmol organ*$^{-1}$	7.01 ± 0.39	5.27 ± 0.21	7.83 ± 0.72	5.71 ± 0.50	6.78 ± 0.17	6.81 ± 0.26	5.55 ± 0.68	5.89 ± 0.27	7.18 ± 1.04	7.54 ± 0.98	0.065	0.851	0.098
aa.p.65	*µmol organ*$^{-1}$	6.94 ± 2.08	10.90 ± 1.14	14.10 ± 2.82	8.79 ± 1.36	11.00 ± 1.46	8.00 ± 1.28	14.2 ± 1.22	9.51 ± 1.09	10.50 ± 1.17	13.00 ± 1.25	0.022	0.500	0.154
aa.a.65	*µmol organ*$^{-1}$	5.55 ± 1.24	4.06 ± 0.15	3.71 ± 0.70	3.94 ± 0.09	5.71 ± 0.91	3.91 ± 0.58	5.24 ± 0.67	3.28 ± 0.13	2.92 ± 0.25	4.24 ± 0.46	0.059	0.105	0.202
aa.g.65	*µmol organ*$^{-1}$	2.16 ± 0.29^{e}	3.21 ± 0.46^{de}	3.97 ± 0.49^{cde}	3.03 ± 0.29^{de}	6.98 ± 0.80^{ab}	3.97 ± 0.54^{cde}	5.59 ± 0.40^{bc}	4.56 ± 0.14^{cd}	4.84 ± 0.40^{bd}	9.06 ± 0.48^{a}	0.000	0.000	0.383
aa.l.65	*µmol organ*$^{-1}$	4.11 ± 0.80^{bc}	3.15 ± 0.54^{c}	4.39 ± 0.45^{ac}	3.51 ± 0.69^{c}	7.26 ± 0.79^{ab}	5.06 ± 0.69^{ac}	7.34 ± 0.29^{a}	4.15 ± 0.41^{bc}	5.22 ± 0.90^{ac}	6.17 ± 0.51^{ac}	0.005	0.012	0.005

Table S7.1. Continued.

Traits	Units	Irrigated					Rainfed					*p*-value		
		MEX	EUR	DRI	KNI	HAR	MEX	EUR	DRI	KNI	HAR	G	W	G×W
aa.b.75	*µmol organ^{-1}*	4.06 ± 0.34^{ab}	5.07 ± 0.25^{ab}	6.70 ± 0.53^{ab}	5.35 ± 0.57^{ab}	7.43 ± 1.84^{a}	3.30 ± 0.28^{ab}	2.87 ± 0.19^{b}	4.92 ± 0.61^{ab}	2.46 ± 0.78^{b}	4.22 ± 1.58^{ab}	0.042	0.001	0.668
aa.s.75	*µmol organ^{-1}*	4.31 ± 0.68	3.34 ± 0.51	5.17 ± 0.75	4.09 ± 0.55	4.05 ± 0.02	5.71 ± 0.05	3.80 ± 0.20	5.33 ± 0.64	5.18 ± 0.63	4.62 ± 0.97	0.073	0.060	0.829
aa.p.75	*µmol organ^{-1}*	11.2 ± 2.0	15.3 ± 1.5	16.1 ± 1.9	17.3 ± 2.2	14.5 ± 2.3	10.2 ± 1.1	9.3 ± 0.6	12.3 ± 1.6	9.5 ± 2.2	9.0 ± 2.6	0.408	0.001	0.477
aa.a.75	*µmol organ^{-1}*	5.64 ± 0.87	5.51 ± 1.15	3.50 ± 0.63	4.70 ± 1.07	6.54 ± 0.85	4.18 ± 0.38	5.04 ± 0.74	3.00 ± 0.43	3.14 ± 0.15	6.13 ± 1.00	0.008	0.094	0.895
aa.g.75	*µmol organ^{-1}*	3.02 ± 0.12^{ab}	2.10 ± 0.38^{b}	3.53 ± 0.72^{ab}	5.30 ± 0.19^{a}	3.48 ± 0.53^{ab}	4.87 ± 0.17^{a}	5.24 ± 0.16^{a}	3.25 ± 0.18^{ab}	4.76 ± 0.62^{a}	4.99 ± 0.87^{a}	0.024	0.001	0.004
aa.l.75	*µmol organ^{-1}*	4.98 ± 0.48^{bc}	3.48 ± 0.99^{c}	4.00 ± 0.86^{bc}	5.42 ± 0.74^{bc}	5.15 ± 1.11^{bc}	6.15 ± 0.49^{ac}	9.51 ± 0.83^{a}	4.35 ± 0.21^{bc}	7.43 ± 0.32^{ab}	6.52 ± 1.05^{ac}	0.044	0.000	0.012
prot.b.65	*mg organ^{-1}*	5.35 ± 0.71^{ac}	5.79 ± 0.83^{ac}	7.31 ± 0.89^{ab}	4.91 ± 0.11^{bc}	8.59 ± 0.10^{a}	4.50 ± 1.38^{bc}	3.43 ± 0.11^{c}	4.59 ± 0.27^{bc}	3.28 ± 0.77^{c}	4.46 ± 0.50^{bc}	0.014	0.000	0.221
