Check for updates

Received: 3 August 2021 | Accepted: 13 December 2021 | Published online: 20 February 2022

DOI: 10.1002/csc2.20691

Crop Science

ORIGINAL RESEARCH ARTICLE

Crop Breeding & Genetics

Variability of the essential oil composition of cultivated populations of *Salvia lavandulifolia* Vahl

Raúl Sánchez-Vioque[1,2] | **David Herraiz-Peñalver[1]** | **Enrique Melero Bravo[1]** | **Gonzalo Ortiz de Elguea-Culebras[1]** | **Baudilio Herrero[3]** | **Yolanda Santiago[4]** | **Marta Bueno[4]** | **Silvia Pérez-Magarino[4]** | **María del Carmen Asensio S. Manzanera[4]**

[1] Instituto Regional de Investigacion y Desarrollo Agroalimentario y Forestal de Castilla La Mancha (IRIAF), CIAF de Albaladejito, Carretera Toledo-Cuenca km 174, Cuenca 16194, Spain

[2] Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT), Fundación Parque Científico y Tecnológico de Castilla-La Mancha, Paseo de la Innovación 1, Albacete 02006, Spain

[3] Dep. de Ciencias Agroforestales, Univ. de Valladolid, Avda. de Madrid, 57, Palencia 34004, Spain

[4] Dep. de Cultivos Herbáceos, Instituto Tecnologico Agrario de Castilla y Leon (ITACyL), Ctra. Burgos km 119, Valladolid 47071, Spain

Correspondence

Raúl Sánchez-Vioque, Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla La Mancha (IRIAF), CIAF de Albaladejito, Carretera Toledo-Cuenca km 174, Cuenca, Spain, 16194.
Email: rsanchezv@jccm.es

Assigned to Associate Editor Marta Lima.

Funding information

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Grant/Award Numbers: RTA2012-00057, RTA2017-00031; European Social Fund

Abstract

Spanish sage or lavender sage (*Salvia lavandulifolia* Vahl) is an aromatic plant with a high economic potential in agriculture because of its rusticity and low chemical inputs. However, the presence of numerous chemotypes hinders the supply of homogeneous crops to the industry and makes the selection and the standardising of the plant material necessary. With this aim, our group achieved an extensive population survey of this species across the Iberian Peninsula to investigate the variability of this species and started a selection program based in multienvironment trials. Thus, 12 wild populations of *S. lavandulifolia* have been grown in two different localities and their dry biomass production, yield, and composition of essential oil have been monitored during 2 yr of cultivation to evaluate the effect of the genotype × environment interaction on these traits. Each population presented a clearly differentiated composition of the essential oil, although camphor (15.3% average), 1,8-cineole (15.0%), α-pinene (11.3%), β-pinene (8.5%), and limonene (7.5%) were the main compounds. Other compounds like *p*-cymene, γ-terpinene, bornyl acetate, spathulenol, or viridiflorol were only present in some samples. The yield of essential oil ranged from 0.9 to 2.3 g per 100 g dry biomass, and the dry biomass from 76 to 322 g plant^{-1}. Despite the high variability of these traits, we inferred that the essential oil composition in *S. lavandulifolia* is chiefly determined by genetics which suggests that individual plants (clones) with a favorable composition for a specific application could be selected and bred with a homogeneous quality over time.

1 | INTRODUCTION

Spanish sage or lavender sage (*Salvia lavandulifolia* Vahl, Labiatae) is an aromatic plant widely distributed in the Western Mediterranean Basin, mainly in the Iberian Peninsula, south of France and north of Africa. It is a rustic species well adapted to relatively poor soils and dryness conditions (Corell et al., 2008), and therefore represents an interesting option

© 2021 The Authors. Crop Science © 2021 Crop Science Society of America

for degraded soils of Spanish rural areas in which other crops are scarcely profitable (Usano-Alemany et al., 2012). Moreover, *S. lavandulifolia* is tolerant of low temperatures and its resistance to pests makes this crop very suitable for organic agriculture. It is mostly used in perfumery and personal care products, but it may find application in other fields like cosmetics, pest control, food industry, or pharmacology due to its numerous biological activities. Many of these activities are attributed to its essential oils and include, among others, spasmolytic, antiseptic, analgesic, the inhibition of cholinesterase enzyme, antioxidant, anti-inflammatory, and central nervous system depressant properties (Porres-Martínez et al., 2013).

