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Phenotypic variation among natural populations of pines. Implications for the management and conservation of genetic resources

Variación fenotípica entre poblaciones naturales de pinos. Implicaciones para la gestión y conservación de recursos genéticos

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Dr. Ricardo Alía Miranda

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Phenotypic variation among natural populations of pines. Implications for the management and conservation of genetic resources

Variación fenotípica entre poblaciones naturales de pinos. Implicaciones para la gestión y conservación de recursos genéticos

PhD Student: Andrés Flores García

Supervisors: Dr. Ricardo Alía Miranda
INIA-CIFOR. Dpto. of Ecology and Forest Genetics
Research Institute on Sustainable Forest Management
Madrid (Spain)

External Reviewers:

Natalia Vizcaíno Palomar
BIOGECO UMR 1202, INRA - Université de Bordeaux

Álvaro Soto de Viana
Department of Systems and Natural Resources
Polytechnic University of Madrid

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I know that I know nothing

Socrates (c. 470 – 399 BC)

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Abstract

The study of variation at different levels is a constant topic of research. In the field of genetics, it is necessary to know the causes and the effects of variation in traits that influence traits of individuals in their natural habitats. Genetic variation is considered the most basic level of biological diversity and a prerequisite for the variability of species, populations, and ecosystems. Populations that lose genetic variation cannot evolve since evolution cannot proceed without genetic variation and populations that are unable to adapt to changing conditions will go extinct. Forest genetics studies have shown that environmental heterogeneity influences the genetic differentiation among tree populations, creating geographic genetic patterns that are consistent with phenotypic traits. One can look at this association to detect climate variables that are shaping the genetic structure of populations or even identify which genes are under pronounced natural selection. Genetic conservation aims to protect and preserve genetic variation, vital for the maintenance of adaptive potential within populations and species. Conserving forest genetic resources (FGR) constitute a unique and irreplaceable resource for the future, including for sustainable economic growth and progress and environmental adaption.

The aim of this thesis is to investigate the phenotypic variation among natural population of pines at local and regional scales, and define its implications in the use and conservation of genetic resources. At first step, we analyzed the relationship between within-population variance in fitness-related phenotypic traits (survival, height and diameter), phenotypic plasticity of these traits, and environmental (climatic) heterogeneity in the region surrounding provenances of *Pinus sylvestris, P. pinaster* and *P. halepensis*. We used multi-site tree provenance tests of Iberian pine species and a model selection approach to infer the relationship between them. It was found that climatic heterogeneity at different spatial scale can explain a significant part of the intrapopulation phenotypic variation in different traits, but the relationships depend on the species and traits considered.

Second, we assessed the inter- and intraspecific genetic variation in seedling drought tolerance in *Pinus oocarpa, P. patula* and *P. pseudostrobus* from the Trans-Mexican Volcanic Belt, a relevant genetic resource management scale. It was evaluated the growth and biomass fractions of pine seedlings in a greenhouse with two highly contrasted watering regimes. We found that even at reduced geographical scales, Mexican pines present differences in the response to water stress. The responses differed among species, including the allometric phenotypic changes in biomass allocation (plasticity), the genetic differences among populations, and the differences in phenotypic plasticity among populations.

Third, we identified areas for gene conservation and proposing measures for the conservation and sustainable use of forest genetic resources for four pines species: *P. greggii, P. oocarpa, P. patula* and *P. pseudostrobus*. It was obtained the most relevant information related to the identification and characterization of forest genetic resources of these species. We used the distribution range of the species, and information for conservation of forest genetic resources and for the sustainable use of forest genetic resources. It was checked gaps considering the distribution area and the genetic zones of the species. We propose recommendations to improve the status of conservation and sustainable use of forest genetic resources in the evaluated species.

Resumen

El estudio de la variación en diferentes niveles es un tema constante de investigación. En el campo de la genética, es necesario conocer las causas y los efectos de la variación en los rasgos que influyen en las características de los individuos en sus hábitats naturales. La variación genética se considera el nivel más básico de diversidad biológica y un requisito previo para la variabilidad de las especies, las poblaciones y los ecosistemas. Las poblaciones que pierden la variación genética no pueden evolucionar ya que la evolución no puede avanzar sin una variación genética y las poblaciones que no pueden adaptarse a las condiciones cambiantes se extinguirán. Los estudios de genética forestal han demostrado que la heterogeneidad ambiental influye en la diferenciación genética entre poblaciones de árboles, creando patrones genéticos geográficos que son consistentes con los rasgos fenotípicos. Se puede observar esta asociación para detectar variables climáticas que están dando forma a la estructura genética de las poblaciones o incluso para identificar qué genes están bajo una pronunciada selección natural. La conservación genética tiene como objetivo proteger y preservar la variación genética, vital para el mantenimiento del potencial de adaptación dentro de las poblaciones y las especies. La conservación de los recursos genéticos forestales (RGF) constituye un recurso único e irremplazable para el futuro, incluido el crecimiento económico sostenible, el progreso y la adaptación al medio ambiente.

El objetivo de esta tesis es investigar la variación fenotípica entre la población natural de pinos a escala local y regional, y definir sus implicaciones en el uso y la conservación de los recursos genéticos. En el primer paso, analizamos la relación entre la varianza dentro de la población en rasgos fenotípicos relacionados con su eficacia biológica (supervivencia, altura y diámetro), la plasticidad fenotípica de estos rasgos y la heterogeneidad ambiental (climática) en la región que rodea procedencias de *Pinus sylvestris, P. pinaster* y *P. halepensis*. Para ello usamos pruebas de procedencia de árboles en sitios múltiples de especies de pino ibérico y la selección de modelos para inferir la relación que había entre ellos. Se encontró que la heterogeneidad climática a diferentes escalas espaciales puede explicar una parte significativa de la variación fenotípica intrapoblacional en diferentes características, pero las relaciones dependen de la especie y los rasgos considerados.

En segundo lugar, evaluamos la variación genética inter e intraespecífica en la tolerancia a la sequía de plántulas en *Pinus oocarpa, P. patula* y *P. pseudostrobus* del Eje Volcánico Transversal de México, que es una escala relevante de manejo de recursos genéticos. Se evaluó el crecimiento y las fracciones de biomasa de plántulas de pino en un invernadero con dos regímenes de riego altamente contrastados. Encontramos que incluso a escalas geográficas reducidas, los pinos

mexicanos presentan diferencias en la respuesta al estrés hídrico. Las respuestas difirieron entre las especies, incluidos los cambios fenotípicos alométricos en la asignación de biomasa (plasticidad), las diferencias genéticas entre las poblaciones y las diferencias en la plasticidad fenotípica entre las poblaciones.

En tercer lugar, identificamos áreas para la conservación genética y propusimos medidas para la conservación y el uso sostenible de los recursos genéticos forestales en cuatro especies de pinos: *P. greggii, P. oocarpa, P. patula* y *P. pseudostrobus*. Se empleó información relevante relacionada a la identificación y caracterización de los recursos genéticos forestales de estas especies. Usamos el rango de distribución de las especies e información para la conservación de los recursos genéticos forestales y del uso sostenible de los recursos genéticos forestales. Se determinaron los huecos considerando el área de distribución y las zonas genéticas de las especie. Proponemos recomendaciones para mejorar el estado de la conservación y el uso sostenible de los recursos genéticos forestales en las especies evaluadas.

Outline of the thesis

This thesis focuses on the understanding of the relationships between environmental heterogeneity and phenotypic variation and plasticity among natural populations of pine species at local and regional scales, and the analyzing what are their implications for their use and conservation of forest genetic resources. The information may provide a valuable insight for forest management and conservation of resources.

The first study (Chapter I) is addressed to test whether under spatially variable environments, gene flow among locally adapted populations is a persistent source of genetic variation at the intrapopulation level for *Pinus sylvestris* L., *P. pinaster* Aiton, and *P. halepensis* Mill. It was found that climatic heterogeneity at different spatial scale can explain a significant part of the intrapopulation phenotypic variation in different traits, but the relationships depend on the species and traits considered.

The second study (Chapter II) is focused on *P. oocarpa* Schiede ex Schltdl., *P. patula* Schiede ex Schltdl. & Cham., and *P. pseudostrobus* Lindl. to assess whether there are inter- and intraspecific genetic variation in seedling drought tolerance at a fine geographical scale. It was found that even at reduced geographical scale, these pines present differences in the response to water stress but responses differed among species.

The last study (Chapter III) proposed measures for the conservation and sustainable use of forest genetic resources, particularly for four pine species: *P. greggii* Engelm. ex Parl., *P. oocarpa* Schiede ex Schltdl., *P. patula* Schiede ex Schltdl. & Cham., and *P. pseudostrobus* Lindl. Based on the most relevant existing information related to the identification and characterization of forest genetic resources it was defined areas for establishing conservation units in genetic zones, and the use of genetic resources by genetic zone.

This thesis has generated three original manuscripts for articles. The second of them is already published in a SCI journal, the other two are in preparation phase for publication.

- Flores, A.; Alía, R.; Robledo-Arnuncio, J.J. Relationship between regional environmental heterogeneity and phenotypic variation and plasticity within Mediterranean pine populations. (In preparation).

- Flores, A.; Climent, J.; Pando, V.; López-Upton, J.; Alía, R. Intraspecific variation in pines from the Trans-Mexican Volcanic Belt grown under two watering regimes: implications for management of genetic resources. Forests. 2018. 9(2), 71; doi: 10.3390/f9020071.

- Flores, A.; García, J.M.; López-Upton, J.; Rullán, C.; Olthoff, A.; Alía, R.; García del Barrio, J.M. Defining priority areas for conservation and use of forest genetic resources in four Mexican pines. (In preparation).

General introduction

1. Introduction to the pine species of study

Overall, the genus *Pinus* is with 113 species the largest genus of conifers. It has the widest distribution of all genera in the family Pinaceae (Figure 1).

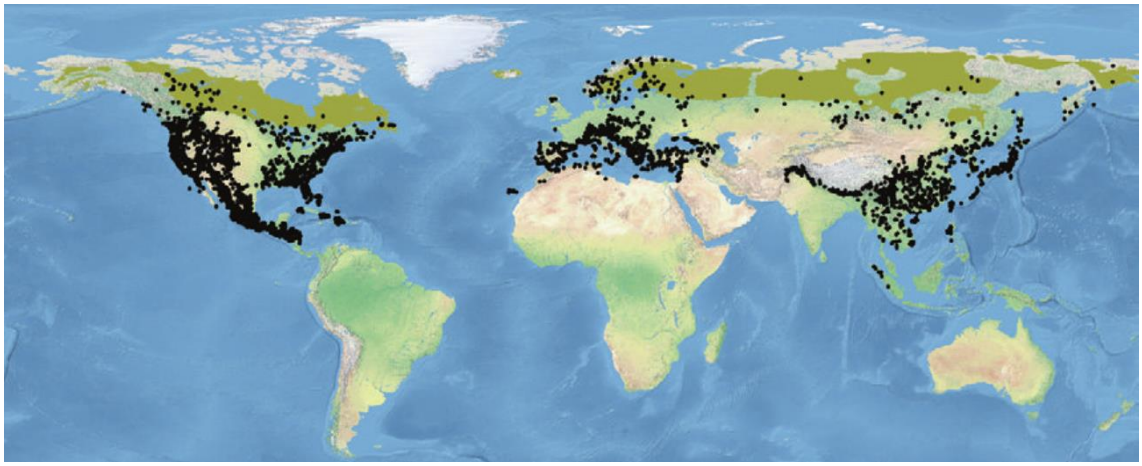

Figure 1. The global distribution of the genus *Pinus*, figure from Aljos Farjon & Filer (2013).

1.1. Pines of Europe and Mexico

For Europe and the Mediterranean, the genus *Pinus* has 13 species (including a species in the Canary Islands). Several of these have been subdivided into subspecies and/or varieties, and several of these have again been raised to species. The Balkans and the Mediterranean have the greatest of this limited diversity, in part reflecting the refugia for tree species that existed south of the Alps and Pyrenees during the Pleistocene glaciations (Farjon and Filer 2013).

The distribution of the genus *Pinus* is very similar to that of all conifer species in Mexico. This is not surprising as the taxonomic diversity of the genus reflects adaptations to virtually all types of habitat in which conifers occur in that country. At the precipitation scale, the genus *Pinus* is rare or absent in the wettest montane rainforests and in lowland tropical rainforest. A total of 46 species of *Pinus* is recognized (Perry 1991), and while this genus is comparatively well studied, new species are being described, often as minor segregates from already known species. A number of these are here recognized as subspecies and varieties, taking the total of taxa in *Pinus* in Mexico to 56 (Farjon 2010; Farjon and Filer 2013).

1.2. Species studied

The Mediterranean species studied during this work were *Pinus sylvestris* L., *P. pinaster* Aiton and *P. halepensis* Mill.; while Mexican species were *P. greggii* Engelm. ex Parl., *P. oocarpa* Schiede ex Schltdl., *P. patula* Schiede ex Schltdl. & Cham., and *P. pseudostrobus* Lindl. Species cover different geographical distribution and a wide range of environments differing in altitude, precipitation, temperature and soil. These species were chosen because of them play an

important role in ecological and economical activities in Spain and Mexico, and due to they are species for using and conservating in both countries.

2. Genetic variation in trees

The study of variation within individuals, among individuals, among populations, and among species is a constant subject to analyze for geneticists. In general, genetic variation is the basis for potential evolutionary change in a taxon and this provides the underpinning of modern biological thought (Falk and Holsinger 1991). For forest, the study of genetics is important precisely because of the unique biological nature of forest trees and also because of the social and economic importance of forests in the world (White et al. 2007).

Genetic variation is considered the most basic level of biological diversity and a prerequisite for the variability of species, populations, and ecosystems (Fox and Wolf 2006; Primack 2006). Diversity is also crucial for the fitness and survival of individuals, the viability of populations, and the ability of species to adapt to environmental change (Allendorf and Luikart 2007). Populations that lose genetic variation cannot evolve since evolution cannot proceed without genetic variation and populations that are unable to adapt to changing conditions will go extinct (Spielman et al. 2004). The presence of sufficient genetic variation in trees is crucial for the persistence of populations. The loss of genetic variation will lead to a lower adaptive ability in response to current and future changes, such as climate change, habitat loss, and new pathogens (Figure 2) (Frankham 2005). For pathogens, for example, variation in resistance to particular pathogen is presumed to be an important selective force for increased genetic variation (Bremermann 1980); the existence of a range of genotypes in a population may result in the survival of a few individuals after pathogen attack (Falk and Holsinger 1991).

When we understand the causes and consequences of phenotypic variation within and among populations, we can detect evolutionary processes operating at a variety of ecological levels: within random-mating populations; within and among subpopulations distributed over a species' geographic range; and even among multispecies associations. These goals, however, require a clear understanding of the nature of phenotypic variation (Fox et al. 2001).

It is impossible to study the impact of the environment on a trait if all organisms experience precisely the same environment, that is the environment does not vary at all from one individual to another. Likewise it is impossible to study the role of genes in producing a phenotype without any genetic variation that is if all individuals are genetically the same. Thus, variation is central, as the differences among individuals serve as markers that allow one to study the genetic and environmental factors responsible for specific traits (Fox and Wolf 2006).

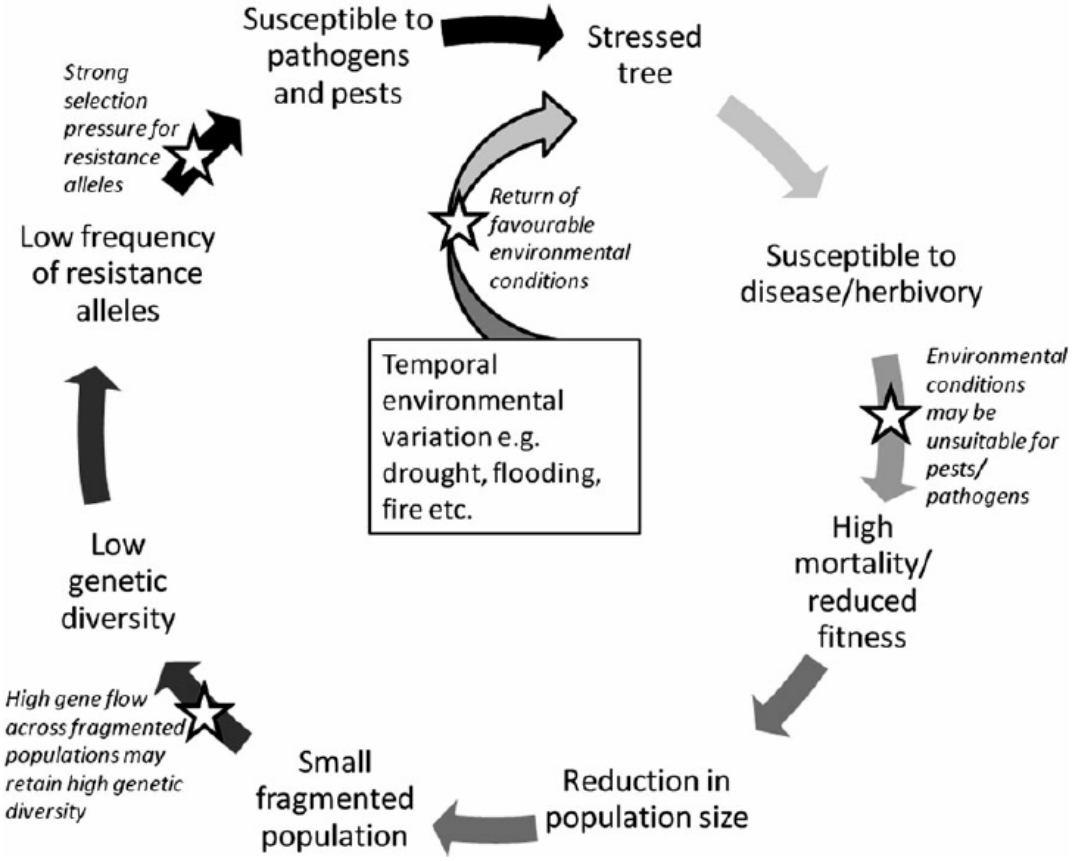

Figure 2. The effects of temporal environmental variation on the susceptibility of tree populations to pests and pathogens, figure from Telford, Cavers, Ennos, & Cottrell (2015).

There are two types of variations based on traits: Qualitative and Quantitative. Qualitative trait is the one which there are a fewer number of discrete phenotypes that can be distinguished by visual observation. A fewer number of genes determine such traits. Data can be put into a few discrete classes. Such traits are less affected by environment (Miglani 2010). Examples of qualitative traits include diseases, seed characteristics, and compositional traits. Because they are amenable to Mendelian analysis, the chi square statistical procedure may be used to determine the inheritance of qualitative genes (Acquaah 2012). Quantitative trait is one for which there is a range of phenotypes differing by degree. Data cannot be put into a few discrete classes. A large number of genes with small additive effect are generally involved in determination of such traits. Quantitative traits are conditioned by many to numerous genes (polygenic inheritance) with effects that are too small to be individually distinguished. They are sometimes called minor genes. Qualitative traits have been found to be more useful for understanding the mechanics of inheritance (Miglani 2010; Acquaah 2012).

Genetic variation can be detected at the molecular as well as the gross morphological level. The availability of biotechnological tools (e.g., DNA markers) allows assessing genetic diversity of their materials at the molecular level. Some genetic variation is manifested as visible variation in morphological traits (e.g., height, color, size), while compositional or chemical traits

(e.g., protein content, sugar content of a plant part) require various tests or devices for evaluating them (Acquaah 2012).

During the past 20 years, different molecular tecniques have been used in trees, such as simple sequence repeats or microsatellites (SSR), chloroplast microsatellites (cpSSR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP), and sequence variation in both mitochondrial DNA (mtDNA), and chloroplast DNA (cpDNA) regions (Wehenkel et al. 2017). Different genetic diversity parameters have been usually calculated, such as the expected heterozygosity (H_e), the percentage of polymorphic loci (*% P*), the Shannon diversity index (*S*), the total haplotype diversity (*H*), the nucleotide diversity (π), the observed degrees of Gregorius' total differentiation (δ_T), and mean genetic diversity ($v_{mean,2}$) among many others (Wehenkel et al. 2017).

Specific genes or sequences of DNA from seeds and needles collected in the field can be examined with electrophoretic surveys of proteins and with a variety of molecular techniques. Surveys of electrophoretically detectable genetic variation of proteins, or allozyme variation, have been used to measure the genetic variation and describe the geographic variation of many species of conifers (Hamrick et al, 1979; Hamrick and Godt, 1990; Loveless and Hamrick, 1984; Mitton, 1983). The DNA from the nucleus, from mitochondria, and from chloroplasts can be sequenced, or examined for restriction fragment length polymorphisms (RFLPs) (Wagner, 1992a,b), including examination of the variable number of tandem repeats (VNTRs), also popularly referred to as DNA fingerprints (Smith and Hinckley 1995).

3. The role of environmental heterogeneity

3.1. Genetic variation and environmental heterogeneity

A central question in evolutionary biology and population genetics is to understand how environmental heterogeneity influences the distribution of genetic variation among natural populations along different spatial scales remains (Manel et al. 2003). Within a plant species, environmental heterogeneity has the potential to influence the distribution of genetic variation among populations (Mitton 2000). Environmental heterogeneity can create genetic heterogeneity through several evolutionary processes (Linhart and Grant 1996). There is a selection balance theory which statement that the niche width variation hypothesis (Van Valen 1965) is generally supported by our genetic-ecological relationships. The hypothesis suggests that the amount of genetic variation may be regarded as an adaptive strategy for increasing population fitness in a spatiotemporally heterogeneous environments (Nevo et al. 1984).

Natural selection can cause populations to adapt to their local environment, resulting in fine-scale microgeographical variation; and genetic heterogeneity may be the result of differential gene exchange, influenced by variation in flowering phenology among local habitats (Gram and Sork 2001). Chance associations caused by genetic drift or founder effects could also create genetic heterogeneity, as founders colonize different sites but gene flow is not sufficient to homogenize differences (Husband and Barrett 1996). If gene flow is locally restricted because of limited pollen and seed dispersal of the species, then the genetic differentiation of populations will show a pattern of isolation-by-distance (Wright 1943), which is considered to be the main force in the establishment of neutral genetic structure in plant populations (Temunović et al. 2012). Theoretically, gene flow is expected to homogenize the distribution of genetic variation unless selection is quite strong (Slarkin 1985). Thus, we cannot assume that environmental heterogeneity will lead to genetic heterogeneity for all traits or for all plant species. Even when selection is strong enough within the range of gene flow to create population differentiation (Antonovics and Bradshaw 1970), other aspects of the genome may remain undifferentiated (Gram and Sork 2001).

Forest genetics studies have shown that environmental heterogeneity influences the genetic differentiation among tree populations, creating geographic genetic patterns that are consistent with phenotypic traits (Savolainen et al. 2007). One can look at this association to detect climate variables that are shaping the genetic structure of populations (Foll and Gaggiotti 2006) or even identify which genes are under pronounced natural selection (Jump et al. 2006; Joost et al. 2008). If we are trying to understand the potential impact of climate change, analyzing the genetic structure of populations overlaid on current and future climate gradients could help identify tree populations that are put at greatest risk by rapid climate change (Sork et al. 2010).

3.2. Phenotypic variation and environmental heterogeneity

Differences in environment cause phenotypic variation at a range of scales. On a small scale, phenotypic variation between neighboring trees in the same stand is caused by differences in microclimate, microsite, competition and exposure to insects and diseases. Large-scale environmental effects on phenotypic expression include differences in elevation, rainfall, temperature regimes and soils that cause tremendous differences in growth rates, form and morphology among populations of the same species growing in different locations (White et al. 2007).

The vast majority of directly observable traits of trees are an outcome of the interaction between the genetic constitution of a plant at a large number of gene loci (genotype, G) and the environmental conditions (E). Thus, it holds for the phenotype (P) that P=G×E, where ×

symbolizes the interaction between environmental and genotypic effects, which often differs from simple additivity of both components (Finkeldey and Hattemer 2007).

Phenotypic traits deserve particular interest if they are important for the adaptation of trees to their environment (adaptive traits) or for the value of a tree (economic traits). Adaptive traits and economic traits are controlled by both genetic and environmental factors for most tree species (Finkeldey and Hattemer 2007).

In line with the definition of genetic structures, the frequency distribution of particular phenotypes is defined as the phenotypic structure of a population. Apart from the direct impact of the environment on the phenotype (P=G×E), there are numerous impacts of the environment on genotypic structures (Figure 3). The environmental conditions influence mutation rates, are crucial for population sizes and, hence, the importance of genetic drift, cause changes of allelic structures owing to fertility selection and genotypic structures owing to viability selection, and have an impact on the mating system. Thus, manifold human alterations of environmental conditions do not only directly affect phenotypes of forest plants, but also change the genotypic structure of populations (Finkeldey and Hattemer 2007).

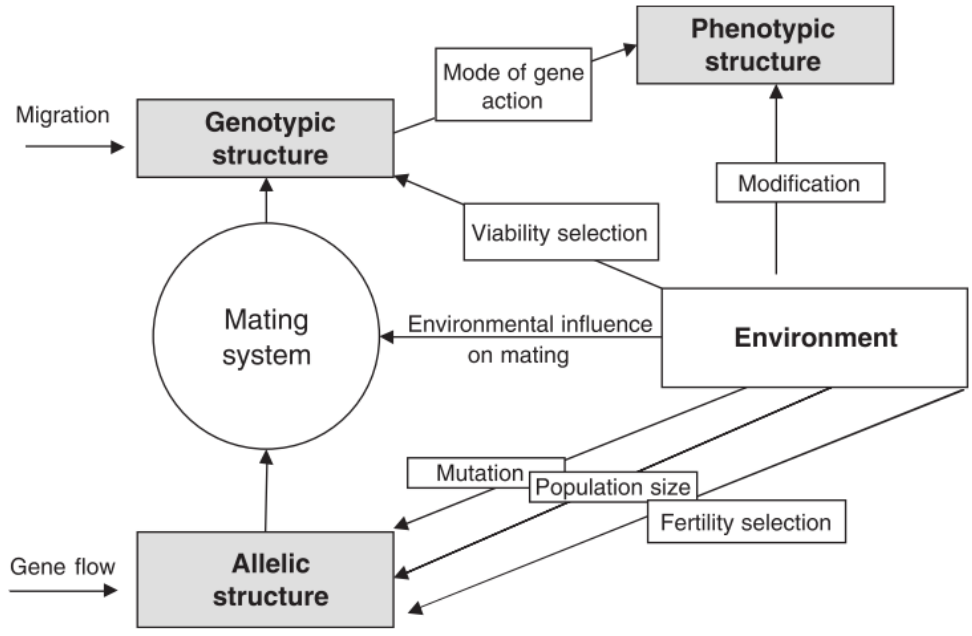

Figure 3. The environmental impact on phenotypic structures, figure from Finkeldey & Hattemer (2007)

3.3. Phenotypic plasticity and environmental heterogeneity

Plasticity allows a single genotype to succeed in a range of environments and may conceal the true extent of genetic differentiation (Falk and Holsinger 1991). When phenotypic responses to environment are functionally adaptive, plasticity allows individual genotypes to maintain fitness under diverse environmental conditions (Travis 2017). Although the definition of plasticity

can expand or contract to accommodate various traits, it is important to keep the definition narrow with respect to which aspects of trait variation we refer to as plasticity (DeWitt and Scheiner 2004). Despite numerous models have been developed to examine the conditions favoring the evolution of plasticity within a population (Scheiner 1993; Tufto 2000), the potential impact of plasticity on population differentiation has not been directly tested (Sultan and Spencer 2002).

Studies of genetic variation in plants are often confounded by phenotypic plasticity. A common definition of phenotypic plasticity is the environmentally sensitive production of alternative phenotypes by given genotypes (Stearns 1989). Phenotypic plasticity embraces genetics, development, ecology, and evolution and can include physics, physiology, and behavioral science (DeWitt and Scheiner 2004). Phenotypic response to environmental change may facilitate the exploitation of some environments and provide protection from others, the level of plasticity in a given trait is thought to be molded by selection (Bradshaw 1965). With recognition of the importance of phenotypic plasticity, in the equation $V_P = V_G + V_E$, the variance partition is expanded to $V_P = V_G + V_E + V_{G\times E} + V_{error}$ (Scheiner and Goodnight 1984), which includes explicit recognition of a systematic environmental effect (V_E) and, perhaps more important, a genotype–environment interaction ($V_{G\times E}$). This interaction specifies that the environment's effect is different for some genotypes relative to others. A number of methods exist to quantify phenotypic plasticity with the use of various indices such as the trait mean, the trait variation coefficient, the trait reaction norm, and the trait extreme values and phenotypic distances (Valladares et al. 2006).

4. Intraspecific variation in forest trees

Although the natural distribution of some forest trees are latitudinally restricted, considerable environmental variation could exist among most of the populations. If the expected variation in some seed traits among populations can be associated with the juvenile growth, it will be of great value for early selection or early identification of outstanding provenances or genotypes (Salazar 1986). Variation in seed quality occurs as a result of climatic conditions, crown position and crown orientation; this variation normally is reflected in early seedling variation (Kamra and Simak 1968).

The simplest method of studying variation in quantitative traits is to compare the growth and morphology of individuals of a species growing in different locations (Zobel and Talbert 1984). Most forest tree species are genetically very variable, and some insights into this variation can be obtained by measuring morphological characteristics such as leaf size and shape, surface texture of the bark, crown form, stem height, etc., in populations sampled throughout the geographic range of a species (Schaal et al. 1991).

The assessment of genetic variation in forest tree species has a long history in forestry (Langlet 1971). Most studies of quantitative genetic variation within and among natural populations of forest trees are based on family analysis (Fins et al. 1992). For this, it could be used seed, which are then sown into one or more nursery or greenhouse environments (i.e. seedling common gardens). Seedling common gardens have advantages in artificial environments (White et al. 2007), such are: a) many provenance locations can be studied since the studies are conducted on young seedlings; b) a large amount of data can be collected in a very short time on morphological, phenological and physiological traits that often effectively discriminates among provenances; c) the experiments are very powerful and are therefore very effective for demonstrating and modeling adaptive variation among provenances; and d) the experimental environments can be manipulated to assess adaptive variation among provenances in resistance to stresses such as frost, drought or disease. These advantages are appropriate for:

- Characterizing genetic patterns of natural geographic variation (clinal, ecotypic or both, e.g. Figure 4)
- Understanding the differential selective forces that have caused observed patterns of adaptive genetic variation (e.g. selection for earlier growth cessation in high elevations)
- Developing preliminary seed transfer guidelines within a region for later verification by long-term field provenance tests (Westfall, 1992)
- Narrowing down the number of promising provenances for a reforestation program to be subsequently tested in long- term field trials

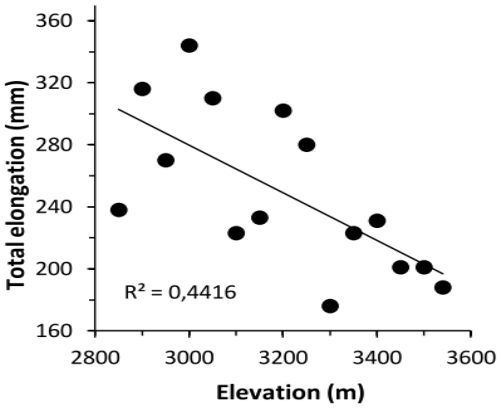

Figure 4. Regression of means per population in 15 populations of *A. religiosa* collected at Cerro de San Andrés, Michoacán, Mexico, against the provenance elevation (masl), for total elongation. Figure from Ortiz-Bibian et al., (2017).

Seedling common gardens may also be subsequently outplanted into one or more field sites for longer-term evaluation. Whether such studies end at the seedling stage or the trees are grown for many years in the field, forest geneticists refer to such experiments as provenance or

seed source studies (White et al. 2007). For each trait measured, the variation among all families in the experiment is partitioned by analysis of variance into variation due to differences among populations in the region and due to families within populations (Namkoong 1979). Patterns of variation among population means are used to examine whether populations are genetically associated with geography or environment (Hernández-Pérez et al. 2001). Variation among families within locations estimates only a portion of the total genetic variation within populations. This is because genetic variation among individuals within families and within individuals is not accounted for (White et al. 2007). The use of several common gardens in contrasting environments allows inferences of how genotypes respond to a diversity of environmental conditions. Although common garden studies reveal differences among genotypes and patterns of performance along environmental gradients, individual genes are not identified (Smith and Hinckley 1995).

A very common approach to both characterizing natural geographic variation and developing seed transfer guidelines is the use of short-term, common garden experiments planted in artificial (non-field) environments (Ying and Yanchuk 2006). The interest often centers on the extent to which differences in provenance means for individual traits, or clusters of traits, are associated with environmental differences across the landscape in order to describe adaptive patterns of variation (White et al. 2007).

5. Conservation of forests genetic resources (FGR)

The total forest area in 2010 was estimated to be 4 billion hectares (FAO 2014), or 31 percent of total land area, and twelve percent of the world's forests are designated for the conservation of biological diversity (Figure 5) (FAO 2010). The five most forest-rich countries (the Russian Federation, Brazil, Canada, the United States of America and China) account for more than half of the total forest area (53 percent), while 64 countries, with a combined population of 2 billion people, have forest on no more than 10 percent of their land area (FAO 2010). Nevertheless, according to the Global Forest Resources Assessment 2015, the global forest area fell by 129 million hectares (3.1 percent) in the period 1990–2015, to just under 4 billion hectares (FAO 2016). Forest loss can have both human and natural causes. The former is far more widespread than the latter, with deforestation occurring when people clear forests and use the land for other purposes, such as agriculture, infrastructure, human settlements and mining. Commercial agriculture accounted for almost 70 percent of the deforestation in Latin America in the period 2000–2010 (FAO 2016). In the Amazon, in particular, agribusiness production for international markets such as cattle ranching, soybean farming and oil-palm plantations has been identified as a main driver of post-1990 deforestation (Rudel et al. 2009).

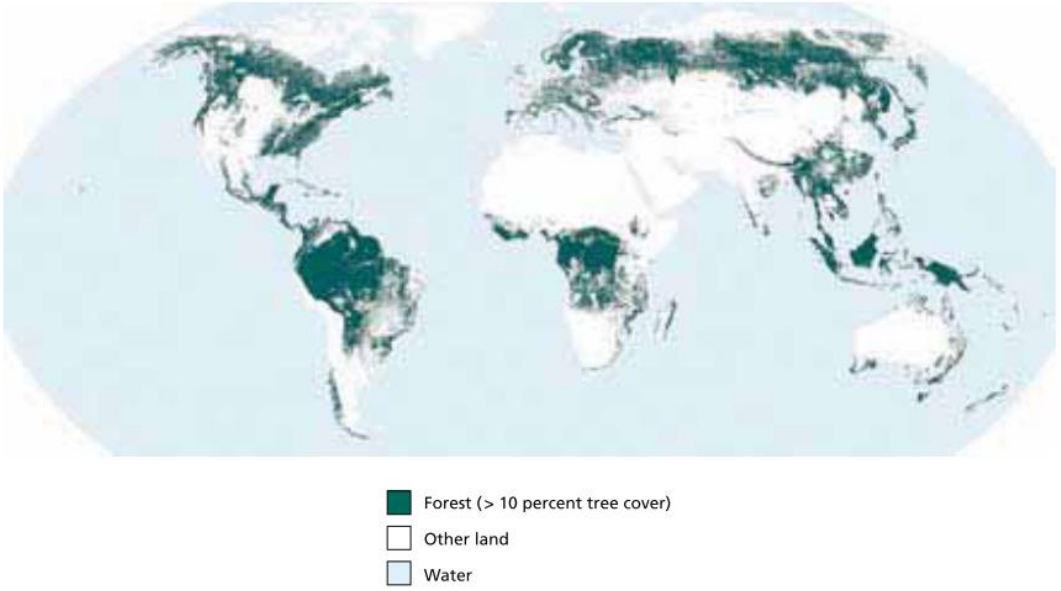

Figure 5. The World Forest, Figure from FAO (2010)

Forest trees are crucial for the maintenance of all biological diversity in terrestrial ecosystems, as well as for the production of fibre-fuel biomass, so the importance and justification of forestry research of all kinds, including genomics, will be a higher priority in the future (Neale and Kremer 2011). The enormous range of goods and services provided by trees and forests trees is both a function of and testimony to the genetic variability contained within them. Conserving FGR is therefore vital, as FGR constitute a unique and irreplaceable resource for the future, including for sustainable economic growth and progress and environmental adaption (Rajora and Mosseler 2001). Forest tree species are generally long lived and extremely diverse. One species can naturally occur in a broad range of ecological conditions. In addition, many forest species have evolved under several periods of major climatic change, and their genetic variability is needed for adaptation to climatic regimes different from those in which they have evolved. FGR have provided the potential for adaptation in the past, and will continue to play this vital role as humankind addresses the challenge of mitigating or adapting to further climate changes (FAO 2014).