prot.s.65	*mg organ^{-1}*	5.48 ± 0.47^{ab}	4.45 ± 0.22^{ab}	5.56 ± 0.23^{a}	4.85 ± 0.27^{ab}	5.28 ± 0.18^{ab}	4.87 ± 0.10^{ab}	3.99 ± 0.15^{b}	4.62 ± 0.27^{ab}	4.40 ± 0.63^{ab}	4.88 ± 0.24^{ab}	0.028	0.009	0.908
prot.p.65	*mg organ^{-1}*	8.44 ± 2.18^{ab}	9.46 ± 0.64^{ab}	10.80 ± 0.86^{a}	10.90 ± 0.76^{a}	6.54 ± 0.29^{ab}	6.48 ± 1.41^{ab}	5.66 ± 0.15^{b}	6.72 ± 0.67^{ab}	6.54 ± 1.02^{ab}	5.43 ± 0.26^{b}	0.074	0.000	0.430
prot.a.65	*mg organ^{-1}*	5.50 ± 1.02^{a}	4.02 ± 0.22^{ab}	3.83 ± 0.71^{ab}	4.11 ± 0.69^{ab}	5.16 ± 0.53^{ab}	4.19 ± 0.13^{ab}	4.38 ± 0.32^{ab}	4.04 ± 0.16^{ab}	2.68 ± 0.14^{b}	3.86 ± 0.29^{ab}	0.087	0.044	0.223
prot.g.65	*mg organ^{-1}*	2.48 ± 0.31^{ac}	1.67 ± 0.06^{c}	2.35 ± 0.23^{ac}	2.01 ± 0.20^{bc}	2.87 ± 0.14^{a}	2.02 ± 0.10^{bc}	2.07 ± 0.06^{ac}	2.40 ± 0.10^{ac}	1.80 ± 0.06^{bc}	2.64 ± 0.22^{ab}	0.000	0.422	0.149
prot.l.65	*mg organ^{-1}*	3.44 ± 0.51^{ab}	2.21 ± 0.14^{bc}	2.75 ± 0.28^{ac}	2.62 ± 0.30^{ac}	3.51 ± 0.09^{a}	2.39 ± 0.21^{ac}	2.39 ± 0.05^{ac}	2.69 ± 0.19^{ac}	1.99 ± 0.18^{c}	2.62 ± 0.24^{ac}	0.017	0.006	0.101
prot.b.75	*mg organ^{-1}*	5.02 ± 0.30^{bcd}	5.50 ± 0.28^{ac}	8.46 ± 0.59^{a}	5.10 ± 0.24^{ad}	6.83 ± 0.97^{ab}	2.25 ± 0.34^{cd}	2.94 ± 0.27^{cd}	3.57 ± 0.10^{cd}	2.05 ± 0.62^{d}	4.15 ± 1.60^{bcd}	0.005	0.000	0.429
prot.s.75	*mg organ^{-1}*	4.04 ± 0.49^{ab}	3.44 ± 0.34^{b}	5.97 ± 0.59^{a}	3.96 ± 0.51^{ab}	4.72 ± 0.27^{ab}	3.67 ± 0.36^{ab}	3.64 ± 0.11^{ab}	5.11 ± 0.36^{ab}	3.42 ± 0.54^{b}	5.24 ± 0.96^{ab}	0.002	0.515	0.646
prot.p.75	*mg organ^{-1}*	8.42 ± 0.89^{ab}	8.23 ± 0.68^{ab}	11.00 ± 0.67^{a}	9.48 ± 1.17^{ab}	10.8 ± 1.63^{a}	4.93 ± 0.33^{b}	5.55 ± 0.31^{b}	7.75 ± 0.61^{ab}	5.66 ± 1.06^{b}	7.02 ± 1.13^{ab}	0.030	0.000	0.969
prot.a.75	*mg organ^{-1}*	3.52 ± 0.24^{ab}	4.30 ± 1.00^{ab}	2.94 ± 0.39^{ab}	3.41 ± 0.33^{ab}	5.80 ± 0.92^{ab}	3.55 ± 0.29^{ab}	4.36 ± 0.19^{ab}	3.74 ± 0.46^{ab}	2.79 ± 0.13^{b}	6.40 ± 1.55^{a}	0.002	0.701	0.865
prot.g.75	*mg organ^{-1}*	1.84 ± 0.08^{b}	1.62 ± 0.26^{b}	2.08 ± 0.22^{ab}	1.88 ± 0.39^{b}	2.77 ± 0.10^{ab}	2.04 ± 0.10^{ab}	2.10 ± 0.13^{ab}	2.49 ± 0.18^{ab}	1.91 ± 0.14^{b}	3.33 ± 0.60^{a}	0.001	0.060	0.862
prot.l.75	*mg organ^{-1}*	2.30 ± 0.01	1.75 ± 0.36	2.32 ± 0.28	1.71 ± 0.32	2.79 ± 0.43	2.02 ± 0.15	2.33 ± 0.07	2.34 ± 0.10	1.87 ± 0.11	2.94 ± 0.56	0.020	0.512	0.699
chla.b.65	*mg organ^{-1}*	0.865 ± 0.123^{ac}	0.906 ± 0.078^{ab}	0.889 ± 0.098^{ab}	0.823 ± 0.067^{ac}	0.966 ± 0.100^{a}	0.525 ± 0.149^{ac}	0.541 ± 0.033^{ac}	0.692 ± 0.019^{ac}	0.428 ± 0.092^{c}	0.495 ± 0.068^{bc}	0.494	0.000	0.660