Discrepancies among Spanish botanists greatly complicate the classification of *Salvia* taxa. We have followed the criteria of Flora Ibérica (Sáez, 2010) and Flora Europaea (Hedge, 1972) which considers the taxon as a species despite *Salvia lavandulifolia* is from the point of view of botanical orthodoxy currently recognized as a subspecies of *Salvia officinalis* L. As occurs with other aromatic plants, *S. lavandulifolia* presents a high variability among populations. Five subspecies have been defined according to their morphological traits only in the Iberian Peninsula, in addition to numerous intermediate groups due to spontaneous intraspecific hybridizations (Sáez, 2010). A hybrid of sage obtained by spontaneous crossing of two close-related species of *Salvia* (*S. officinalis* and *S. lavandulifolia* subsp. *lavandulifolia*) has been also described, which shows intermediate essential oil production and composition in comparison with the parental species (Herraiz-Peñalver et al., 2015). Essential oil composition is also highly variable (Herraiz-Peñalver et al., 2010; Jordán et al., 2009; Méndez-Tovar et al., 2016) and several chemotypes have been identified in *Salvia lavandulifolia* subsp. *vellerea* (Jordán et al., 2009). In general, α- and β-pinene, 1,8-cineole, and camphor are the predominant terpenes in the essential oil but the proportions of these and other constituents are very variable and some terpenes like camphene, camphor, and viridiflorol may be absent in some populations. Moreover, most of the crops of *S. lavandulifolia* in Spain are original wild plants collected and cultivated by the farmers (Herraiz-Peñalver, Asensio-Manzanera et al., 2017), which further hampers the supply of a homogeneous and well-characterised plant material of *S. lavandulifolia* that ensures the quality and quantity of the essential oils in the end-uses. Standardising the plant material, thus providing maximum homogeneity, is the main target for production sector.

It is well known that the phenotype of an individual plant is the result of its genetic constitution (genotype) and the influence of the environment in which it is grown. In this balance, a predominance of the genetic heritability implies a reduction of the environmental variations and a phenotypic stability that favours the selection and propagation of those clones with desirable characteristics (Falconer & MacKay, 1996). The variability of Spanish sage essential oil seems mainly related to the genotype since chemical variation among years of harvest is very small (Méndez-Tovar et al., 2016). However, other environmental factors must be taken into account in order to select suitable genotypes for commercial production for both producers and industries.

Core Ideas

- Wild populations (12) of *Salvia lavandulifolia* were investigated for their adaptation to cultivation.
- Biomass and yield and composition of essential oil were monitored 2 yr in two locations .
- High variability of traits mostly explained by the origin of populations (genetics).
- Minor influence of year and location (environmental factor).
- Promising results for the selection and breeding of homogeneous plants over time.

To address plant breeding and clone selection in *S. lavandulifolia*, our groups have achieved during the last years an extensive population survey of this species across the Iberian Peninsula in order to investigate the variability of this species (Herraiz-Peñalver et al., 2010; Mendez-Tovar et al., 2016). Subsequently, some of these wild populations were selected and grown in our experimental fields to evaluate their behaviour under cultivation. Morphological traits showed a good stability during 2 yr of cultivation in two different locations and, despite some intrapopulation variability was observed, most of the variability was explained by the geographical origin of the populations (Herraiz-Peñalver, Asensio-Manzanera et al., 2017). In the present study, agronomic traits like the dry biomass and essential oil yielded per plant, and the composition of the essential oil of 12 wild populations of *S. lavandulifolia* have been monitored during 2 yr under cultivation in two different localities to evaluate the effect of the genotype × environment interaction on these traits. Heritability in a broad sense of the evaluated traits was calculated in order to assess the stability of the selected genotypes.

2 | MATERIALS AND METHODS

2.1 | Plant material and distillation of essential oils

A previous survey on more than 50 populations of the Iberian Peninsula showed a high variability in terms of the chemical composition of the essential oil and the phenols of the

14350653, 2022, 2, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.1002/csc2.20691 by Universidad De Valladolid, Wiley Online Library on [10/01/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 Geographical data of the original collecting locations in the Iberian Peninsula of the cultivated populations of S. lavandulifolia