In conserving FGR, it has been stablished primarily conserving genetic diversity within and among populations of a species. The priorities in conservation are normally based on the economic and environmental importance of the species, its ecological functions, the level of risk, or other special features that contribute to the importance of a species (Rajora and Mosseler 2001). Marginal populations may be of special interest to conservation because they have a higher probability of containing genetic resources that may be of special interest in terms of adaptation in stressful environments (Petit et al. 2005). Such populations may experience a unique

combination of selective forces, increased levels of inbreeding and genetic drift due to small population size and isolation that may foster the development/origin of interesting genotypes (Rajora and Mosseler 2001).

5.1. Conservation strategies

FGR can be conserved *in situ* and *ex situ* (Ledig 1986). *In situ* conservation represents a more evolutionarily dynamic approach compared with the more static *ex situ* conservation approach (Brush 2000). *In situ* conservation is commonly the preferred approach for maintaining the genetic diversity of forest trees. Genetic material of forest trees is also conserved *ex situ* in seed banks, seed orchards, clone collections, provenance trials, planted conservation stands and botanical gardens to complement *in situ* conservation efforts (Schueler et al. 2013). *In situ* conservation of forest trees has several advantages as compared to *ex situ* conservation (Rotach 2005), such as: a) *in situ* conservation is dynamic allowing temporal and spatial changes in genetic diversity while *ex situ* conservation is mostly static maintaining the once-sampled genetic diversity, b) trees within *in situ* conservation units remain exposed to evolutionary processes, as they continue interacting with their environment and competing with individuals of the same or other species c) it is easier and cheaper to conserve tree populations in their natural habitat than under *ex situ* conditions, and d) larger population sizes can be managed *in situ* than *ex situ* (Schueler et al. 2013).

The overall objective of an *in situ* conservation program is to ensure that the maximum possible range of genetic diversity is represented within the minimum number and size of reserves, established and run with a minimum of costs (Maxted et al. 2000). Since genetic conservation is a long term task for the benefit of future generations, reserve sites as well as site conditions should be sustainable for the foreseeable future. In order to minimize the need for interventions and thus running costs, populations selected as *in situ* reserves should possibly be growing under optimal habitat conditions, in sufficiently large, viable populations and in ecosystems with a maximum of intact natural processes and functions (Rotach 2005). For these objectives, the next information is required:

a) Population structure with its spatial and temporal dynamics
b) Eco-geographic distribution of the species and its genetic structure
c) Autochthony of populations, value and potential of the genetic resources
d) Habitat requirements and habitat breadth of the species, availability and quality of habitats
e) Life history traits - biological and ecological characteristics of the species

f) Relevant biotic and abiotic factors of the natural ecosystem, including interactions and natural processes, dynamics of relevant processes, their sensibility to human impact and their actual status
g) Threats to the species and its environment, causes and intensities, current conservation status
h) Socio-economic value, importance of resources from an international perspective
i) Existing protected areas, ownership; stakeholders, land use planning, legal and financial factors and other relevant information.

5.2. Utilization of forest genetic resources

FGR have been utilized extensively in systematic way only for about 100 years. The oldest form is the testing of tree species and their provenances for different uses and under different environmental conditions (Koskela et al. 2014). The main purpose of provenance research has been, and still is, the identification of well-growing and sufficiently-adapted tree populations to serve as seed sources for reforestation (König 2005). Due to the long timeframe to reach recommendations it has been challenging for many countries and research organizations to maintain trials, and to continue measuring them. Unfortunately, several important trials have been abandoned and some collected data lost (Geburek and Konrad 2008). Furthermore, there are old trial data sets sometimes dating back decades that have not yet been thoroughly analysed and published (FAO 2014). As provenance trials are costly to establish and maintain, new approaches, such as short-term common garden tests in nurseries and molecular analyses in laboratories, are increasingly used for testing provenances (FAO, 2014). However, while usefully complementary, these approaches cannot fully substitute for provenance trials, which are still needed for studying long-term growth performance, including the plastic and adaptive responses of tree populations to climate change (Alfaro et al. 2014).

In addition to maintaining old provenance trials, it is necessary to invest in establishing new ones. Often, existing trials have not been established in marginal sites that would be particularly useful for analyzing climate change related tree responses. Furthermore, many trials were established long before climate change became a research topic and the traits that were or are being measured may not be the most important ones in this context (Koskela et al. 2014; Alfaro et al. 2014).

The results of provenance research have been crucial for tree breeding programs, which mostly aim at gradual improvement of breeding populations rather than the development of new varieties. The International Tree Breeding and Conservation Program (Camcore) focused on

Mesoamerican pines, and it has had a major role in transferring tree germplasm for breeding purposes. From the 1980s, it undertook range-wide seed collections of 191 provenances of six Mesoamerican pines (*P. tecunumanii, P. oocarpa, P. caribaea, P. maximinoi, P. patula* and *P. greggii*) and it has established provenance or progeny trials at 823 locations in ten countries (Dvorak et al. 2018).

6. Objectives of the thesis

This thesis is designed to investigate the phenotypic variation among natural population of pines at local and regional scales, and define its implications in the management and conservation of genetic resources, with the following specific objectives:

- To assess the relationships between environmental heterogeneity and phenotypic variation and plasticity among natural populations of pine species (Chapter I).
- To evaluate the inter- and intraspecific genetic variation of natural populations of pine species drought tolerance (Chapter II).
- To propose measures for the conservation and sustainable use of forest genetic resources for natural populations of pine (Chapter III).

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Chapter I

Relationship between regional environmental heterogeneity and phenotypic variation and plasticity within Mediterranean pine populations

1. Abstract

Intrapopulation genetic variation of forest tree species is a major driver for *in situ* adaptation under climatic change conditions, but we need to determine at what extent the potential positive correlation between environmental heterogeneity and within-population genetic diversity may be modulated by the extent of phenotypic plasticity of the traits under consideration. We used multi-site tree provenance tests and a model selection approach to infer the relationship between within-population variance in fitness-related phenotypic traits (survival, height and diameter), phenotypic plasticity of these traits, and environmental (climatic) heterogeneity in the region surrounding provenances of three Iberian pine species: *Pinus sylvestris*, *P. pinaster* and *P. halepensis*. We show that climatic heterogeneity at different spatial scale can explain a significant part of the intrapopulation phenotypic variation for the different traits, but the relationships depend on the species and traits considered, reflecting that the role of genotype–environment interactions, population structure and stabilizing selection also plays an important role in determining the levels of intrapopulation genetic variation.

Keywords: Adaptation, genetic variation, Provenance tests, Mediterranean environment, *Pinus pinaster*, *Pinus sylvestris*, *Pinus halepensis*

2. Introduction

Understanding how the environment influences the interacting effects of natural selection, gene flow and genetic drift on genetic diversity is a major question in evolutionary ecology. Genetic differentiation among populations depends on population demographic structure, the scale and magnitude of environmental heterogeneity and the balance between divergent selection and interpopulation gene flow (Savolainen et al. 2007). Within populations, the level of genetic variation is determined by migration-mutation-drift balance and, in the case of selected traits, by local selection. Forest tree species typically exhibit high levels of intrapopulation genetic variation at both molecular markers and quantitative traits (Savolainen et al. 2004; Alberto et al. 2013). Why this is the case even for putatively strongly selected traits (Houle 1992), in which the variance-depleting action of stabilizing selection could be expected to prevail over mutation, remains an important open question (Yeaman et al. 2010). Theoretical explanations include pleiotropic selection (Gillespie 1984; Turelli and Barton 2004), genetic isolation by distance (Goldstein and Holsinger 1992), temporally fluctuating selection (Bürger and Gimelfarb 2002), genotype-environment interactions (Rose 1982; Gillespie and Turelli 1989), and single-trait empirical measures being unable to reveal actually low levels of multivariate genetic variation on the axes on which (multivariate) selection could be operating (Walsh and Blows 2009; Kirkpatrick 2009). An additional mechanism of within-population genetic variation maintenance is gene flow among populations subject to spatially varying selection across heterogeneous environments. Early theoretical models have shown that this mechanism can increase additive genetic variance within populations, provided that (i) the scale of gene flow is large relative to that of environmental variation, and that (ii) the amount of gene flow is not so high that it prevents local adaptation (Slatkin 1978; Barton 1999).

Many forest tree species could meet these two requirements because, especially wind-pollinated ones, they exhibit potential for long-distance gene dispersal across typically large and environmentally heterogeneous ranges (Kremer et al. 2012), while showing clear evidences of adaptive genetic differentiation among populations (Savolainen et al. 2007). Strong selective pressures acting on ample genetic variation during early life stages has been invoked as one of the factors potentially explaining local adaptation under substantial long-distance gene flow in forest trees (Petit and Hampe 2006). Indeed, maybe the only experimental study that has investigated the potential association between gene flow and within-population quantitative genetic variation in the wild has focused in a wind-pollinated temperate tree (Yeaman & Jarvis, 2006). The authors showed significant and substantial correlation between regional climatic heterogeneity and quantitative genetic variation within *Pinus contorta* populations, consistently with the hypothesis that gene flow and heterogeneous selection play an important role in

maintaining additive genetic variation. It remains unclear though to what extent these results are generalizable to other species because, among other factors, the scale and rates of both regional environmental variation and gene flow could differ (Yeaman and Jarvis 2006; Yeaman et al. 2010).

On the other hand, there is little empirical knowledge on how phenotypic plasticity may influence the interaction between among-population gene flow and within-population genetic variance. Long-range dispersal among populations distributed across heterogeneous environments, which can tend to increase genetic variance within populations, could also favor the evolution of adaptive phenotypic plasticity, analogously to the effect of temporal variation on selective regimes (Sultan and Spencer 2002). Adaptive phenotypic plasticity might in turn enhance effective gene flow among divergent populations, by allowing dispersers to persist in new environments (Crispo 2008), while it could either hamper adaptive genetic divergence (by enabling rapid phenotypic changes that dampen natural selection) or enhance genetic divergence (by buffering the demographic effects of maladaptation without preventing adaptive evolution) (Price et al. 2003; Crispo 2008; Chevin et al. 2012; Alberto et al. 2013). It is thus difficult to make general predictions about the potential associations between phenotypic plasticity, gene flow and genetic variance within natural populations. Environmental heterogeneity could be positively correlated with both phenotypic plasticity and within-population genetic variation if gene flow across selective environments is simultaneously increasing population genetic variance and selecting for phenotypically plastic individuals, without historical levels of adaptive phenotypic plasticity being strong enough to prevent adaptive genetic differentiation between populations in the region. By contrast, the levels of phenotypic plasticity and genetic variance within populations might be negatively correlated if higher values of the former have resulted in lower adaptive genetic differentiation between recipient populations and their respective sources of regional gene flow. Alternatively, there might be no apparent association between phenotypic plasticity and genetic variance, either because of insufficient statistical power or because their determinant factors are uncoupled (Scheiner and Goodnight 1984).

In this work we used multi-site tree provenance tests to infer the relationship between within-population variance in fitness-related phenotypic traits (survival, height and diameter), phenotypic plasticity of these traits, and environmental (climatic) heterogeneity in the region surrounding provenances of three Iberian pine species: *Pinus sylvestris*, *P. pinaster* and *P. halepensis*. Environmental heterogeneity is ubiquitous in natural systems, and especially in Mediterranean environments, where conspecific populations can display significant differences in their local habitat. we used several regional neighborhood sizes for measuring climatic heterogeneity, because the ability to detect the latter depends on the scale of measurement (Wiens

1989), and especially because we ignore the actual spatial scale of historical gene exchange among populations. Our main hypothesis is that under spatially variable environments, gene flow among locally adapted populations is a persistent source of genetic variation at the intrapopulation level, but the potential positive correlation between environmental heterogeneity and within-population genetic diversity may be modulated by the extent of phenotypic plasticity of the traits under consideration.

3. Materials and Methods

3.1. Plant material and experimental design

We used information from multi-site provenance tests with a different number of populations and sites by species: 22 populations planted in 6 sites for *Pinus sylvestris*, 54 populations planted in 6 sites for *P. pinaster*, and 56 populations planted in 5 sites for *P. halepensis* (See Figure 1). Populations cover the distribution range of each of the species, and seedlots from each population were obtained from at least 25 trees separated 50 meters to each other. The provenance tests were established using a random complete block design, each site was selected in a wide range of ecological conditions under different environments (See details of design, spacing, plot size and environmental conditions in Supp. Information, Table S1).

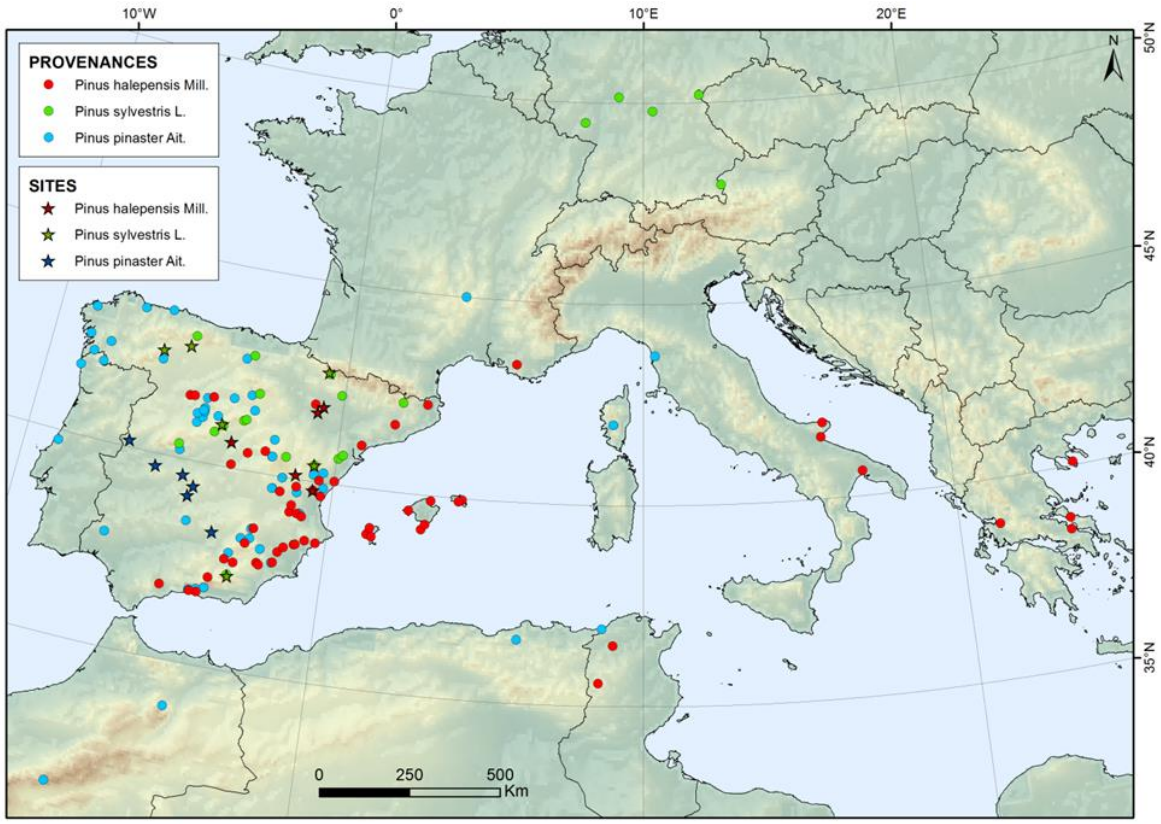

Figure 1. Populations and common garden experimental sites of *Pinus sylvestris*, *Pinus pinaster* and *Pinus halepensis* used in the study.

3.2. Within population phenotypic variation and plasticity

Three phenotypic traits were measured, at ages 13 or 18 depending on the species (Supplementary information Table S1): diameter at breast height (*d*, in mm), total height (*h*, in cm) and survival (*s*, presence/absence) that were obtained from the GENFORD database (data and details can be accessed in www.genfored.es). At these ages, intrapopulation competition still does not affect greatly the differences among populations (Alia et al. 2001; Vizcaíno-Palomar et al. 2016).

For each phenotypic trait and experimental site, a mixed model using a restricted maximum likelihood method was adjusted, with provenance as a fixed effect and blocks as a random effect. For survival a binomial linking function was used (Gilmour et al. 1985). The BLUE (Best linear unbiased predictor) was obtained from the model for each trait *x*, provenance *i*, and site *j* (Px_i^j).

For each provenance and trait, we calculated the intrapopulation phenotypic variance pooled across sites (V*d*, V*h*, V*s*). We also computed a plasticity index (PI*d*, PI*h* and PI*s*) following (Valladares et al. 2006) as:

$$\mathrm{PI}x_i = (Px_i^{max} - Px_i^{min})/Px_i^{max}$$

where, for the trait *x* (*d*, *h*, *s*), the plasticity index of population *i* is a function of the maximum and minimum value of Px_i^j across sites (1 to j).

3.3. Regional climatic heterogeneity

The environmental data correspond to a 1x1 km grid of climatic variables taken from Gonzalo's (2008) climatic model for the Iberian Peninsula, and from Worldclim model (Hijmans et al. 2005) for the provenances from other areas. Data were standardized and converted to z-scores to have the same scale to facilitate comparisons among variables. For each species, four climatic variables were chosen (Table 1), with the highest explained deviance scores (D^2) when individually fitted in a generalized linear model (McCullagh and Nelder 1989) of the distribution of each species (Serra-Varela et al. 2015). The regional environmental heterogeneity for each climatic variable and population was estimated at two different spatial scales (10 and 100 km^2), by computing the standard deviation of the target climatic variable across 1x1 km cells in a circular grid of the corresponding size centered at the target population. These scales were chosen to broadly represent short and long distance airborne regional gene flow. We also considered a 50 km^2 scale, but the results were consistently similar to those for 100 km^2 (see Supplementary

information, Table S2), and were excluded from further analysis. All the variables were checked for outliers by a descriptive statistical analysis and their distributions (Belsley et al. 1980).

Table 1. Climatic variables selected for the three species.

		Species		
Code	**Variable**	***P. sylvestris***	***P. pinaster***	***P. halepensis***
TS	Temperature Seasonality	+	+	+
TJ	Min Temperature of Coldest Month	+	-	-
TW	Mean Temperature of Coldest Quarter	-	+	+
PS	Precipitation of Wettest Quarter	+	-	-
PA	Annual Precipitation	-	+	+
PW	Precipitation of Warmest Quarter	+	+	+

3.4. Relationships between phenotypic and regional climatic heterogeneity variables

We used a model selection approach (Johnson and Omland 2004) as a way to draw inferences from a set of multiple competing models simultaneously. We obtained a set of 15 competing linear models, when including from one to four regional climatic heterogeneity variables, being the full model:

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \beta_4 x_{4i} + \varepsilon_i$$

where, y_i is the value of the phenotypic variable considered for the provenance i, β_o is the constant term, β_j is the regression coefficient for the regional climatic heterogeneity variable x_{ji} (with j varying from 1 to 4), and ε_i is the error term.

All the 15 models were sorted by their Akaike Information Criterion (AIC), and the differences between the AIC of each model minus the lowest AIC across models was computed ($D_i = AIC_i - AIC_{min}$). Akaike weights were computed as the model likelihood values normalized across all R= 15 models, so that they sum to 1 (Burnham and Anderson 2002), which provide a relative weight of evidence for each model (probability that model i is the best model for the observed data, given the candidate set of models):

$$w_i = \frac{exp\left(-\frac{D_i}{2}\right)}{\sum_{r=1}^{R} exp\left(-\frac{D_r}{2}\right)}$$

We compute for each phenotypic variable (intrapopulation phenotypic variance and plasticity index), species and regional scale (10 or 100 km^2) a weighted average of the coefficient of determination (R^2) with the regional climatic heterogeneity, and their respective relative importance (RI), their regression coefficients (β_i) and the variance of these estimates to check for the importance of the relationships among phenotypic variation and regional environmental heterogeneity.

The relative importance (RI) of a predictor variable was calculated as the sum of the Akaike weights over all of the models in which the parameter of interest appears (Burnham and Anderson 2002).

The weighted average of coefficient of determination (R^2), the regression coefficients (β) across all models, and the variance of these estimates, were calculated respectively as:

$$R^2 = \sum w_i R_i^2$$

$$\beta = \sum w_i \beta_i$$

$$var(\beta) = \left[\sum_{i=1}^{R} w_i \sqrt{var(\beta_i|g_i) + (\beta_i - \beta)^2}\right]^2$$

where, var($\beta_i|g_i$) denotes the conditional sampling variance of the parameter given model g_i. The variance estimator assesses the precision of the estimate over the set of models considered.

The model estimation and selection were performed using the SAS software (SAS Institute 2004) and the regional climatic heterogeneity was computed using the ArcGIS 10.3 Desktop Software (Esri 2014).

4. Results

Climatic heterogeneity was able to explain a significant portion of the total intrapopulation phenotypic variation at different spatial scales, as the weighted R^2 of the different averaging models range from 0.006 to 0.560 (Table 2). The values are higher in Scots pine (0.120 to 0.560) than in Maritime pine (0.008 to 0.304) and Aleppo pine (0.006 to 0.203). The phenotypic variance of survival, in Aleppo and Maritime pine, present 1-fold higher R^2 than plasticity index, being the only trait with a clear differences when comparing intrapopopulation variance and plasticity.

There are slight differences among scales, with the 100 km^2 scale is presented higher R^2 values in most of the traits. Six traits (V*d*, V*s* in Scots pine, V*d*, PI*h* and V*s* in maritime pine, PI*d* in Aleppo pine) present a higher value in the 100 km^2 scale, and three traits (V*h* and PI*h* in Scots pine and V*s* in Aleppo pine) present a higher R^2 in the 10 km^2 scale.

However, some of the variables present models with a poor goodness of fit. The variables and scales for the different species with a clear support of the data are V*d*, V*s*, PI*d* and PI*h* in Scots pine and V*d*, V*h*, V*s*, PI*d*, PI*h* in Maritime pine (all at the 100 km^2 scale, but V*s* also at the

10 km^2 in Maritime pine), and V*s*, PI*h* and PI*d* in Aleppo pine (the first two at the 10 km^2 scale and the last one at the 100 km^2 scale).

When comparing the intrapopulation genetic variability and phenotypic plasticity for the different traits, for diameter and height in Scots and Maritime pine plasticity present models with higher explained variance than intrapopulation variation. In Aleppo pine the values are similar for both traits. In survival, in all the species intrapopulation variation present models with higher variance explained than plasticity.

4.1. Relationship among intrapopulation phenotypic variance and environmental heterogeneity

When analyzing the models, only few variables present a high relative importance in the models (value higher than 0.70), and in most of the models there are no a single variable with a clear support of the data. For Scots pine none of the intrapopulation phenotypic variance present variables with high relative importance, but all the phenotypic plasticity have some regional climatic heterogeneity variable with a high relative importance. In Maritime pine, there are opposite results (intrapopulation phenotypic variance present different significant regional climatic variables). The case of Aleppo pine is intermediate, as it is vary depending on the trait (Table 2).

There are different patterns depending of the species and traits. PS environmental heterogeneity in Scots pine present a clear negative relationship with the variables PI*d* and PI*h*, and PW environmental heterogeneity a clear positive relationship with the variable PI*h* (Table 2). However, in *Pinus pinaster*, TS and PA environmental heterogeneity present a clear negative relationship with the variables V*s* and V*d* respectively, whereas TW and PW environmental heterogeneity present a clear positive relationship with the variables V*s* and V*d*, and with V*h* and V*s* respectively. In *Pinus halepensis*, TW environmental heterogeneity presents a clear positive relationship with the variables V*s*, PI*d* an PI*h*.

Table 2. Weighted average of the parameters across all models, including the coefficient of determination (R^2), the regression coefficients (β) and standard deviation (*sd*), and relative importance of each regional climatic heterogeneity variable (RI).

Phenotypic variable	Scale (km²)	R^2	TS		TJ[1]/TW[2]		PS[1]/PA[2]		PW	
			β (*sd*)	RI	β (*sd*)	RI	β (*sd*)	RI	β (*sd*)	RI
P. sylvestris										
V*d*	10	0.136	1.42E+01 (1.19E+01)	0.616	-8.00E-01 (6.95E+00)	0.346	1.10E+01 (1.12E+01)	0.525	-1.40E+01 (1.41E+01)	0.542
	100	0.275	4.95E+00 (7.39E+00)	0.379	4.57E+00 (7.77E+00)	0.381	-2.70E+00 (6.79E+00)	0.374	-2.54E+01 (1.30E+01)	0.412
V*h*	10	0.189	7.27E+01 (8.05E+01)	0.470	1.21E+02 (1.05E+02)	0.583	-1.82E+01 (5.14E+01)	0.331	5.84E+00 (6.17E+01)	0.330
	100	0.120	7.49E+01 (8.60E+01)	0.490	5.78E+01 (8.00E+01)	0.451	7.30E+00 (5.05E+01)	0.328	1.31E+01 (5.34E+01)	0.334
V*s*	10	0.249	-2.54E-03 (2.02E-03)	0.596	-2.43E-03 (2.15E-03)	0.552	1.81E-03 (1.80E-03)	0.480	-2.39E-04 (1.36E-03)	0.332
	100	0.444	-2.96E-03 (2.17E-03)	0.604	-2.97E-03 (2.15E-03)	0.610	-1.34E-04 (7.69E-04)	0.154	-5.79E-05 (7.79E-04)	0.149
PI*d*	10	0.185	4.93E-03 (7.52E-03)	0.394	6.48E-03 (9.11E-03)	0.419	-2.60E-02 (1.61E-02)	0.782	1.12E-02 (1.31E-02)	0.496
	100	0.222	2.71E-03 (7.31E-03)	0.352	3.79E-03 (7.79E-03)	0.372	-1.98E-02 (1.49E-02)	0.660	2.49E-02 (1.63E-02)	0.733
PI*h*	10	0.560	1.93E-02 (1.20E-02)	0.721	-6.74E-04 (5.54E-03)	0.295	-5.32E-02 (1.70E-02)	0.980	3.92E-02 (1.80E-02)	0.871
	100	0.441	1.47E-02 (1.22E-02)	0.571	-4.93E-04 (1.01E-02)	0.384	-1.37E-02 (1.25E-02)	0.551	3.29E-02 (1.60E-02)	0.859
PI*s*	10	0.131	2.73E-03 (1.70E-02)	0.332	-3.29E-02 (2.82E-02)	0.588	-6.51E-03 (1.65E-02)	0.356	-6.29E-03 (1.86E-02)	0.360
	100	0.201	2.10E-02 (2.48E-02)	0.452	-7.02E-04 (2.16E-02)	0.365	5.09E-02 (3.83E-02)	0.666	-5.83E-02 (4.05E-02)	0.707
P. pinaster										
V*d*	10	0.067	4.76E+01 (5.45E+01)	0.490	5.86E+01 (5.62E+01)	0.540	-5.01E+01 (5.72E+01)	0.491	-4.34E+01 (3.93E+01)	0.538
	100	0.304	-3.47E+01 (4.69E+01)	0.462	1.16E+02 (5.83E+01)	0.865	-9.37E+01 (4.27E+01)	0.841	-4.28E+01 (3.28E+01)	0.582
V*h*	10	0.074	-3.89E+02 (4.65E+02)	0.461	-2.41E+02 (3.97E+02)	0.408	2.06E+01 (3.77E+02)	0.361	9.05E+02 (5.34E+02)	0.780
	100	0.077	7.23E+02 (4.58E+02)	0.719	2.17E+01 (2.99E+02)	0.378	-6.69E+01 (1.83E+02)	0.327	6.22E+01 (1.82E+02)	0.326
V*s*	10	0.094	5.92E-04 (3.93E-03)	0.323	2.06E-04 (3.68E-03)	0.312	-4.36E-03 (5.80E-03)	0.429	1.33E-02 (7.08E-03)	0.828
	100	0.195	-1.81E-02 (1.01E-02)	0.788	2.41E-02 (1.08E-02)	0.929	4.57E-03 (4.56E-03)	0.492	5.57E-03 (4.45E-03)	0.564
PI*d*	10	0.040	2.33E-03 (1.49E-02)	0.345	-5.18E-03 (1.49E-02)	0.352	-1.28E-04 (1.51E-02)	0.358	2.59E-02 (2.01E-02)	0.635
	100	0.101	2.59E-02 (2.09E-02)	0.588	2.19E-02 (1.90E-02)	0.558	-3.09E-03 (8.40E-03)	0.319	-8.80E-03 (1.13E-02)	0.391
PI*h*	10	0.032	-1.35E-03 (1.31E-02)	0.309	1.48E-03 (1.25E-02)	0.344	-2.90E-03 (1.40E-02)	0.367	2.13E-02 (1.76E-02)	0.607

Phenotypic variable	Scale (km^2)	R^2	TS		TJ^1/TW^2		PS^1/PA^2		PW	
			β (*sd*)	RI	β (*sd*)	RI	β (*sd*)	RI	β (*sd*)	RI
PI*s*	100	0.164	1.95E-02 (1.85E-02)	0.498	3.60E-02 (2.04E-02)	0.722	1.10E-03 (7.06E-03)	0.268	-9.37E-03 (1.05E-02)	0.410
	10	0.008	-1.31E-02 (2.37E-02)	0.414	-3.20E-03 (2.10E-02)	0.382	2.49E-03 (2.03E-02)	0.378	1.05E-02 (1.76E-02)	0.421
	100	0.014	1.03E-02 (1.76E-02)	0.423	8.29E-03 (1.60E-02)	0.410	2.09E-03 (1.18E-02)	0.338	-1.12E-02 (1.61E-02)	0.437
P. halepensis										
V*d*	10	0.006	-2.10E+00 (7.20E+00)	0.379	2.38E+00 (7.89E+00)	0.381	-1.08E+00 (7.67E+00)	0.374	-3.85E+00 (7.30E+00)	0.412
	100	0.049	8.54E+00 (1.01E+01)	0.471	1.22E+01 (1.20E+01)	0.527	-3.77E+00 (6.68E+00)	0.370	-2.18E+00 (6.30E+00)	0.343
V*h*	10	0.050	1.94E+01 (4.88E+01)	0.363	9.07E+01 (8.11E+01)	0.561	2.20E+01 (5.94E+01)	0.381	-2.89E+01 (4.57E+01)	0.382
	100	0.095	5.87E+01 (6.46E+01)	0.463	6.44E+01 (7.59E+01)	0.461	4.63E+01 (5.38E+01)	0.429	-1.38E+02 (8.85E+01)	0.713
V*s*	10	0.143	-3.28E-04 (2.10E-03)	0.313	1.15E-02 (5.44E-03)	0.869	-1.09E-03 (3.12E-03)	0.360	4.31E-05 (1.44E-03)	0.218
	100	0.033	-1.12E-03 (2.80E-03)	0.391	4.85E-03 (4.33E-03)	0.581	-8.67E-04 (1.99E-03)	0.366	-1.96E-03 (2.70E-03)	0.438
PI*d*	10	0.022	5.09E-04 (1.70E-03)	0.271	2.71E-03 (2.87E-03)	0.516	-1.17E-03 (2.39E-03)	0.367	2.61E-04 (1.40E-03)	0.222
	100	0.203	-5.80E-03 (3.97E-03)	0.675	1.40E-02 (6.17E-03)	0.946	7.30E-04 (1.34E-03)	0.299	-5.86E-03 (3.40E-03)	0.748
PI*h*	10	0.104	-4.95E-04 (2.64E-03)	0.308	3.70E-03 (4.18E-03)	0.431	1.50E-03 (3.50E-03)	0.350	8.93E-03 (5.53E-03)	0.716
	100	0.125	-7.21E-03 (6.48E-03)	0.562	1.83E-02 (9.46E-03)	0.879	5.68E-04 (2.19E-03)	0.236	-2.92E-03 (3.77E-03)	0.402
PI*s*	10	0.008	-4.97E-03 (9.88E-03)	0.406	-3.71E-03 (1.06E-02)	0.389	6.52E-03 (1.16E-02)	0.417	-6.82E-04 (7.72E-03)	0.316
	100	0.038	-1.80E-03 (1.03E-02)	0.371	2.04E-02 (1.70E-02)	0.608	3.17E-03 (7.66E-03)	0.356	-3.99E-03 (8.81E-03)	0.293

[1] *P. sylvestris*; [2] *P. pinaster* and [3] *P. halepensis*

5. Discussion

This work analyze the relationship between within-population variance in fitness-related phenotypic traits (survival, height and diameter), their phenotypic plasticity, and environmental (climatic) heterogeneity in the region surrounding provenances of three Iberian pine species. In most of the cases, we can find a clear relationship among phenotypic variance and phenotypic plasticity and environmental heterogeneity, but only few cases have a clear relationship with some of the environmental variables. The coefficient of determination in the models indicate that there spatial heterogeneity explain a significant part of the intrapopulation genetic variation and phenotypic plasticity of the populations, but depending on the trait under consideration and the species. We need to take into consideration that AIC is a relative measure of how good a model is among a candidate set of models given the data, and it is particularly prone to poor choices of model formulation, and therefore, only variables with support of the data should be analysed (Symonds and Moussalli 2011). These results demonstrate that gene flow is not the only driver in shaping the genetic variation under heterogeneous environments and that, according to the theory, adaptive phenotypic plasticity evolves under spatial variation in natural selection.

Overall, phenotypic variability is better explained at large scale of environmental heterogeneity (100 km^2) than a medium one (10 km^2), as effects of spatial variability is more important in large neighborhoods (Moudrý and Šímová 2012). Mostly, variability at small scales would not be able to detect the large variability found at these scales in Mediterranean species (Linhart and Grant 1996). Moreover, in the Mediterranean there is a general trend of increasing heterogeneity with spatial scale, but the extent of variation depend on the environmental variable (Quilchano et al. 2008). The differences among scales of the different environmental variables will play a major role in different processes as regeneration or competition that influence the adaptability of the species at the long term. Gene flow in these landscapes are mediated by the different patches composing the landscape, and also the extent of long-distance gene flow. Therefore the complexity of these interactions at the species and trait level, determine differences in adaptability of populations of the species from close regions (Rodriguez-Quilon et al. 2016) or even at the intrapopulation level (Alía et al. 2014a).

According to our results, the lack of any general and consistent pattern among species and traits for the relationship among specific climatic variables and intrapopulation genetic variation and plasticity suggest that a simple connection among the two factors is unlikely. The relative importance of other putative mechanisms, related to the history of the species, and the ecological context (species and trait under consideration) must be taken into account. Precipitation (PS, PW) has more influence

on the variability in *P. sylvestris* specially with Plasticity indexes. The negative relationship with PI*d* and PI*h* indicates that under environments with heterogeneous dry conditions, scots pine population present a reduce plasticity, as a avoidance mechanism for drought. It has been reported that populations from dry conditions has a lower level of phenotypic plasticity in growth for scots pine (Alía et al. 2001). In *P. pinaster* and *P. halepensis* both temperature and precipitation have a significant effect on intrapopulation genetic variation. The importance of these climatic effects are in accordance with the influence on allometry in these species (Vizcaíno-Palomar et al.2016), in determining the climate-growth relationships (Olivar et al. 2015), and the adaptation to future conditions (Benito Garzón et al. 2011).

A clear relationship among intrapopulation genetic diversity and environmental heterogeneity has been reported in some specific cases (Yeaman and Jarvis 2006), but it is not general (Yeaman et al. 2010). According to our results, under spatially variable environments, gene flow among locally adapted populations is a persistent source of genetic variation at the intrapopulation level, but the correlation between environmental heterogeneity and within-population genetic diversity is modulated by the extent of phenotypic plasticity of the trait under consideration, and also by other putative factors as the history of the species or the ecological context of the trait under consideration. The species, and the three traits under consideration present different level of quantitative vs neutral differentiation in the species (Gonzalez-Martinez et al. 2001, Alia et al. 2001, Alia and Bastien, 2000).

6. Conclusion

We show that climatic heterogeneity at different spatial scale can explain a significant part of the intrapopulation phenotypic variation in different traits, but the relationships depend on the species and traits considered, reflecting the role mutation– selection balance, genotype–environment interactions, population structure and stabilizing selection, temporally fluctuating selection pressures and pleiotropic overdominance in determining the levels of intrapopulation genetic variation.