chla.s.65	*mg organ^{-1}*	0.891 ± 0.063	0.939 ± 0.039	1.110 ± 0.027	0.827 ± 0.040	0.984 ± 0.160	0.786 ± 0.039	1.140 ± 0.179	1.070 ± 0.108	0.951 ± 0.089	1.290 ± 0.187	0.057	0.181	0.340
chla.p.65	*mg organ^{-1}*	0.872 ± 0.191	0.949 ± 0.064	1.220 ± 0.221	1.070 ± 0.015	0.568 ± 0.040	0.748 ± 0.102	1.140 ± 0.220	0.722 ± 0.042	0.700 ± 0.044	0.869 ± 0.152	0.155	0.241	0.029
chla.a.65	*mg organ^{-1}*	0.855 ± 0.175^{a}	0.650 ± 0.060^{ab}	0.611 ± 0.128^{ab}	0.652 ± 0.083^{ab}	0.671 ± 0.061^{ab}	0.455 ± 0.008^{ab}	0.624 ± 0.052^{ab}	0.503 ± 0.035^{ab}	0.320 ± 0.032^{b}	0.427 ± 0.070^{b}	0.287	0.000	0.195
chla.g.65	*mg organ^{-1}*	0.260 ± 0.033^{ac}	0.188 ± 0.002^{cd}	0.307 ± 0.021^{a}	0.241 ± 0.017^{ad}	0.293 ± 0.021^{ab}	0.189 ± 0.011^{cd}	0.211 ± 0.019^{bcd}	0.267 ± 0.013^{ac}	0.159 ± 0.012^{d}	0.293 ± 0.017^{ab}	0.000	0.008	0.040
chla.l.65	*mg organ^{-1}*	0.417 ± 0.066^{a}	0.289 ± 0.026^{ab}	0.380 ± 0.035^{a}	0.353 ± 0.026^{ab}	0.381 ± 0.041^{a}	0.258 ± 0.019^{ab}	0.311 ± 0.026^{ab}	0.370 ± 0.032^{a}	0.194 ± 0.016^{b}	0.294 ± 0.025^{ab}	0.066	0.002	0.044
chla.b.75	*mg organ^{-1}*	0.621 ± 0.046^{ab}	0.734 ± 0.063^{ab}	1.170 ± 0.033^{a}	0.828 ± 0.140^{ab}	1.180 ± 0.417^{a}	0.345 ± 0.077^{b}	0.543 ± 0.156^{ab}	0.462 ± 0.057^{ab}	0.204 ± 0.052^{b}	0.384 ± 0.151^{ab}	0.178	0.000	0.271
chla.s.75	*mg organ^{-1}*	0.765 ± 0.051	0.753 ± 0.054	1.001 ± 0.066	0.723 ± 0.097	1.450 ± 0.365	0.893 ± 0.122	0.946 ± 0.136	1.220 ± 0.342	0.890 ± 0.225	0.962 ± 0.085	0.117	0.833	0.363
chla.p.75	*mg organ^{-1}*	1.82 ± 0.13^{ab}	2.06 ± 0.30^{ab}	2.35 ± 0.47^{ab}	2.02 ± 0.03^{ab}	2.99 ± 0.85^{a}	0.89 ± 0.03^{b}	1.29 ± 0.46^{ab}	1.38 ± 0.26^{ab}	1.33 ± 0.42^{ab}	1.50 ± 0.24^{ab}	0.290	0.001	0.869
chla.a.75	*mg organ^{-1}*	0.475 ± 0.018	0.581 ± 0.124	0.479 ± 0.035	0.527 ± 0.050	0.644 ± 0.183	0.441 ± 0.052	0.476 ± 0.062	0.468 ± 0.100	0.252 ± 0.044	0.647 ± 0.163	0.155	0.193	0.620
chla.g.75	*mg organ^{-1}*	0.186 ± 0.005^{ab}	0.157 ± 0.020^{b}	0.228 ± 0.031^{ab}	0.179 ± 0.022^{ab}	0.276 ± 0.031^{ab}	0.185 ± 0.021^{ab}	0.209 ± 0.008^{ab}	0.247 ± 0.037^{ab}	0.137 ± 0.011^{b}	0.313 ± 0.060^{a}	0.001	0.486	0.533
chla.l.75	*mg organ^{-1}*	0.244 ± 0.004	0.193 ± 0.034	0.269 ± 0.038	0.193 ± 0.020	0.301 ± 0.041	0.204 ± 0.007	0.249 ± 0.015	0.257 ± 0.042	0.166 ± 0.019	0.300 ± 0.056	0.014	0.819	0.614
chlb.b.65	*mg organ^{-1}*	0.351 ± 0.036	0.298 ± 0.035	0.356 ± 0.049	0.282 ± 0.018	0.397 ± 0.045	0.235 ± 0.051	0.386 ± 0.040	0.425 ± 0.062	0.268 ± 0.041	0.245 ± 0.024	0.092	0.360	0.031