Populations	Locality	Province	Latitude	Longitude	Altitude
					masl
1	Letur	Albacete	38°14'27" N	02°10'12" W	1,185
2	Moratalla	Murcia	38°11'35" N	02°11'22" W	1,165
3	La Sentiú de Sió	Lérida	41°48'24" N	00°52'47" E	248
4	Tuixent	Lérida	42°14'37" N	01°31'51" E	1,031
5	Guixers	Lérida	42°09'03" N	01°41'55" E	1,388
6	Vertavillo	Palencia	41°49'17" N	04°19'53" W	823
7	Cifuentes	Guadalajara	40°46'52" N	02°40'36" W	990
8	Olmeda de la Cuesta	Cuenca	40°19'33" N	02°27'29" W	820
9	Serón de Nágima	Soria	41°30'15" N	02°11'54" W	951
10	Saelices de la Sal	Guadalajara	40°53'53" N	02°20'25" W	1,016
11	Aliaguilla	Cuenca	39°44'46" N	01°21'10" W	1,090
12	Tendilla	Guadalajara	40°32'31" N	02°57'03" W	858

hydrodistilled residue. The populations best representing this variability, in addition to those showing a distinct essential oil yield, geographical distribution, and general appearance were selected for this work (Table 1). These populations have distinguishing morphological characteristics (Herraiz-Peñalver, Asensio-Manzanera et al., 2017), and were occasionally difficult to adjust to the taxonomic classification of *S. lavadulifolia* subspecies proposed by Sáez (2010). Twenty-five individual plants representative of the genotypic variability of each selected wild population were vegetatively propagated and rooted under greenhouse conditions. Each population was planted in an experimental plot which consisted of one specimen of those 25 different plants planted in a row. Plants were placed in the field with separations of 2 m between rows and 0.5 m among plants. The experimental design was a randomized block design with three replications settled in 2012 in two different locations:

- Centro de Investigación Agroforestal-CIAF de Albaladejito (40° 04′ 31′′ N, 02° 12′ 18′′ W, Cuenca, Spain), 902 masl, on a basic soil (pH: 8.43) with franc texture, medium content of organic matter (1,77%), and low salinity. From July 2013 to June 2014, monthly average temperature was 14.1 ˚C and annual rain 441 mm, and from July 2014 to June 2015, monthly average temperature was 14.5 ˚C and annual rain 450 mm.
- Instituto Tecnológico Agrario de Castilla y León-ITACYL (41° 42′ 08′′ N, 04° 42′ 31′′ W, Valladolid, Spain), 695 masl, on a typic Xerofluvent soil, characterized by a silty texture with fine detritic deposits and calcilulites as parent material. From July 2013 to June 2014, monthly average temperature was 13.3 ˚C and annual rain 404 mm, and from July 2014 to June 2015, monthly average temperature was 13.4 ˚C and annual rain 411 mm.

The trial was carried out in rainfed conditions, and cultural practises were limited to weed control. All aerial parts (inflorescences, leaves, and stems) of each plot were collected during the seasons 2014 and 2015 from the experimental fields at the flowering period and dried at room temperature prior to hydrodistillation. Dry biomass per plant was calculated as the ratio between the total weight of each plot and the number of living plants. Around 180 g of a representative dry sample of each plot was hydrodistilled (2.5 L) for 4 h using a Clevenger type apparatus (Boland et al., 1991).

2.2 | Analysis of the essential oils

The identification of the chemical compounds of the essential oils was carried out by gas chromatography–mass detector (GC–MS) in a HP-6890N GC coupled to a HP-5973 inert MS detector equipped with a HP-5MS capillary column (30-m length, 0.25-mm i.d., 0.25-µm film thickness). The injection temperature was 250 ˚C and 1 µl of sample (previously diluted 1:33 in acetone) was injected in split mode (100:1). The carrier gas was helium at a flow rate of 1.2 ml min^{-1} and the oven column program was set to 70 ˚C (held for 5 min), raised to 100 ˚C at 2 ˚C min^{-1}, raised to 154 ˚C at 3 ˚C min^{-1} and raised to 280 ˚C at 100 ˚C min^{-1}. Detection was in electron ionization (EI) mode (70 eV), and identification was carried out using spectra obtained from commercial standard compounds and from National Institute of Standards and Technology (NIST) library. Standards compounds were provided by Sigma-Aldrich and Fluka. Fifty-two compounds were identified, and their relative peak area was calculated in relation to the total chromatogram area (Supplemental Table S1). However, only the compounds with a percentage higher than 1% were considered and discussed.