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Chapter II

Intraspecific variation in pines from the Trans-Mexican Volcanic Belt grown under two watering regimes: implications for management of genetic resources

1. Abstract

Management of forest genetic resources requires experimental data related to the genetic variation of the species and populations under different climatic conditions. Foresters also demand to know how the main selective drivers will influence the adaptability of the genetic resources. To assess the inter- and intraspecific variation and plasticity in seedling drought tolerance at a relevant genetic resource management scale, we tested the changes in growth and biomass allocation of seedlings of *Pinus oocarpa, P. patula* and *P. pseudostrobus* under two contrasting watering regimes. We found general significant intraspecific variation and intraspecific differences in plasticity, since both population and watering x population interaction were significant for all three species. All the species and populations share a common general avoidance mechanism (allometric adjustment of shoot/root biomass). However, the intraspecific variation and differences in phenotypic plasticity among populations modify the adaptation strategies of the species to drought. Some of the differences are related to the climatic conditions of the location of origin. We confirmed that even at reduced geographical scales, Mexican pines present differences in the response to water stress. The differences among species and populations are relevant in afforestation programs as well as in genetic conservation activities.

Keywords: drought stress; genetic variation; early testing; adaptive variation; genecology; phenotypic plasticity.

2. Introduction

In the last decades, there has been an increasing concern about the consequences of climate change on the future distribution and productivity of forest species. Many forest areas have experienced a decrease in rainfall and a subsequent increase in drought severity. In particular, Mexico will experience, on average, an increase of 1.5 ºC in mean annual temperature, and a decrease of 6.7% in annual precipitation by 2030 (Sáenz-Romero et al. 2010). This is already posing practical problems in the management of many forest tree species, derived from the shifts in species distribution (Thuiller et al. 2005), and the future requirements in terms of adaptation and productivity.

We are far from having enough experimental data to address important aspects related to the adaptability, i.e., the potential or ability of a population to adapt to changes in environmental conditions through changes in its genetic structure (Hubert and Cottrell 2007). For example we lack information about the roles of genetic variation, phenotypic plasticity (Chevin et al. 2010), and of phenotype changes derived from the trade-offs among life-history traits, among others (Santos-del-Blanco et al. 2015). This information is essential at scales that are meaningful for forest management (i.e., at forest or forest-landscape scales), as it is necessary to make decisions when selecting the species and the basic material to use in afforestation and restoration programs (e.g., local vs non local), or to suggest changes in silvicultural systems (e.g., regeneration methods, selection of parent trees) to increase forest resilience. Therefore, the evaluation of local genetic resources at fine scales is essential for the management of local genetic resources, complementing information at larger scales.

Low water availability has been identified, particularly in conifers, as one of the major abiotic stressors, conducive to stomatal closure, reduced photosynthesis and death due to carbon starvation (Pallardy 2008). Tolerance to low water availability is an important selective factor, involving quite different traits, such as rooting depth, transpiration area of leaves and shoots, and size and number of shoots (Stebbins 1950). There are, therefore, important adaptive differences in the response at different levels, from species to individuals (Barton and Teeri 1993; Valladares and Sánchez-Gómez 2006).

Intra-specific genetic variation is crucial in forest trees species, which must endure both abiotic and biotic stressors for long periods of time (Holt 1990). Particularly, it is necessary to develop management options for the genetic resources of target species, and to determine if genotypes would be able to grow efficiently under future stressful conditions. However, testing drought-tolerant genotypes amongst mature trees growing in the field is cumbersome, due to the previously mentioned

complexity of plant responses to drought and the lack of control of watering treatments (Jones 2007). An alternative approach is to develop controlled experimental conditions to test genotypes at early stages (López et al. 2009). Early developmental stages in plants are the most critical in the survival of forest trees, and are related to the future adaptability of the species (Alía et al. 2014a) depending on the genetic intraspecific variation in these genetic traits. Evaluating morphological and physiological changes in response to low water availability at early ages is a recognized way to know their adaptive responses (i.e., leaf water potential and gas exchange (Wright et al. 1992) and changes in growth and survival (Engelbrecht and Kursar 2003)). Inter- and intraspecific variation among populations of different pines species, when cultivated under contrasting water availability, reveal high population divergence for phenotypic changes and marked allocational shifts, a plastic response (Chambel et al. 2007). Moreover, different works have addressed some of the features involved in the growth process that can skew the results of early testing in plants, e.g., pot size, water quality and salinity (Levy and Syvertsen 2004; Poorter et al. 2012a).

Pinus is the largest genus of the *Pinaceae* family, with 114 species widely distributed in the Northern Hemisphere (Farjon and Filer 2013). Mexico presents the highest specific diversity (46 species), with contrasting geographical and intraspecific genetic patterns, as a result of adaptive responses to climate changes in the past (Perry 1991). Among them, *Pinus oocarpa* Schiede ex Schltdl., *P. patula* Schiede & Schltdl. & Cham. and *P. pseudostrobus* Lindl. are three economically important Mexican pines, used in highly productive forest plantations established in the tropics and subtropics (Cambrón-Sandoval et al. 2012). These pines occupy diverse habitats in the country, and present a variety of ecological roles and life histories. Specifically, the Trans-Mexican Volcanic Belt (TMVB) covers a wide range of environments differing in altitude, precipitation, temperature and soil. Thus, the Volcanic Belt constitutes a good model area to check for intraspecific differences in growth and performance to drought stress in Mexican pines, as a way to improve some recommendations for the management of genetic resources under climate change scenarios.

The objective of this study was to assess the inter- and intraspecific genetic variation in seedling drought tolerance in *Pinus oocarpa*, *P. patula* and *P. pseudostrobus* from the TMVB. We tested the seedlings under two contrasted controlled watering regimes and we measured different adaptive morphological and allocation traits. Our hypotheses were that at a fine geographical scale: i) both seedling growth and biomass are affected by low water availability, a potential adaptive response and ii) these responses differ due to species intraspecific variation.

This information is essential to implement breeding and conservation programs under climate change scenarios.

3. Materials and Methods

3.1. Plant material

The natural distribution of the study species covers small and large areas throughout the north, center and south of Mexico. *Pinus oocarpa* (OC) and *P. pseudostrobus* (PS) are usually found in fragmented mixed stands, while *P. patula* (PA) occurs in pure stands. We sampled populations of the three species from the TMVB. The number of sampling sites (populations) was different for each species (Figure 1 and Table 1): *P. oocarpa*, two populations (OC01, OC02), *P. patula*, 10 populations (PA01, PA02, PA03, PA04, PA06, PA07, PA08, PA09, PA11, PA12), and *P. pseudostrobus*, five populations (PS01 to PS05). Seedlots were either samples provided by academic institutions (15 out of 17 samples) or commercial seedlots provided by a private seed supplier (two out of 17), and were composed of seeds from at least 20 mother trees per population. The sampling for *P. oocarpa* was limited to areas where the taxonomic identification of the species was clear, to avoid biases in the comparisons. This is particularly important in the eastern area of the study, where three new species have been recently described but assigned to *P. oocarpa* by the National Forest Inventory.

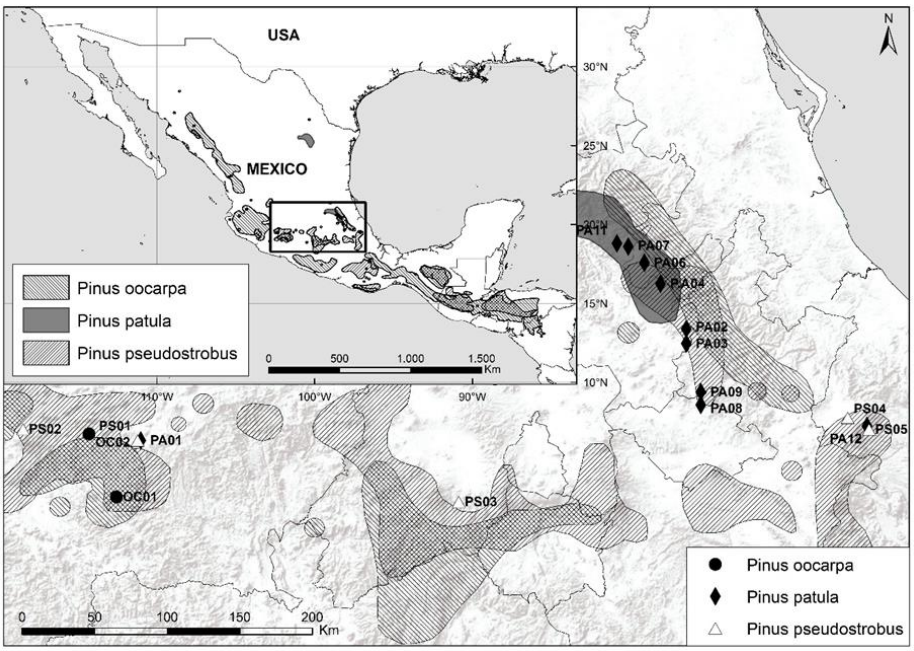

Figure 1. Location of sampled populations and distribution range of the species (Farjon and Filer 2013) for *Pinus oocarpa*, *P. patula* and *P. pseudostrobus*.

Table 1. Location and general characteristics of the *Pinus* spp. populations sampled in Mexico.

Code[1]	Population, State	Supplier[2]	Latitude and longitude	Altitude (m)	MAT[3] (ºC)	MAP[4] (mm)
OC01	Ario de Rosales, Mich.	INIFAP	19° 04' / 101° 44'	1490	20.7	1112
OC02	San Ángel Zurumucapio, Mich.	INIFAP	19° 27' / 101° 54'	1700	17.0	1299
	Range[4]				13.8 to 21.3	891 to 1422
PA01	Casas Blancas, Mich.	Colpos	19° 25' / 101° 35'	2258	15.7	1060
PA02	Acaxochitlán, Hgo.	Colpos	20° 06' / 98° 12'	2190	13.8	962
PA03	Ahuazotepec, Pue.	Colpos	20° 01' / 98° 12'	2250	13.8	847
PA04	Apulco, Hgo.	Colpos	20° 23' / 98° 22'	2200	15.2	909
PA06	Huayacocotla, Ver.	Colpos	20° 31' / 98° 28'	2050	16.1	1099
PA07	Tlahuelompa, Hgo.	Colpos	20° 37' / 98° 34'	2020	16.2	1234
PA08	Tlaxco, Tlax.	Colpos	19° 38' / 98° 07'	2800	12.1	764
PA09	Villa Cuauhtémoc, Pue.	Colpos	19° 43' / 98° 07'	2720	12.4	730
PA11	Zacualtipán, Hgo.	Colpos	20° 38' / 98° 38'	2030	16.1	1199
PA12	Xico, Ver.	Asoc. For.	19º 30’ / 97º 05’	2839	11.5	1019
	Range				11.1 to 17.7	615 to 1223
PS01	Casas Blancas, Mich.	INIFAP	19° 25' / 101° 36'	2244	15.7	1054
PS02	Nvo San Juan Parangaricutiro, Mich.	INIFAP	19° 29' / 102° 19'	2245	15.2	1173
PS03	Tenango del Valle, Ver.	INIFAP	19° 02' / 99° 37'	2990	11.3	1156
PS04	Perote, Ver.	Colpos	19° 33' / 97° 12'	3200	9.5	1322
PS05	Xico, Veracruz.	Asoc. For.	19° 30' / 97° 05’	2839	11.5	1019
	Range				9.0 to 16.9	717 to 1415

[1] OC: *P. oocarpa*; PA: *P. patula*, PS: *P. pseudostrobus*; [2] INIFAP Michoacán; Colpos: Colegio de Postgraduados en Ciencias Agrícolas; Asoc. For.: Asociación Forestal Especializada AC; [3] MAT= Mean annual temperature; [4] MAP= Mean annual precipitation; [5] Range: MAT and MAP ranges in the TMVB region. All values calculated with ANUSPLIN software (Sáenz-Romero et al. 2010; Crookston 2017).

3.2 Experiment description and experimental design

Three hundred seeds per population were sowed in trays containing moistened rock wool and covered with plastic film (see Appendix A for details in the experimental set-up). Trays were placed inside a germination chamber at 25±1ºC, 60±5% relative humidity and an eight-hour photoperiod. The germination was recorded three times a week and then used to calculate the germination curve parameters (total germination in %, speed) based on a sample of 60 seeds per population. Germination for the three species started at three days. *P. oocarpa* and *P. pseudostrobus* had a higher germination rate than *P. patula* (Supplementary information Figure S1).

We transplanted fifty seedlings into individual plastic containers, except for three *P. patula* populations (PA02, PA07 and PA08) that had a low germination rate, for which we transplanted, respectively, 38, 26 and 35 seeds. The total number of seedlings used were 786. We used individual plastic containers with a mixture of peat moss and vermiculite substrate (3:1 v/v) whose size was big enough (250 cm^3) to avoid root restriction, given the short duration of the experiment (Poorter et al. 2012a). The trial was established in a greenhouse under controlled conditions (Appendix A). The trial was set up with a randomized complete blocks design, with five seedlings per experimental unit, and

five blocks in each of the two watering treatments. Seedlings were maintained in a slow-growth phase during 135 days from November to March to allow the material to harden. Then plants were cultivated in a normal-growing phase (April to June). Fifty seedlings per population were submitted to two watering treatments during 90 days (25 seedlings per watering regime): Field Capacity (FC) and Drought-Stress (DS). For those populations with lower seed germination rate we set an equal number of seedlings per treatment (PA02, PA07 and PA08). The watering regimes were based on the mean saturation level of the substrate: 90–100% on FC and 35-45% on DS treatments. We determined the amount of water for each watering event every two days by weighing plants randomly chosen from each treatment.

3.3 Variables measured

We periodically recorded the survival, height (mm) and ontogenetic stage of all seedlings (Chambel et al. 2007). Species were in the epicotyl elongation and formation of axillary buds phase at the beginning of the experimental phase, and had dwarf shoots by the end of it (Appendix A). We obtained the height growth increment (**HG** in mm) during the watering experiment as the difference between height at the beginning and the end of the watering experiment (H_t-H_0). At the end of the experiment (90 days of watering treatment, 225 days old), all plants were harvested and partitioned in roots, stems, and leaves. They were dried (65 ºC / 72 h) and weighed (g, ±0.01) (Poorter and Nagel 2000) to assess total dry mass (**TDM** in mg) and that of its components: roots, stems and needles (**RDM**, **SDM**, and **NDM**, respectively, in mg). The root mass fraction (**RMF**, roots dry mass to total dry mass), stem mass fraction (**SMF**, stem dry mass to total dry mass) and needle mass fraction (**NMF**, needles dry mass to total dry mass) were also computed. The specific leaf area (**SLA** in cm^2/g) was estimated from 10 needles randomly chosen from each plant (Alía et al. 2014b).

3.4 Data analysis

3.4.1. Seedling survival

For seedling survival, we performed a logistic regression analysis using a maximum likelihood method:

$$p_{ik(j)} = 1 / [1 + \exp(-z_{ik(j)})] \quad (1)$$

$$z_{ik(j)} = \log[p_{ik(j)} / (1 - p_{ik(j)})] = \mu + W_i + S_j + P_{k(j)} \quad (2)$$

where $p_{ik(l)}$ is the survival probability in the i^{th} watering regime of the k^{th} population within the j^{th} species; $z_{ik(j)}$ is the logit estimation in the i^{th} watering treatment of the k^{th} population within the j^{th} species; μ is the grand mean; W_i is the effect of the i^{th} watering regime (1 to 2), S_j is the effect of the j^{th} species (1 to 3), and $P_{k(j)}$ is the effect of the k^{th} population within the j^{th} species (1 to 10). The WS_{ij} interaction was not included in the model due to its lack of significance.

3.4.2. Mixed model

For the other variables, we conducted an inter-species variance analysis according to the following mixed model:

$$y_{ijkl} = \mu + W_i + S_j + WS_{ij} + PS_{k(j)} + BW_{l(i)} + c\, x_{ijkl} + \varepsilon_{ijkl}\,, \qquad (3)$$

where y_{ijkl} is the value of observation in the l^{th} block of the k^{th} population of the j^{th} species in the i^{th} watering treatment; μ is the general mean; W_i is the fixed effect of the i^{th} watering treatment (1 to 2); S_j is the fixed effect of the j^{th} species (1 to 3); WS_{ij} is the interaction fixed effect of the i^{th} treatment with the j^{th} species; $PS_{k(j)}$ is the random effect of the k^{th} population within the j^{th} species; $BW_{l(i)}$ is the random effect of the l^{th} block (1 to 5) within the i^{th} treatment; c is the lineal effect of the covariate x_{ijkl} (seedling height at the beginning of the watering regimen), and ε_{ijkl} is the experimental error.

In order to examine the intra-species variation a variance analysis was performed for each species, using the follow model:

$$y_{ijk} = \mu + W_i + P_j + WP_{ij} + BW_{k(i)} + c\, x_{ijkl} + \varepsilon_{ijk}\,, \qquad (4)$$

where y_{ijk} is the value of observation in the k^{th} block of the j^{th} population of the i^{th} watering regime; μ is the general mean; W_i is the fixed effect of the i^{th} treatment (1 to 2); P_j is the fixed effect of the j^{th} population (2 to 10); WP_{ij} is the interaction fixed effect of i^{th} treatment with the j^{th} population; $BW_{k(i)}$ is the random effect of the k^{th} block (1 to 5) within the i^{th} treatment; c is the lineal effect of the covariate x_{ijk} (seedling height at the beginning of the watering regimen), and ε_{ijk} is the experimental error.

We analyzed the variation of dry masses and mass fractions including the initial height as a covariate to correct the bias due to differences in the initial growth (South and Larsen 1988). Consequently, the experimental error of the models was reduced in each case.

3.4.3. Phenotypic plasticity

For each species, we calculated the plasticity index of a trait due to drought stress effect as (Hernández-Pérez et al. 2001):

$$PI=(V_1-V_2)/V_1 * 100 \quad (5)$$

where V_1 is the trait mean under the FC treatment; V_2 is the trait mean under the DS treatment.

In species with significant treatment x population interaction (*WP*), a plasticity analysis for each population was conducted, plotting the mean value trait by population on a dimensional plane where the x-axis was the drought stress treatment (DS) and the y-axis was the field capacity treatment (FC) (Pigliucci and Schlichting 1996).

3.4.4. Allometric analysis

We further used allometric analysis based in log-transformed data to study the changes in root dry mass compared to the sum of stem and needle dry mass. Differences between the two watering regimes in slopes and intercepts for the three species with their populations were assessed by parallelism test using watering regimes (Poorter and Nagel 2000; Poorter et al. 2012b).

3.4.5. Factor analysis

In order to display the overall performance of the populations tested we performed, for *P. patula* and *P. pseudostrobus* (species with more than two populations), a factor analysis using a maximum likelihood method and a Varimax rotation to maximize the variation of factor loadings and to facilitate the interpretation of the factors. We used variables with highly significant differences in the watering treatment (model 1): **HG**, **RDM**, **SDM**, **SMF** and **SLA**. A Biplot using the values of the factors for the FC and DS treatments were considered for each population. A correlation coefficient was computed for the mean values of the populations of the two axes, the plasticity (differences among FC/DS treatment), and the altitude and rainfall.

All the statistical analysis were performed using the SAS software (SAS Institute, Cary, NC, USA).

4. Results

4.1 Response to watering regimes

Water stress treatment significantly affected species survival, irrespective of seed origin (Table S1). Mortality (from the beginning to the end of the drought experiment) ranged from 30% for *Pinus oocarpa* to 4% for *P. pseudostrobus*, *P. patula* offering an intermediate value, 12% (Table S2).

Watering produced significant differences for all three pine species in seedling phenotypic changes (Table 2, Table S3). Most traits, with the exception of relative biomass allocation to roots (**RMF**) and needle biomass (**NDM**), showed distinct phenotypic changes (i.e., plasticity) in response to drought, indicating the importance of the watering treatment. We also found differences in the plastic responses of the species (species by watering interactions). Moreover, data confirmed a general significant intraspecific variation and intraspecific differences in plasticity, since both population and watering x population interaction were significant for all the three species.

Table 2. Mean squares and level of significance[1] in the inter-specific analysis estimated for all species for different functional traits in three Mexican pines.

Trait[2]	W	S	WxS	c	P(S)	B(W)
df	**1**	**2**	**2**	**1**	**14**	**8**
HG	800,680**	174,440**	31,469**	-	13,167**	5,394**
RDM	588,837*	122,126*	103,861**	4,820,150**	31,919**	148,087**
SDM	1,611,569**	112,624*	88,819**	3,649,888**	24,332**	47,780**
NDM	1,936,624ns	3,957,069**	168,257ns	26,807,653**	240,005**	1,031,535**
TDM	11,731,955*	4,778,621**	936,486**	86,216,460**	461,308**	2,379,984**
RMF	0.003ns	0.055*	0.010*	0.022**	0.011**	0.024**
SMF	0.214**	0.198**	0.025**	0.020**	0.013**	0.007**
NMF	0.167**	0.467**	0.032**	0.086**	0.024**	0.014**
SLA	33,146**	72,346**	1,693ns	33,228**	7,498**	3,819**

[1] Mean squares, and Level of significance: **significant differences (p<0.01); *significant differences (p<0.05); ns, not significant (p<0.05). [2] **HG**: height growth increment; **RDM**: root dry mass; **SDM**: stem dry mass; **NDM**: needle dry mass; **TDM**: total dry mass; **RMF**: root mass fraction; **SMF**: stems mass fraction; **NMF**: needles mass fraction; **SLA**: specific leaf area. **W**: Watering. **S**: Species. **WxS**: Watering x Species interaction. **c**: Covariate: Initial height for all traits except for HG. **P(S)**: Population within species. **B(W)**: Block within treatment. **df**: degrees of freedom.

4.2 Allocation patterns

Overall, regression models between root dry mass with stem plus needles dry mass, representing relative allocation to roots, had a positive relationship with low watering regime. For FC and DS, regression lines did not share a common trajectory (p<0.0001) for all three species. However, intercepts were different for *P. patula* and *P. pseudostrobus* (p<0.0001) but not for *P. oocarpa* (p=0.344) (Figure 2, Table S4).

4.3 Intraspecific variation

Height growth increment significantly varied with watering treatment for all study species, the extent of the change significantly varying for *P. patula* and *P. pseudostrobus* populations, but not for *P. oocarpa*'s (Table 3). The more plastic traits were related to height growth increment, stem and needle biomass and specific leaf area (Table 4).

We found differences among populations in many of the analyzed traits, especially those related to the biomass components, but not for allocation fractions: stem and total biomass in *Pinus oocarpa*, height growth increment, total biomass and biomass components and specific leaf area in *P. patula*, and all the traits except stem biomass in *P. pseudostrobus*. For many of those traits that showed a significant population effect, significant differences in population phenotypic plasticity were detected, indicating differences among species and populations in response to drought, e.g., population phenotypic changes in stem and needle biomass in *Pinus oocarpa* and *P. patula*, or biomass allocation and specific leaf area in *P. pseudostrobus*.

The patterns of phenotypic plasticity among populations were quite contrasting depending on the trait (Figure 3). The height growth increment showed sharp differences in phenotypic plasticity for two of the species (*P. patula* and *P. pseudostrobus*), with a higher variation for the first species. It is interesting to notice that for **SDM** (Figure 3b), *Pinus patula* populations were quite homogeneous in allocating biomass to stems despite the differences in height, while the two *P. oocarpa* populations had quite different patterns. *P. pseudostrobus* populations showed differences in phenotypic plasticity for **SMF** and **SLA**, being populations PS05 and PS04 the most interactive for the two traits (Figure 3d, 3e).

Table 3. Mean squares and level of significance[1] in the intra-specific analysis per species for different functional traits in three Mexican pines.

Trait[2]	W	P	WxP	c	B(W)
*P. oocarp*a					
df	1	1	1	1	8
HG	193,937**	517ns	3,106ns	-	4,504**
RDM	411,344ns	122,364*	71,980ns	1,543,789**	92,363**
SDM	515,777*	122,994**	164,325**	1,506,868**	66,208**
NDM	1,218,092ns	385,868ns	601,087*	5,944,773**	574,158**
TDM	6,067,398ns	1,747,874*	2,099,484**	24,086,763**	1,575,038**
RMF	0.008ns	0.001ns	0.004ns	0.001ns	0.010ns
SMF	0.012ns	0.002ns	0.005ns	0.007*	0.003*
NMF	0.040*	0.005ns	0.000ns	0.013ns	0.005ns
SLA	2.156ns	4 ns	19.ns	2,077ns	502.ns
P. patula					
df	1	9	9	1	8
HG	736,102**	3,460**	5,617**	-	2,882*
RDM	329,833ns	10,358ns	16,031*	1,319,554**	94,360**
SDM	1,239,102**	14,035**	16,812**	691,007**	16,274**
NDM	584,235ns	85,401**	61,937ns	9,253,456**	436,834**
TDM	6,153,728*	214,427*	225,448**	25,668,546**	1,086,400**
RMF	0.006ns	0.003ns	0.002ns	0.007ns	0.019**
SMF	0.372**	0.004*	0.003ns	0.001ns	0.007**
NMF	0.280**	0.004ns	0.003ns	0.003ns	0.016**
SLA	178,790**	2,949**	676.ns	39,736**	4,007**
P. pseudostrobus					
df	1	4	4	1	8
HG	173,685**	34,691**	3,742*	-	3,135*
RDM	25,352ns	75,630**	18,592ns	1,940,741**	18,583ns
SDM	272,247**	4,556ns	12,154ns	1,109122**	18,678**
NDM	486,907ns	665,570**	175,298ns	11,061,214**	353,560**
TDM	1,872,890ns	924,259**	304,728ns	33,341,014**	665,116**
RMF	0.023ns	0.035**	0.007*	0.054**	0.006**
SMF	0.075**	0.022**	0.006*	0.037**	0.003ns
NMF	0.013ns	0.057**	0.008ns	0.180**	0.006ns
SLA	64,286**	11,042**	2,603*	345ns	1,286ns

[1]Mean Squares and level of significance: **significant differences (p<0.01); *significant differences (p<0.05); ns, not significant (p<0.05). [2]**HG**: height growth increment; **RDM**: root dry mass; **SDM**: stem dry mass; **NDM**: needle dry mass; **TDM**: total dry mass; **RMF**: root mass fraction; **SMF**: stems mass fraction; **NMF**: needles mass fraction; **SLA**: specific leaf area. **W**: Watering. **P**: Population. **WxP**: Watering x Population interaction. **c**: Covariate: Initial height for all traits except for HG. **B(W)**: Block within treatment. **df**: degrees of freedom

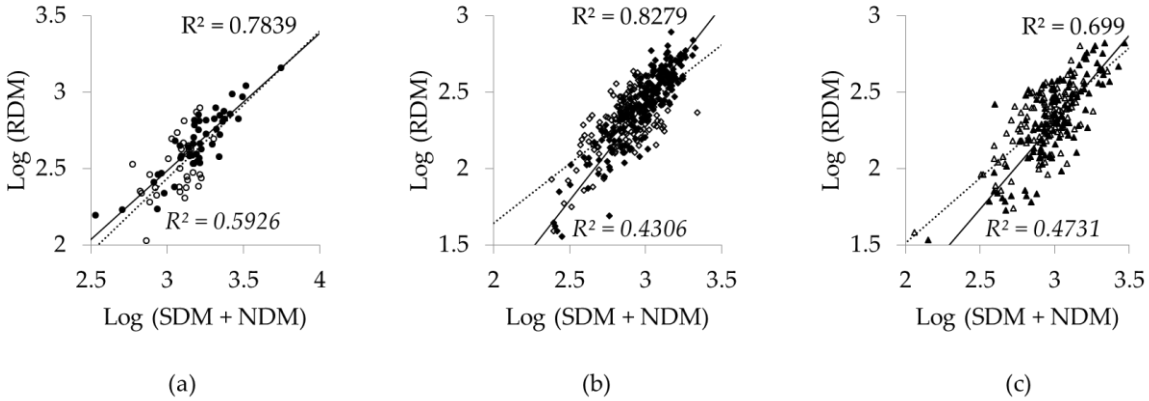

Figure 2. Allometric regression between RDM with SDM+NDM for FC and DS regimes: (a) *P. oocarpa*, (b) *P. patula* and (c) *P. pseudostrobus*. Solid lines, full symbols and R^2 for FC treatment while dotted lines, empty symbols and *italics* R^2 for DS treatment.

Table 4. Plasticity Index of the traits under the drought stress treatment at the species level (only variables with a Watering significant effect are included).

Trait[1]	*P. oocarpa*	*P. patula*	*P. pseudostrobus*
HG	73.74	57.60	60.08
SDM	43.03	48.94	35.04
TDM	-	24.11	-
SMF	-	32.60	26.88
NMF	-7.74	-10.09	-
SLA	-	20.30	19.92

[1] **HG**: height growth increment; **RDM**: root dry mass; **SDM**: stem dry mass; **NDM**: needle dry mass; **TDM**: total dry mass; **RMF**: root mass fraction; **SMF**: stems mass fraction; **NMF**: needles mass fraction; **SLA**: specific leaf area.

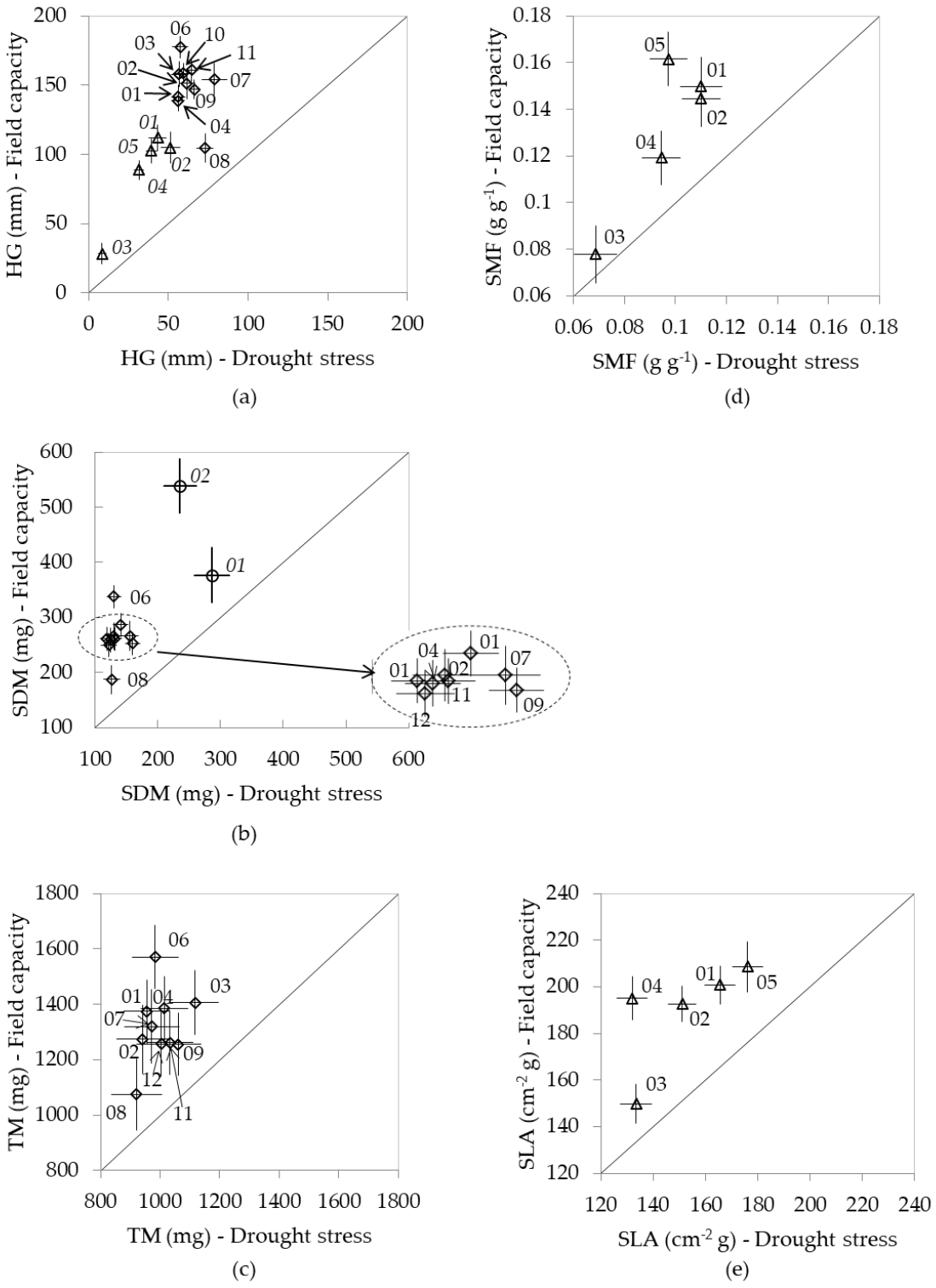

Figure 3. Analysis of plasticity by species for growth variables and biomass components with significant response to watering treatment (a) **HG**: height growth increment; (b) **SDM**: stems dry mass; (c) **TDM**: total dry mass; (d) **SMF**: stem mass fraction; and (e) **SLA**: specific leaf area (*P. oocarpa*=○, *P. patula*=◊ y *P. pseudostrobus*=Δ). Bars indicate the standard errors in the two treatments.

4.4 Phenotypic variation of the Mexican species under full capacity and drought stress treatments

The two first factors explained 86.09% of the total variation, with the first factor (PC1), related to stem growth and **SLA,** explaining 59.85% of the total variation, and the second factor, related to root and stem dry biomass, explaining 26.24% of it (Table S5). The two treatments clearly differed, all populations analyzed showing a similar pattern, mainly due to an increment in stem mass under the full capacity treatment, although they differed either in the extent of the variation or in the allocation pattern (expressed in the two axes). The differences were higher for *Pinus patula* than for *P. pseudostrobus*. *P. pseudostrobus* populations showed a similar performance, PS03 and PS04 behaving similarly under the two treatments (Figure S2). *Pinus patula* showed a significant correlation (r=0.634*) between rainfall of the origin and plasticity in PC2 (Figure 4a). In the case of *Pinus pseudostrobus*, the value was significant (r=0.823*) in the case of PC2 (Figure 4b).

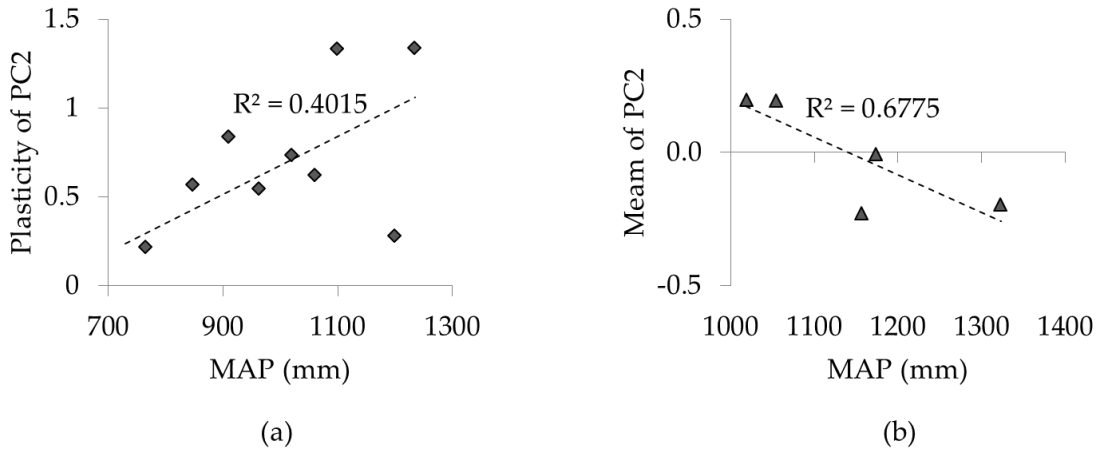

Figure 4. Relationships among rainfall (MAP, data from Table 1) with a) plasticity of Principal Component 2 for *P. patula* (◆), and b) mean of Principal Component 2 for *P. pseudostrobus* (▲).

5. Discussion

This paper evaluates the variation in growth and biomass allocation in seedlings of three Mexican pines grown under two contrasting watering regimes. The results showed inter- and intraspecific variation in seedling drought tolerance, which confirms our hypothesis that the watering regime had a significant effect in phenotypic changes for plants of *Pinus oocarpa, P. patula* and *P. pseudostrobus*. All species and populations shared a common general avoidance mechanism (increasing water uptake and reducing water loss (Poorter and Markesteijn 2008)) in relation to changes in their allocation patterns, but the intraspecific variation and differences in phenotypic plasticity among populations modified the adaptation strategies of the species to drought. The

sampling scheme allowed us to detect differences among geographically close populations, with strong implications for forest management.