chlb.s.65	*mg organ^{-1}*	0.493 ± 0.009^{c}	0.628 ± 0.045^{bc}	0.706 ± 0.060^{bc}	0.442 ± 0.024^{c}	0.790 ± 0.266^{ac}	0.643 ± 0.079^{bc}	1.310 ± 0.218^{ab}	1.050 ± 0.203^{ac}	0.985 ± 0.016^{ac}	1.520 ± 0.293^{a}	0.016	0.000	0.371
chlb.p.65	*mg organ^{-1}*	0.653 ± 0.168^{ab}	0.748 ± 0.044^{ab}	0.917 ± 0.122^{ab}	0.797 ± 0.029^{ab}	0.599 ± 0.006^{b}	0.651 ± 0.105^{ab}	1.290 ± 0.275^{a}	0.518 ± 0.008^{b}	0.740 ± 0.053^{ab}	1.170 ± 0.223^{ab}	0.097	0.146	0.008
chlb.a.65	*mg organ^{-1}*	0.327 ± 0.046^{ab}	0.247 ± 0.025^{bc}	0.219 ± 0.033^{bc}	0.219 ± 0.029^{bc}	0.312 ± 0.019^{ab}	0.211 ± 0.007^{bc}	0.397 ± 0.025^{a}	0.246 ± 0.020^{bc}	0.142 ± 0.005^{c}	0.285 ± 0.0252^{ab}	0.000	0.597	0.001
chlb.g.65	*mg organ^{-1}*	0.178 ± 0.0215^{bc}	0.128 ± 0.004^{bc}	0.176 ± 0.014^{bc}	0.141 ± 0.015^{bc}	0.186 ± 0.018^{bc}	0.140 ± 0.016^{bc}	0.181 ± 0.019^{bc}	0.188 ± 0.007^{b}	0.114 ± 0.002^{c}	0.262 ± 0.015^{a}	0.000	0.110	0.003
chlb.l.65	*mg organ^{-1}*	0.279 ± 0.046^{a}	0.200 ± 0.014^{ab}	0.246 ± 0.018^{ab}	0.207 ± 0.025^{ab}	0.255 ± 0.031^{ab}	0.206 ± 0.025^{ab}	0.318 ± 0.033^{a}	0.316 ± 0.039^{a}	0.137 ± 0.008^{b}	0.283 ± 0.022^{a}	0.008	0.417	0.009
chlb.b.75	*mg organ^{-1}*	0.294 ± 0.046	0.415 ± 0.084	0.511 ± 0.050	0.328 ± 0.038	1.160 ± 0.769	0.333 ± 0.159	0.589 ± 0.274	0.418 ± 0.129	0.175 ± 0.065	0.212 ± 0.060	0.542	0.265	0.298
chlb.s.75	*mg organ^{-1}*	0.653 ± 0.123	0.731 ± 0.129	0.730 ± 0.0816	0.520 ± 0.0971	1.690 ± 0.571	1.090 ± 0.335	1.370 ± 0.241	1.420 ± 0.566	1.160 ± 0.326	1.020 ± 0.124	0.510	0.097	0.191
chlb.p.75	*mg organ^{-1}*	2.13 ± 0.41	2.27 ± 0.53	2.22 ± 0.66	2.00 ± 0.10	3.48 ± 0.98	0.96 ± 0.09	1.57 ± 0.69	1.53 ± 0.33	1.60 ± 0.43	2.14 ± 0.57	0.219	0.021	0.903
chlb.a.75	*mg organ^{-1}*	0.229 ± 0.007	0.267 ± 0.032	0.187 ± 0.014	0.205 ± 0.019	0.392 ± 0.087	0.250 ± 0.035	0.331 ± 0.051	0.319 ± 0.093	0.169 ± 0.022	0.437 ± 0.102	0.009	0.223	0.680
chlb.g.75	*mg organ^{-1}*	0.132 ± 0.0057^{b}	0.115 ± 0.006^{b}	0.140 ± 0.013^{b}	0.121 ± 0.018^{b}	0.209 ± 0.017^{ab}	0.138 ± 0.007^{b}	0.171 ± 0.016^{ab}	0.197 ± 0.042^{ab}	0.132 ± 0.008^{b}	0.243 ± 0.035^{a}	0.001	0.019	0.619
chlb.l.75	*mg organ^{-1}*	0.175 ± 0.000	0.145 ± 0.012	0.167 ± 0.015	0.128 ± 0.020	0.253 ± 0.036	0.171 ± 0.013	0.255 ± 0.024	0.244 ± 0.070	0.165 ± 0.025	0.275 ± 0.068	0.044	0.046	0.547