14350653, 2022, 2, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.1002/csc2.20691 by Universidad De Valladolid, Wiley Online Library on [10/01/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 Means of the essential oil composition, essential oil yield, and dry biomass of populations of S. lavandulifolia corresponding to both seasons and both locations. Quantification of compounds is expressed as their relative peak areas in relation to the total chromatogram area, essential oil yield in g per 100 g of dry plant, and dry biomass in g per plant. Means between populations followed by a common letter are not significantly different according to Tukey's test at $p < .05$

	Populations											
	Aliaguilla	Cifuentes	Guixers	La Sentiú de Sió	Letur	Moratalla	Olmeda de la Cuesta	Saelices de la Sal	Serón de Nágima	Tendilla	Tuixent	Vertavillo
α-Thujene	0.2c	0.4cb	11.8a	0.5c	0.2c	0.2c	0.4c	0.5c	0.6c	0.2c	4.9b	0.7c
α-Pinene	5.1e	16.4ab	7.9de	8.4cde	9.5cd	7.7de	12.3bc	16.6a	15.9ab	15.6ab	11.6cd	9.9cd
Camphene	4.9cd	3.1e	5.5c	7.0b	4.1d	8.7a	5.3c	2.5e	2.4e	4.8d	4.3d	5.6c
Sabinene	2.4c	1.1d	4.0a	0.3f	0.7de	1.1d	0.3ef	1.1d	0.9d	0.2f	3.1b	0.7de
β-Pinene	9.3bc	12.0a	9.0bc	3.9f	4.5ef	4.0f	9.8b	12.1a	5.7e	7.0d	13.4a	7.9cd
β-Myrcene	2.6ef	5.1cd	2.8ef	4.4d	2.1f	3.2e	4.4d	6.0b	5.5bc	6.9a	5.5bc	4.9cd
p-Cymene	0.2g	1.2def	1.4de	1.8bc	1.1f	0.2g	2.5a	1.5cd	2.0b	0.9f	1.0f	1.4de
D-Limonene	3.7f	11.7a	6.5ed	5.3ef	3.9f	6.4ed	10.1ab	10.9ab	5.9def	8.7bc	7.8cd	5.3ef
1,8-Cineole	38.7a	14.7d	4.2g	10.4f	18.7bc	10.2f	13.9ed	18.7bc	21.3b	17.5c	5.9g	11.7ef
γ-Terpinene	0.4f	2.8bc	3.1ab	2.3cd	0.3f	0.4f	3.6a	3.0b	3.3ab	1.6e	2.0ed	2.2d
Camphor	12.3de	9.7ef	11.5def	22.0b	24.4b	32.4a	17.9c	7.0g	6.7g	13.9d	8.8fg	11.1ef
Borneol	4.9bc	2.7de	2.1e	5.8ab	6.2a	5.1bc	3.1d	2.1e	1.8e	2.3de	2.0e	4.8c
Bornyl acetate	1.6bcd	1.9bc	0.5e	2.4ab	3.4a	1.9bc	1.5cd	1.2cde	1.7bcd	1.4cd	0.9de	3.3a
Trans-caryophyllene	1.7ef	2.5d	3.7bc	3.7abc	2.2de	1.3f	3.6bc	3.4c	4.2ab	3.3c	4.4a	3.9abc
Spathulenol	1.4b	0.9cd	2.6a	0.4ef	1.1bc	0.6de	0.2ef	1.2bc	1.1bc	0.1f	2.4a	0.4ef
Caryophyllene oxide	1.0e	1.5d	1.9c	0.6f	1.3de	0.5f	0.5f	2.3b	2.7ab	0.4f	2.9a	2.1bc
Viridiflorol	0.1e	0.4e	0.3e	4.1c	3.2cd	0.3e	2.0d	0.3e	0.5e	5.6b	0.4e	12.4a
Essential oil yield	2.3a	1.2de	1.1ef	1.5cd	1.6c	2.1ab	1.6c	1.2de	1.2de	2.0b	0.9f	1.7c
Dry biomass	201bcd	227bc	169cde	203bcd	186bcd	99ef	322a	249b	76f	244bc	143def	178bcd

2.3 | Statistical analyses

All statistical analyses were carried out using the IBM SPSS Statistics ver. 22 package. Analysis of variance (ANOVA) was performed fixing as independent factors the parameters "Year", "Environment", "Population", and their interactions. Sum of squares were used to determine the proportion of the total variation explained by the regression model.