Our study is limited to moderately stressful experimental conditions, as we were dealing with species and populations that differ in their tolerance to water stress, but in accordance to the climatic scenarios predicted by 2030 (Sáenz-Romero et al. 2010). Our results evidenced the existence of an avoidance mechanism in the face of drought stress at the seedling stage, which is the most critical in both the natural and artificial regeneration methods. The existence of watering x population interaction in many traits implies differences in the genetic responses of the populations that are important for the in situ adaptation of the species, due to the possible selection of reaction norms. Experiments under more intense water stress, that is, more stressful conditions than those predicted for the next generation, could result in hidden reaction norms, i.e., responses of the populations not described previously (Schlichting 2008). Another caveat of the study is that maternal environmental effects at the seedling stage significantly modulate variability in the trees growing in the stressful environment (Zas et al. 2013). However, we minimized the impact of these effects by using the initial height as a covariate. Finally, we focused our experiment in a restricted area, using a limited number of samples (in the case of *P. oocarpa*, only two, to avoid biases due to taxonomic errors in the identification, see Material and Methods). The sampled populations, however, cover the range of mean temperature and rainfall of the study area (Table 1). We addressed the level and patterns of variation of close together populations in the same region as a means to infer genetic resources management recommendations in the study area. We are not able, however, to provide estimates of the level of genetic variation of the species, which is largely dependent on the sampling scheme.

The adjustment to drought stress treatment in the Mexican pines analyzed mainly involved allometric changes by reduction of aerial biomass, although it is interesting to point out that root allocation was not significantly affected, and neither was needle dry biomass. Seedling allometric changes, linked to low water availability in the soil (Sáenz-Romero et al. 2012), are associated to particular physiological processes, including changes in photosynthetic and transpirational capacities, that depend on the level of stress (Pallardy 2008). A reduction in **SLA**, an important functional trait related to leaf assimilation capacity (Niinemets 1999), was also observed. Such reduction in **SLA** under water-stress conditions has been repeatedly reported in seedling experiments (e.g. *P. canariensis* (Climent et al. 2006), *P. halepensis* (Baquedano and Castillo 2006)), under similar experimental conditions. **SLA** changes were due to shifts in the watering regime (Reich et al. 2003), and seedlings from drought-tolerant seed sources showed greater reductions in needle size, area per needle and stomata per needle than seedlings from non-tolerant sources (Cregg 1994).

The three species analyzed did not behave similarly, and presented significant differences in the level of intraspecific variation and phenotypic plasticity under water stress treatment. *P. oocarpa* showed the highest mortality, growth reduction and needle biomass fraction increment. *P. oocarpa* seemed the least tolerant to water stress, while *P. pseudostrobus* was the most tolerant. The climatic information from the sampled populations (and from the species in the area of study) is not exactly coherent with this behavior, since *P. oocarpa* lives under higher annual temperatures (18.8 ºC) and rainfall (1,205 mm) than the other two species (14.3 ºC and 982 mm for *P. patula*, and 12.6 ºC and 1,145 mm for *P. pseudostrobus*). Therefore, climatic data (temperature and rainfall) cannot be solely relied upon in predicting drought tolerance in forest species, especially when dealing with populations from a restricted area, where other factors and climatic variables could have shaped local adaptation, determining the behavior of each species (Leimu and Fischer 2008; Bansal et al. 2015; Warwell and Shaw 2017).

For the three species, several patterns have been described for the relationship between the ecological conditions and the performance in field or in greenhouse experiments of the species, indicating that these relationships depend both on the species and the experimental conditions (sampled material and site). In many cases there is a maximum (or minimum) of the performance at a given ecological (rainfall, altitude) value. For *P. oocarpa* seedlings, the occurrence of a seedling stage was high whenever the rainfall at the seed origin was less than 1,250 mm. The ability to form a lignotuber (storage root typical from seedling-stage pines) is probably an adaptation to dry, fire-frequented environments (Greaves 1980). Height growth was related to the altitude, rainfall and dry season of the seed origin (Greaves 1980), and the greatest growth would occur in populations originating from 1,255 *masl*, with populations from either lower or higher altitudes having a lower growth (Sáenz-Romero et al. 2006). *Pinus patula* provenances from lower altitudes showed higher growth and a larger number of shoots cycles than provenances from higher altitudes (Salazar-García et al. 1999; Sáenz-Romero et al. 2011a). However, in a greenhouse-provenance trial, seedlings showed slightly higher growth potential in provenances from mid-altitude (2,700 *masl*) than those provenances originated in altitudinal extremes (2,400 and 3,000 *masl*) (Sáenz-Romero et al. 2011b). *Pinus pseudostrobus* populations from lower altitudes (2,300-2,400 *masl*) presented poorer health than populations from intermediate altitudes (2,700 *masl*), and those populations from altitudinal extremes (2,300 and 2,900 *masl*) presented the lowest percentages of germination, while the highest germination rate corresponded to 2,700 *masl* (Lopez-Toledo et al. 2017).

Intraspecific variation will influence the strategies of the species at two main levels: genetic variation and differences in the plastic response of the populations. The three species showed

significant levels of intraspecific variation within the sampled area, with *P. oocarpa*, for which only two populations were sampled, having a largest level of genetic variation, the two populations differing in phenotypic plasticity in response to drought stress. It has been reported that populations from low altitudes tend to show higher growth potential than trees from populations originating at higher altitudes (Sáenz-Romero et al. 2006), and that populations from altitudes of origin above 1,000 *masl* are less drought-tolerant than those of below 1,000 *masl* (Masuka and Gumbie 1998). It is quite likely that populations (and species) from low altitudes have a more conservative growth strategy, related to the avoidance of drought stress (Barton and Teeri 1993; Poulos and Berlyn 2007). However, at our sampling scale, the population from the high altitude (OC02) was more tolerant to drought stress, as indicated by its lower mortality, and its better stem biomass adjustment under our two watering regimes.

Pinus patula populations showed a significant among-population variation in most of the traits related to stem growth and **SLA**, and they also differed in levels of phenotypic plasticity for those traits, although not for **SLA**. It has been reported that *P. patula* provenances from lower altitudes have a higher growth (Salazar-García et al. 1999). In our study, there is a correlation among seed origin rainfall and the mean value of the first factor (r=0.65), related to stem mass fraction, and **SLA** and the plasticity in the second factor (r=0.63), related to root and stem dry biomass, indicating that even at local scales there is an adaptive pattern to climate of the integrated phenotypes.

P. pseudostrobus also showed intraspecific differences in traits related to stem biomass, and **SLA**, but also significant differences in phenotypic plasticity among populations. We found a correlation of the mean value of the populations in the factor 2 (r=0.82*) related to root and stem dry biomass. The low sampling size (5 populations), could influence the lack of significance of the factor 1 (r=0.65 ns) and the plasticity of the factor 2 (r=0.68 ns). The linear relationships described in this study can also be caused for the sampling area, as we cannot discard a more complex performance (as the one described in the studies previously mentioned), when expanding the study area. It is interesting to notice that populations from western Mexico did not have significant genotype-environment interaction (Viveros-Viveros et al. 2005; Castellanos-Acuña et al. 2013), when tested in close-by test sites. Therefore, estimating intraspecific differences in terms of adaptability at local scales will require an estimate of among-population genetic differences in terms of genetic phenotypic plasticity (Chambel et al. 2005) in a larger number of populations.

The implications for forest genetic resources management are related to the natural and artificial regeneration of the species and conservation of genetic resources. In the TMVB, the

populations of the species differ in adaptability to drought stress, and our ability to predict the responses requires a sufficient sample size, that is, at spatial scales significant for forest management we can detect differences in genetic variation and patterns of performance related to the climate of origin. In the case of *P. oocarpa*, even two very close populations performed differently and, for the other two species, the existence of intraspecific variation (population and drought-by-population interaction) justifies the use of local material in afforestation programs (McKay et al. 2005). More productive allochthonous basic materials could be used in the region ensuring that native populations were not introgressed with this potentially non-adapted material (IUCN 2004). This study also shows the importance of the area for the genetic conservation of the species, as some conservation units can be selected having differential value in terms of adaptation for the future climatic conditions (Rodriguez-Quilon et al. 2016). Also, our results show that, at early developmental stages, genetic differences in survival are important depending on the species, and therefore silvicultural treatments must be taken into consideration to favor different biomass allocation (e.g, by reducing competition or light) (Nocentini et al. 2017). Managing the genetic resources within a region, therefore, needs not only information at the species level, but a more precise information about major variation patterns of their populations, as the effects will affect the future adaptation and performance of the species in the area considered.

6. Conclusion

We confirmed that even at reduced geographical scales, Mexican pines present differences in the response to water stress. The responses differed among species, including the allometric phenotypic changes in biomass allocation (plasticity), the genetic differences among populations, and the differences in phenotypic plasticity among populations. Testing three different species that presented differences in water stress tolerance, allowed us to detect different strategies of avoidance (mainly changes in allometry, but also changes in needle structure for some of the populations), and some patterns of species response. These differences are relevant not only in afforestation programs, but also in genetic conservation activities.

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Chapter III

Defining areas for conservation and use of forest genetic resources in four Mexican pines

1. Abstract

Forest tree species provide a wide range of goods and services, such as sustainable economic growth. The conservation and sustainable use of the genetic resources should benefit of new developments for monitoring adaptive gene diversity. In some countries, that have great species diversity, it is necessary to identify geographical areas for conservation. Mexico harbors around 49 of the approximately 120 species of pines in the world, for this reason it is urgent to prioritize areas for conservation and sustainable use of their forest genetic resources. The aims of this work were: i) to identify areas for gene conservation and ii) to propose measures for the conservation and sustainable use of forest genetic resources of four species: *P. greggii*, *P. oocarpa, P. patula* and *P. pseudostrobus*. Overall, there were prioritized 52 areas for establishing conservation units based on the existence of genetic data and species distribution, and there were identified the most important genetic zones for the use of forest genetic resources. However, the current conservation efforts for the four Mexican pines are still insufficient. It is still necessary to develop genetic studies in order to advance in the conservation of the species in the long-term face current climatic changes.

Keywords: Gene conservation unit, Dynamic conservation, Forest reproductive material, Seed zones

2. Introduction

Forest tree species are long lived and widely distributed species, with usually a low degree of domestication. They are essential for the maintenance of biological diversity in terrestrial ecosystems and provide a wide range of goods and services, including genetic resources that are indispensable and constitute a unique and irreplaceable resource for the future, and the sustainable economic growth, progress and environmental adaption (Rajora and Mosseler 2001). However, many activities (e.g., forest management, seed transfer) or large perturbation events (e.g. climatic change) are affecting, or can affect in the near future, the genetic diversity of the species and their distribution over space and time. Therefore, actions have been requested at the global scale (FAO 2014), as within and among populations genetic diversity have provided the potential for adaptation in the past, and will continue to play this vital role as humankind addresses the challenge of mitigating or adapting to further climate changes. The conservation and sustainable use of the genetic resources should benefit of new developments for monitoring adaptive gene diversity, and for the stewardship of natural populations and the genetic improvement (Neale and Kremer 2011). A first step needed is the characterization of areas for conservation and use of forest genetic resources to define priority actions.

Conservation activities and management for economic purposes for most of the forest tree species are disconnected, from the use of their genetic resources. However, these two aspects should be considered in forest management decisions as, they take place at a landscape level, where, for example, the use of the genetic resources (by breeding or planting) can affect the conservation of genetic resources (Yanchuk 2009) by favoring the introgression with non-adapted material or by substitution with more productive material (Koskela et al. 2014). These actions could affect the ecosystem, making it unable to provide the genetic services of the forest ecosystem they replace (Rymer 1981; Ehrlich and Mooney 1983). The adequate conservation of existing resources can provide new sources of diversity for breeding programs in the future. To our knowledge, only few programs have considered both the conservation and sustainable use of forest genetic resources as a part of the same objectives (Jiménez et al. 2009).

Usually populations are the main objective for conservation and/or breeding of forest genetic resources. Forest tree species present remarkable phenotypic differences among populations in important traits, despite high levels of gene flow (Savolainen et al. 2007). These differences are essential both for breeding, i.e. selection of the best material according to the objective (Baliuckas, Pliura, & Eriksson, 2004; Gray et al., 2016) and conservation programs when the species as a whole are rarely endangered (Ledig 1986; Aravanopoulos 2016). Therefore a “bioregion” approach for the use of genetic resources (deployment and procurement zones) (van Buijtenen 1992), and conservation

(Kanowski 2001) are being applied as the best geographic basis for planning and implementation. Therefore, it is necessary to consider as a starting point the geographical areas with significant values for conservation or production of forest reproductive material.

The priorities in genetic conservation are based on the economic and environmental importance of the species, its ecological functions, the level of risk, or other special features that contribute to the relevance of a species (Rajora and Mosseler 2001). Although genetic studies of forest species have increased enormously throughout this century (Alberto et al. 2013), we lack information for most species. This means that, for the moment, we should take decisions in absence of genetic knowledge (Eriksson et al. 1993). Therefore, we should use some surrogates to identify geographical areas significant for conservation. For example, the climatic regions (European conservation program, (Lefèvre et al. 2013), areas with similar genetic background (Rodriguez-Quilon et al. 2016), or even the regions of provenance of the species (Alía et al. 2009). Within these areas it would be desirable to establish gene conservation units for a single species or a group of species (García del Barrio et al. 2013; de Vries et al. 2015). Practical experience for forestry suggests that sound management of genetic resources must include conservation efforts based on two overlapping strategies: management of natural forests with due respect to their genetic resources, and the establishment of networks of smaller gene conservation areas (FAO et al. 2001; Koskela et al. 2013).

For the sustainable use of forest genetic resources, the procurement zones (seed zones/breeding zones) can be considered as the first level of stratification of the species, as they define areas with a similar performance, and therefore with a similar interest for breeding or other non-intensive activities. The information from provenance test would help to define the value of each of these regions, as they can differ depending of the trait of interest. The establishment of seed stands, seed orchards or the management of breeding populations are key aspects for prioritizing the areas. Also, it is necessary to take into account the deployment zones of the reproductive material, but at initial stages, usually we lack the information to define precisely the seed transfer functions, and therefore we can assume that are equivalent to the procurement zone.

Mexico is a world source of pine biodiversity. In this context, Mexican pines are a good case study for prioritizing areas for conservation and sustainable use of their forest genetic resources. In Mexico there are present 49 of the approximately 120 species of pines in the world (Gernandt and Pérez-de la Rosa 2014). These pines have a different level of genetic knowledge, but in most of the cases is still incomplete. Genetic variation have been studied in only 1.2% forest tree species, for which 58% belong to the genus *Pinus*, 15% to *Quercus*, 10% to *Abies*, and 6% to *Picea*; most of genetic diversity studied come from Pinacea family but only includes 2% of all Mexican tree taxa

(Wehenkel et al. 2017). However, they are actively managed e.g. 60% are of commercial importance and provide timber, resin and pulp for the paper industry (Sánchez-González 2008), and constitute a valuable source of genetic resources for the genera (Dvorak 1990; Wehenkel et al. 2017). Therefore, it is necessary to advance in the conservation and sustainable use of the species, and specially to define priority actions. We have selected four species, with contrasting distribution range and importance in the country (Perry 1991): *P. greggii* Engelm. ex Parl. (*P. greggii* Engelm. ex Parl. var. *greggii* and *P. greggii* Engelm. ex Parl. var. *australis* Donahue & Lopez), *P. oocarpa* Schiede ex Schltdl., *P. patula* Schiede ex Schltdl. & Cham. (*P. patula* Schiede ex Schltdl. & Cham. var. *patula* and *P. patula* var. Schiede ex Schltdl. & Cham. *longipedunculata* Loock ex Martínez) and *P. pseudostrobus* Lindl. At national level, these species are among the main pines for timber production (Sánchez-González 2008) and play an important role for sawmill industry and resin production (Fuentes et al., 2006). Additionally, species have been used for plantations in the country (López-Upton et al., 2005) and different continents due to their fast-growing and volume production, e.g. *P. oocarpa* in Europe, Asia, Africa, America and Oceania; or *P. patula* in Africa (W. Dvorak, 2012; Gwaze et al. 2000). These species are considered highly valuable for *ex situ* gene conservation (FAO 2012), i.e. gene-conservation banks and genetic test on lands (Dvorak 1990, 2012).

The aims of this work are: i) to identify areas for gene conservation and ii) to propose measures for the conservation and sustainable use of forest genetic resources of *P. greggii, P. oocarpa, P. patula* and *P. pseudostrobus.* Firstly, we identify the most relevant existing information related to the identification and characterization of forest genetic resources of these species. We used concerning the distribution range of the species, information for conservation of forest genetic resources (identification of genetic conservation units, protected areas) and for the sustainable use of forest genetic resources (forest germplasm production units, or populations used at different stages in breeding). We check for gaps considering the distribution area and the genecological zones of the species, and we propose recommendations to improve the status of conservation and sustainable use of forest genetic resources in the considered species.

3. Material and methods

We considered four pines species: *P. greggii* (*P. greggii* var. *greggii* and *P. greggii* var. *australis*), *P. oocarpa*, *P. patula* (*P. patula* var. *patula* and *P. patula* var. *longipedunculata*) and *P. pseudostrobus*. The species are distributed along temperate mountain systems (Sierra Madre Oriental, Sierra Madre Occidental, Sierra Madre del Sur, Sistema Neovolcánico Transversal and Sierra Madre Centroamericana y Altos de Chiapas). They constitute pure and mixed coniferous forests, and pine-

oak forests. Main populations of these species occur in a wide altitudinal range (600-3000 m a.s.l.) (see Table S1).

The distribution range of the species was established based on the National Forest and Land Inventory [NFLI, 2004 to 2007, (CONAFOR 2017)] using the plots considered as natural forests (Figure 1), after removing the plots considered as plantations (CONAFOR 2012). We have not included plots where to our knowledge it is difficult to contrast the origin of the population, and should be confirmed that they are native populations as they could have been classified wrongly. This database recorded and measured all the species across the country in a systematic stratified random sampling with a grid of 5 km.

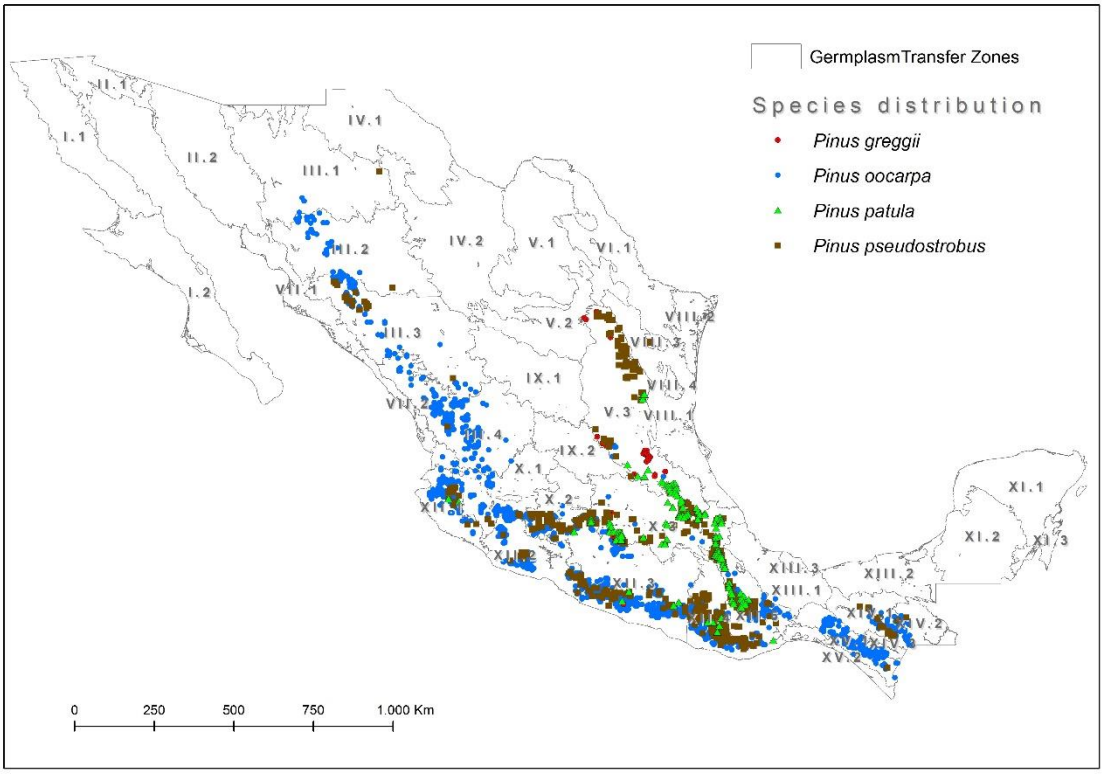

Figure 1. Distribution of the pine species studied following NFLI, 2004 to 2007, (CONAFOR 2017) and Germplasm Transfer Zones defined in México (CONAFOR, 2016).

3.1. Genetic zones

Seed zones are areas with similar ecological and climatic characteristics that harbor populations with relatively uniform genotypic or phenotypic characteristics (Ledig 1988; Flores Flores et al. 2014; CONAFOR 2016; Secretaria de Economía 2016). The existing genetic studies for the four species (Tables S2, S3 and S4) do not allow a precise definition of genetic zones, and

therefore we used the germplasm transfer zones (equivalent to seed zones) of the species as a *proxy*. In general, for Mexico it has been defined, under to a coarse approach, 41 zones, grouped in 15 Mexican Physiographic Provinces (Figure 1, Table S5). The genetic zones for each of the four pines were defined by overlapping the tree species distribution data according to the NFLI with the germplasm transfer zones (CONAFOR 2016). Genetic zones with less than 20 individual trees registered for a species were excluded due to the possible difficulty of finding a population that meets the minimum conservation requirements or that would be used in seed collection.

3.2. Conservation units

We have followed a set of minimum requirements (Table S6) for defining genetic conservation units based on population size, management, monitoring and ownership (according to Koskela et al. (2013)). Each conservation unit should cover a multiple objective (e.g. source of germplasm, *in situ* conservation) and meet some requirements for its management. In this sense, the objective of conservation compatible with the management of the population should be meet under any of the Protected Areas (Figure S1) established by the Mexican Commission for Biodiversity (CONABIO, information for 2016 available at www.gob.mx/conabio), or private ownership.

Three main selection criteria (Table 1) are used. Firstly, we prioritized populations characterized in genetic studies and/or provenance or progeny trials. Secondly, protected areas for biodiversity conservation, instead private ownership, were chosen. Finally, we prioritized extensive and centered populations in the genetic zone when the species distribution had isolated patches. In general, a conservation unit was selected by each genetic zone and species except in cases of clearly fragmented distribution (e.g. high distance between population cores, existing barriers to dispersion) in which two and up to three conservation units (CUs) were selected by species and genetic zone. The georeferenced information were mapped using the QGIS Software (http://qgis.osgeo.org) (QGIS Development Team 2015).

Table 1. Criteria for selection of genetic conservation units within a genetic zone.

Condition 1	Condition 2	Criteria
The population has genetic information (molecular or phenotypic studies) at a large scale of the species range.	Located in a Protected Area	C1.1
	Located outside a Protected Area	C1.2
The population has genetic information (molecular or phenotypic studies) at a local scale of the species range.	Located in a Protected Area	C2.1
	Located outside a Protected Area	C2.2
The population do not have any genetic information. It is identified in a NFI plot.	Located in a Protected Area	C3.1
	Located outside a Protected Area	C3.2

3.3. Use and conservation of genetic resources

In absence of information on the demand of forest genetic resources by seed zone, we measured the importance of a genetic zone for the use of genetic resources of the species by the presence of forest germplasm production units (seed stands and seed area), under the assumption that the investment in the establishment and maintenance of such units, is a clear indication of the economic relevance of that specie use. We also used information from the existing material in genetic trials, i.e. provenance and progeny tests, established by different institutions (Tables S2, S3), as they provide information of populations identified for selection of forest reproductive material with well-known genetic background. These populations have been evaluated, and therefore different genetic information is available from papers and reports (Table S4).

For each genetic zone of the three species, we collected the number of trees sampled by the NFLI (n_i), the number of populations with molecular data (n_{mk}), the number of populations with seed stands (seed stand plus seed area) (n_{st}), the number of individuals selected for progeny test (n_{is}), the number of populations present in provenance test (n_{pt}), and the number of seed orchards (n_{so}). Finally, we calculate the number of minimum gene conservation units (n_g).

The importance of the pines species for timber production was based on the production by state (Table S7) that included different pine species. We made a proportional assignment to each genetic zone based on the distribution of the four species and those whose are harbored in genetic zones. A similar approach was followed for plantation area. We then estimated the productivity and degradation areas based on land zoning established by National Forest Commission (CONAFOR 2018). We defined also a value for conservation and a value for breeding of the populations

(subjective scale based on expert knowledge), and by recommendations established by the papers or reports analyzed previously (Table S8).

We assessed the status of each species using indicators derived and adapted from the EUFORGEN program (EUFORGEN, 2017) (Table 2).

Table 2. Effort and importance of the genetic zones in conservation and management of genetic resources.

Indicator		Description
I1	Number of genetic zones	Number of genetic zones with presence of the species
I2	Molecular characterization effort	% of genetic zones with at least 1 sample in molecular studies
I3	Provenance characterization effort	% of genetic zones with at least 1 population in provenance test
I4	Progeny characterization effort	% of genetic zones with plus trees in progeny tests
I5	Seed stands index	% of genetic zones with at least 1 forest seed production units
I6	Seed orchard index	% of genetic zones with at least 1 seed orchard
I7	Genetic conservation index	% of genetic zones with at least 1 conservation units identified

Based on this information we identified the priority regions for conservation, priority areas for production of FRM, and priority areas for genetic characterization.

4. Results

4.1. Genetic zones

Sixteen genetic zones (defined as the overlapping areas of presence of selected species inside the CONAFOR (2016) germplasm transfer zones), with effective presence of at least one of the four pine species were identified (four with one species, six with two species, four with three species and two with all the species). Attending indicator I1, *Pinus oocarpa* has the widespread representation (15 genetic zones) followed by *Pinus pseudostrobus* (13 genetic zones). On the other hand, *Pinus patula* (6 genetic zones) and *Pinus greggii* (2 genetic zones) have a more restricted distribution (Table 3).

Table 3. Status of target species.

	Indicator	*P. pseudostrobus*	*P. oocarpa*	*P. patula*	*P. greggii*
I1	Number genetic zones	13	15	6	2
I2	Molecular characterization effort	38.46	86.66	66.66	50.00
I3	Provenance characterization effort	0.00	6.66	33.33	100.00
I4	Progeny characterization effort	7.69	0.00	16.66	50.00
I3	Seed stands index	46.15	40.00	16.66	0
I6	Seed orchard index	0	0	16.66	0
I7	Genetic conservation index *	100.1	153.3	180.0	200.0

*Based on CUs proposed for each species (see Table S9)

4.2. Conservation units

We have identified 52 areas for establishing CUs for the target species (Figure 2 and Table S9): 23 for *P. oocarpa*, 9 for *P. patula*, 4 for *P. greggii*, and 16 for *P. pseudostrobus*. In relation with the selection criteria, near 60% of the CUs were chosen because the existence of genetic data (criterion C1 or C2; Table 1). The 34% of the units where located in Protected Areas. Despite this, three protected areas were identified where it was possible to describe units for more than one species. If all that CUs were defined (Table S9), values over 100% in I7 (Table 3) will be reached for all species which would be an important step in the genetic conservation of these species.

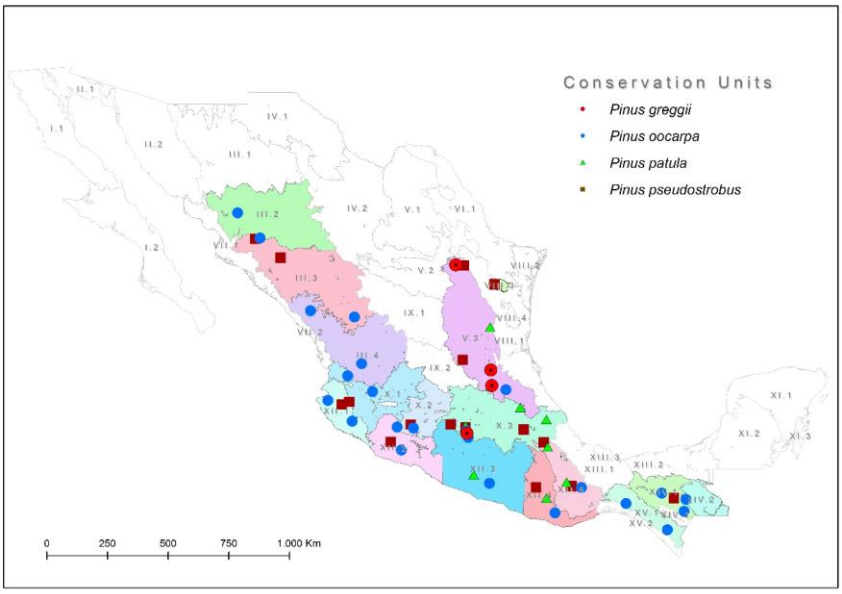

Figure 2. Conservation units and genetic zones (polygons colored) for four Mexican pine species.

4.3. Use and conservation of genetic resources

P. patula was the only species with seed orchard established (I6 >0, Table 3). About molecular characterization effort, *P. oocarpa* and *P. greggii* had most effort than *P. patula* or *P. pseudostrobus* (I2, Table 3). Also, *P. greggii* and *P. patula* had more generalized provenance (I3, Table 3) and progeny (I4, Table 3) characterization efforts than the other species with indicator values 0 or near to 0. Although *P. greggii* had more forest reproductive material production efforts units than the rest the species, it covers just a few states for timber production (Table 4).

The efforts aiming the use of forest reproductive material are quite reduced. In all the cases, the activities are of low intensity and based on the selection of seed stands (Table 4). There are many gaps for all the species, with many regions without any source of identified forest reproductive material to be used as a local source.

This is especially important in several states, as they differ in their importance in timber production (Table S10). Overall, for volume of timber, three states had higher production (>40,000 m^3) from 9 genetic zones: Chiapas (XIV.1, XIV.2 and XV.1), Jalisco (X.1, XII.1, XII.2), and Oaxaca (XII.4, XII.5 and XIII.1). For plantation activity, two states had most of the area established (>20 ha) in five genetic zones: Oaxaca (XII.4, XII.5 and XIII.1) and Veracruz (V.3, X.3 and XII.5).

With respect to productivity of forest land (Table 5), nine zones (III.2, III.3, III.4, X.1, XII.1, XII.2, XII.3, XII.4 and XII.5) presented large areas with high productivity while eight zones (III.2, III.3, V.3, X.1, X.3, XII.5, XIV.1 and XV.1) possessed potential for afforestation. This aspect demands a constant seed source from a forest reproductive material production unit. For forest restoration, we identified four zones (III.2, V.3, XII.2 and XII.3) for afforestation as they have large areas with high soil degradation, and three zones (XII.2, XII.3 and XII.4) that possess severe erosion. By consequence, it is necessary to supply an adequate amount of seed for the establishment of production (e.g. timber production or breeding) and conservation (e.g. forest restoration) activities.

Table 4. Conservation efforts and use of genetic resources by genetic zone and species (PS: *P. pseudsotrobus*, OC: *P. oocarpa*, PA: *P. patula*, GR: *P. greggii*).

Genetic Zone	# Trees National Forest Inventory (n_i)				# Gene Conservation Units (n_g)				Molecular characterization (n_{mk})				# Seed stands (n_{st})				Progeny testing (n_{is})				Provenance testing (n_{pt})				# Seed orchards (n_{so})			
	PS	**OC**	**PA**	**GR**	**PS**	**OC**	**PA**	**GR**	**PS**	**OC**	**PA**	**GR**	**PS**	**OC**	**PA**	**GR**	**PS**	**OC**	**PA**	**GR**	**PS**	**OC**	**PA**	**GR**	**PS**	**OC**	**PA**	**GR**
III.2	55	587	-	-	1	1	-	-	0	1	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
III.3	199	593	-	-	1	2	-	-	0	1	-	-	0	1	-	-	0	0	-	-	0	0	-	-	0	0	-	-
III.4	-	1201	-	-	-	2	-	-	-	3	-	-	-	1	-	-	-	0	-	-	-	0	-	-	-	0	-	-
V.3	1136	56	538	308	2	1	2	3	0	2	3	19	1	0	0	0	0	0	0	0	0	0	5	22	0	0	0	0
VIII.3	27	-	-	-	1	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-
X.1	61	699	-	-	1	2	-	-	0	1	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
X.2	777	581	126	-	1	1	1	-	2	8	0	-	2	1	0	-	13	0	0	-	0	5	0	-	0	0	0	-
X.3	1262	177	1028	23	2	1	3	1	4	2	1	0	8	0	5	0	0	0	36	120	0	0	4	2	0	0	1	0
XII.1	240	1222	-	-	1	2	-	-	0	1	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
XII.2	268	1503	-	-	1	2	-	-	1	1	-	-	1	2	-	-	0	0	-	-	0	0	-	-	0	0	-	-
XII.3	608	2650	81	-	1	2	1	-	1	2	1	-	0	2	0	-	0	0	0	-	0	0	0	-	0	0	0	-
XII.4	1944	1534	66	-	1	1	1	-	0	1	0	-	0	0	0	-	0	0	0	-	0	0	0	-	0	0	0	-
XII.5	1122	924	820	-	2	1	2	-	0	0	7	-	1	0	0	-	0	0	0	-	0	0	0	-	0	0	0	-
XIV.1	88	404	-	-	1	2	-	-	1	4	-	-	1	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
XIV.2	-	312	-	-	-	1	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-
XV.1	-	1123	-	-	-	2	-	-	-	3	-	-	-	1	-	-	-	0	-	-	-	0	-	-	-	0	-	-
Total	7787	13566	2659	331	16	23	10	4	9	30	12	19	14	8	5	0	13	0	36	120	0	5	9	24	0	0	1	0

n_i: # of trees sampled by the NFLI, n_g: # of gene conservation units, n_{mk}: # of populations per zone with molecular data, n_{st}: # of populations with seed stands, n_{is}: # of individuals selected for progeny testing, n_{pt}: # of populations present in provenance testing, n_{so}: # of seed orchards.

Table 5. Importance for conservation and use of forest genetic resources by genetic zone and species (PS: *P. pseudsotrobus*, OC: *P. oocarpa*, PA: *P. patula*, GR: *P. greggii*).

Genetic Zone	Presence of the pine species	Wood production (%)[1]	Plantation (%)[2]	Productivity of forest land[3]		Soil degradation[3]		Value for conservation (1 to 4)				Value for breeding (1 to 4)			
				I.A	I.B	II.A	II.B	PS	OC	PA	GR	PS	OC	PA	GR
III.2	50	-	-	2,333.6	1,399.7	109.8	12.9	4	2	-	-	1	2	-	-
III.3	88	-	-	2,744.4	1,292.2	36.7	0.4	3	2	-	-	2	2	-	-
III.4	137	0.73	0.04	2,349.9	615.8	47.6	11.1	-	2	-	-	-	3	-	-
V.3	143	1.47	10.15	872.9	1,988.0	388.6	8.2	1	4	4	4	3	1	4	4
VIII.3	2	2.27	-	7.9	102.7	0.0	0.0	4	-	-	-	1	-	-	-
X.1	66	6.13	0.31	1,693.5	1,305.2	34.1	4.1	3	2	-	-	1	2	-	-
X.2	66	-	-	706.3	939.5	64.7	19.9	2	4	2	-	3	4	2	-
X.3	144	2.48	19.40	782.0	1,332.4	16.5	18.5	1	4	4	4	3	1	4	4
XII.1	93	12.68	0.64	1,621.6	344.0	56.4	10.0	3	2	-	-	2	3	-	-
XII.2	114	5.12	0.23	1,738.9	443.8	231.3	55.7	3	2	-	-	2	3	-	-
XII.3	300	1.55	0.71	3,126.0	471.9	417.4	159.4	2	2	4	-	2	3	4	-
XII.4	213	17.27	25.51	1,733.4	150.1	50.7	67.3	1	2	4	-	3	3	4	-
XII.5	188	18.15	43.03	1,602.3	1,524.0	0.8	1.1	1	2	4	-	3	3	4	-
XIV.1	60	6.88	-	175.3	2,310.1	2.1	4.5	4	3	-	-	1	2	-	-
XIV.2	18	10.88	-	49.2	674.0	0.0	0.0	-	3	-	-	-	2	-	-
XV.1	109	14.38	-	279.9	1,798.6	0.0	0.0	-	2	-	-	-	3	-	-
Total	1791	100.00	100.0	21,817.0	16,692.1	1,456.8	373.1	-	-	-	-	-	-	-	-

[1] Relative value of total timber production, [2] Relative value of total plantation area, [3] Thousands of hectares, I.A: Forest land with high productivity, I.B: Land suitable for afforestation, II.A: Forest land with high soil degradation, II.B: Forest land with severe erosion.