chltot.b.65	*mg organ^{-1}*	1.56 ± 0.34^{ab}	1.21 ± 0.06^{ab}	1.11 ± 0.12^{ab}	1.15 ± 0.14^{ab}	1.31 ± 0.13^{ab}	1.04 ± 0.03^{ab}	1.57 ± 0.11^{a}	1.40 ± 0.19^{ab}	0.81 ± 0.05^{b}	0.97 ± 0.08^{ab}	0.099	0.270	0.021
chltot.s.65	*mg organ^{-1}*	0.73 ± 0.08^{b}	0.69 ± 0.06^{b}	1.15 ± 0.12^{ab}	0.64 ± 0.07^{b}	1.00 ± 0.26^{ab}	0.67 ± 0.08^{b}	1.41 ± 0.27^{ab}	1.44 ± 0.24^{ab}	0.93 ± 0.04^{b}	1.80 ± 0.20^{a}	0.001	0.001	0.092
chltot.p.65	*mg organ^{-1}*	0.595 ± 0.079^{ab}	0.407 ± 0.058^{b}	0.612 ± 0.116^{ab}	0.449 ± 0.056^{b}	0.513 ± 0.029^{b}	0.549 ± 0.113^{ab}	1.070 ± 0.187^{a}	0.610 ± 0.089^{ab}	0.406 ± 0.024^{b}	0.905 ± 0.168^{ab}	0.056	0.009	0.008
chltot.a.65	*mg organ^{-1}*	1.18 ± 0.22^{a}	0.90 ± 0.08^{ab}	0.83 ± 0.16^{ab}	0.87 ± 0.11^{ab}	0.98 ± 0.08^{ab}	0.67 ± 0.01^{ab}	1.02 ± 0.08^{a}	0.75 ± 0.05^{ab}	0.46 ± 0.0358^{b}	0.71 ± 0.10^{ab}	0.098	0.003	0.057
chltot.g.65	*mg organ^{-1}*	0.439 ± 0.054^{abc}	0.317 ± 0.006^{cd}	0.483 ± 0.034^{ab}	0.382 ± 0.031^{bd}	0.479 ± 0.038^{ab}	0.329 ± 0.026^{bd}	0.392 ± 0.038^{bd}	0.455 ± 0.020^{abc}	0.273 ± 0.013^{d}	0.555 ± 0.031^{a}	0.000	0.359	0.012
chltot.l.65	*mg organ^{-1}*	0.696 ± 0.112^{a}	0.489 ± 0.040^{ab}	0.626 ± 0.052^{ab}	0.560 ± 0.050^{ab}	0.636 ± 0.070^{ab}	0.465 ± 0.043^{ab}	0.630 ± 0.059^{ab}	0.686 ± 0.070^{a}	0.331 ± 0.023^{b}	0.577 ± 0.046^{ab}	0.032	0.114	0.019

Table S7.1. Continued.

Traits	Units	Irrigated					Rainfed					*p*-value		
		MEX	EUR	DRI	KNI	HAR	MEX	EUR	DRI	KNI	HAR	G	W	G×W
chltot.b.75	*mg organ*$^{-1}$	0.92 ± 0.09	1.15 ± 0.15	1.68 ± 0.08	1.16 ± 0.18	2.34 ± 1.17	0.68 ± 0.23	1.13 ± 0.43	0.88 ± 0.18	0.38 ± 0.12	0.60 ± 0.21	0.409	0.014	0.321
chltot.s.75	*mg organ*$^{-1}$	1.42 ± 0.15	1.48 ± 0.18	1.83 ± 0.11	1.24 ± 0.19	3.14 ± 0.93	1.99 ± 0.46	2.32 ± 0.38	2.64 ± 0.91	2.04 ± 0.55	1.98 ± 0.20	0.346	0.252	0.241
chltot.p.75	*mg organ*$^{-1}$	3.95 ± 0.53	4.33 ± 0.82	4.57 ± 1.12	4.02 ± 0.11	6.47 ± 1.81	1.85 ± 0.06	2.86 ± 1.15	2.91 ± 0.58	2.93 ± 0.84	3.64 ± 0.81	0.246	0.005	0.899
chltot.a.75	*mg organ*$^{-1}$	0.704 ± 0.024	0.848 ± 0.155	0.666 ± 0.047	0.732 ± 0.067	1.040 ± 0.266	0.691 ± 0.087	0.807 ± 0.111	0.787 ± 0.191	0.421 ± 0.056	1.080 ± 0.258	0.049	0.687	0.673
chltot.g.75	*mg organ*$^{-1}$	0.318 ± 0.010^{ab}	0.272 ± 0.024^{b}	0.368 ± 0.043^{ab}	0.299 ± 0.040^{b}	0.485 ± 0.048^{ab}	0.323 ± 0.028^{ab}	0.380 ± 0.023^{ab}	0.443 ± 0.078^{ab}	0.268 ± 0.018^{b}	0.556 ± 0.093^{a}	0.001	0.144	0.594
chltot.l.75	*mg organ*$^{-1}$	0.419 ± 0.004	0.337 ± 0.046	0.437 ± 0.053	0.321 ± 0.039	0.555 ± 0.072	0.375 ± 0.020	0.505 ± 0.038	0.501 ± 0.112	0.332 ± 0.039	0.576 ± 0.123	0.021	0.302	0.583