Heritability in broad sense (h^2BS) is the ratio of genotypic variance to the phenotypic variance (Lal et al., 2013). This parameter ranges between 0 and 1, the closer is to 1 the higher is the heritability. Mean sum of squares were used to estimate genotypic and phenotypic variances. The mean contents of dry biomass and essential oil yield and composition were calculated for each population and average multiple comparisons were established by using Tukeyt's test. In order to assess the patterns of variation, principal component analysis (PCA) was done using the most significant compounds, that is, the compounds with average percentage values higher than 1% and those statistically significant by population and by year (ANOVA results).

3 | RESULTS

The composition of the essential oil of Spanish sage showed a great variability among populations (Table 2; Supplemental Table S2). Many of these differences were quantitative as in the case of α-pinene whose mean contents ranged from 5.1 to 16.6%, those of β-pinene from 3.9 to 13.4%, limonene from 3.7 to 11.7%, 1,8-cineole from 4.2 to 38.7%, or camphor from 6.7 to 32.4%. In addition to these differences among major terpenes, others like *p*-cymene, γ-terpinene, bornyl acetate, spathulenol, or viridiflorol were only present in some samples. The mean yield of essential oil ranged from 0.9 to 2.3 g per 100 g of dry biomass, and the dry biomass from 76 to 322 g plant^{-1}. It is important to remark that the essential oils of our populations did not contain either α- or β-thujone, two toxic and undesirable compounds present in significant proportions in *S. officinalis* essential oil (Herraiz-Peñalver et al., 2010).

The data of the ANOVA performed to evaluate the influence of the parameters "Population", "Year", "Environment", and their interactions on the main essential oil compounds

14350653, 2022, 2, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.1002/csc2.20691 by Universidad De Valladolid, Wiley Online Library on [10/01/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 3 Percentages of the sum of squares obtained in the analysis of variance of the essential oil compounds and essential oil and dry biomass yields

	Model (R^2)	Population (P)	Year (Y)	Environment (E)	P × Y	P × E	Y × E	P × Y × E	h^2BS
α-Thujene	93.57***	95.21***	0.00	0.31	0.20	2.01*	0.07	0.56	.8764
α-Pinene	64.06***	80.55***	0.06	1.71	2.98	3.67	3.13*	3.47	.4963
Camphene	85.61***	84.63***	1.40*	1.85**	1.60	3.18	1.42*	3.18	.7331
Sabinene	94.02***	83.92***	0.00	6.20*	0.17	5.38*	0.00	0.59	.7898
β-Pinene	92.01***	70.82***	0.09	12.03*	1.16	4.79*	0.99**	2.48*	.7372
β-Myrcene	89.59***	56.01***	7.38***	14.51***	6.04***	3.85**	1.31**	3.79**	.5922
p-Cymene	90.40***	56.11***	1.01**	17.68***	1.23	11.12***	4.06***	2.23	.5150
D-Limonene	73.60***	75.87***	0.00	2.66*	1.21	5.00	1.41	1.61	.5598
1,8-Cineole	94.94***	87.69***	1.66***	1.14***	0.84	1.69**	1.71***	1.18*	.8790
γ-Terpinene	87.71***	84.54***	2.31***	0.52	2.07	2.19	1.76**	1.15	.7613
Camphor	92.86***	93.98***	0.00	0.16	0.50	1.73	0.22	0.79	.8684
Borneol	85.09***	70.15***	0.05	5.21***	0.89	7.06**	0.23	2.33	.6203
Bornyl acetate	72.48***	44.34***	2.37*	12.68***	3.46	11.10*	1.84	2.11	.2807
trans-Caryophyllene	85.67***	56.35***	0.09	29.09***	2.37	6.78**	0.03	1.37	.5406
Spathulenol	90.49***	83.17***	0.00	11.56***	0.53	5.33***	0.01	0.74	.6697
Caryophyllene oxide	94.06***	64.05***	0.49*	11.51***	1.71	9.30***	1.90***	3.05***	.6233
Viridiflorol	86.98***	81.50***	0.59	0.78*	2.76	1.74	0.49	1.23	.7444
Essential oil yield	85.30***	75.53***	0.00	11.50***	1.83	3.91	7.54***	2.09	.6855
Dry biomass	83.50***	20.20***	0.04	46.48***	2.59	7.14**	0.16	5.50**	.2503

Note. h^2BS, broad sense heritability.
*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.