Based on these gaps, as well as on the importance of the genetic zones for conservation and use of forest genetic resources (Table 3), six genetic zones (V.3, X.2, X.3, XII.3, XII.4 and XII.5) could be defined as the most suitable sources for use for germplasm for commercial plantation (Figure 3, and Table S10). These zones have the highest potential for conservation and breeding. Considering a subjective scales, there are two genetic zones (V.3 and X.3) with the highest values for conservation for most of the species, and four (X.2, XII.3, XII.4 and XII.5) with significant importance. For a breeding value, two zones (V.3 and X.3) have higher importance, while four zones (X.2, XII.3, XII.4 and XII.5) significant importance.

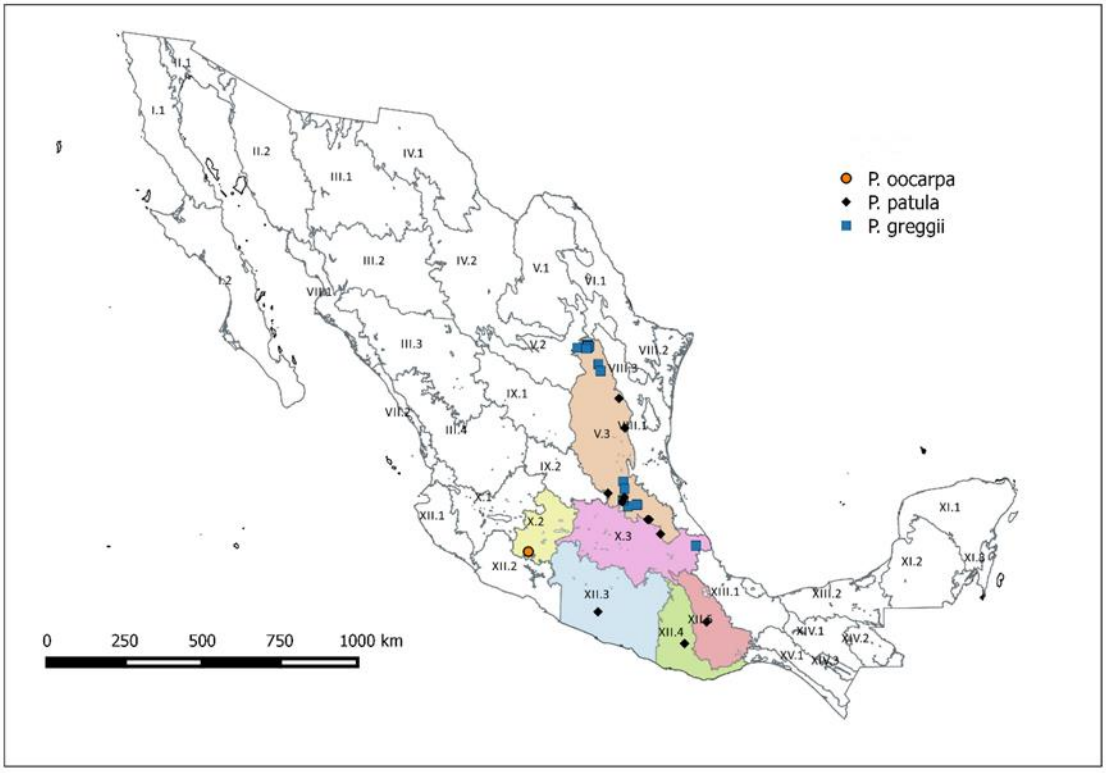

Figure 3. Populations and their zones considered as the most suitable source for use for reforestation.

Two genetic zones are target for conservation and use of the four pines studies (regions V.3 y X.3). Although they are not the genetic zones with a greater number of trees inventoried, they are the most diverse in species number and those in which the actions dedicated to the conservation and use of genetic resources are more intensive (see Table 4).

In summary, the efforts for conservation, characterization and use of forest genetic resources in the four Mexican pines (Table 3) are still insufficient. Most of the genetic zones do not have still enough data for characterization of forest genetic resources, or even the production of seeds.

5. Discussion

In this paper we have identified some priority actions and zones in order to advance in the conservation and use of forest genetic resources in four Mexican pine species. Using Forest Germplasm Procurement Zones defined by CONAFOR (2016) as a proxy for genetic zones, we identify 52 areas for delimiting conservation units in 16 genetic zones. Also, based on the existing data, we considered that six genetic zones should be the most suitable sources for use in reforestation for these species.

One of the main aspects to develop is considering the conservation and sustainable use of forest resources as part of forest management in Mexico, currently, the main conservation program (defined by CONABIO) does not include forest tree species. The election of areas for conservation of genetic resources could be efficiently established by using information of different sources. Usually, in absence of genetic information, it will be necessary to cover the ecological distribution range of the species (Lefèvre et al. 2013). In our case, we are able to cover the different areas of the species by using the forest germplasm procurement zones, where some genetic information is available (Wehenkel et al. 2017). Most of the Mexican pine species should have high genetic diversity, relatively low genetic differentiation among populations for neutral or nearly neutral markers (Galicia et al., 2015 in Wehenkel et al. 2017), e.g. *P. oocarpa* has high genetic diversity (Wehenkel et al. 2017), and high genetic differentiation among populations along altitudinal gradients for adaptive traits, such as growth potential and frost resistance (Sáenz-Romero et al. 2006, 2012; Viveros-Viveros et al. 2009; Loya-Rebollar et al. 2013; Ruiz-Talonia et al. 2014; Ortiz-Bibian et al. 2017) while many exceptions with low genetic diversity figures also exist, such as some endemics and taxa with fragmented distributions (e.g. *P. greggii* (Parraguirre Lezama et al. 2002; Wehenkel et al. 2017)). Although, some works for *P. greggii* have reported the opposite (Ramírez-Herrera et al. 2005; López-Upton et al. 2005a) and it have been pointed that moving seed among populations increase maladaptation (Hernández Martínez et al. 2007). Therefore the definition of genetic zones based on the germplasm transfer zones is very conservative, in terms of not including in the same area populations that might differ genetically.

Within these areas, some of the minimum requirements can be based on the existing information, but there are two main topics that need further consideration. Firstly, the minimum population size and the demography of the population need a more precise evaluation. In a second step, it would be necessary to include in the management plan of the area three important aspects (National Research Council 1993): a) an explicit objective to maintain population variation, b) an established protocol for providing information on and access to the protected resources by *ex situ* collections, breeders, researchers, and other germplasm users, including a procedure for the sustainable collection of reproductive material by authorized agencies and individuals, and c) a

procedure for monitoring the status of the populations conserved as part a of a national genetic resources information system. Also, monitoring of these conservation units should be taken into consideration in order to maintain the genetic diversity of the species at the long term (Aravanopoulos 2016).

Additionally, considering climatic change, is needed to consider ex-situ conservation units at places where suitable climatic habitat for priority species will occur in the future, for which present forest tree populations are adapted after a long evolutionary history at in-situ contemporary reserves. A climatic zonification for contemporary climate and an homologous zonification future climatic change scanerios, can be used as guideline to consider sites of ex-situ conservation units that will serve as germplasm sources under future (likely warmer and dryer) climate (Sáenz-Romero et al. 2010, 2016; Castellanos-Acuña et al. 2017).

The indicators for the use of forest reproductive material indicates, that despite the economical importance of the species, most of the activities should be based on low-input strategies. These strategies focus on allowing the production of local genetic resources for use in forest management activities, however have at present many gaps. Only some of the areas have enough seed areas.

The identification of only few areas with interest in production of forest reproductive material will allow a more precise planning of breeding activities, and also including the *in situ* conservation as a main target for forest management. A broader vision for *in situ* forest conservation recognises that achieving and sustaining forest conservation also requires the integration of social and economic goals into conservation planning processes.

On the other hand, in Mexico there is a continuous challenge to improve the use and conservation of genetic resources face next climatic changes or changes in land use (e.g. conversion from *P. oocarpa* and *P. pseudostrobus* forests to avocado orchards (Chávez-León et al. 2012; Bravo-Espinosa et al. 2012)). It was predicted a loss of forest coverage for *P. oocarpa* (50.4 %), *P. patula* (23.4 %) and *P. pseudostrobus* (12.8 %) for 2050 (Villers-Ruiz and Trejo-Vázquez 1998). This situation had motivated to propose conservation areas (Rojas-Soto et al. 2012; Sáenz-Romero et al. 2003) but neither governmental authorities nor nongovernmental agencies have set or promoted an intense *in situ* conservation, e.g. the Mexican National Council on Natural Protected Areas has not considered species vulnerability to climate change as part of its conservation priorities (Villers-Ruiz and Trejo-Vázquez 1998). In order to respond to the challenges posed by the rapid erosion of biodiversity and global climate change, forests conservation and protection policies must rely on polycentric and democratic governance schemes (Merino-Perez 2013). Although few attempts have done for official conservation policies but are largely perceived by rural societies as an unfair governmental imposition, resulting in

frequent processes of land use change and forest deterioration (Merino-Perez 2013). Cooperation between communal groups and state and federal governments in Mexico will be a key requirement in order for conservation of genetic resources to be successful.

The use of genetic resources for management programs is a major problem in the country. For example, in reforestation is still necessary to reduce main problems for high seedling mortality such as frost damage (e.g. 14% (Sáenz-Romero and Tapia-Olivares 2008)), drought and poor seedling quality (e.g. 66% (Burney et al. 2015)), inappropriate time of planting (e.g. 50% (FAO-CONAFOR 2012)), marginal seedling transportation and storage facilities (e.g. 32.8% of seedlings are lost in this stage), and inadequate infrastructure (Valtierra Pacheco and Magaña Torres 2008; Burney et al. 2015). In some cases, the problems are associated to incorrect selection and lack of knowledge of seed source e.g. 33% seed source used is unknown (Burney et al. 2015), though it could be more, and poor adaptation to local environmental and climatic conditions on the reforestation site (Sáenz-Romero and Lindig-Cisneros 2004). Other problems for species are reduction on fitness due to outbreeding depression (McKay et al. 2005), confusing on infraspecific taxa, e.g. *P. pseudostrobus* (Stead and Styles 1984). Overall, the number of forest germplasm production units used in Mexico is little significant and in most of the cases seed for seedling production is from natural stands, and sometimes seed quality information is unknown (Valtierra Pacheco and Magaña Torres 2008). However, perhaps the bottom line of this low-survival seedling problem, is that reforestation programs in México actually are temporal labour-paid job programs, to aminorate poverty in rural areas. In other words, federal and state subsidies are spend on that basis of number of seedlings produced in the forest nurseries and planted in the field, rather than on actual survival rate after, say, 5-years-old or so.

The establishment of genetic conservation units could be able to recover deforested areas (i.e. around 190,000 ha/year in southeastern Mexico, 96,000 ha/year in the northwest, and 62,000 ha/year in the west (INEGI 2010; Guerra-De la Cruz and Galicia 2017)), and to help government strategies for forestry sector at national level, e.g. further intensification of harvesting of natural forests, the execution of aggressive reforestation programs, and the establishment of commercial plantations (Comisión Nacional Forestal 2001). These strategies can be summarized as a transition from natural forests to planted forests in Mexico, since all of them lead to the establishment of forested and reforested areas at various scales, intensities, and objectives (Guerra-De la Cruz and Galicia 2017). The National Strategy for Sustainable Forest Management and the 2025 Strategic Forest Program, considers that sustainable forest management in Mexico must harness and increase the productivity of forest ecosystems through improved silvicultural practices but must also recognize and generate alternatives for other benefits (e.g. biodiversity conservation).

Government and academic institutions are proposed to establisht some conservation units in protected areas whose objectives are ensuring preservation of habitats for endangered fauna and flora, but this proposal does not rely on genetic conservation criteria. Protected areas alone do not fulfill all specific requirements for the conservation of forest genetic (Koski et al., 1997). Also, these areas are affected by illegal extraction of various forest products (e.g. logging, conversion of forest timber into charcoal (Wallace et al. 2015)) that alter their status. This areas must receive a special attention, during conservation unit management, in order to be viable.

In Mexico, most of forested land (75 %) is under collective tenure, and more than 50 % of all collective holdings are forest communities (Merino-Perez 2013). Overall, 39,600,000 hectares are collectively owned by ejidos and comunidades agrarias (Madrid et al. 2009). An ejido is a land owned and managed by a group (i.e. between 50 and 150 people or exceptionally more than 1000) (Silva-Flores et al. 2016) whereas the comunidades agrarias are areas that were restituted to indigenous communities after that their historical rights were officially recognized (Merino-Perez 2013). It is considering that deforestation problem is due to collective property and use of natural resources by the rural poor, as the main drivers (Merino-Perez 2013); and it is showing that woody cover gains have occurred in regions where migration have been important (Rudel 2008).

6. Conclusion

We identified genetic conservation areas for delimiting Genetic Conservation Units in four important Mexican pine species, and defined principal steps for their conservation and sustainable use in this country. The number of areas proposed were different among species and relied on a minimum requirements that must be follow for conservation. The seed procurement zones were the hub of conservation proposal which ensured the uniform genotypic or phenotypic characteristics for conservations units. However, it is necessary to develop genetic studies in order to advance in the conservation of the species in the long term, and stablishing Conservation Units particularly in procurement seed zones with high forest species diversity. We found that the efforts for use of forest reproductive material are limited and they are mainly focused on forest germplasm production units (seed stands and seed area). Actions must be initiated to improve the use and conservation of genetic resources, specially considering the challenges imposed by the ongoing face current climatic changes and pressure for land-use change to other uses, like agriculture, grazing and urban development.

7. References

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Conclusions / Conclusiones

Conclusions

1. The European and American pines studied display important phenotypic variations among their natural populations –at local and regional scales-, which will play an important role during decision-making for the next the climatic changes (Chapters I, II and III).
2. Environmental heterogeneity is a factor which shapes part of the intrapopulation phenotypic variation for Mediterranean pines. Environmental heterogeneity must be modelled for the management of genetic resources based on phenotypic responses in field trials, e.g. height, depend on species (Chapter I).
3. Mediterranean pines do not have a clear relationship among phenotypic variance and phenotypic plasticity and environmental heterogeneity. Nonetheless, there are specific signs that precipitation plays an important role for *P. sylvestris*, and temperature and precipitation for *P. pinaster* and *P. halepensis* (Chapter I).
4. Early testing of Mexican pine seedlings under stressful conditions can provide useful information for evaluating the genetic resources under future environmental changes (e.g. decrease in annual precipitation). Their performance would provide insight for management of forst genetic resoucres at a local scale (Chapter II).
5. The intraspecific variation and phenotypic plasticity are important factors for conservation activities at local scales. Also, different provenances can be used in selection and or breeding programs e.g. improvement drought tolerance/resistance, and increase the growth of selections for reforestation programs (Chapter II and III).
6. We provide information to efficiently integrate breeding and conservation of genetic resouces in forest management activities in Mexico. It would be necessary to develop an effective network for conservation of genetic resources linked with susteinable forest management and forest restoration programs. This action must ensure the adaptive capacity of the species at the long term (Chapter III).
7. It is necessary to increase the efforts for conservation of Mexican pines and include a more intense conservation program in current government institutions. However, there are advances conservation works at national level but it is recommend to increase them in order to improve the use of forest genetic resources (Chapter III).

Conclusiones

1. Los pinos europeos y americanos estudiados muestran importantes variaciones fenotípicas entre sus poblaciones naturales, a escala local y regional, que desempeñarán un papel importante durante la toma de decisiones para los próximos cambios climáticos (Capítulos I, II y III).
2. La heterogeneidad ambiental es un factor que forma parte de la variación fenotípica intrapoblacional de los pinos mediterráneos. La heterogeneidad ambiental debe modelarse para el manejo de recursos genéticos con base en respuestas fenotípicas en ensayos de campo, como por ejemplo la altura que depende de la especie (Capítulo I).
3. Los pinos mediterráneos no tienen una relación clara entre la varianza fenotípica y la plasticidad fenotípica y la heterogeneidad ambiental. No obstante, hay signos específicos de que la precipitación juega un papel importante para *P. sylvestris*, y la temperatura y precipitación para *P. pinaster* y *P. halepensis* (Capítulo I).
4. Las pruebas tempranas de plántulas de pino mexicano bajo condiciones estresantes pueden proporcionar información útil para evaluar los recursos genéticos para futuros cambios ambientales (por ejemplo, disminución en la precipitación anual). Su respuesta proporcionaría información para el manejo de recursos genéticos a escala local (Capítulo II).
5. La variación intraespecífica y la plasticidad fenotípica son factores importantes para las actividades de conservación a escala local. Además, se pueden usar diferentes procedencias en programas de selección y/o memora, por ejemplo mejorar la tolerancia/resistencia a la sequía y aumentar el crecimiento de las selecciones para los programas de reforestación (Capítulo II y III).
6. Brindamos información para integrar de manera eficiente la mejora genética y la conservación de los recursos genéticos en las actividades de manejo forestal en México. Sería necesario desarrollar una red efectiva para la conservación de los recursos genéticos vinculados con el manejo forestal sostenible y los programas de restauración forestal. Esta acción debe garantizar la capacidad de adaptación de las especies a largo plazo (Capítulo III).
7. Es necesario aumentar los esfuerzos para la conservación de los pinos mexicanos e incluir un programa de conservación más intenso en las instituciones gubernamentales actuales. Sin embargo, existen avances en trabajos de conservación a nivel nacional, pero se recomienda aumentarlos para mejorar el uso de los recursos genéticos forestales (Capítulo III).

Annex

Supplementary material (Chapter I)

Table S1. Description of provenance tests by species.

Table S2. Summary of models obtained for each phenotypic variable, geographical spatial scale and species.

Table S3. Summary of models for different phenotypic variable, based on its level of empirical support for the 50 km2 scale.

Table S1. Description of provenance tests by species.

Site	Species	Longitude	Latitude	Altitude	Age	# Blocks	Plot size (# trees)	Spacing (m)
Aragües	*P. sylvestris*	0º37' W	42º44' N	1370	13	4	16	2.5 x 2.5
Baza	*P. sylvestris*	2º56' W	37º21' N	1850	13	4	16	2.5 x 2.5
Curueño	*P. sylvestris*	5º21' W	42º46' N	1150	13	4	16	2.5 x 2.5
Gúdar	*P. sylvestris*	0º35' W	40º27' N	1680	13	4	16	2.5 x 2.5
Manzanal	*P. sylvestris*	6º09' W	42º29' N	1350	13	4	16	2.5 x 2.5
Navafría	*P. sylvestris*	3º49' W	41º02' N	1600	13	4	16	2.5 x 2.5
Acebo	*P. pinaster*	6º41'W	40º10' N	530	18	4	16	2.5 x 2.5
Cabañeros	*P. pinaster*	4º23' W	39º22' N	1020	18	4	16	2.5 x 2.5
Calderona	*P. pinaster*	3º33' W	38º21' N	753	18	4	16	2.5 x 2.5
Espinoso	*P. pinaster*	4º49' W	30º36' N	830	18	4	16	2.5 x 2.5
Miravete	*P. pinaster*	5º42' W	39º42' N	660	18	4	16	2.5 x 2.5
Ríofrío	*P. pinaster*	4º30' W	39º05' N	730	18	4	16	2.5 x 2.5
Ademuz	*P. halepensis*	1º17' W	40º30' N	850	12	4	4	2.5 x 2.5
Cucalón	*P. halepensis*	0º34' W	39º49' N	640	12	4	4	2.5 x 2.5
Valdeolmos	*P. halepensis*	3º26' W	40º38' N	730	12	4	4	3.0 x 2.5
Zaragoza	*P. halepensis*	0º49' W	41º43' N	215	12	4	4	2.5 x 3.0
Zuera	*P. halepensis*	0º38' W	41º52' N	350	12	4	4	2.5 x 2.0

Table S2. Summary of models obtained for each phenotypic variable, geographical spatial scale and species.

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
P. sylvestris										
V*d*	10	327.282	28.2836		28.4413	-41.8348	0.306	124.720	0.000	0.157
		327.282	13.8197				0.084	125.160	0.440	0.126
		327.282	27.0246			-18.8737	0.165	125.691	0.971	0.097
		327.282			10.6865		0.050	125.741	1.021	0.094
		327.282		5.4202			0.013	126.360	1.640	0.069
		327.282			26.8324	-20.7973	0.126	126.409	1.689	0.068
		327.282	31.9108	-8.5789	30.3919	-39.4729	0.317	126.468	1.748	0.066
		327.282				0.0338	0.000	126.569	1.849	0.062
		327.282	21.3239	-10.2268			0.106	126.783	2.063	0.056
		327.282	11.3259		4.7906		0.092	127.030	2.310	0.050
		327.282	27.3732	-0.8448		-18.486	0.165	127.689	2.969	0.036
		327.282		-2.9241	12.6199		0.053	127.705	2.985	0.035
		327.282		12.2323		-9.1116	0.029	128.096	3.376	0.029
		327.282	20.455	-17.0831	11.3337		0.137	128.204	3.484	0.028
		327.282		7.601	25.2869	-25.2803	0.137	128.209	3.489	0.027
	100	273.865				-9.040	0.009	339.089	0.000	0.157
		273.865	-4.546				0.002	339.344	0.255	0.138
		273.865			-2.753		0.001	339.399	0.310	0.135
		273.865		1.806			0.000	339.417	0.328	0.133
		273.865		4.881		-10.473	0.012	340.997	1.908	0.061
		273.865	-2.168			-8.429	0.010	341.071	1.982	0.058
		273.865			1.603	-9.756	0.009	341.080	1.991	0.058
		273.865	-9.984	8.327			0.007	341.178	2.089	0.055
		273.865		9.832	-10.366		0.005	341.237	2.148	0.054
		273.865	-4.521		-0.041		0.002	341.344	2.255	0.051
		273.865	-8.466	10.165		-9.636	0.016	342.827	3.738	0.024
		273.865		8.635	-5.334	-9.194	0.013	342.956	3.867	0.023
		273.865	-4.305		4.151	-9.679	0.011	343.030	3.941	0.022
		273.865	-8.615	13.891	-8.341		0.010	343.066	3.977	0.022
		273.865	-7.995	12.453	-3.669	-8.803	0.017	344.807	5.718	0.009
V*h*	10	3854.580		200.716			0.204	194.365	0.000	0.196
		3854.580	187.843				0.179	194.868	0.503	0.153
		3854.580		262.712	-93.762		0.230	195.852	1.487	0.093
		3854.580	87.878	136.233			0.223	195.997	1.632	0.087
		3854.580		236.613		-48.013	0.210	196.261	1.896	0.076
		3854.580				128.887	0.084	196.616	2.251	0.064
		3854.580	200.582		-24.472		0.181	196.825	2.460	0.057
		3854.580	191.318			-4.967	0.179	196.867	2.502	0.056
		3854.580	95.527	196.587	-99.768		0.251	197.404	3.039	0.043
		3854.580			79.944		0.032	197.498	3.133	0.041
		3854.580	116.664	180.878		-87.967	0.238	197.678	3.313	0.037
		3854.580		255.398	-102.564	17.567	0.230	197.842	3.477	0.035
		3854.580			-50.636	168.198	0.089	198.525	4.160	0.025
		3854.580	189.554		-39.853	27.207	0.182	198.804	4.439	0.021

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
		3854.580	103.832	202.752	-85.953	-28.613	0.252	199.381	5.016	0.016
	100	3854.580	166.511				0.141	195.599	0.000	0.188
		3854.580		158.841			0.128	195.833	0.234	0.167
		3854.580				102.246	0.053	197.153	1.554	0.086
		3854.580			88.789		0.040	197.372	1.773	0.077
		3854.580	112.082	65.029			0.147	197.479	1.880	0.073
		3854.580	177.292		-17.913		0.142	197.579	1.980	0.070
		3854.580	159.399			12.654	0.141	197.588	1.989	0.069
		3854.580		148.449	23.701		0.130	197.791	2.192	0.063
		3854.580		155.709		5.016	0.128	197.831	2.232	0.061
		3854.580			28.912	80.829	0.055	199.121	3.522	0.032
		3854.580	121.112	62.519	-11.513		0.148	199.471	3.872	0.027
		3854.580	112.378	66.183		-2.245	0.147	199.478	3.879	0.027
		3854.580	170.568		-41.197	36.894	0.145	199.524	3.925	0.026
		3854.580		157.185	37.316	-23.549	0.131	199.773	4.174	0.023
		3854.580	126.204	54.376	-20.055	12.213	0.148	201.467	5.868	0.010
Vs	10	0.038	-0.004				0.224	-151.821	0.000	0.154
		0.038		-0.006	0.004		0.294	-151.328	0.493	0.120
		0.038	-0.006		0.003		0.283	-151.076	0.745	0.106
		0.038		-0.004			0.187	-151.065	0.756	0.106
		0.038	-0.003	-0.004	0.004		0.358	-150.857	0.964	0.095
		0.038	-0.003	-0.002			0.240	-150.144	1.677	0.067
		0.038	-0.005			0.001	0.232	-149.982	1.839	0.061
		0.038		-0.006	0.005	-0.002	0.310	-149.686	2.135	0.053
		0.038	-0.005		0.004	-0.002	0.293	-149.311	2.510	0.044
		0.038		-0.005		0.001	0.193	-149.199	2.622	0.042
		0.038				-0.002	0.072	-148.955	2.866	0.037
		0.038	-0.003	-0.004	0.004	-0.001	0.360	-148.890	2.931	0.036
		0.038	-0.004	-0.003		0.002	0.268	-148.758	3.063	0.033
		0.038			0.004	-0.005	0.143	-148.235	3.586	0.026
		0.038			0.000		0.002	-147.786	4.035	0.020
	100	0.038	-0.006				0.439	-156.998	0.000	0.194
		0.038		-0.006			0.437	-156.958	0.040	0.191
		0.038	-0.003	-0.003			0.477	-156.125	0.873	0.126
		0.038		-0.006	-0.001		0.447	-155.251	1.747	0.081
		0.038	-0.006			-0.001	0.441	-155.075	1.923	0.074
		0.038	-0.006		0.000		0.439	-155.013	1.985	0.072
		0.038		-0.006		0.000	0.437	-154.958	2.040	0.070
		0.038	-0.003	-0.003		0.000	0.477	-154.132	2.866	0.046
		0.038	-0.003	-0.003	0.000		0.477	-154.126	2.872	0.046
		0.038		-0.006	-0.002	0.001	0.455	-153.469	3.529	0.033
		0.038	-0.006		0.001	-0.001	0.446	-153.207	3.791	0.029
		0.038	-0.003	-0.004	0.000	0.000	0.478	-152.152	4.846	0.017
		0.038				-0.004	0.172	-150.785	6.213	0.009
		0.038			-0.003		0.145	-150.266	6.732	0.007
		0.038			-0.001	-0.003	0.184	-149.016	7.982	0.004

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
PI*d*	10	0.844			-0.040	0.033	0.256	-95.647	0.000	0.179
		0.844		0.025	-0.031		0.223	-94.941	0.705	0.125
		0.844	0.021		-0.025		0.206	-94.602	1.044	0.106
		0.844		0.015	-0.043	0.024	0.294	-94.486	1.161	0.100
		0.844			-0.014		0.080	-94.255	1.392	0.089
		0.844	0.010		-0.039	0.025	0.278	-94.128	1.519	0.084
		0.844	0.012	0.017	-0.032		0.247	-93.460	2.187	0.060
		0.844	0.008				0.024	-93.303	2.344	0.055
		0.844		0.005			0.009	-93.060	2.586	0.049
		0.844				0.002	0.002	-92.945	2.702	0.046
		0.844	0.005	0.012	-0.042	0.022	0.298	-92.584	3.063	0.039
		0.844	0.012			-0.006	0.032	-91.436	4.211	0.022
		0.844	0.009	-0.002			0.025	-91.315	4.332	0.020
		0.844		0.007		-0.003	0.011	-91.088	4.559	0.018
		0.844	0.011	0.002		-0.007	0.032	-89.442	6.205	0.008
	100	0.844			-0.033	0.042	0.314	-96.945	0.000	0.258
		0.844	0.011		-0.037	0.039	0.341	-95.598	1.347	0.132
		0.844		0.006	-0.033	0.037	0.323	-95.163	1.782	0.106
		0.844				0.017	0.119	-94.940	2.005	0.095
		0.844		0.015			0.094	-94.491	2.454	0.076
		0.844	0.017	-0.007	-0.040	0.042	0.346	-93.707	3.238	0.051
		0.844	0.010				0.040	-93.574	3.371	0.048
		0.844		0.007		0.013	0.133	-93.189	3.757	0.039
		0.844		0.020	-0.011		0.132	-93.179	3.766	0.039
		0.844	0.001			0.017	0.119	-92.942	4.004	0.035
		0.844			-0.002		0.002	-92.941	4.004	0.035
		0.844	-0.009	0.023			0.104	-92.673	4.272	0.030
		0.844	0.018		-0.013		0.082	-92.277	4.668	0.025
		0.844	-0.011	0.016		0.013	0.147	-91.456	5.489	0.017
		0.844	-0.001	0.021	-0.011		0.132	-91.181	5.765	0.014
PI*h*	10	0.690	0.023		-0.057	0.040	0.614	-101.782	0.000	0.421
		0.690			-0.058	0.057	0.517	-100.192	1.590	0.190
		0.690	0.026	-0.007	-0.055	0.042	0.619	-100.023	1.759	0.175
		0.690	0.040		-0.034		0.461	-98.455	3.327	0.080
		0.690		0.006	-0.059	0.054	0.522	-98.389	3.393	0.077
		0.690	0.039	0.002	-0.035		0.462	-96.469	5.313	0.030
		0.690	0.022				0.164	-93.436	8.346	0.006
		0.690		0.029	-0.033		0.225	-92.633	9.149	0.004
		0.690	0.036	-0.019			0.224	-92.613	9.169	0.004
		0.690			-0.014		0.065	-91.633	10.149	0.003
		0.690	0.026			-0.006	0.171	-91.559	10.223	0.003
		0.690				0.012	0.051	-91.406	10.376	0.002
		0.690		0.007			0.017	-90.846	10.936	0.002
		0.690	0.035	-0.021		0.004	0.225	-90.651	11.131	0.002

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
		0.690		-0.005		0.016	0.054	-89.460	12.322	0.001
	100	0.690	0.022		-0.028	0.041	0.536	-98.847	0.000	0.198
		0.690	0.041	-0.023	-0.037	0.052	0.574	-98.222	0.625	0.145
		0.690				0.033	0.378	-98.172	0.675	0.141
		0.690			-0.019	0.047	0.433	-97.639	1.208	0.108
		0.690	0.015			0.025	0.429	-97.527	1.320	0.102
		0.690		0.011		0.026	0.404	-96.837	2.010	0.072
		0.690		0.010	-0.018	0.040	0.455	-96.273	2.574	0.055
		0.690	0.029				0.283	-95.878	2.969	0.045
		0.690	0.016	-0.002		0.025	0.429	-95.534	3.313	0.038
		0.690		0.027			0.258	-95.348	3.499	0.034
		0.690	0.019	0.011			0.296	-94.180	4.667	0.019
		0.690	0.030		-0.002		0.283	-93.893	4.954	0.017
		0.690		0.025	0.005		0.266	-93.503	5.344	0.014
		0.690	0.020	0.011	-0.001		0.296	-92.182	6.665	0.007
		0.690			0.016		0.089	-92.062	6.785	0.007
PIs	10	0.448		-0.051			0.163	-66.318	0.000	0.216
		0.448				-0.036	0.084	-64.881	1.437	0.105
		0.448			-0.035		0.079	-64.805	1.514	0.101
		0.448	0.026	-0.070			0.183	-64.708	1.611	0.097
		0.448		-0.048	-0.003		0.163	-64.326	1.992	0.080
		0.448		-0.053		0.003	0.163	-64.324	1.994	0.080
		0.448	-0.025				0.040	-64.129	2.189	0.072
		0.448			-0.018	-0.022	0.092	-63.025	3.293	0.042
		0.448	0.001			-0.037	0.084	-62.881	3.437	0.039
		0.448	-0.009		-0.031		0.083	-62.871	3.447	0.039
		0.448	0.028	-0.067		-0.006	0.184	-62.727	3.591	0.036
		0.448	0.027	-0.067	-0.005		0.184	-62.726	3.593	0.036
		0.448		-0.052	-0.008	0.008	0.164	-62.351	3.968	0.030
		0.448	0.000		-0.018	-0.022	0.092	-61.025	5.293	0.015
		0.448	0.028	-0.066	-0.003	-0.004	0.184	-60.731	5.587	0.013
	100	0.448			0.094	-0.089	0.279	-66.708	0.000	0.216
		0.448	0.036		0.080	-0.098	0.328	-65.847	0.861	0.140
		0.448		0.031	0.096	-0.110	0.317	-65.575	1.133	0.122
		0.448	0.057			-0.051	0.166	-64.390	2.318	0.068
		0.448	0.029				0.052	-64.337	2.372	0.066
		0.448			0.029		0.052	-64.333	2.375	0.066
		0.448	0.030	0.007	0.082	-0.101	0.329	-63.860	2.848	0.052
		0.448				-0.019	0.023	-63.850	2.858	0.052
		0.448	0.082	-0.063			0.129	-63.684	3.024	0.048
		0.448		0.005			0.002	-63.505	3.203	0.043
		0.448	0.087	-0.042		-0.042	0.196	-62.968	3.740	0.033
		0.448	0.018		0.018		0.065	-62.555	4.154	0.027
		0.448		-0.009	0.033		0.056	-62.409	4.299	0.025
		0.448		0.028		-0.036	0.052	-62.340	4.369	0.024
		0.448	0.073	-0.061	0.012		0.134	-61.782	4.926	0.018

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
P. pinaster										
V*d*	10	1475.480		103.466		-96.002	0.087	499.421	0.000	0.124
		1475.480	104.757			-100.176	0.083	499.594	0.173	0.113
		1475.480		162.448	-139.925		0.083	499.621	0.200	0.112
		1475.480	160.054		-140.146		0.073	500.100	0.679	0.088
		1475.480		42.172			0.021	500.477	1.056	0.073
		1475.480		159.286	-87.963	-65.006	0.105	500.571	1.150	0.070
		1475.480	37.594				0.017	500.674	1.253	0.066
		1475.480	162.677		-87.140	-72.996	0.100	500.785	1.364	0.062
		1475.480				-29.943	0.011	500.951	1.530	0.057
		1475.480	78.684	109.502	-163.169		0.092	501.197	1.776	0.051
		1475.480	46.450	64.356		-102.174	0.090	501.257	1.836	0.049
		1475.480			-0.290		0.000	501.429	2.008	0.045
		1475.480	88.566	99.519	-111.291	-68.553	0.116	502.023	2.602	0.034
		1475.480			52.551	-69.753	0.025	502.317	2.896	0.029
		1475.480	-10.576	51.974			0.022	502.469	3.048	0.027
	100	1475.480		108.871	-104.117	-72.987	0.337	487.365	0.000	0.264
		1475.480		80.487	-133.802		0.293	488.176	0.811	0.176
		1475.480	-65.717	157.665	-80.797	-66.926	0.349	488.552	1.187	0.146
		1475.480	-83.324	145.343	-101.107		0.313	488.914	1.549	0.122
		1475.480	-134.621	219.272		-87.583	0.305	489.434	2.069	0.094
		1475.480			-133.699		0.215	490.776	3.411	0.048
		1475.480	50.515		-153.570		0.241	491.297	3.932	0.037
		1475.480	-184.175	223.762			0.238	491.463	4.098	0.034
		1475.480		128.174		-122.901	0.232	491.829	4.464	0.028
		1475.480	74.208		-140.590	-54.763	0.265	491.860	4.495	0.028
		1475.480			-124.645	-22.233	0.220	492.497	5.132	0.020
		1475.480		80.316			0.078	497.875	10.510	0.001
		1475.480				-72.989	0.064	498.515	11.150	0.001
		1475.480	39.376			-93.695	0.078	499.876	12.511	0.001
		1475.480	-9.895				0.001	501.377	14.012	0.000
V*h*	10	14349.050	-986.000			1302.800	0.107	703.736	0.000	0.190
		14349.050		-878.271		1202.500	0.098	704.171	0.435	0.153
		14349.050				641.750	0.047	704.625	0.889	0.122
		14349.050			-790.860	1240.860	0.077	705.210	1.474	0.091
		14349.050	-802.320	-202.735		1309.100	0.108	705.704	1.968	0.071
		14349.050	-1072.430		130.040	1262.240	0.108	705.719	1.983	0.070
		14349.050		-855.074	-36.560	1215.380	0.098	706.169	2.433	0.056
		14349.050			149.150		0.003	706.615	2.879	0.045
		14349.050	-112.540				0.001	706.663	2.927	0.044
		14349.050		-110.521			0.001	706.665	2.929	0.044
		14349.050	-1027.070		1046.610		0.031	707.351	3.615	0.031
		14349.050		-914.178	934.950		0.027	707.511	3.775	0.029
		14349.050	-875.780	-264.068	194.120	1250.450	0.109	707.668	3.932	0.027
		14349.050	-71.680	-44.090			0.001	708.661	4.925	0.016