Table S7.2. Effect of genotypic variability (G), water regime (W) and their interaction (G×W) on agronomic traits, carbon and nitrogen isotope composition, grain quality traits, grain mineral content, spectral vegetation indices, leaf relative water content, and organ-specific fresh and dry weights, water content, and carbon (glucose, glucose-6-phosphate, fructose, sucrose, starch, and malate) and nitrogen (glutamate, total amino acids, proteins, chlorophylls a, b and total) metabolites. The numbers in the traits represent the Zadoks scale when they were measured. The organ-specific traits were expressed as concentration in dry weight (DW) and as total organ content. The means in each row with different letters are statistically different (p<0.05; two-way ANOVA, TUKEY test; yellow colour indicates the significance of a factor). The colour scale in the means shows the minimum (red) and maximum (blue) values per trait. The rest of the abbreviations are described throughout the text. MEX, Mexa; EUR, Euroduro; DRI, Don Ricardo; KNI, Kiko Nick; HAR, Haristide; b, blade; s, sheath; p, peduncle; a, awn; g, glume; l, lemma; e, the whole ear.

Traits	Units	blade		sheath		peduncle		awn		glume		lemma		*p*-value		
		irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	organ	water	O×W
glc.65	µmol organ^{-1}	1.97 ± 0.57^{c}	2.31 ± 0.36^{c}	6.78 ± 0.78^{c}	6.94 ± 0.84^{c}	66.16 ± 4.87^{a}	43.63 ± 6.68^{b}	9.24 ± 1.32^{c}	7.94 ± 1.37^{c}	6.68 ± 0.50^{c}	5.06 ± 0.47^{c}	12.66 ± 1.31^{c}	7.72 ± 0.89^{c}	0.000	0.001	0.000
glc.75	µmol organ^{-1}	4.65 ± 1.09^{b}	1.80 ± 0.23^{b}	3.70 ± 0.66^{b}	2.58 ± 0.25^{b}	14.03 ± 2.57^{a}	6.19 ± 1.56^{b}	5.81 ± 1.48^{b}	3.40 ± 0.90^{b}	3.62 ± 0.47^{b}	1.96 ± 0.27^{b}	4.44 ± 0.70^{b}	2.20 ± 0.35^{b}	0.000	0.000	0.038
glc6P.65	nmol organ^{-1}	180.1 ± 9.7^{cde}	67.9 ± 4.1^{h}	204.7 ± 8.0^{be}	91.2 ± 3.4^{gh}	481.7 ± 33.7^{a}	252 ± 23.0^{b}	248.2 ± 19.4^{bc}	167.9 ± 8.9^{def}	150.9 ± 9.5^{eg}	103.7 ± 5.2^{fgh}	233.1 ± 14.3^{bd}	138.6 ± 6.8^{eg}	0.000	0.000	0.000
glc6P.75	nmol organ^{-1}	160 ± 10.6^{bce}	75.9 ± 11.3^{f}	107.2 ± 6.8^{def}	93.7 ± 9.2^{ef}	233.9 ± 18.8^{a}	179.3 ± 21.1^{ac}	188.2 ± 21.4^{ab}	165.6 ± 26.5^{acd}	105.9 ± 6.4^{def}	118.8 ± 10.3^{cf}	118.9 ± 6.7^{bcef}	129.7 ± 10.1^{bcef}	0.000	0.004	0.007