and on essential oil and biomass yields are shown in Table 3. The model was statistically highly significant ($p < .001$) for all terpenes as well as for the yields of biomass and essential oil. The R^2 of the model was particularly high in 1,8-cineole (94.9%), caryophyllene oxide (94.1%), sabinene (94.0%), and α-thujene (93.6%), whereas α-pinene (64.1%), bornyl acetate (72.5%), and limonene (73.6%) showed the lowest values. "Population" was the most explicative parameter and highly significant ($p < .001$) for all the terpenes, with values especially high for α-thujene (95.2%) and camphor (94.0%). In contrast, bornyl acetate (44.3%), β-myrcene (56.0%), and *p*-cymene (56.1%) were the terpenes less influenced by this parameter, in addition to the biomass production (20.2%). The composition of the essential oils was scarcely affected by the parameter Year, and only in the case of β-myrcene (7.4%) had a certain importance. The effect of Environment was noticeable on *trans*-caryophyllene (29.1%), *p*-cymene (17.7%), β-myrcene (14.5%), bornyl acetate (12.7%), β-pinene (12.0%), spathulenol (11.6%), and caryophyllene oxide (11.5%). The biomass production was especially sensitive to the influence of environment (46.5%), whereas this parameter had a low to moderate effect on the essential oil yield (11.5%). The interaction Population × Year was only significant ($p < .001$) for β-myrcene (6.0%). A relative influence, mainly on *p*-cymene (11.1%), bornyl acetate (11.1%), and caryophyllene oxide (9.3%) was seen for the interaction Population × Environment, whereas the interaction Year × Environment preferentially affected to the essential oil yield (7.5%). Finally, the interaction of Population, Environment, and Year showed a negligible influence on the essential oil composition as well as on the essential oil and biomass yields. Terpenes showing the highest degree of heritability (h^2BS) were 1,8-cineole (.8790), α-thujene (.8764), camphor (.8684), sabinene (.7898), and γ-terpinene (.7613), whereas bornyl acetate (.2807) and α-pinene (.4963) showed the lowest values. Essential oil yield had a good heritability (.6855) in contrast to the dry biomass production (.2503).

The PCA resulted in four components that accounted for 78.4% of the total variability (Table 4). Principal Component 1 (33.0% of variability) was moderate and positively correlated with the contents of β-pinene (.323) and γ-terpinene (.300), and inversely with borneol (−.361) and camphor (−.354). Principal Component 2 (20.2% variability) was positively correlated with α-pinene (.335) and β-myrcene (.305) and negatively with sabinene (−.456), spathulenol (−.410), and α-thujene (−.378). Principal Component 3 (15.1% variability) was positively correlated with caryophyllene (.468) and *p*-cymene (.431) and negatively with 1,8-cineole (−.331).

14350653, 2022, 2, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.1002/csc2.20691 by Universidad De Valladolid, Wiley Online Library on [10/01/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14350653, 2022, 2, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.1002/csc2.20691 by Universidad De Valladolid, Wiley Online Library on [10/01/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 4 Principal component analysis (PCA) of the main essential oil compounds (≥1 %), their Kovats retention index (RI), and method of identification

			Components			
	RI[a]	IdC[b]	1	2	3	4
α-Thujene	920	T	.187	−.378	.160	.281
α-Pinene	926	S	.257	.335	−.122	−.008
Camphene	942	S	−.288	−.118	.024	.466
Sabinene	966	S	.165	−.456	−.102	−.077
β-Pinene	971	S	.323	−.039	−.252	−.031
β-Myrcene	974	S	.202	.305	−.190	.142
p-Cymene	1017	S	.128	.202	.431	.039
D-Limonene	1,021	S	.255	.257	−.178	.225
1,8-Cineole	1,023	S	−.103	.020	−.331	−.507
γ-terpinene	1,053	S	.300	.160	.239	.184
Camphor	1,133	S	−.354	−.015	.030	.298
Borneol	1,154	S	−.361	−.009	.160	−.124
Bornyl acetate	1,274	S	−.247	.176	.240	−.283
Trans-Caryophyllene	1,407	S	.197	.076	.468	−.053
Spathulenol	1,565	T	.184	−.410	.180	−.179
Caryophyllene oxide	1,570	S	.243	−.149	.250	−.338
Viridiflorol	1,579	S	−.113	.260	.259	.009

[a]RI, Kovats retention index relative to n-alkanes on nonpolar column HP-5MS.
[b]IdC, identification compound; S, standard compound; T, tentatively identified compound.