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
		14349.050	-695.530	-446.163	1140.420		0.034	709.218	5.482	0.012
	100	14349.050	890.730				0.090	702.585	0.000	0.260
		14349.050	990.760		-254.272		0.096	704.285	1.700	0.111
		14349.050	1062.780	-220.892			0.092	704.480	1.895	0.101
		14349.050	882.270			16.091	0.090	704.584	1.999	0.096
		14349.050		606.865			0.042	704.852	2.267	0.084
		14349.050				480.047	0.026	705.563	2.978	0.059
		14349.050	1525.470	-580.674	-463.871		0.107	705.755	3.170	0.053
		14349.050	950.700		-276.215	92.581	0.097	706.253	3.668	0.041
		14349.050	1057.150	-220.382		9.948	0.092	706.479	3.894	0.037
		14349.050		494.991		287.293	0.050	706.487	3.902	0.037
		14349.050			135.464		0.002	706.634	4.049	0.034
		14349.050		606.693	134.688		0.044	706.758	4.173	0.032
		14349.050			-71.941	509.342	0.027	707.541	4.956	0.022
		14349.050	1488.810	-606.330	-506.162	139.356	0.108	707.683	5.098	0.020
		14349.050		499.101	22.166	276.667	0.050	708.485	5.900	0.014
Vs	10	0.082				0.012	0.094	-287.116	0.000	0.234
		0.082			-0.010	0.020	0.123	-286.558	0.558	0.177
		0.082		-0.004		0.015	0.101	-285.438	1.678	0.101
		0.082	-0.004			0.015	0.100	-285.422	1.694	0.100
		0.082	0.006		-0.015	0.020	0.129	-284.851	2.265	0.076
		0.082		0.005	-0.015	0.020	0.128	-284.806	2.310	0.074
		0.082	0.006				0.022	-283.726	3.390	0.043
		0.082		0.005			0.018	-283.555	3.561	0.040
		0.082	-0.002	-0.003		0.015	0.101	-283.452	3.664	0.038
		0.082			0.005		0.015	-283.413	3.703	0.037
		0.082	0.004	0.002	-0.016	0.020	0.130	-282.877	4.239	0.028
		0.082	0.007		-0.001		0.022	-281.737	5.379	0.016
		0.082	0.006	-0.001			0.022	-281.729	5.387	0.016
		0.082		0.004	0.001		0.018	-281.562	5.554	0.015
		0.082	0.007	0.000	-0.001		0.022	-279.737	7.379	0.006
	100	0.082	-0.021	0.025		0.012	0.226	-290.032	0.000	0.228
		0.082	-0.029	0.032	0.009	0.009	0.259	-289.949	0.083	0.219
		0.082	-0.027	0.033	0.012		0.222	-289.829	0.203	0.206
		0.082	-0.014	0.024			0.162	-288.567	1.465	0.109
		0.082		0.013			0.108	-287.813	2.219	0.075
		0.082		0.010		0.006	0.129	-286.831	3.201	0.046
		0.082		0.013	0.002		0.110	-285.915	4.117	0.029
		0.082				0.010	0.067	-285.838	4.194	0.028
		0.082		0.010	-0.001	0.006	0.129	-284.851	5.181	0.017
		0.082			-0.003	0.011	0.072	-284.042	5.990	0.011
		0.082	-0.001			0.011	0.068	-283.883	6.149	0.011
		0.082	0.004				0.012	-283.307	6.725	0.008
		0.082			0.002		0.002	-282.858	7.174	0.006
		0.082	-0.001		-0.003	0.012	0.072	-282.054	7.978	0.004
		0.082	0.004		0.000		0.012	-281.307	8.725	0.003

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
PI*d*	10	0.424				0.032	0.051	-170.648	0.000	0.217
		0.424		-0.019		0.044	0.062	-169.140	1.508	0.102
		0.424			-0.021	0.048	0.060	-169.068	1.580	0.098
		0.424	-0.014			0.042	0.056	-168.897	1.751	0.090
		0.424			0.016		0.012	-168.868	1.780	0.089
		0.424	0.014				0.009	-168.757	1.891	0.084
		0.424		0.009			0.004	-168.527	2.121	0.075
		0.424	0.013	-0.030		0.043	0.063	-167.194	3.454	0.039
		0.424		-0.013	-0.009	0.048	0.062	-167.175	3.473	0.038
		0.424	-0.001		-0.020	0.048	0.060	-167.068	3.580	0.036
		0.424		-0.016	0.029		0.015	-167.008	3.640	0.035
		0.424	0.037	-0.025			0.014	-166.952	3.696	0.034
		0.424	0.001		0.015		0.012	-166.868	3.780	0.033
		0.424	0.019	-0.026	-0.014	0.047	0.064	-165.269	5.379	0.015
		0.424	0.025	-0.033	0.022		0.019	-165.176	5.472	0.014
	100	0.424		0.044			0.096	-172.765	0.000	0.176
		0.424	0.042				0.089	-172.425	0.340	0.149
		0.424	0.057			-0.028	0.117	-171.819	0.946	0.110
		0.424		0.051		-0.018	0.109	-171.430	1.335	0.090
		0.424	0.051		-0.021		0.107	-171.319	1.446	0.086
		0.424	0.021	0.028			0.104	-171.167	1.598	0.079
		0.424		0.044	-0.001		0.096	-170.768	1.997	0.065
		0.424	0.036	0.027		-0.028	0.131	-170.508	2.257	0.057
		0.424	0.061		-0.015	-0.024	0.126	-170.275	2.490	0.051
		0.424		0.053	0.008	-0.022	0.112	-169.548	3.217	0.035
		0.424	0.036	0.016	-0.015		0.111	-169.503	3.262	0.034
		0.424	0.042	0.021	-0.007	-0.026	0.132	-168.583	4.182	0.022
		0.424				0.002	0.000	-168.347	4.418	0.019
		0.424			-0.001		0.000	-168.341	4.424	0.019
		0.424			-0.002	0.003	0.000	-166.355	6.410	0.007
PI*h*	10	0.518				0.027	0.043	-178.900	0.000	0.208
		0.518			-0.022	0.043	0.055	-177.477	1.423	0.102
		0.518			0.011		0.007	-177.294	1.606	0.093
		0.518		0.011			0.007	-177.286	1.614	0.093
		0.518	0.010				0.006	-177.239	1.661	0.091
		0.518	-0.015			0.037	0.050	-177.231	1.669	0.090
		0.518		-0.011		0.034	0.047	-177.091	1.809	0.084
		0.518		0.007	-0.029	0.044	0.056	-175.517	3.383	0.038
		0.518	0.000		-0.022	0.043	0.055	-175.477	3.423	0.038
		0.518		0.005	0.006		0.007	-175.313	3.587	0.035
		0.518	0.001		0.010		0.007	-175.296	3.604	0.034
		0.518	-0.001	0.011			0.007	-175.286	3.614	0.034
		0.518	-0.021	0.007		0.036	0.050	-175.249	3.651	0.033
		0.518	-0.011	0.015	-0.026	0.044	0.057	-173.558	5.342	0.014
		0.518	-0.005	0.009	0.008		0.008	-173.321	5.579	0.013
	100	0.518		0.052			0.161	-184.708	0.000	0.218

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
		0.518		0.059		-0.017	0.176	-183.513	1.195	0.120
		0.518	0.047				0.132	-183.194	1.514	0.102
		0.518	0.017	0.039			0.167	-183.046	1.662	0.095
		0.518		0.052	0.007		0.164	-182.853	1.855	0.086
		0.518	0.061			-0.026	0.162	-182.758	1.950	0.082
		0.518	0.031	0.038		-0.025	0.195	-182.547	2.161	0.074
		0.518		0.062	0.017	-0.026	0.190	-182.278	2.430	0.065
		0.518	0.052		-0.014		0.141	-181.679	3.029	0.048
		0.518	0.016	0.039	0.000		0.167	-181.047	3.661	0.035
		0.518	0.063		-0.008	-0.024	0.165	-180.916	3.792	0.033
		0.518	0.023	0.045	0.009	-0.028	0.198	-180.693	4.015	0.029
		0.518			0.007		0.003	-177.111	7.597	0.005
		0.518				0.006	0.002	-177.068	7.640	0.005
		0.518			0.006	0.003	0.003	-175.135	9.573	0.002
PIs	10	0.628	-0.013				0.004	-141.922	0.000	0.137
		0.628				0.011	0.003	-141.864	0.058	0.133
		0.628		-0.009			0.002	-141.828	0.094	0.131
		0.628			-0.003		0.000	-141.736	0.186	0.125
		0.628	-0.036			0.035	0.023	-140.734	1.188	0.076
		0.628		-0.027		0.028	0.015	-140.384	1.538	0.064
		0.628	-0.044		0.036		0.012	-140.279	1.643	0.060
		0.628			-0.026	0.030	0.011	-140.196	1.726	0.058
		0.628	-0.030	0.019			0.006	-139.981	1.941	0.052
		0.628		-0.026	0.020		0.005	-139.949	1.973	0.051
		0.628	-0.045		0.013	0.031	0.023	-138.772	3.150	0.028
		0.628	-0.050	0.015		0.035	0.023	-138.770	3.152	0.028
		0.628		-0.025	-0.004	0.029	0.015	-138.387	3.535	0.023
		0.628	-0.049	0.007	0.034		0.013	-138.286	3.636	0.022
		0.628	-0.053	0.011	0.010	0.032	0.024	-136.792	5.130	0.011
	100	0.628	0.020				0.011	-142.199	0.000	0.139
		0.628		0.020			0.011	-142.195	0.004	0.139
		0.628				-0.014	0.005	-141.964	0.235	0.124
		0.628			0.005		0.001	-141.754	0.445	0.111
		0.628	0.038			-0.034	0.033	-141.210	0.989	0.085
		0.628		0.030		-0.026	0.026	-140.873	1.326	0.072
		0.628	0.011	0.011			0.012	-140.256	1.943	0.053
		0.628		0.020	0.005		0.011	-140.222	1.977	0.052
		0.628	0.021		-0.004		0.011	-140.213	1.986	0.051
		0.628			0.013	-0.019	0.009	-140.121	2.078	0.049
		0.628	0.030	0.009		-0.034	0.034	-139.251	2.948	0.032
		0.628	0.037		0.005	-0.035	0.034	-139.231	2.968	0.032
		0.628		0.033	0.019	-0.035	0.033	-139.222	2.977	0.031
		0.628	0.011	0.011	0.000		0.012	-138.256	3.943	0.019
		0.628	0.021	0.018	0.012	-0.037	0.036	-137.343	4.856	0.012
P. halepensis										
V*d*	10	273.865				-9.040	0.009	339.089	0.000	0.157

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
		273.865	-4.546				0.002	339.344	0.255	0.138
		273.865			-2.753		0.001	339.399	0.310	0.135
		273.865		1.806			0.000	339.417	0.328	0.133
		273.865		4.881		-10.473	0.012	340.997	1.908	0.061
		273.865	-2.168			-8.429	0.010	341.071	1.982	0.058
		273.865			1.603	-9.756	0.009	341.080	1.991	0.058
		273.865	-9.984	8.327			0.007	341.178	2.089	0.055
		273.865		9.832	-10.366		0.005	341.237	2.148	0.054
		273.865	-4.521		-0.041		0.002	341.344	2.255	0.051
		273.865	-8.466	10.165		-9.636	0.016	342.827	3.738	0.024
		273.865		8.635	-5.334	-9.194	0.013	342.956	3.867	0.023
		273.865	-4.305		4.151	-9.679	0.011	343.030	3.941	0.022
		273.865	-8.615	13.891	-8.341		0.010	343.066	3.977	0.022
		273.865	-7.995	12.453	-3.669	-8.803	0.017	344.807	5.718	0.009
	100	272.564		22.317			0.050	337.527	0.000	0.168
		273.063	20.445				0.045	337.708	0.181	0.153
		272.144		29.041		-13.113	0.065	338.926	1.399	0.083
		272.703		26.063	-12.065		0.065	338.944	1.417	0.083
		273.274	24.236		-12.143		0.060	339.125	1.598	0.076
		272.588	10.853	14.617			0.057	339.260	1.733	0.071
		274.006			-4.775		0.003	339.336	1.809	0.068
		273.865				-0.140	0.000	339.430	1.903	0.065
		273.033	21.033			-3.482	0.047	339.655	2.128	0.058
		272.750	13.619	16.889	-13.640		0.076	340.525	2.998	0.038
		272.330		30.970	-9.918	-10.869	0.075	340.544	3.017	0.037
		272.207	7.199	23.177		-11.637	0.068	340.814	3.287	0.032
		273.271	24.252		-12.080	-0.212	0.060	341.125	3.598	0.028
		274.024			-5.263	1.508	0.003	341.327	3.800	0.025
		272.455	10.609	22.665	-11.652	-8.302	0.081	342.310	4.783	0.015
V*h*	10	2307.260		150.210			0.061	475.208	0.000	0.197
		2307.260			102.744		0.028	476.459	1.251	0.105
		2307.260		174.625		-83.155	0.078	476.532	1.324	0.102
		2307.260	97.646				0.026	476.564	1.356	0.100
		2307.260		176.431	-33.865		0.062	477.159	1.951	0.074
		2307.260	-0.810	150.739			0.061	477.208	2.000	0.072
		2307.260				-31.884	0.003	477.424	2.216	0.065
		2307.260			146.109	-97.122	0.049	477.677	2.469	0.057
		2307.260	115.860			-64.568	0.036	478.170	2.962	0.045
		2307.260	56.257		68.997		0.034	478.251	3.043	0.043
		2307.260	12.484	166.833		-84.389	0.078	478.522	3.314	0.038
		2307.260		165.192	13.402	-86.370	0.078	478.525	3.317	0.037
		2307.260	4.940	174.104	-35.026		0.062	479.158	3.950	0.027
		2307.260	58.443		111.516	-98.163	0.055	479.447	4.239	0.024
		2307.260	11.059	159.911	11.098	-86.910	0.078	480.518	5.310	0.014
	100	2295.180		199.055		-220.416	0.121	474.736	0.000	0.139
		2300.580	161.554			-157.167	0.114	475.048	0.312	0.119

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
		2292.950		175.932	118.888	-247.308	0.154	475.321	0.585	0.104
		2302.570			145.336	-176.999	0.099	475.686	0.950	0.087
		2306.970				-131.498	0.048	475.713	0.977	0.085
		2301.970	135.038				0.048	475.725	0.989	0.085
		2298.450	132.770		108.015	-186.411	0.140	475.949	1.213	0.076
		2295.900	83.186	131.293		-203.364	0.130	476.354	1.618	0.062
		2304.650			88.025		0.021	476.751	2.015	0.051
		2302.250		86.030			0.018	476.860	2.124	0.048
		2293.550	50.817	136.153	110.584	-235.013	0.158	477.180	2.444	0.041
		2301.060	118.839		51.893		0.054	477.471	2.735	0.035
		2302.560	147.042	-18.292			0.048	477.708	2.972	0.032
		2301.440		64.283	70.043		0.030	478.406	3.670	0.022
		2301.910	136.024	-27.339	54.315		0.055	479.434	4.698	0.013
Vs	10	0.080		0.011			0.152	-264.850	0.000	0.301
		0.080		0.016	-0.005		0.166	-263.455	1.395	0.150
		0.080	-0.003	0.013			0.157	-263.079	1.771	0.124
		0.080		0.012		-0.001	0.153	-262.866	1.984	0.112
		0.080	-0.002	0.016	-0.005		0.169	-261.567	3.283	0.058
		0.080		0.016	-0.006	0.001	0.167	-261.483	3.367	0.056
		0.080	-0.003	0.013		0.000	0.158	-261.084	3.766	0.046
		0.080			0.007		0.052	-260.713	4.137	0.038
		0.080	0.006				0.040	-260.251	4.599	0.030
		0.080	-0.002	0.017	-0.005	0.001	0.169	-259.602	5.248	0.022
		0.080				0.003	0.009	-259.083	5.767	0.017
		0.080	0.003		0.005		0.058	-258.959	5.891	0.016
		0.080			0.007	0.000	0.052	-258.714	6.136	0.014
		0.080	0.005			0.001	0.042	-258.317	6.533	0.011
		0.080	0.003		0.005	0.000	0.058	-256.961	7.889	0.006
	100	0.080		0.005			0.028	-259.775	0.000	0.152
		0.079		0.008		-0.006	0.059	-259.002	0.773	0.103
		0.080				-0.002	0.006	-258.958	0.817	0.101
		0.080			-0.002		0.004	-258.893	0.882	0.098
		0.080	0.001				0.000	-258.754	1.021	0.091
		0.080	-0.005	0.009			0.044	-258.402	1.373	0.076
		0.080		0.006	-0.004		0.042	-258.315	1.460	0.073
		0.079	-0.007	0.014		-0.007	0.091	-258.283	1.492	0.072
		0.080		0.009	-0.003	-0.005	0.066	-257.274	2.501	0.043
		0.080			-0.001	-0.002	0.008	-257.024	2.751	0.038
		0.080	0.001			-0.002	0.007	-256.999	2.776	0.038
		0.080	0.001		-0.002		0.006	-256.958	2.817	0.037
		0.080	-0.005	0.009	-0.003		0.054	-256.794	2.981	0.034
		0.079	-0.007	0.014	-0.001	-0.007	0.093	-256.365	3.410	0.028
		0.080	0.001		-0.002	-0.002	0.010	-255.104	4.671	0.015
PId	10	0.870		0.004			0.027	-281.067	0.000	0.170
		0.870	0.002				0.009	-280.408	0.659	0.123
		0.870				0.001	0.002	-280.147	0.920	0.108

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
		0.870			0.001		0.001	-280.091	0.976	0.105
		0.870		0.008	-0.006		0.053	-280.067	1.000	0.103
		0.870	0.000	0.004			0.027	-279.075	1.992	0.063
		0.870		0.004		0.000	0.027	-279.067	2.000	0.063
		0.870	0.003		-0.001		0.011	-278.471	2.596	0.047
		0.870	0.002			0.000	0.010	-278.422	2.645	0.045
		0.870		0.008	-0.006	0.001	0.056	-278.209	2.858	0.041
		0.870			0.000	0.001	0.002	-278.148	2.919	0.040
		0.870	0.001	0.008	-0.006		0.053	-278.079	2.988	0.038
		0.870	0.000	0.004		0.000	0.027	-277.075	3.992	0.023
		0.870	0.003		-0.002	0.001	0.012	-276.522	4.545	0.018
		0.870	0.000	0.008	-0.006	0.001	0.056	-276.217	4.850	0.015
	100	0.870	-0.009	0.018		-0.008	0.258	-287.087	0.000	0.350
		0.870	-0.010	0.018	0.003	-0.009	0.273	-285.874	1.213	0.191
		0.870		0.011		-0.006	0.173	-285.108	1.979	0.130
		0.870		0.007			0.105	-284.188	2.899	0.082
		0.870	-0.007	0.012			0.151	-284.146	2.941	0.081
		0.870		0.010	0.001	-0.007	0.177	-283.261	3.826	0.052
		0.870	-0.007	0.012	0.001		0.153	-282.201	4.886	0.030
		0.870		0.007	0.000		0.105	-282.188	4.899	0.030
		0.870			0.002		0.010	-280.423	6.664	0.013
		0.870				-0.002	0.005	-280.269	6.818	0.012
		0.870	0.001				0.004	-280.223	6.864	0.011
		0.870			0.003	-0.003	0.022	-278.880	8.207	0.006
		0.870	0.002			-0.002	0.011	-278.493	8.594	0.005
		0.870	0.001		0.002		0.011	-278.472	8.615	0.005
		0.870	0.001		0.003	-0.003	0.024	-276.955	10.132	0.002
PI*h*	10	0.666				0.013	0.111	-239.087	0.000	0.224
		0.666		0.007		0.012	0.136	-238.101	0.986	0.137
		0.666			0.004	0.012	0.120	-237.429	1.658	0.098
		0.666	0.001			0.013	0.112	-237.103	1.984	0.083
		0.666		0.010			0.061	-237.027	2.060	0.080
		0.666			0.009		0.053	-236.725	2.362	0.069
		0.666	-0.005	0.010		0.012	0.145	-236.513	2.574	0.062
		0.666		0.008	-0.003	0.012	0.137	-236.168	2.919	0.052
		0.666	-0.002		0.005	0.012	0.121	-235.484	3.603	0.037
		0.666	0.005				0.013	-235.191	3.896	0.032
		0.666	-0.003	0.012			0.065	-235.184	3.903	0.032
		0.666		0.007	0.004		0.064	-235.180	3.907	0.032
		0.666	-0.002		0.010		0.054	-234.763	4.324	0.026
		0.666	-0.005	0.011	-0.002	0.012	0.146	-234.538	4.549	0.023
		0.666	-0.004	0.009	0.005		0.070	-233.414	5.673	0.013
	100	0.665	-0.013	0.022			0.150	-238.745	0.000	0.197
		0.665		0.013			0.098	-238.535	0.210	0.178
		0.665	-0.016	0.029		-0.009	0.188	-238.407	0.338	0.167
		0.665		0.016		-0.006	0.115	-237.223	1.522	0.092

Phenotypic variable	Scales	Int	TS	TJ/TW	PS/PA	PW	R^2	AIC	Di	w_i
		0.665	-0.013	0.022	0.001		0.151	-236.783	1.962	0.074
		0.665	-0.017	0.029	0.004	-0.010	0.195	-236.723	2.022	0.072
		0.665		0.013	0.000		0.098	-236.537	2.208	0.065
		0.665		0.016	0.001	-0.006	0.115	-235.243	3.502	0.034
		0.666			0.004		0.008	-234.994	3.751	0.030
		0.666	0.002				0.002	-234.794	3.951	0.027
		0.666				0.001	0.001	-234.764	3.981	0.027
		0.666	0.001		0.003		0.008	-233.010	5.735	0.011
		0.666			0.003	0.000	0.008	-232.997	5.748	0.011
		0.666	0.002			0.001	0.003	-232.827	5.918	0.010
		0.666	0.001		0.003	0.000	0.008	-231.013	7.732	0.004
PIs	10	0.315	-0.009				0.006	-156.547	0.000	0.144
		0.315			0.006		0.003	-156.426	0.121	0.136
		0.315		-0.005			0.002	-156.393	0.154	0.133
		0.315				-0.001	0.000	-156.333	0.214	0.129
		0.315	-0.019		0.018		0.021	-155.101	1.446	0.070
		0.315		-0.024	0.024		0.019	-155.047	1.500	0.068
		0.315	-0.010	0.002			0.006	-154.551	1.996	0.053
		0.315	-0.009			0.001	0.006	-154.551	1.996	0.053
		0.315			0.008	-0.005	0.004	-154.480	2.067	0.051
		0.315		-0.005		0.000	0.002	-154.393	2.154	0.049
		0.315	-0.015	-0.017	0.028		0.028	-153.374	3.173	0.029
		0.315	-0.019		0.020	-0.005	0.022	-153.149	3.398	0.026
		0.315		-0.024	0.028	-0.007	0.022	-153.142	3.405	0.026
		0.315	-0.010	0.001		0.001	0.006	-152.554	3.993	0.020
		0.315	-0.014	-0.018	0.031	-0.006	0.030	-151.451	5.096	0.011
	100	0.314		0.027			0.048	-158.160	0.000	0.202
		0.313		0.035		-0.016	0.063	-156.724	1.436	0.098
		0.315			0.011		0.010	-156.689	1.471	0.097
		0.315	0.010				0.007	-156.585	1.575	0.092
		0.314	-0.015	0.037			0.057	-156.486	1.674	0.087
		0.315				0.000	0.000	-156.329	1.831	0.081
		0.314		0.026	0.004		0.050	-156.208	1.952	0.076
		0.313	-0.021	0.052		-0.020	0.079	-155.357	2.803	0.050
		0.313		0.034	0.008	-0.017	0.067	-154.877	3.283	0.039
		0.315	0.007		0.009		0.013	-154.804	3.356	0.038
		0.315			0.013	-0.004	0.011	-154.731	3.429	0.036
		0.315	0.010			-0.002	0.007	-154.593	3.567	0.034
		0.314	-0.016	0.036	0.006		0.059	-154.584	3.576	0.034
		0.313	-0.024	0.053	0.012	-0.023	0.087	-153.696	4.464	0.022
		0.315	0.007		0.011	-0.005	0.014	-152.857	5.303	0.014

Table S3. Summary of models for different phenotypic variable, based on its level of empirical support for the 50 km^2 scale.

Phenotypic variable	Scale (km^2)	Weighted R^2	TS		TJ[1]/TW[2]		PS[1]/PA[2]		PW	
			β (*sd*)	RI	β (*sd*)	RI	β (*sd*)	RI	β (*sd*)	RI
P. sylvestris										
V*d*	50	0.192	2.157 (6.108)	0.349	7.17 (8.212)	0.466	-2.568 (5.979)	0.356	-19.007 (11.618)	0.756
V*h*	50	0.171	49.778 (72.716)	0.423	107.34 (91.496)	0.581	-19.858 (48.871)	0.334	38.305 (58.347)	0.378
V*s*	50	0.410	-0.003 (0.002)	0.625	-0.003 (0.002)	0.612	-0.001 (0.0007)	0.081	-0.001 (0.0007)	0.216
PI*d*	50	0.434	0.012 (0.01)	0.561	-0.007 (0.008)	0.433	-0.033 (0.013)	0.902	0.035 (0.014)	0.924
PI*h*	50	0.573	0.024 (0.012)	0.835	-0.006 (0.007)	0.412	-0.025 (0.011)	0.839	0.037 (0.013)	0.941
PI*s*	50	0.116	0.029 (0.027)	0.569	-0.004 (0.018)	0.362	0.011 (0.019)	0.410	-0.032 (0.027)	0.596
P. pinaster										
V*d*	50	0.279	-7.404 (20.939)	0.309	151.402 (50.965)	0.974	-123.414 (43.686)	0.952	-28.633 (28.461)	0.454
V*h*	50	0.035	285.092 (324.16)	0.469	-206.089 (314.51)	0.417	19.591 (183.11)	0.337	333.33 (321.54)	0.529
V*s*	50	0.187	-0.017 (0.008)	0.824	0.018 (0.0088)	0.848	-0.003 (0.0029)	0.364	0.006 (0.0049)	0.573
PI*d*	50	0.038	0.016 (0.015)	0.528	0.007 (0.012)	0.392	-0.004 (0.009)	0.349	0.005 (0.01)	0.365
PI*h*	50	0.033	0.006 (0.012)	0.413	0.015 (0.016)	0.543	-0.004 (0.009)	0.359	-0.001 (0.009)	0.341
PI*s*	50	0.013	0.007 (0.015)	0.396	-0.001 (0.013)	0.359	0.012 (0.016)	0.456	-0.002 (0.012)	0.360
P. halepensis										
V*d*	50	0.024	0.456 (7.21)	0.366	11.704 (11.53)	0.544	-3.579 (7.17)	0.387	-2.385 (6.88)	0.371
V*h*	50	0.109	66.139 (66.59)	0.483	74.509 (76.19)	0.483	73.46 (66.02)	0.517	-75.301 (68.46)	0.513
V*s*	50	0.043	-0.001 (0.0023)	0.284	0.004 (0.0037)	0.552	0.002 (0.0026)	0.442	-0.002 (0.0023)	0.303
PI*d*	50	0.214	-0.006 (0.0036)	0.692	0.013 (0.0053)	0.967	-0.001 (0.0011)	0.180	-0.003 (0.0024)	0.479
PI*h*	50	0.167	-0.009 (0.0064)	0.614	0.017 (0.0083)	0.889	0.0008 (0.0023)	0.302	0.0007 (0.0027)	0.318
PI*s*	50	0.043	-0.01 (0.01)	0.447	0.019 (0.01)	0.59	0.006 (0.0093)	0.400	-0.004 (0.009)	0.372

Supplementary material (Chapter II)

Appendix A. Experimental details

Table S1. Analysis of survival in the drought experiment for the three species.

Table S2. Values of survival and ontogenic stages recorded per population.

Table S3. Mean (± standard errors) for growth variables and biomass fractions for the two watering treatments (FC / DS).

Table S4. Analysis of unequal slope and intercept estimated among watering regimes by species.

Table S5. Percentage of the total variance explained by components and weights obtained among their variables.

Figure S1. Germination speeds for *P. oocarpa* (OC), *P. patula* (PA) and *P. pseudostrobus* (PS) and among their populations (OC01-02; PA01-04, 06-09, 11-12; PS01-05).

Figure S2. Biplot off the variables (**X**) and populations on the plane defined by the two Principal Components, for *P. patula* (♦) and *P. pseudostrobus (*▲). Full symbols and population's number represent FC treatment while empty symbols and underlined population's number represent DS treatment.

Appendix A. Experimental details

Populations: Are described in Table 1 and located in Figure 1.

Germination: Three hundred seeds per population were soaked in Hydrogen Peroxide (H2O2, 1:5v/v) during 15 min, then rinsed twice and soaked with distilled water during 24 hours. Seeds were then sowed in trays containing moistened rock wool and covered with plastic film. Trays were placed inside a germination chamber at 25±1ºC and 60±5% relative humidity and 8-hour photoperiod. The germination was recorded three times a week and then used to calculate the germination curve parameters (total germination in %, speed) based on a sample of 60 seeds per population. Germination for three species started at 3 days, *P. oocarpa* and *P. pseudostrobus* having a higher germination rate than *P. patula.*

Container characteristics: Fifty seedlings from each population were transplanted into individual plastic containers, except from *P. patula* populations (PA02, PA07 and PA08), which had a low germination rate. We used 250 cm^3 individual plastic containers with a mixture of peat moss and vermiculite substrate (3:1 v/v). All the containers were equally filled. This container size was big enough to avoid root restriction, given the short duration of the experiment (Poorter et al. 2012a).

Experiment design: The seedlings were installed in a greenhouse with temperature control (see next section for details). Plants were arranged in a randomized complete blocks design, with 5 seedlings per block, and 5 blocks in each of the two watering treatments (25 seedlings per treatment). Two different growing phases were established to hasten ontogenetic changes, differing in temperature and photoperiod. Seedlings were maintained in a slow-growth phase during 135 days from November to March (8±2 ºC and 60±5% relative humidity), and then cultivated in a normal-growing phase from April to June (24±2 ºC and 80±5% relative humidity). Both growth phases were implemented at the greenhouse. During this second growing phase, seedlings were submitted to two watering treatments during 90 days.

Greenhouse controlled conditions:

	Slow growth phase	**Normal growth phase**
Duration	135 days. Nov-March	90 days. April to June
Position of the trays with respect to the solar angle	west to east	west to east
Temperature	8±2 ºC	24±2 ºC
Photoperiod	Short days	Long days
Irrigation system	misting nozzle	misting nozzle
Watering amount	Full capacity	Two watering regimes
Relative humidity	60±5%	80±5%
Heat shield	40-50% reflecting radiant heat	40-50% reflecting radiant heat
Fertilization	Peter's 20-20-20 -N-P-K	Peter's 20-20-20 -N-P-K

Watering was determined by weighing every second day 25 pots randomly chosen from each treatment (50 in the first phase). The two watering regimes were established based on the mean saturation level of the substrate: 90–100% on FC and 35-45% on DS treatments.

Table S1. Analysis of survival in the drought experiment for the three species.

Effect	**df**	**Wald Chi-Square**	**Pr > ChiSq**
W[1]	1	44.107	<0.0001
S[2]	2	12.1902	0.0023
P(S)[3]	14	11.4100	0.6536

[1]Watering. [2]Species. [3]Population within species.

Table S2. Values of survival and ontogenic stages recorded per population.

Code[1]	W[2]	Survival (%)[3]		Ontogenic score[4] (B, %)							Ontogenic score (E, %)						
		B	E	7	6	5	4	3	2	1	7	6	5	4	3	2	1
OC01	FC	100	100	0	4	0	16	44	32	4	0	46	0	50	4	0	0
	DS	100	60	0	0	0	0	84	16	0	0	60	0	40	0	0	0
OC02	FC	100	100	0	0	0	12	64	24	0	4	64	0	32	0	0	0
	DS	80	60	0	0	0	12	60	24	4	0	63	0	37	0	0	0
Mean	*FC*	*100*	*100*	*0*	*2*	*0*	*14*	*54*	*28*	*2*	*2*	*55*	*0*	*41*	*2*	*0*	*0*
Mean	*DS*	*90*	*60*	*0*	*0*	*0*	*6*	*72*	*20*	*2*	*0*	*62*	*0*	*38*	*0*	*0*	*0*
PA01	FC	100	100	0	0	0	0	29	71	0	50	50	0	0	0	0	0
	DS	100	80	0	4	0	0	21	71	4	38	62	0	0	0	0	0
PA02	FC	80	80	0	0	0	0	6	94	0	25	75	0	0	0	0	0
	DS	60	40	0	0	0	0	0	100	0	27	73	0	0	0	0	0
PA03	FC	80	80	0	0	0	4	26	70	0	48	52	0	0	0	0	0
	DS	80	60	0	0	0	0	29	71	0	35	65	0	0	0	0	0
PA04	FC	60	60	0	5	0	0	23	73	0	73	27	0	0	0	0	0
	DS	60	40	0	4	0	0	29	67	0	47	53	0	0	0	0	0
PA06	FC	100	100	0	0	0	0	24	76	0	68	32	0	0	0	0	0
	DS	100	80	0	0	0	0	13	88	0	42	58	0	0	0	0	0
PA07	FC	40	40	0	0	0	0	42	58	0	50	50	0	0	0	0	0
	DS	60	60	0	0	0	0	25	75	0	40	60	0	0	0	0	0
PA08	FC	60	60	0	0	0	0	0	87	13	7	93	0	0	0	0	0
	DS	60	60	0	0	0	0	0	100	0	14	86	0	0	0	0	0
PA09	FC	100	100	0	0	0	0	16	84	0	40	60	0	0	0	0	0
	DS	100	80	0	4	0	0	40	56	0	74	26	0	0	0	0	0
PA11	FC	100	100	0	0	0	0	4	96	0	21	79	0	0	0	0	0
	DS	100	100	0	0	0	0	12	88	0	21	79	0	0	0	0	0
PA12	FC	100	100	0	0	0	4	36	60	0	48	52	0	0	0	0	0
	DS	100	100	0	0	0	4	40	52	4	13	88	0	0	0	0	0
Mean	*FC*	*82*	*82*	*0*	*0*	*0*	*1*	*21*	*77*	*1*	*43*	*57*	*0*	*0*	*0*	*0*	*0*
Mean	*DS*	*82*	*70*	*0*	*1*	*0*	*0*	*21*	*77*	*1*	*35*	*65*	*0*	*0*	*0*	*0*	*0*
PS01	FC	100	100	0	43	0	0	4	48	4	63	33	0	0	4	0	0
	DS	80	80	0	38	0	0	8	54	0	77	23	0	0	0	0	0
PS02	FC	100	100	0	40	0	0	0	56	4	67	33	0	0	0	0	0
	DS	100	100	0	20	0	0	0	80	0	86	14	0	0	0	0	0
PS03	FC	100	100	0	46	0	0	29	25	0	8	92	0	0	0	0	0
	DS	80	60	0	79	0	0	0	17	4	25	75	0	0	0	0	0
PS04	FC	100	100	0	24	0	0	16	60	0	20	76	0	4	0	0	0
	DS	80	80	0	29	0	0	4	67	0	46	50	0	0	4	0	0
PS05	FC	100	100	0	4	0	0	32	64	0	32	68	0	0	0	0	0
	DS	100	100	0	4	0	0	24	68	4	39	61	0	0	0	0	0
Mean	*FC*	*100*	*100*	*0*	*31*	*0*	*0*	*16*	*51*	*2*	*38*	*61*	*0*	*1*	*1*	*0*	*0*
Mean	*DS*	*88*	*84*	*0*	*34*	*0*	*0*	*7*	*57*	*2*	*55*	*44*	*0*	*0*	*1*	*0*	*0*

[1] OC: *P. oocarpa*; PA: *P. patula*, PS: *P. pseudostrobus*. [2] W: Watering regime; FC: Field capacity; DS: Drought stress;
[3] B: Beginning of the experiment; E: End of the experiment. [4] Ontogenic score following (Chambel et al. 2007).

Table S3. Mean (± standard errors) for growth variables and biomass fractions for the two watering treatments (FC / DS).