fru.65	µmol organ^{-1}	1.98 ± 0.43^{c}	1.72 ± 0.29^{c}	4.33 ± 0.52^{c}	3.29 ± 0.37^{c}	35.58 ± 2.44^{a}	21.59 ± 2.92^{b}	1.64 ± 0.22^{c}	1.55 ± 0.21^{c}	1.91 ± 0.14^{c}	2.10 ± 0.20^{c}	5.51 ± 0.53^{c}	3.64 ± 0.28^{c}	0.000	0.000	0.000
fru.75	µmol organ^{-1}	5.40 ± 1.30^{b}	3.31 ± 0.62^{b}	7.83 ± 1.78^{b}	4.94 ± 0.81^{b}	17.76 ± 2.95^{a}	7.90 ± 1.64^{b}	3.43 ± 1.03^{b}	2.61 ± 0.70^{b}	2.64 ± 0.52^{b}	1.96 ± 0.41^{b}	3.36 ± 0.61^{b}	2.53 ± 0.39^{b}	0.000	0.000	0.003
suc.65	µmol organ^{-1}	16.7 ± 2.0^{e}	14.7 ± 1.1^{e}	26.3 ± 1.8^{cd}	29.2 ± 1.3^{bc}	38.6 ± 4.3^{a}	37.1 ± 2.4^{ab}	15.6 ± 1.3^{e}	19.1 ± 1.7^{de}	10.2 ± 1.3^{e}	13.9 ± 1.1^{e}	12.8 ± 1.0^{e}	17.0 ± 1.2^{e}	0.000	0.098	0.389
suc.75	µmol organ^{-1}	17.3 ± 1.7^{cd}	14.9 ± 2.0^{d}	27.8 ± 1.4^{c}	28.2 ± 1.9^{c}	103.0 ± 6.4^{a}	66.5 ± 4.7^{b}	13.5 ± 1.6^{d}	21.2 ± 2.2^{cd}	9.6 ± 1.1^{d}	14.0 ± 1.0^{d}	11.2 ± 0.9^{d}	16.2 ± 0.8^{cd}	0.000	0.022	0.000
starch.65	µmol organ^{-1}	2.01 ± 0.43^{ad}	1.51 ± 0.31^{bcd}	0.91 ± 0.15^{d}	1.45 ± 0.27^{bcd}	2.14 ± 0.21^{ad}	2.33 ± 0.34^{ac}	2.49 ± 0.30^{ac}	3.26 ± 0.43^{a}	0.83 ± 0.09^{d}	1.20 ± 0.13^{cd}	2.75 ± 0.25^{ab}	3.32 ± 0.38^{a}	0.000	0.060	0.341
starch.75	µmol organ^{-1}	1.23 ± 0.27^{bc}	0.98 ± 0.26^{be}	0.39 ± 0.07^{e}	0.75 ± 0.09^{be}	0.48 ± 0.06^{de}	0.55 ± 0.06^{cde}	1.31 ± 0.18^{b}	2.65 ± 0.19^{a}	0.57 ± 0.07^{cde}	1.20 ± 0.09^{bd}	1.12 ± 0.11^{be}	2.19 ± 0.18^{a}	0.000	0.000	0.000
malate.65	µmol organ^{-1}	0.971 ± 0.081^{b}	0.623 ± 0.065^{cd}	1.508 ± 0.079^{a}	1.050 ± 0.072^{b}	1.558 ± 0.128^{a}	0.740 ± 0.120^{bc}	0.318 ± 0.048^{de}	0.187 ± 0.017^{e}	0.086 ± 0.012^{e}	0.128 ± 0.014^{e}	0.313 ± 0.035^{de}	0.214 ± 0.021^{e}	0.000	0.000	0.000
malate.75	µmol organ^{-1}	1.046 ± 0.082^{ab}	0.588 ± 0.077^{c}	1.204 ± 0.061^{a}	0.887 ± 0.062^{b}	0.308 ± 0.039^{de}	0.200 ± 0.029^{de}	0.361 ± 0.046^{d}	0.168 ± 0.041^{de}	0.097 ± 0.010^{e}	0.094 ± 0.005^{e}	0.161 ± 0.018^{de}	0.132 ± 0.012^{e}	0.000	0.000	0.000
Glu.65	µmol organ^{-1}	2.50 ± 0.15^{b}	1.21 ± 0.11^{cd}	2.13 ± 0.08^{b}	1.50 ± 0.07^{c}	3.11 ± 0.27^{a}	2.28 ± 0.13^{b}	1.23 ± 0.080^{cd}	0.86 ± 0.05^{d}	0.85 ± 0.07^{d}	0.88 ± 0.04^{d}	1.28 ± 0.08^{cd}	1.03 ± 0.06^{cd}	0.000	0.000	0.000
Glu.75	µmol organ^{-1}	2.19 ± 0.16^{b}	0.84 ± 0.12^{d}	1.43 ± 0.10^{c}	1.03 ± 0.07^{cd}	3.31 ± 0.23^{a}	2.12 ± 0.15^{b}	1.15 ± 0.11^{cd}	0.95 ± 0.10^{cd}	0.84 ± 0.08^{d}	0.98 ± 0.05^{cd}	1.01 ± 0.09^{cd}	1.04 ± 0.05^{cd}	0.000	0.000	0.000
aa.65	µmol organ^{-1}	6.82 ± 0.46^{b}	4.47 ± 0.36^{bc}	6.52 ± 0.30^{b}	6.59 ± 0.34^{b}	10.35 ± 0.96^{a}	11.04 ± 0.76^{a}	4.59 ± 0.37^{bc}	3.92 ± 0.28^{c}	3.87 ± 0.48^{c}	5.61 ± 0.51^{bc}	4.48 ± 0.46^{bc}	5.59 ± 0.37^{bc}	0.000	0.745	0.001