Finally, PC4 (10.1% variability) was positively correlated with camphene (.466) and negatively with 1,8-cineole (−.507) and caryophyllene oxide (−.338).

When means of samples corresponding to each season and location were labelled according to their original collecting locations (Population factor) and displayed in a scatterplot generated from the PCA analysis, they mostly grouped together regardless the season (Year factor) or location (Environment factor) of cultivation (Figure 1). Populations Tuixent and Guixers from The Pyrenees, characterized by a significant amount of α-thujene (4.9% and 11.8%, respectively; Table 2), grouped together. Samples from Aliaguilla with high contents in 1,8-cineole (38.7%) grouped distinctly. Moratalla, with the highest content in camphor (32.4%), also formed a separated cluster. The rest of the samples showed a more heterogeneous distribution.

4 | DISCUSSION

The main compounds present in the essential oils of these populations of Spanish sage were α-pinene, β-pinene, limonene, 1,8-cineole, and camphor (Table 2), which agree with those described in the literature for this species (Guillen & Ibargoitia, 1995; Herraiz-Peñalver et al., 2010; Méndez-Tovar et al., 2016; Usano-Alemany et al., 2016). Nevertheless, these terpenes, as well as others less abundant, showed a remarkable variability in their contents. Some terpenes like α-thujene, *p*-cymene, γ-terpinene, bornyl acetate, spathulenol, or viridiflorol were in negligible amounts (~0.1%) in some populations but were relatively significant in others. A similar variability has been observed in wild plants from Iberian Peninsula (Herraiz-Peñalver et al., 2010) despite the fact that some of them lacked camphor unlike samples analysed in this work, in which it was always identified. Likewise, some authors did not detect camphene in naturally grown or cultivated plants of *S. lavandulifolia* (Rzepa et al., 2009), in contrast to the samples analysed in this work. Thus, the presence or absence of some of these terpenes could be considered as potential chemotaxonomic markers to discriminate species, subspecies, or populations of the genus *Salvia* in general, and *S. lavandulifolia* in particular. For example, α-thujene was present in percentages higher than 1% only in samples from Guixers (11.8%) and Tuixent (4.9%), and viridiflorol in Vertavillo (12.4%), Tendilla (5.6%), La Sentiú de Sió (4.1%), Letur (3.2%), and Olmeda de la Cuesta (2.0%).

In the classical quantitative genetic framework, an observed phenotype results of the sum of genetic and environmental effects plus an interaction between both. From a breeding point of view, the bigger the genetic effect, the easier the selection of cultivars is. Traditionally, quantitative traits are largely affected by environmental effects, and therefore, they are more difficult to select than qualitative traits which are mainly determined by genetics. Since *S. lavandulifolia* wild populations show a great variability (Herraiz-Peñalver et al., 2010; Jordán et al., 2009; Méndez-Tovar et al., 2016), multilocation assays must be conducted in order to minimize environmental effects that may mislead the selection of suitable genotypes for commercial production of sage. In agreement with a previous wild population survey (Méndez-Tovar et al., 2016), most of the variability observed in this work for the yield and the composition of the essential oils was related with the original collecting locations of plants (Population factor) as deduced from the multivariate analysis of variance, which demonstrated that this factor was largely the most important (Table 3). The year of cultivation had a minimal influence on the composition of the essential oils as well as in the yields of biomass and essential oil, and only in the content of β-myrcene had a limited weight. In general, the Environmental factor, namely the location where the trial was conducted, had a much major influence than the Year factor, however its percentages—with the exception of the dry biomass yield—were rather low as compared with those due to genotype (Population factor). The prevalence of the Population factor over the other factors analysed resulted in quite high values of heritability (h^2BS ≥ .5) for most of the terpenes. Only the content of bornyl acetate, poorly correlated with the genotype, showed a low heritability. Likewise,