	HG	RDM	SDM	NDM	TM	RMF	SMF	NMF	SLA
P. oocarpa									
OC01	120.04±9.81a* / 36.60 ±4.03a	446.55 ±50.01b / 338.65 ±55.05a	376.52 ±49.56b / 286.09 ±27.98a	1,110.15 ±151.84b / 989.81 ±85.96a	1,933.51 ±246.12b / 1,613.99 ±140.12a	0.24±0.01a / 0.20±0.03a	0.18 ±0.01b / 0.18 ±0.02a	0.58 ±0.01a / 0.62 ±0.02a	153.93 ±12.72a / 156.44 ±0.00a*
OC02	133.60±8.89a* / 30.11 ±2.31a	580.22 ±49.64a* / 331.46 ±51.68a	538.97 ±49.23a* / 235.46 ±25.91a	1,414.58 ±150.89a* / 896.92 ±81.86a	2,533.68 ±244.73a* / 1,463.78 ±132.31a	0.23±0.01a / 0.23±0.03a	0.21 ±0.01a* / 0.16 ±0.01a	0.56 ±0.01a / 0.61 ±0.02a	151.12 ±0.00b* / 121.90 ±0.00b
P. patula									
PA01	141.71±8.80ab* / 56.33 ±4.44a	311.30 ±32.42a / 210.84 ±25.45a	261.36 ±20.80abc* / 118.83 ±10.79a	802.41 ±68.88ab / 626.16 ±51.30a	1,374.89 ±114.49ab* / 954.78 ±76.14a	0.22 ±0.01a / 0.22 ±0.02a	0.20 ±0.01a* / 0.12 ±0.01b	0.58 ±0.01ab / 0.66 ±0.02a*	222.90 ±8.90a / 176.34 ±6.83ab
PA02	151.38±11.02ab* / 61.82 ±5.11a	284.38 ±35.26a / 205.72 ±29.12a	266.35 ±24.40abc* / 130.36 ±13.46a	722.23 ±74.43ab / 613.03 ±59.53a	1,273.71 ±124.27ab / 940.79 ±87.50a	0.22 ±0.01a / 0.22 ±0.03a	0.21 ±0.01a* / 0.14 ±0.01ab	0.57 ±0.01b / 0.66 ±0.02a	232.53 ±10.79a* / 184.98 ±8.20ab
PA03	157.83±9.10ab* / 56.71 ±4.60a	301.97 ±32.71a / 250.33 ±26.30a	286.59 ±21.17ab* / 141.02 ±11.56a	817.68 ±69.43ab / 729.42 ±53.62a	1,405.78 ±115.47ab / 1,117.95 ±79.35a	0.21 ±0.01a / 0.23 ±0.02a	0.21 ±0.01a* / 0.12 ±0.01b	0.59 ±0.01ab / 0.65 ±0.02a*	220.02 ±9.05a* / 162.53 ±7.08b
PA04	138.91±7.49ab* / 56.29 ±4.19a	303.37 ±33.09a* / 206.02 ±26.31a	258.89 ±21.66abc* / 125.41 ±11.57a	823.56 ±70.17ab / 684.82 ±53.64a	1,384.76 ±116.77ab* / 1,014.60 ±79.37a	0.21 ±0.01a / 0.21 ±0.02a	0.19 ±0.01a* / 0.12 ±0.01b	0.60 ±0.01ab / 0.67 ±0.02a*	226.08 ±9.26a* / 169.54 ±7.23ab
PA06	177.60±7.92a* / 57.53 ±4.86a	326.10 ±32.24a* / 211.41 ±25.69a	338.37 ±20.55a* / 130.00 ±11.01a	906.70 ±68.51a* / 643.68 ±51.95a	1,570.91 ±113.85a* / 983.56 ±77.04a	0.21 ±0.01a / 0.22 ±0.02a	0.22 ±0.01a* / 0.13 ±0.01b	0.58 ±0.01b / 0.65 ±0.02a*	204.39 ±8.77a / 173.70 ±6.83ab
PA07	154.33±11.87ab* / 79.00 ±8.03a	294.24 ±37.73a / 187.86 ±29.77a	266.47 ±27.36abc / 155.46 ±14.49a	758.83 ±79.29ab / 627.36 ±62.84a	1,319.76 ±132.79ab / 971.89 ±92.12a	0.21 ±0.01a / 0.19 ±0.03a	0.21 ±0.01a / 0.19 ±0.01a	0.59 ±0.01ab / 0.62 ±0.02a	215.29 ±13.48a / 176.09 ±8.44ab
PA08	104.67±10.36c* / 73.14 ±5.03a	253.21 ±36.55a / 190.61 ±27.64a	187.26 ±25.97c / 126.85 ±12.73a	633.35 ±76.98b / 600.52 ±57.22a	1,075.00 ±128.74b / 920.84 ±84.29a	0.20 ±0.01a / 0.21 ±0.03a	0.17 ±0.01a* / 0.14 ±0.01ab	0.63 ±0.01a / 0.65 ±0.02a	226.52 ±13.78a / 194.49 ±7.63a
PA09	146.96±6.89ab* / 66.47 ±3.69a	266.93 ±32.21a / 228.05 ±25.73a	252.69 ±20.51bc* / 160.19 ±11.32a	735.45 ±68.46ab / 705.17 ±52.89a	1,255.25 ±113.76b / 1,060.48 ±77.21a	0.21 ±0.01a / 0.26 ±0.02a	0.20 ±0.01a* / 0.14 ±0.01ab	0.59 ±0.01ab / 0.64 ±0.02a	231.03 ±9.06a* / 177.54 ±6.97ab
PA11	161.04±8.45ab* / 64.74 ±4.19a	278.36 ±32.59a / 242.23 ±25.76a	261.12 ±21.01abc* / 131.75 ±11.07a	721.88 ±69.20b / 657.69 ±52.15a	1,261.79 ±115.07b / 1,033.13 ±77.30a	0.22 ±0.01a / 0.23 ±0.02a	0.21 ±0.01a* / 0.13 ±0.01b	0.58 ±0.01b / 0.64 ±0.02a	237.60 ±9.65a* / 188.85 ±7.13a
PA12	158.48±7.20ab* / 59.56 ±6.36a	297.54 ±32.77a / 248.33 ±26.90a	249.46 ±21.25bc* / 122.17 ±12.09a	710.31 ±69.55b / 633.86 ±55.23a	1,256.38 ±115.68b / 1,003.03 ±81.58a	0.22 ±0.01a / 0.24 ±0.02a	0.20 ±0.01a* / 0.13 ±0.01ab	0.58 ±0.01b / 0.64 ±0.02a*	237.52 ±9.15a* / 192.17 ±7.49a
P. pseudostrobus									

	HG	RDM	SDM	NDM	TM	RMF	SMF	NMF	SLA
PS01	112.13±9.46a* / 43.45 ±5.48ab	210.20 ±24.82ab / 174.76 ±22.73b /	177.73 ±22.53a* / 119.95 ±14.05a	817.94 ±83.78b / 781.29 ±79.29ab	1,204.03 ±117.30b / 1,075.33 ±103.47a	0.16 ±0.01b / 0.18 ±0.01b	0.15 ±0.01a* / 0.11 ±0.01a	0.69 ±0.02ab / 0.72 ±0.02ab*	200.84 ±8.27a* / 165.66 ±5.84ab
P202	104.92±11.31a* / 51.41 ±6.09a	211.24 ±23.68ab / 241.32 ±21.28ab	194.61 ±21.73a / 137.12 ±13.34a	859.44 ±80.64b / 874.47 ±76.17ab	1,265.07 ±112.98b / 1,253.17 ±99.18a	0.15 ±0.01b / 0.19 ±0.01ab	0.14 ±0.01a* / 0.11 ±0.01a	0.70 ±0.02ab / 0.70 ±0.01ab	192.60 ±7.64a* / 151.10 ±5.44bC
PS03	28.40±7.38b* / 8.65 ±2.43c	305.76 ±25.24a / 238.13 ±22.93ab	172.54 ±22.47a / 102.31 ±14.16a	1,219.94 ±83.55a / 916.07 ±79.76a	1,689.83 ±116.98a / 1,256.34 ±104.10a	0.17 ±0.01b / 0.18 ±0.01ab	0.08 ±0.01b / 0.07 ±0.01b	0.76 ±0.02a / 0.75 ±0.02a	149.81 ±8.49b / 133.55 ±6.03C
PS04	88.88±6.87a* / 31.78 ±3.04b	289.91 ±23.18ab / 278.05 ±21.19a	159.27 ±21.38a / 120.49 ±13.29a	785.11 ±79.30b / 849.47 ±75.94ab	1,233.41 ±111.13b / 1,248.58 ±98.86a	0.24 ±0.01a / 0.23 ±0.01a	0.12 ±0.01ab / 0.09 ±0.01ab	0.64 ±0.02b / 0.68 ±0.01b	195.13 ±9.19a* / 132.03 ±5.72C
PS05	102.64±8.87a* / 39.48 ±3.15ab	205.38 ±23.21b / 221.16 ±20.78ab	193.48 ±21.40a* / 103.25 ±13.09a	786.90 ±79.38b / 685.46 ±75.06b	1,184.88 ±111.24b / 1,010.25 ±97.65a	0.16 ±0.01b / 0.22 ±0.01ab*	0.16 ±0.01a* / 0.10 ±0.01ab	0.68 ±0.02b / 0.68 ±0.01b	208.71 ±10.82a / 176.11 ±5.78a

HG: height growth increment (mm); **RDM**: root dry mass (mg); **SDM**: stem dry mass (mg); **NDM**: needles dry mass (mg); **TDM**: total dry mass (mg); **RMF**: root mass fraction ($g\ g^{-1}$); **SMF**: stem mass fraction ($g\ g^{-1}$) **NMF**: needles mass fraction ($g\ g^{-1}$); **SLA**: specific leaf area (cm^2/g). Different letters indicate statistical differences for provenance and asterisk indicate statistical differences for treatment (both at $p<0.05$).

Table S4. Analysis of unequal slope and intercept estimated among watering regimes by species.

Effect	Ndf	Ddf	F	P
P. oocarpa				
Intercept	2	79	1.08	0.344
Slope	2	79	83.32	<0.0001
P. patula				
Intercept	2	370	53.99	<0.0001
Slope	2	370	480.54	<0.0001
P. pseudostrobus				
Intercept	2	228	16.29	<0.0001
Slope	2	228	193.96	<0.0001

Ndf: numerator degree of freedom; Ddf: denominator degree of freedom; F: F-value; P: p-value

Table S5. Percentage of the total variance explained by components and weights of the variables.

Variables	Principal components	
	PC1 (59.85%)	PC2 (26.24%)
HG	0.82768	0.31010
RDM	-0.06602	0.84983
SDM	0.42444	0.90545
SMF	0.87379	0.29773
SLA	0.69885	-0.17294

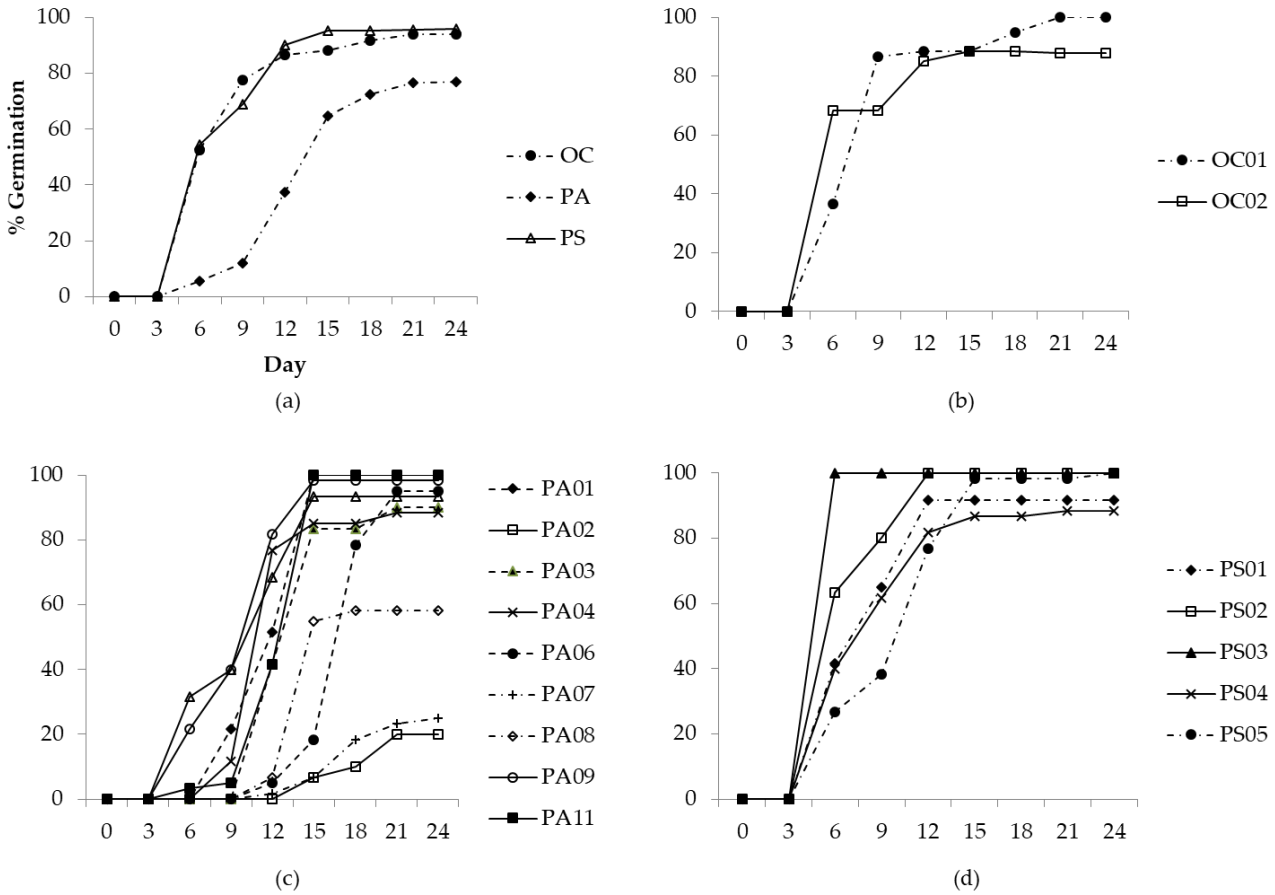

Figure S1. Germination speeds for *P. oocarpa* (OC), *P. patula* (PA) and *P. pseudostrobus* (PS) and among their populations (OC01-02; PA01-04, 06-09, 11-12; PS01-05).

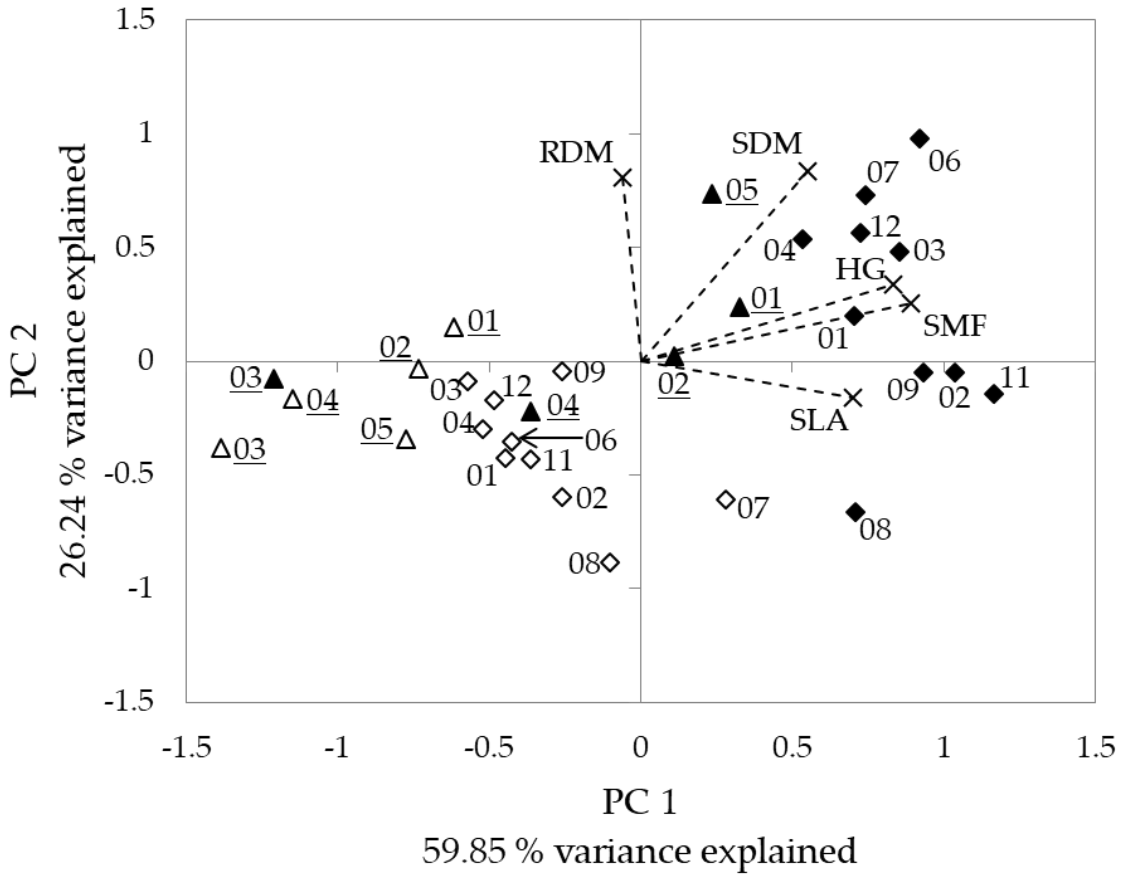

Figure S2. Biplot off the variables (**X**) and populations on the plane defined by the two Principal Components, for *P. patula* (♦) and *P. pseudostrobus (*▲). Full symbols and population's number represent FC treatment while empty symbols and underlined population's number represent DS treatment.

Supplementary material (Chapter III)

Figure S1. Protected areas for biodiversity (CONABIO, 2016).

Table S1. General climatic and edaphic patterns of target species.

Table S2. Test sites and origin sites for provenance trials.

Table S3. Test sites and origin sites for progeny trials.

Table S4. Populations for genetic diversity analysis.

Table S5. Physiographic Provinces and Germplasm Transfer Zones (CONAFOR 2016).

Table S6. Minimum requirements for genetic conservation units (Koskela et al. 2013).

Table S7. Volume of timber for target species per state, across their natural distribution.

Table S8. Population and genetic zone considered as the most suitable source for use in reforestation.

Table S9. Conservation units for *P. greggii, P. oocarpa, P. patula* and *P. pseudostrobus*.

Table S10. Importance of timber production, plantation area and species by genetic zone.

Figure S1. Protected areas for biodiversity (CONABIO, 2016).

Table S1. General climatic and edaphic patterns of target species.

Species	General characteristics of the populations
P. greggii	Trees from the northern populations occur in degraded stands on shallow calcareous soils with pH 6.8 to 7.7 (Donahue and López-Upton 1996). These populations exist at elevations from 1900 to 2600 m with annual rainfall between 650 and 750 mm. The southern populations of *P. greggii* occur in stands on predominantly acidic soils with pH 4.2 to 6.1 (Hernández Martínez et al. 2007). Trees in these populations are found at elevations of 1250 to 2380 m and receive between 1465 to 2380 mm of annual precipitation (Dvorak 2002a; Hernández Martínez et al. 2007).
P. oocarpa	This species occurs from 350 to 2500 m elevation in Mexico and Central America but reaches its best development between 1200 to 1800 m. Along the northwest coast of Mexico it occurs in areas with as little as 600 to 800 mm of annual rainfall. In southern and eastern Mexico and most of Central America it generally occurs in areas of 1000 to 1500 mm of annual precipitation with dry seasons of up to 5 months. In some locations where *Pinus oocarpa* is most often found on shallow, sandy clay soils of moderate soil acidity (pH 4.0 to 6.5) that are well drained (Dvorak 2002c).
P. patula	The specie grows on fertile, well-drained soils on mountain ridges and slopes in cloud forest environments at elevations between 1490 and 3100 m but is most common between 2100 and 2800 m (Perry 1991). It generally occupies sites that receive between 1000 and 2000 mm of annual precipitation with distinct dry seasons of up to 4 months (Dvorak 2002b).
P. pseudostrobus	The species grows at elevations from 1600 to 3250 m, but the best stands are found at 2500 m on deep volcanic soils (López-Upton 2002). This tree can also be found in swallow and calcareous soils. This pine grows in temperate to temperate-warmer climates, where temperatures may drop to freezing during the coldest winter months. The species is found where temperatures range from -9 to 40 °C and annual rainfall from May to October is 600 to 2000 mm (Martínez 1948; Perry 1991).

Table S2. Test sites and origin sites for provenance trials.

Species	Locality	State	LN	LO	Alt	Area (ha)	Site	Author
P. greggii var. greggii & australis	**Tlacotepec Plumas, Coixtlahuaca**	Oaxaca	17.86667	-97.43333	2120		**Test site**	(Cruz, 2011)
P. greggii var. greggii	Pto. Los Conejos	Coahuila	25.46667	-100.56667	2450		Provenance	
P. greggii var. greggii	Santa Anita	Coahuila	25.45000	-100.56667	2500		Provenance	
P. greggii var. greggii	Puerto San Juan	Coahuila	25.41667	-100.55000	2650		Provenance	
P. greggii var. greggii	Los Lirios	Coahuila	25.38333	-100.56667	2400		Provenance	
P. greggii var. greggii	Jamé	Coahuila	25.35000	-100.60000	2450		Provenance	
P. greggii var. greggii	Ej. 18 de Marzo, Galeana	Nuevo León	24.43333	-100.16667	2100		Provenance	
P. greggii var. australis	El Madroño	Querétaro	21.26667	-99.16667	1650		Provenance	
P. greggii var. australis	El Piñón	Hidalgo	20.93333	-99.20000	1830		Provenance	
P. greggii var. australis	Molango	Hidalgo	20.81667	-98.76667	1200		Provenance	
P. greggii var. australis	Xochicoatlán	Hidalgo	20.78333	-98.66667	1700		Provenance	
P. greggii var. australis	Comunidad Durango	Hidalgo	20.76667	-99.38333	1850		Provenance	
P. greggii var. greggii & australis	**Magdalena Zahuatlán, Nochixtlán**	Oaxaca	19.40000	-97.20000	2160		**Test site**	(Villegas-Jiménez et al., 2013)
P. greggii var. greggii	Pto. Los Conejos	Coahuila	25.46667	-100.56667	2450		Provenance	
P. greggii var. greggii	Santa Anita	Coahuila	25.45000	-100.56667	2500		Provenance	
P. greggii var. greggii	Puerto San Juan	Coahuila	25.41667	-100.55000	2650		Provenance	
P. greggii var. greggii	Los Lirios	Coahuila	25.38333	-100.56667	2400		Provenance	
P. greggii var. greggii	Jamé	Coahuila	25.35000	-100.60000	2450		Provenance	
P. greggii var. greggii	Ej. 18 de Marzo, Galeana	Nuevo León	24.43333	-100.16667	2100		Provenance	
P. greggii var. australis	El Madroño	Querétaro	21.26667	-99.16667	1650		Provenance	
P. greggii var. australis	El Piñón	Hidalgo	20.93333	-99.20000	1830		Provenance	
P. greggii var. australis	Molango	Hidalgo	20.81667	-98.76667	1200		Provenance	
P. greggii var. australis	Xochicoatlán	Hidalgo	20.78333	-98.66667	1700		Provenance	
P. greggii var. australis	Comunidad Durango	Hidalgo	20.76667	-99.38333	1850		Provenance	
P. greggii var. greggii	**Ej. 18 de Marzo**	Nuevo León	24.88333	-100.18333			**Test site**	(Rodríguez et al., 2008)
P. greggii var. greggii	Puerto Los Conejos	Coahuila/N.L.	25.46667	-100.58333	2520	70	Provenance	
P. greggii var. greggii	Santa Anita	Coahuila	25.45000	-100.56667	2560	30	Provenance	
P. greggii var. greggii	Agua Fría	Coahuila/N.L.	25.43333	-100.50000	2400	30	Provenance	
P. greggii var. greggii	Puerto San Juan	Coahuila	25.41667	-100.55000	2650	25	Provenance	
P. greggii var. greggii	Los Lirios	Coahuila	25.38333	-100.51667	2420	300	Provenance	
P. greggii var. greggii	El Penitente	Coahuila	25.36667	-100.90000	2405	230	Provenance	

Species	Locality	State	LN	LO	Alt	Area (ha)	Site	Author
P. greggii var. greggii	Jamé	Coahuila	25.35000	-100.56667	2552	35	Provenance	
P. greggii var. greggii	Las Placetas	Nuevo León	24.91667	-100.18333	2450	210	Provenance	
P. greggii var. greggii	La Tapona	Nuevo León	24.71667	-100.10000	2130	170	Provenance	
P. greggii var. australis	**Ejido Cerro de León**	Veracruz	19.65000	-97.23333	2400		**Test site**	(Gutiérrez, 2012)
P. greggii var. australis	Carrizal Chico	Veracruz	20.43333	-98.35000	1670		Provenance	
P. greggii var. australis	Naolinco	Veracruz	19.65000	-96.86667	1540		Provenance	
P. greggii var. australis	**Patoltecoya**	Puebla	20.21667	-98.20000	1415		**Test site**	(López-Upton et al., 2005)
P. greggii var. australis	Valle Verde	Querétaro	21.48333	-99.21667	1490		Provenance	
P. greggii var. australis	El Madroño	Querétaro	21.26667	-99.16667	1650		Provenance	
P. greggii var. australis	Laguna Seca	Hidalgo	21.06667	-99.16667	1720	1201.9	Provenance	
P. greggii var. australis	San Joaquín	Querétaro	20.93333	-99.56667	2350		Provenance	
P. greggii var. australis	El Piñón	Hidalgo	20.93333	-99.20000	1830		Provenance	
P. greggii var. australis	Pemuxtitla	Hidalgo	20.80000	-98.73333	1400		Provenance	
P. greggii var. australis	Xochicoatlán	Hidalgo	20.75000	-98.66667	1800		Provenance	
P. greggii var. australis	Xodhé	Hidalgo	20.80417	-99.34333	1950	63.2	Provenance	
P. greggii var. australis	Cieneguilla	Hidalgo	20.74361	-99.03333	2000	138.2	Provenance	
P. greggii var. australis	Patoltecoya	Puebla	20.21667	-98.20000	1415		Provenance	
P. oocarpa	**Las Víboras**	Veracruz	19.65333	-96.88444	900		**Test site**	(Mendizabal, 1999)
P. oocarpa	Los Tuxtlas	Veracruz	18.05000	-94.93333	550		Provenance	
P. oocarpa	La Lagunilla	Guatemala	14.70000	89.95000	1600		Provenance	
P. oocarpa	San Lorenzo	Guatemala	15.08333	89.81667	1740		Provenance	
P. oocarpa	San Jerónimo	Guatemala	15.05000	90.30000	1425		Provenance	
P. oocarpa	**Canalejas**	Michoacán	19.06667	-101.73333	1490		**Test site**	(Sáenz-Romero et al., 2006)
P. oocarpa	Uruapan	Michoacán	19.35000	-102.10000	1505		Provenance	
P. oocarpa	Matanguarán	Michoacán	19.33333	-102.08333	1430		Provenance	
P. oocarpa	El Catorce	Michoacán	19.30000	-102.08333	1325		Provenance	
P. oocarpa	La Tinaja	Michoacán	19.28333	-102.08333	1220		Provenance	
P. oocarpa	Charapendo	Michoacán	19.26667	-102.10000	1075		Provenance	
P. patula	**Los Ayacahuites**	Puebla	20.21667	-98.05000	1440		**Test site**	(Salazar-García et al., 1999)
P. patula	Pinal de Amoles	Querétaro	21.01667	-99.16667	2400		Provenance	
P. patula	Encarnación	Hidalgo	20.51667	-99.11667	2400		Provenance	
P. patula	Zacualtipan	Hidalgo	20.38333	-98.40000	2220		Provenance	

Species	Locality	State	LN	LO	Alt	Area (ha)	Site	Author
P. patula	Tlahuelompan	Hidalgo	20.38333	-98.35000	2020		Provenance	
P. patula	Huayacocotla	Veracruz	20.31667	-98.28333	2030		Provenance	
P. patula	Estación Apulco	Hidalgo	20.21667	-98.21667	2190		Provenance	
P. patula	Acaxochitlán	Hidalgo	20.10000	-98.11667	2290		Provenance	
P. patula	Ahuazotepec	Puebla	2-000	-98.11667	2460		Provenance	
P. patula	Ayehualulco	Puebla	19.56667	-97.58333	2000		Provenance	

Table S3. Test sites and origin sites for progeny trials.

Species	Locality	State	LN	LO	Alt	Area (ha)	Site	Author
P. greggii var. australis	**Cuatzetzenco**	Hidalgo	19.94892	-98.35183	2790		**Test site**	(Ruíz Farfán, 2014)
P. greggii var. australis	El Piñón	Hidalgo	20.94417	-99.20472	1830	353.2	Provenance	
P. greggii var. australis	Mesa de la Cebada	Hidalgo	20.90667	-99.18333	2090	16.2	Provenance	
P. greggii var. australis	El Cobre-Mesa de Andrade	Hidalgo	20.88778	-99.17278	2100	707.2	Provenance	
P. greggii var. australis	Zacualpan	Veracruz	20.43333	-98.33333	1600		Provenance	
P. greggii var. australis	Valle Verde	Querétaro	21.48333	-99.21667	1490		Provenance	
P. greggii var. australis	**Toluca de Guadalupe**	Tlaxcala	19.46319	-97.96550	2680		**Test site**	(Ruíz Farfán, 2014)
P. greggii var. australis	El Piñón	Hidalgo	20.94417	-99.20472	1830	353.2	Provenance	
P. greggii var. australis	Mesa de la Cebada	Hidalgo	20.90667	-99.18333	2090	16.2	Provenance	
P. greggii var. australis	El Cobre-Mesa de Andrade	Hidalgo	20.88778	-99.17278	2100	707.2	Provenance	
P. greggii var. australis	Zacualpan	Veracruz	20.43333	-98.33333	1600		Provenance	
P. greggii var. australis	Valle Verde	Querétaro	21.48333	-99.21667	1490		Provenance	
P. patula	**Aquixtla**	Puebla	19.71997	-97.98925	2930		**Test site**	(Gómez, 2013)
P. patula	Conrado Castillo	Tamaulipas	23.93330	-99.45000	1780		Provenance	
P. patula	Pinal de Amoles	Querétaro	21.11667	-99.68333	2465		Provenance	
P. patula	La Encarnación	Hidalgo	20.88333	-99.21667	2525		Provenance	
P. patula	Zacualtipán	Hidalgo	20.65000	-98.66667	2090		Provenance	
P. patula	Potrero de Monroy	Veracruz	20.40000	-98.41667	2400		Provenance	
P. patula	Cumbre de Muridores	Hidalgo	20.31667	-98.35000	2430		Provenance	
P. patula	La Cruz	Hidalgo	20.28333	-98.30000	2375		Provenance	
P. patula	Acaxochitlán	Hidalgo	20.15000	-98.16667	2475		Provenance	
P. patula	Tlacotla	Tlaxcala	19.66667	-98.08333	2832		Provenance	
P. patula	Ingenio del Rosario	Veracruz	19.51667	-97.10000	2820		Provenance	
P. patula	Corralitla	Veracruz	18.63333	-97.10000	2115		Provenance	
P. patula	Santa María Pápalo	Oaxaca	17.81667	-96.80000	2495		Provenance	
P. patula	Carrizal de Bravo	Guerrero	17.56667	-99.88333	2210		Provenance	
P. patula	Ixtlán	Oaxaca	17.40000	-96.45000	2735		Provenance	
P. patula	Cuajimoloyas	Oaxaca	17.16667	-96.35000	2610		Provenance	
P. patula	El Tlacuache	Oaxaca	16.73333	-97.15000	2460		Provenance	

Species	Locality	State	LN	LO	Alt	Area (ha)	Site	Author
P. patula	Zacualtipan	Hidalgo	20.65000	-98.66667	2220		Provenance	
P. patula	Estación Apulco	Hidalgo	20.40000	-98.36667	2200		Provenance	
P. patula	Zacualpan	Veracruz	20.33333	-98.41667	1850		Provenance	
P. patula	Acaxochitlán	Hidalgo	20.10000	-98.20000	2190		Provenance	
P. patula	Ayehualulco	Puebla	19.95000	-97.91667	2120		Provenance	
P. patula	**Acaxochitlán**	Hidalgo	20.16444	-98.22528	2260		**Test site**	(Gómez, 2013)
P. patula	Conrado Castillo	Tamaulipas	23.93330	-99.45000	1780		Provenance	
P. patula	Pinal de Amoles	Querétaro	21.11667	-99.68333	2465		Provenance	
P. patula	La Encarnación	Hidalgo	20.88333	-99.21667	2525		Provenance	
P. patula	Zacualtipán	Hidalgo	20.65000	-98.66667	2090		Provenance	
P. patula	Potrero de Monroy	Veracruz	20.40000	-98.41667	2400		Provenance	
P. patula	Cumbre de Muridores	Hidalgo	20.31667	-98.35000	2430		Provenance	
P. patula	La Cruz	Hidalgo	20.28333	-98.30000	2375		Provenance	
P. patula	Acaxochitlán	Hidalgo	20.15000	-98.16667	2475		Provenance	
P. patula	Tlacotla	Tlaxcala	19.66667	-98.08333	2832		Provenance	
P. patula	Ingenio del Rosario	Veracruz	19.51667	-97.10000	2820		Provenance	
P. patula	Corralitla	Veracruz	18.63333	-97.10000	2115		Provenance	
P. patula	Santa María Pápalo	Oaxaca¶	17.81667	-96.80000	2495		Provenance	
P. patula	Carrizal de Bravo	Guerrero	17.56667	-99.88333	2210		Provenance	
P. patula	Ixtlán	Oaxaca	17.40000	-96.45000	2735		Provenance	
P. patula	Cuajimoloyas	Oaxaca	17.16667	-96.35000	2610		Provenance	
P. patula	El Tlacuache	Oaxaca	16.73333	-97.15000	2460		Provenance	
P. patula	Zacualtipan	Hidalgo	20.65000	-98.66667	2220		Provenance	
P. patula	Estación Apulco	Hidalgo	20.40000	-98.36667	2200		Provenance	
P. patula	Zacualpan	Veracruz	20.33333	-98.41667	1850		Provenance	
P. patula	Acaxochitlán	Hidalgo	20.10000	-98.20000	2190		Provenance	
P. patula	Ayehualulco	Puebla	19.95000	-97.91667	2120		Provenance	
P. pseudostrobus	**Morelia**	Michoacán	19.76872	-101.14906			**Test site**	(Cambrón-Sandoval et al., 2014)
P. pseudostrobus	Nuevo San Juan Parangaricutiro	Michoacán	19.46667	-102.18333			Provenance	

Table S4. Populations for genetic diversity analysis.