aa.75	µmol organ^{-1}	5.73 ± 0.47^{cd}	3.55 ± 0.40^{d}	4.19 ± 0.26^{d}	4.93 ± 0.29^{cd}	14.87 ± 0.94^{a}	10.05 ± 0.74^{b}	5.18 ± 0.45^{cd}	4.30 ± 0.39^{d}	3.49 ± 0.33^{d}	4.62 ± 0.27^{cd}	4.60 ± 0.38^{cd}	6.79 ± 0.52^{c}	0.000	0.026	0.000
protein.65	mg organ^{-1}	6.39 ± 0.44^{b}	4.05 ± 0.32^{cd}	5.12 ± 0.16^{bc}	4.55 ± 0.16^{c}	9.21 ± 0.61^{a}	6.16 ± 0.35^{b}	4.52 ± 0.32^{c}	3.83 ± 0.18^{cd}	2.28 ± 0.13^{e}	2.19 ± 0.09^{e}	2.91 ± 0.18^{de}	2.41 ± 0.10^{e}	0.000	0.000	0.000
protein.75	mg organ^{-1}	6.18 ± 0.41^{b}	2.99 ± 0.37^{cd}	4.43 ± 0.29^{c}	4.22 ± 0.29^{c}	9.58 ± 0.51^{a}	6.18 ± 0.40^{b}	4.00 ± 0.37^{c}	4.17 ± 0.43^{c}	2.04 ± 0.14^{d}	2.38 ± 0.18^{d}	2.18 ± 0.16^{d}	2.30 ± 0.14^{d}	0.000	0.000	0.000
chla.65	mg organ^{-1}	0.890 ± 0.038^{ac}	0.537 ± 0.040^{de}	0.951 ± 0.040^{ab}	1.047 ± 0.068^{a}	0.937 ± 0.078^{ab}	0.835 ± 0.066^{bc}	0.688 ± 0.048^{cd}	0.466 ± 0.031^{ef}	0.258 ± 0.014^{fg}	0.224 ± 0.014^{g}	0.364 ± 0.019^{eg}	0.286 ± 0.018^{fg}	0.000	0.000	0.000
chla.75	mg organ^{-1}	0.906 ± 0.097^{bc}	0.388 ± 0.051^{d}	0.957 ± 0.100^{b}	0.983 ± 0.084^{b}	2.248 ± 0.205^{a}	1.277 ± 0.133^{b}	0.541 ± 0.043^{cd}	0.457 ± 0.049^{d}	0.205 ± 0.014^{d}	0.218 ± 0.020^{d}	0.240 ± 0.016^{d}	0.235 ± 0.018^{d}	0.000	0.000	0.000
chlb.65	mg organ^{-1}	0.337 ± 0.018^{d}	0.312 ± 0.027^{d}	0.612 ± 0.058^{c}	1.102 ± 0.107^{a}	0.743 ± 0.047^{bc}	0.873 ± 0.102^{ab}	0.265 ± 0.017^{d}	0.256 ± 0.024^{d}	0.162 ± 0.008^{d}	0.177 ± 0.014^{d}	0.237 ± 0.014^{d}	0.252 ± 0.021^{d}	0.000	0.001	0.000
chlb.75	mg organ^{-1}	0.541 ± 0.157^{de}	0.346 ± 0.072^{de}	0.864 ± 0.153^{cd}	1.211 ± 0.139^{bc}	2.420 ± 0.271^{a}	1.561 ± 0.204^{b}	0.256 ± 0.025^{e}	0.301 ± 0.035^{de}	0.143 ± 0.010^{e}	0.176 ± 0.015^{e}	0.174 ± 0.014^{e}	0.222 ± 0.021^{e}	0.000	0.184	0.000
chltotal.65	mg organ^{-1}	1.27 ± 0.08^{a}	1.16 ± 0.09^{a}	0.84 ± 0.074^{bc}	1.25 ± 0.13^{a}	0.52 ± 0.035^{de}	0.71 ± 0.08^{be}	0.95 ± 0.06^{ab}	0.72 ± 0.05^{bd}	0.42 ± 0.02^{de}	0.40 ± 0.03^{e}	0.60 ± 0.03^{cde}	0.54 ± 0.04^{cde}	0.000	0.440	0.000
chltotal.75	mg organ^{-1}	1.45 ± 0.24^{cd}	0.73 ± 0.12^{de}	1.82 ± 0.25^{c}	2.19 ± 0.22^{bc}	4.67 ± 0.47^{a}	2.84 ± 0.33^{b}	0.80 ± 0.06^{de}	0.76 ± 0.08^{de}	0.35 ± 0.02^{e}	0.39 ± 0.03^{e}	0.41 ± 0.03^{e}	0.46 ± 0.04^{e}	0.000	0.004	0.000

10. CHAPTER 10: BIBLIOGRAPHY

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