Species	Locality	State	LN	LO	Alt	Area (ha)	Ho	He	Author
P. greggii var. australis	Zacualpan	Veracruz	20.43333	-98.33333	1600		0.090	0.120	(Parraguirre et al., 2002)
P. greggii var. australis	Molango	Hidalgo	20.81667	-98.76667	1400		0.064	0.112	
P. greggii var. australis	Pemuxtitla	Hidalgo	20.80000	-98.73333	1400		0.074	0.129	
P. greggii var. australis	Xochicoatlán	Hidalgo	20.75000	-98.66667	1800		0.127	0.104	
P. greggii var. australis	Cieneguilla	Hidalgo	20.74361	-99.03333	2000	138.2	0.057	0.092	
P. greggii var. australis	El Piñón	Hidalgo	20.93333	-99.20000	1830		0.084	0.097	
P. greggii var. australis	Laguna Seca	Hidalgo	21.06667	-99.16667	1720	1201.9	0.100	0.146	
P. greggii var. australis	Valle Verde	Querétaro	21.48333	-99.21667	1490		0.044	0.096	
P. greggii var. australis	El Madroño	Querétaro	21.26667	-99.16667	1650		0.133	0.123	
P. greggii var. australis	San Joaquín	Querétaro	20.93333	-99.56667	2350		0.101	0.127	
P. greggii var. greggii	Jamé	Coahuila	25.35000	-100.56667	2552	35	0.083	0.154	
P. greggii var. greggii	Los Lirios	Coahuila	25.38333	-100.51667	2420	300	0.038	0.068	
P. greggii var. greggii	Puerto San Juan	Coahuila	25.41667	-100.55000	2650		0.131	0.209	
P. greggii var. greggii	Santa Anita	Coahuila	25.45000	-100.56667	2560	30	0.061	0.117	
P. greggii var. greggii	El Penitente	Coahuila	25.36667	-100.90000	2405	230	0.093	0.157	
P. greggii var. greggii	Agua Fría	Coahuila/N.L.	25.43333	-100.50000	2400	30	0.053	0.053	
P. greggii var. greggii	Puerto Los Conejos	Coahuila/N.L.	25.46667	-100.58333	2520	70	0.103	0.162	
P. greggii var. greggii	Las Placetas	Nuevo León	24.91667	-100.18333	2450	210	0.089	0.089	
P. greggii var. greggii	La Tapona	Nuevo León	24.71667	-100.10000	2130	170	0.137	0.191	
Mean							***0.087***	***0.123***	
P. oocarpa	Uruapan	Michoacán	19.35000	-102.10000	1505		-	-	(Sáenz-Romero & Tapia-Olivares, 2003)
P. oocarpa	Matanguarán	Michoacán	19.33333	-102.08333	1430		-	-	
P. oocarpa	El Catorce	Michoacán	19.30000	-102.08333	1325		-	-	
P. oocarpa	La Tinaja	Michoacán	19.28333	-102.08333	1220		-	-	
P. oocarpa	Charapendo	Michoacán	19.26667	-102.10000	1075		-	-	
Mean							***0.1147***	***0.1020***	
P. oocarpa	Chinipas	Chihuahua	27.31000	-108.59700	1460		-	0.606	(Dvorak et al., 2009)
P. oocarpa	Mesa de los Leales	Chihuahua	26.37600	-107.76500	1305		-	0.533	
P. oocarpa	Duraznito Picachos	Durango	23.68000	-105.89400	1615		-	0.63	

Species	Locality	State	LN	LO	Alt	Area (ha)	Ho	He	Author
P. oocarpa	Capilla del Taxte	Sinaloa	23.42100	-105.86500	1260		-	0.620	
P. oocarpa	La Petaca	Sinaloa	23.41800	-105.80400	1635		-	0.628	
P. oocarpa	El Tuito	Jalisco	20.35800	-105.24500	950		-	0.678	
P. oocarpa	Ocotes Altos	Nayarit	21.26900	-104.51300	1450		-	0.437	
P. oocarpa	El Durazno	Jalisco	19.36700	-102.68300	750		-	0.575	
P. oocarpa	Taretan/Uruapan	Michoacán	19.41700	-102.06700	1610		-	0.664	
P. oocarpa	Tzararacua	Michoacán	19.41700	-102.03300	1400		-	0.642	
P. oocarpa	Los Negros	Michoacán	19.21700	-101.75000	1710		-	0.689	
P. oocarpa	El Llano	Michoacán	19.25000	-100.41700	1760		-	0.622	
P. oocarpa	Valle de Bravo	Edo. Méx.	19.23300	-100.11700	1870		-	0.697	
P. oocarpa	Tenería	Edo. Méx.	18.98300	-100.05000	1760		-	0.694	
P. oocarpa	El Campanario	Guerrero	17.28400	-99.26600	1528		-	0.670	
P. oocarpa	Chinameca	Hidalgo	20.75000	-98.65000	1550		-	0.672	
P. oocarpa	Huayacocotla	Veracruz	20.50000	-98.41700	1300		-	0.670	
P. oocarpa	San Sebastián Coatlán	Oaxaca	16.18300	-96.83300	1750		-	0.689	
P. oocarpa	Ocotal Chico	Veracruz	18.25000	-94.86700	550		-	0.598	
P. oocarpa	San Pedro Solteapán	Veracruz	18.25000	-94.85000	602		-	0.589	
P. oocarpa	El Jícaro	Oaxaca	16.53300	-94.20000	1000		-	0.623	
P. oocarpa	La Cascada	Chiapas	16.83300	-93.83300	900		-	0.604	
P. oocarpa	Cienega de Leon	Chiapas	16.75000	-93.75000	1100		-	0.605	
P. oocarpa	El Sanibal	Chiapas	16.83300	-92.91700	1180		-	0.631	
P. oocarpa	La Florida	Chiapas	16.91700	-92.88300	1625		-	0.617	
P. oocarpa	La Codicia	Chiapas	16.91700	-92.11700	1200		-	0.698	
P. oocarpa	La Trinitaria	Chiapas	16.25000	-92.05000	1450		-	0.640	
Mean								***0.630***	
P. patula	Capulálpam de Méndez	Oaxaca	17.38956	-96.51469	2783		0.696	0.790	(Alfonso-Corrado et al., 2014)
P. patula	Jaltianguis	Oaxaca	17.57994	-96.51461	2634		0.669	0.845	
P. patula	Santiago Comaltepec	Oaxaca	17.32658	-96.40250	2782		0.554	0.838	
P. patula	Sitio de un año	Oaxaca	17.30783	-96.38283	2618		0.746	0.769	
P. patula	Sitio de cinco años	Oaxaca	17.30586	-96.38475	2266		0.579	0.775	

Species		Locality	State	LN	LO	Alt	Area (ha)	Ho	He	Author
P. patula		Sitio de 18 años	Oaxaca	17.32011	-96.38925	2324		0,648	0,765	
	Mean							***0.648***	***0.797***	
P. patula		Conrado Castillo	Tamaulipas	23.93300	-99.46700	1780		-	0.583	(Dvorak et al., 2009)
P. patula		El Cielo	Tamaulipas	23.06700	-99.23300	1665		-	0.568	
P. patula		Llano de Carmonas	Puebla	19.80000	-97.90000	2705		-	0.529	
P. patula		Cruz Blanca	Veracruz	19.65000	-97.15000	2500		-	0.509	
P. patula		Corralitla	Veracruz	18.63300	-97.10000	2115		-	0.570	
P. patula		Yextla	Guerrero	17.59800	-99.84300	2295		-	0.599	
	Mean								***0.559***	
P. pseudostrobus		Santa Rosa	Michoacán	19.82417	-100.06361	900		0.29	0.277	(Delgado et al., 2013)
	Mean								***0.277***	
P. pseudostrobus		Malacatepec	Edo. Méx.	19.35444	-100.15111	3100		-	0.272	(Delgado & Piñero, 2002)
P. pseudostrobus		Angangueo	Michoacán	19.53944	-100.37917	2850		-	0.298	
P. pseudostrobus		Temascaltepec	Edo. Méx.	19.47028	-100.70083	3100		-	0.54	
P. pseudostrobus		Zitácuaro	Michoacán	2-000	-100.63333	1700		-	0.512	
P. pseudostrobus		Aguililla	Michoacán	18.82139	-102.92667	2370		-	0.413	
P. pseudostrobus		San Cristobal	Chiapas	16.74028	-92.43333	2440		-	0.345	
	Mean								***0.396***	
P. pseudostrobus		Joya del Durazno	Michoacán	19.46333	-102.15000	2200		0.12	0.12	(Viveros-Viveros et al., 2014)
P. pseudostrobus		Cerro Pario	Michoacán	19.47333	-102.18333	2910		0.1	0.09	
	Mean								***0.105***	

Table S5. Physiographic Provinces and Germplasm Transfer Zones (CONAFOR 2016).

Code	Name
I.1, I.2	Península de Baja California 1 and 2
II.1, II.2	Llanura Sonorense 1 and 2
III.1, III.2, III.3, III.4	Sierra Madre Occidental 1, 2, 3, and 4
IV.1, IV.2	Sierras y Llanuras del Norte 1 and 2
V.1, V.2, V.3	Sierra Madre Oriental 1, 2 and 3
VI.1	Grandes Llanuras de Norteamérica 1
VII.1, VII.2	Llanura Costera del Pacífico 1 and 2
VIII.1, VIII.2, VIII.3, VIII.4	Llanura Costera del Golfo Norte 1, 2, 3 and 4
IX.1, IX.2	Mesa del Centro 1 and 2
X.1, X.2, X.3	Eje Neovolcánico 1, 2, 3
XI.1, XI.2, XI.3	Península de Yucatán 1, 2 and 3
XII.1, XII.2, XII.3, XII.4, XII.5	Sierra Madre del Sur 1, 2, 3, 4 and 5
XIII.1, XIII.2, XIII.3	Llanura Costera del Golfo Sur 1, 2 and 3
XIV.1, XIV.2, XIV.3	Sierras de Chiapas y Guatemala 1, 2 and 3
XV.1, XV.2, XV.3	Cordillera Centroamericana 1, 2 and 3

Table S6. Minimum requirements for genetic conservation units (Koskela et al. 2013)**.**

Requirement	Description
Species	The species has been recognized as target tree species for conservation.
	There is one of the following conservation proposes for the unit:
	Propose 1: maintain genetic diversity in large tree populations
	Propose 2: conserve specific adaptive or other traits in marginal or scattered tree populations
	Propose 3: conserve rare or endangered tree species with populations consisting of a small number of remaining individuals
Population size	This requirement depends on the conservation objective. The number of trees is verified based on NFLI data:
	Objective 1: If the unit is to maintain genetic diversity of species, the conservation unit must consists of 500 or more reproducing trees.
	Objective 2: If the unit is to conserve specific adaptive or other traits in marginal or scattered tree populations, the unit must harbor a minimum of 50 reproducing trees.
	Objective 3: If the unit is to conserve remaining populations of rare or endangered species, it must harbor a minimum of 15 unrelated reproducing trees.
Management	Forest management is applied for target species within the unit for:
	Propose 1: ensure the continued existence of tree populations.
	Propose 2: provide favorable conditions for growth and natural regeneration
Monitoring	Regeneration success is assessed every five or ten years, and to update the management plan
	Monitoring the units to evaluate their objective status
Ownership	Case 1: the unit corresponding to private landowners
	Case 2: the unit corresponding to communal land owners

Table S7. Volume of timber for target species per state, across their natural distribution.

State	Volume		Plantation		Comment
	Year[1]	m3r	Year[2]	ha	
P. greggii					
Coahuila	2013	-	-	-	
Hidalgo	2014	-	-	-	
Edo. México	-	-	-	-	
Nuevo León	2013	-	-	-	
Puebla	2014	-	-	-	
Querétaro	2014	14,460	2010	28.64	For volume value include *P. greggii, P. patula, P. oocarpa and P. teocote.* For plantation species are unknown.
San Luis Potosí	2014	-	-	-	
P. oocarpa					
Chiapas	2013	186,023	2010	0	For volume value include *P. oocarpa, P. oocarpa var ochoterenae, P. maximinoi, P. teocote, P. devoniana* and *P. oaxacana.* For plantation species are unknown.
Chihuahua	2014	-	-	-	
Colima	2013	926	2010	0	For volume value *include P. oocarpa, P. pseudostrobus* and *P. douglasiana.* For plantation species are unknown.
Durango	2014	-	-	-	
Guanajuato	2014	-	-	-	
Guerrero	2013	-	-	-	
Hidalgo	2014	-	-	-	
Jalisco	2013	245,925	2010	3.35	For volume value include *P. leiophylla, P. devoniana, P. oocarpa, P. pseudostrobus, P. douglasiana, P. lumholtzi* and *P. maximinoi.* For plantation species are unknown.
Edo. México	-	-	-	-	
Michoacán	2012	-	-	-	
Nayarit	2012	-	-	-	
Oaxaca	2014	339,854	2010	136.20	For volume value include *P. patula, P. pseudostrobus, P. devoniana, P. montezumae, P. rudis, P. leiophylla, P. teocote, P. oaxacana, P. oocarpa, P. ayacahuite, P. herrerae, P. pringlei, P. michoacana, P. douglasiana, P. maximinoi, P. lawsonii, P. chiapensis* and *P. caribaea.* For plantation species are unknown.
Puebla	2014	-	-	-	
Querétaro	2014	14,460	2010	28.64	For volume value include *P. greggii, P. patula, P. oocarpa* and *P. teocote.* For plantation species are unknown.
San Luis Potosí	2014	-	-	-	
Sinaloa	2013	-	-	-	
Sonora	2014	-	-	-	
Zacatecas	2014	-	-	-	

State	Volume		Plantation		Comment
	Year[1]	m3r	Year[2]	ha	
P. patula					
Edo. México	-	-	-	-	
Guerrero	2013	-	-	-	
Hidalgo	2014	111,109	2010	4.53	For volume value include *P. patula, P. teocote, P. leiophilla, P. montezumae, P. rudis, P. pseudostrobus* and *P. ayacahuite.* For plantation species are unknown.
Jalisco	2013	-	-	-	
Michoacán	2012	-	-	-	
Morelos	2013	13,181	2010	4.00	For volume value include *P. pseudostrobus, P. montezumae, P. patula*, and *P. leiophylla.* For plantation species are unknown.
Oaxaca	2014	339,854	2010	136.20	For volume value include *P. patula, P. pseudostrobus, P. devoniana, P. montezumae, P. rudis, P. leiophylla, P. teocote, P. oaxacana, P. oocarpa, P. ayacahuite, P. herrerae, P. pringlei, P. douglasiana, P. maximinoi, P. lawsonii, P. chiapensis* and *P. caribaea.* For plantation species are unknown.
Puebla	2014	171,204	2010	35.82	For volume value include *P. ayacahuite, P. chiapensis, P. hartwegii, P. leiophylla, P. montezumae, P. patula, P. pseudostrobus* and *P. teocote.* For plantation species are unknown.
Querétaro	2014	14,460	2010	28.64	For volume value include *P. greggi, P. patula, P. oocarpa* and *P. teocote.* For plantation species are unknown.
Tamaulipas	2014	22,541	2010	0	For volume value include *P. teocote, P. pseudostrobus, P. montezumae, P. patula, P. ayacahuite, P. cembroides* and *P. nelsonii.* For plantation species are unknown.
Tlaxcala	2014	14,216	2010	198.36	For volume value include *P. pseudostrobus, P. ayacahuite, P. teocote, P. rudis, P. leiophylla* and *P. patula.* For plantation species are unknown.
Veracruz	2014	134,068	2010	704.56	For volume value include *P. ayacahuite, P. montezumae, P. patula, P. rudis, P. pseudostrobus*, and *P. teocote.* For plantation species are unknown.
P. pseudostrobus					
Chiapas	2013	-	-	-	
Chihuahua	2014	-	-	-	
Coahuila	2013	-	-	-	
Durango	2014	-	-	-	
Edo. México	-	-	-	-	
Guanajuato	2014	-	-	-	
Guerrero	2013	110,906	2010	0	For volume value include *P. ayacahuite, P. herrerae, P. maximinoi, P. pseudostrobus, P. teocote* and *P. greggii.* For plantation species are unknown.

State	Volume		Plantation		Comment
	Year[1]	m3r	Year[2]	ha	
Jalisco	2013	245,925	2010	3.35	For volume value include *P. leiophylla, P. devoniana, P. oocarpa, P. pseudostrobus, P. douglasiana, P. lumholtzi* and *P. maximinoi.* For plantation species are unknown.
Michoacán	2012	-	-	-	
Morelos	2013	13,181	2010	4.00	For volume value include *P. pseudostrobus, P. montezumae, P. patula,* and *P. leiophylla.* For plantation species are unknown.
Nayarit	2013	-	-	-	
Nuevo León	2013	-	-	-	
Oaxaca	2014	339,854	2010	136.20	For volume value include *P. patula, P. pseudostrobus, P. devoniana, P. montezumae, P. rudis, P. leiophylla, P. teocote, P. oaxacana, P. oocarpa, P. ayacahuite, P. herrera, P. pringlei, P. douglasiana, P. maximinoi, P. lawsonii, P. chiapensis* and *P. caribaea.* For plantation species are unknown.
Puebla	2014	171,204	2010	35.82	For volume value *include P. ayacahuite, P. chiapensis, P. hartwegii, P. leiophylla, P. montezumae, P. patula, P. pseudostrobus* and *P. teocote.* For plantation species are unknown.
Querétaro	2014	-	-	-	
San Luis Potosí	2014	-	-	-	
Sinaloa	2013	-	-	-	
Tamaulipas	2014	22,541	2010	0	For volume value include *P. teocote, P. pseudostrobus, P. montezumae, P. patula, P. ayacahuite, P. cembroides* and *P. nelsonii.* For plantation species are unknown.
Tlaxcala	2014	14,216	2010	198.36	For volume value include *P. pseudostrobus, P. ayacahuite, P. teocote, P. rudis, P. leiophylla* and *P. patula.* For plantation species are unknown.
Veracruz	2014	134,068	2010	704.56	For volume value include *P. ayacahuite, P. montezumae, P. patula, P. rudis, P. pseudostrobus* and *P. teocote.* For plantation species are unknown.

[1]According data from the Mexico National Institute of Statistics, Geography and Informatics (INEGI 2018), [2]according data from 2010 (SEMARNAT 2018).

Table S8. Population and genetic zone considered as the most suitable source for use in reforestation of forest plantations.

Number of species populations / Trial / Test site	Significant populations	State	LON	LAT	Evaluated variable	Main conclusion	Authors	Genetic zone
6 populations - *P. greggii* var. *greggii* 6 populations – *P. greggii* var. *australis* Provenance test in Patoltecoya, Pue	El Madroño Molango Zimapán	Qro Hgo Hgo	21.266667 20.816667 20.75	-99.166667 -98.766667 -99.033333	Survival, diameter, and height (plants 33 months old)	Provenances Zimapán, Molango and El Madrono had higher growth in diameter and height than others.	(López-Ayala et al. 1999)	V.3 V.3 V.3
2 populations - *P. greggii* var. *australis* Provenance test in Ejido Cerro de León, Ver	Naolinco	Ver	19.65	-96.866667	Diameter and height growth (plants 3 years old)	The progeny from Naolinco presented a better development both in height and in diameter.	(Gutiérrez Valencia et al. 2012)	X.3
10 populations - *P. greggii* var. *australis* Provenance test in Patoltecoya, Pue	Valle Verde Pemuxtitla	Qro Hgo	21.483333 20.8	-99.216667 -98.733333	Survival, diameter, height, and volume (plants 6 years old)	Provenances from Querétaro and Hidalgo grew best than the rest.	(López-Upton et al. 2005)	V.3 V.3
9 populations - *P. greggii* var. *greggii* Provenance test in Ej. 18 de Marzo, Nuevo León	Agua Fría La Tapona	Coah/N.L. N. León	25.433333 24.716667	-100.5 -100.1	Diameter and height (tress 10.9 years old).	Trees from Agua Fría maintained the largest growth in total height and was slightly exceeded by La Tapona, as to the normal diameter.	(Rodriguez-Laguna et al. 2013;	V.3 V.3
6 populations - *P. greggii* var. *greggii* 7 populations - *P. greggii* var. *australis* Provenance test in	Los Lirios El Piñón	Coah Hgo	25.383333 20.933333	-100.566667 -99.2	Aboveground biomass	The highest leaf biomass accumulation	(Villegas-Jiménez et al. 2013)	V.3 V.3

Number of species populations / Trial / Test site	Significant populations	State	LON	LAT	Evaluated variable	Main conclusion	Authors	Genetic zone
Magdalena Zahuatlán, Nochixtlán, Oax	Molango	Hgo	20.816667	-98.766667	allocation (trees 14 year old)	was for El Piñón and Molango provenances. Los Lirios accumulated the highest biomass in wood + bark.		V.3
6 populations - *P. greggii* var. *greggii* 6 populations - *P. greggii* var. *australis* Provenance test in Tlacotepec Plumas, Coixtlahuaca, Oax	Molango	Hgo	20.816667	-98.766667	Wood density (trees 14 years old)	The Molango provenance was significantly higher than other provenances	(Cruz Mejía 2011)	V.3
6 populations - *P. greggii* var. *greggii* 3 populations - *P. greggii* var. *australis* Seedling provenance test under cold conditions at greenhouse / lab New Mexico State, USA*	Jamé Puerto San Juan Santa Anita El Conejo	Coah Coah Coah Coah	25.35 25.416667 25.45 25.466667	-100.6 -100.533333 -100.566667 -100.566667	Seedling cold hardiness, bud set, and bud break (seedlings <1 year old)	For cold hardiness, Jamé, San Juan, Sta. Anita and El Conejo provenances were significant than the rest. For bud set, Jamé was significant different than others.	(Aldrete, Mexal, and Burr 2008)	V.3 V.3 V.3 V.3
6 populations - *P. greggii* var. greggii 6 populations - *P. greggii* var. *australis* Seedling provenance test to different pH water at nursery Texcoco, Edo. Méx.	El Madroño	Qro	21.266667	-99.166667	Speed germination, survival, foliage color, height (seedlings 16 months old)	El Madroño provenance was significantly different in height than others.	(López-Upton et al. 2000)	V.3
3 populations - *P. greggii* var. *greggii* 6 populations - *P. greggii* var. *australis*	El Piñón	Hgo	20.933333	-99.2	Carbon composition,	El Piñón provenance had		V.3

Number of species populations / Trial / Test site	Significant populations	State	LON	LAT	Evaluated variable	Main conclusion	Authors	Genetic zone
Seedling provenance test to two water regimes at greenhouse Texcoco, Edo. Méx.					biomass (seedlings 24 months old)	the greatest biomass accumulation in both treatments; thus, it is considered as the one with the largest potential.	(García García et al. 2003)	
9 populations - *P. greggii* var. *greggii* 6 populations - *P. greggii* var. *australis* Seedling provenance test to two water regimes at greenhouse Texcoco, Edo. Méx.	Pto. Los Conejos	Coah	25.485767	-100.581733	Diameter, height, number of roots, and biomass (seedlings 1.5 years old)	Populations from north were more tolerant of drought stress.	(Hernández-Pérez et al. 2001)	V.3
	Santa Anita	Coah	25.450171	-100.569618				V.3
	Agua Fría	Coah/N.L.	25.433333	-100.5				V.3
	Los Lirios	Coah	25.383333	-100.566667				V.3
	Puerto San Juan	Coah	25.416667	-100.55				V.3
	El Penitente	Coah	25.366667	-100.9				V.3
	Jamé	Coah	25.35	-100.6				V.3
	Las Placetas	N. León	24.916667	-100.183333				V.3
	La Tapona	N. León	24.716667	-100.1				V.3
5 populations - *P. oocarpa* Provenance test on the field Canalejas, Mich	La Tinaja	Mich	19.283333	-102.083333	Seedling height (plants 2.5 years old)	The largest difference was in La Tinaja population, its height was 23% superior.	(Sáenz-Romero, Guzmán-Reyna, and Rehfeldt 2006)	X.2
11 populations - *P. patula* Provenance test on the field Los Ayacahuites, Pue	Pinal de Amoles	Qro	21.016667	-99.166667	Height growth and number of growth cycles (plants 18 months old). Survival, diameter and height (plants 5 years old).	Provenances from Zacualtipan, Tlahuelompan and Zacatlán Nte, and Pinal de Amoles grew best than other ones.	Salazar-García et al. (1999); López-Upton et al. (2000)	V.3
	Zacualtipan	Hgo	20.383333	-98.4				V.3
	Tlahuelompan	Hgo	20.383333	-98.35				V.3
	Zacatlán Nte	Pue	19.9666667	-97.9833333				X.3

Number of species populations / Trial / Test site	Significant populations	State	LON	LAT	Evaluated variable	Main conclusion	Authors	Genetic zone
13 populations - *P. patula* Seedling provenance test at forest nursery Ixtlán de Juárez, Oax and Quebec, Canada*	Populations at 2650 masl	Oax	17.3860333	-96.4823	Seedling height (seedlings 6 months old)	The population sampled at 2650 m of altitude (middle) had the largest value of total seedling height.	(Ruiz-Talonia et al. 2014)	XII.5
20 populations - *P. patula* Seedling provenance test at lab North Carolina, USA*	La Encarnación	Hgo	20.883333	-99.216667	Resistance to pitch canker (Fusarium circinatum) (seedlings 21 weeks old)	The most resistant provenances occur for El Cielo, Yextla, Conrado Castillo, El Tlacuache, La Encarnación and Pinal Amoles.	(Hodge and Dvorak 2006)	V.3
	El Tlacuache	Oax	16.733333	-97.15				XII.4
	Yetla	Oax	17.6	-99.85				XII.3
	Pinal de Amoles	Qro	21.116667	-99.683333				V.3
	Conrado Castillo	Tamps	23.933333	-99.466667				V.3
	El Cielo	Tamps	23.066667	-99.233333				V.3

* Test in a foreign country

Table S9. Conservation units for *P. greggii, P. oocarpa, P. patula* and *P. pseudostrobus*.

Genetic zone	Criteria	Protected area	Locality	State
P. greggii				
V.3	C1a	C.A.D.N.R. 026 Bajo Río San Juan	Puerto San Juan	Coahuila
V.3	C1a	Sierra Gorda	Valle Verde	Querétaro
V.3	C1a	Los Mármoles	El Piñón	Hidalgo
X.3	C3a	Z.P.F.T.C.C. de los ríos Valle de Bravo, Malacatepec, Tilostoc y Temascaltepec	Valle de Bravo	Edo. México
P. oocarpa				
III.2	C2b	-	Chinipas	Chihuahua
III.3	C2b	-	Mesa de los Leales	Chihuahua
III.3	C3a	La Michilía	Suchil	Durango
III.4	C2b	-	Duraznito Picachos	Durango
III.4	C3a	C.A.D.N.R. 043 Estado de Nayarit	La Yesca	Nayarit
V.3	C2b	-	Chinameca	Hidalgo
X.1	C2b	-	Ocotes Altos	Nayarit
X.1	C3a	La Primavera	Zapopán	Jalisco
X.2	C1b	-	Matanguarán	Michoacán
X.3	C2a	Z.P.F.T.C.C. de los ríos Valle de Bravo, Malacatepec, Tilostoc y Temascaltepec	Valle de Bravo	Edo. México
XII.1	C2b	-	EL Tuito	Jalisco
XII.1	C3a	Sierra de Manantlán	Cuautitlán de García Barragán	Jalisco
XII.2	C2b	-	El Durazno	Jalisco
XII.2	C3b	-	Tumbiscatio	Michoacán
XII.3	C2b	-	Tenería	Edo. México
XII.3	C2b	-	El Campanario	Guerrero
XII.4	C2b	-	San Sebastián Coatlán	Oaxaca
XII.5	C3b	-	Santa María Alolepec	Oaxaca
XIV.1	C2b	-	La Florida	Chiapas
XIV.1	C2b	-	La Tinitaria	Chiapas
XIV.2	C3b	-	Altamirano	Chiapas
XV.1	C2b	-	El Jícaro	Oaxaca
XV.1	C3a	El Triunfo	Siltepec	Chiapas
P. patula				
V.3	C2b	-	El Cielo	Tamaulipas
V.3	C1a	Los Mármoles	Zimapán	Hidalgo
X.3	C1a	Z.P.F.V. la Cuenca Hidrográfica del Río Necaxa	Acaxochitlán	Hidalgo
X.3	C2b	-	Cruz Blanca	Veracruz
X.3	C3a	Bosencheve	San José Villa de Allende	Edo. México
XII.3	C2b	-	Yextla	Guerrero
XII.4	C1b	-	El Tlacuache	Oaxaca
XII.5	C1b	-	Corralitla	Veracruz
XII.5	C2b	-	Santiago Comaltepec	Oaxaca

Genetic zone	Criteria	Protected area	Locality	State
P. pseudostrobus				
III.2	C3b	-	Sinaloa	Sinaloa
III.3	C3b	-	Badiraguato	Sinaloa
V.3	C3a	Cumbres de Monterrey	Santiago	Nuevo León
V.3	C3a	El Potosí	Rio averde	San Luis Potosí
VIII.3	C3b	-	San Carlos	Tamaulipas
X.1	C3a	C.A.D.N.R. 043 Estado de Nayarit	Atenguillo	Jalisco
X.2	C1b	-	Nuevo San Juan Parangaricutiro	Michoacán
X.3	C2a	Z.P.F.T.C.C. de los ríos Valle de Bravo, Malacatepec, Tilostoc y Temascaltepec	Malacatepec	Edo. México
X.3	C3a	La Montaña Malinche	Huamantla	Tlaxcala
XII.1	C3b	-	Talpa de Allende	Jalisco
XII.2	C2b	-	Aguililla	Michoacán
XII.3	C2b	-	Temescaltepec	Edo. México
XII.4	C3b	-	San Mateo Peñasco	Oaxaca
XII.5	C3a	Cañon del Río Blanco	Maltrata	Veracruz
XII.5	C3b	-	San Francisco Cajonos	Oaxaca
XIV.1	C2b	-	San Cristobal de las Casas	Chiapas

Table S10. Importance of timber production, plantation area and species by genetic zone.

Genetic zone	State	Total Volume[1] (m3)	Total Plantation[2] (ha)	Importance (%)[3]	Importance for timber production (m3)	Importance for plantation area (ha)
P. greggii						
V.3	Coahuila	-	-	0.55	-	-
V.3	Hidalgo	-	-	0.83	-	-
V.3	Nuevo León	-	-	0.55	-	-
V.3	Querétaro	14,460	28.64	2.20	318	0.63
V.3	S.L. Potosí	-	-	2.48	-	-
X.3	Edo. México	-	-	0.55	-	-
X.3	Puebla	-	-	0.28	-	-
Mean					***159***	***0.32***
P. oocarpa						
III.2	Chihuahua	-	-	2.05	-	-
III.2	Sinaloa	-	-	0.05	-	-
III.2	Sonora	-	-	0.05	-	-
III.3	Chihuahua	-	-	0.71	-	-
III.3	Durango	-	-	1.25	-	-
III.3	Sinaloa	-	-	0.92	-	-
III.3	Zacatecas	-	-	0.04	-	-
III.4	Durango	-	-	3.79	-	-
III.4	Jalisco	245,925	3.35	2.96	7,279	0.10
III.4	Nayarit	-	-	7.46	-	-
III.4	Sinaloa	-	-	0.83	-	-
III.4	Zacatecas	-	-	1.18	-	-
V.3	Guanajuato	-	-	0.28	-	-
V.3	Hidalgo	-	-	0.28	-	-
V.3	S. L. Potosí	-	-	0.83	-	-
X.1	Jalisco	245,925	3.35	23.36	57,448	0.78
X.1	Michoacán	-	-	2.80	-	-
X.1	Nayarit	-	-	2.34	-	-
X.2	Michoacán	-	-	15.43	-	-
X.3	Edo. México	-	-	1.10	-	-
X.3	Michoacán	-	-	1.10	-	-
X.3	Puebla	-	-	0.55	-	-
X.3	Querétaro	14,460	28.64	0.28	40	0.08
XII.1	Colima	926	0	1.18	11	0.00
XII.1	Jalisco	245,925	3.35	42.94	105,600	1.44
XII.1	Nayarit	-	-	2.35	-	-
XII.2	Guerrero	-	-	9.13	-	-
XII.2	Jalisco	245,925	3.35	17.79	43,750	0.60
XII.2	Michoacán	-	-	18.27	-	-
XII.3	Guerrero	-	-	39.68	-	-
XII.3	Edo. México	-	-	3.41	-	-
XII.3	Michoacán	-	-	2.00	-	-
XII.3	Oaxaca	339,854	136.20	1.20	4,078	1.63
XII.4	Oaxaca	339,854	136.20	23.46	79,730	31.95
XII.5	Oaxaca	339,854	136.20	25.71	87,376	35.02
XIII.1	Oaxaca	339,854	136.20	20.00	67,971	27.24
XIV.1	Chiapas	186,023	0	36.75	68,363	0.00
XIV.2	Chiapas	186,023	0	58.06	108,005	0.00

Genetic zone	State	Total Volume[1] (m3)	Total Plantation[2] (ha)	Importance (%)[3]	Importance for timber production (m3)	Importance for plantation area (ha)
XV.1	Chiapas	186,023	0	76.76	142,791	0.00
Mean					***48,278***	***6.18***
P. patula						
V.3	Hidalgo	111,109	4.53	2.75	3,055	0.12
V.3	Puebla	171,204	35.82	1.38	2,363	0.49
V.3	Querétaro	14,460	28.64	0.28	40	0.08
V.3	Tamaulipas	22,541	0	1.10	248	0.00
V.3	Veracruz	134,068	704.56	3.58	4,800	25.22
X.2	Michoacán	-	-	0.53	-	-
X.3	Cd México	-	-	0.28	-	-
X.3	Hidalgo	111,109	4.53	1.66	1,844	0.08
X.3	Edo. México	-	-	5.25	-	-
X.3	Michoacán	-	-	0.28	-	-
X.3	Morelos	13,181	4.00	0.28	37	0.01
X.3	Puebla	171,204	35.82	3.59	6,146	1.29
X.3	Tlaxcala	14,216	198.36	0.83	118	1.65
X.3	Veracruz	134,068	704.56	3.87	5,188	27.27
XII.1	Jalisco	-	-	1.18	-	-
XII.3	Guerrero	-	-	0.60	-	-
XII.3	Edo. México	-	-	0.20	-	-
XII.3	Michoacán	-	-	0.40	-	-
XII.3	Oaxaca	339,854	136.20	0.20	680	0.27
XII.4	Oaxaca	339,854	136.20	25.59	86,969	34.85
XII.5	Oaxaca	339,854	136.20	6.58	22,362	8.96
XII.5	Puebla	171,204	35.82	1.88	3,219	0.67
XII.5	Veracruz	134,068	704.56	3.76	5,041	26.49
Mean					***20,301***	***18.21***
P. pseudostrobus						
III.2	Durango	-	-	0.05	-	-
III.2	Sinaloa	-	-	0.09	-	-
III.3	Durango	-	-	0.04	-	-
III.3	Sinaloa	-	-	0.71	-	-
III.4	Nayarit	-	-	0.12	-	-
V.3	Coahuila	-	-	0.55	-	-
V.3	Guanajuato	-	-	0.28	-	-
V.3	Nuevo León	-	-	11.57	-	-
V.3	Puebla	171,204	35.82	2.20	3,766	0.79
V.3	Querétaro	-	-	0.28	-	-
V.3	S. L. Potosí	-	-	2.20	-	-
V.3	Tamaulipas	22,541	0	5.23	1,179	0.00
X.1	Jalisco	245,925	3.35	1.40	3,443	0.05
X.1	Michoacán	-	-	0.93	-	-
X.2	Michoacán	-	-	19.15	-	-
X.3	Cd México	-	-	0.28	-	-
X.3	Guanajuato	-	-	0.28	-	-
X.3	Edo. México	-	-	3.87	-	-
X.3	Michoacán	-	-	5.80	-	-
X.3	Morelos	13,181	4.00	1.38	182	0.06
X.3	Puebla	171,204	35.82	4.42	7,567	1.58
X.3	Tlaxcala	14,216	198.36	1.38	196	2.74

Genetic zone	State	Total Volume[1] (m3)	Total Plantation[2] (ha)	Importance (%)[3]	Importance for timber production (m3)	Importance for plantation area (ha)
X.3	Veracruz	134,068	704.56	2.49	3,338	17.54
XII.1	Jalisco	245,925	3.35	8.24	20,264	0.28
XII.2	Guerrero	110,906	0	5.29	5,867	0.00
XII.2	Jalisco	245,925	3.35	0.48	1,180	0.02
XII.2	Michoacán	-	-	3.85	-	-
XII.3	Guerrero	110,906	0	9.62	10,669	0.00
XII.3	Edo. México	-	-	0.40	-	-
XII.3	Michoacán	-	-	2.20	-	-
XII.3	Oaxaca	339,854	136.20	0.20	680	0.27
XII.4	Oaxaca	339,854	136.20	1.42	4,826	1.93
XII.5	Oaxaca	339,854	136.20	16.30	55,396	22.20
XII.5	Puebla	171,204	35.82	1.57	2,688	0.56
XII.5	Veracruz	134,068	704.56	3.13	4,196	22.05
XIII.1	Oaxaca	339,854	136.20	20.00	67,971	27.24
XIV.1	Chiapas	-	-	14.53	-	-
XIV.2	Chiapas	-	-	3.23	-	-
XV.1	Chiapas	-	-	0.70	-	-
Mean					***11,377***	***5.72***

[1]According 2013/2014 data from the Mexico National Institute of Statistics, Geography and Informatics (INEGI 2018); [2]according data from 2010 (SEMARNAT 2018); [3]according distribution of target species and other species (*P. teocote, P. maximinoi, P. devoniana, P. oaxacana, P. leiophylla, P. lumholtzi, P. montezumae, P. rudis, P. ayacahuite, P. herrerai, P. pringlei, P. lawsonii, P. chiapensis, P. caribaea, P. cembroides, P. nelsonii, P. arizonica, P. douglasiana, P. hartwegii* and *P. engelmannii*) that harboured in genetic zones, data from NFLI (CONAFOR 2017).