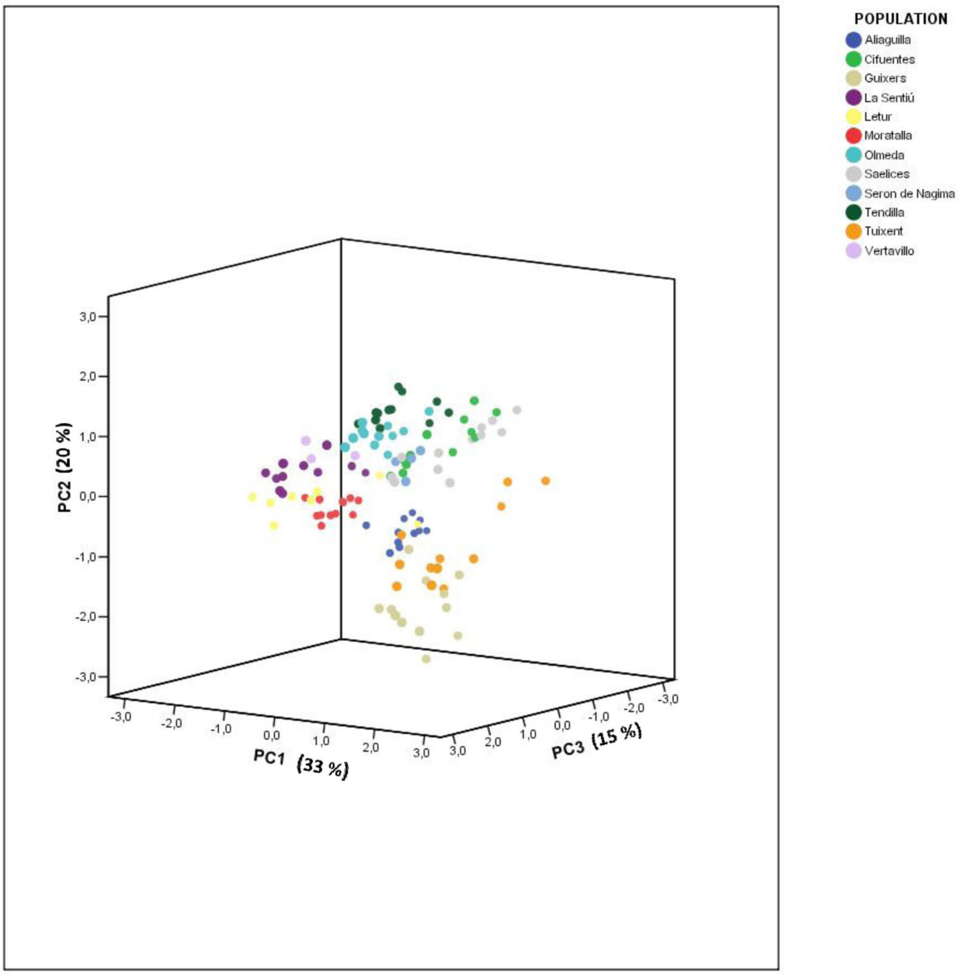

FIGURE 1 Distribution of samples labelled by population in a scatterplot of the three first principal components of the principal component analysis

the production of biomass also showed a scarce heritability. Overall, these data are a starting point to the accomplishment of potential plant-breeding programs to select homogeneous crops.

The predominance of the geographic origin of the plants on the composition of the essential oils was confirmed from the distribution of the samples in a scatterplot of the first three principal components generated from the PCA analysis (Figure 1). Overall, this analysis showed a grouping of samples according to their origin independently of the year or location of cultivation, which again points to a prevalence of the genetic factors on the composition of the essential oil. This is in agreement with the previous study of the morphological variability of these plants, which was also heavily influenced by the geographical origin of the populations (Herraiz-Peñalver, Asensio-Manzanera et al., 2017). Previous studies on wild populations of *S. lavandulifolia* (Herraiz et al., 2010; Jordán et al., 2009; Méndez-Tovar et al., 2016) and other species of *Salvia* (Rajabi et al., 2014) have shown the importance of the geographical origin on the composition of essential oil. However, these studies were performed with wild plants from different locations, and therefore from different climatic conditions and soils, hence the authors conclude that differences observed among populations could be a priori attributed to genetic and/or environmental factors. The results of this work, in which the location has been fixed (Cuenca or Valladolid) for all populations and the climatic conditions have been the same within each year and location, suggest that the essential oil composition in *S. lavandulifolia* is chiefly determined by genetics, and environmental factors have a minor influence. These results are consistent with those observed in individual plants of *S. lavandulifolia* collected from different locations which preserved their essential-oil chemical profiles when they were cultivated in an experimental field during 3 yr (Usano-Alemany et al., 2014). A very similar study carried out by our group on the essential oil variability of cultivated rosemary has also shown a pattern clustering strongly dependent on the collecting location of the original populations (Herraiz-Peñalver, Fernández-Sestelo et al., 2017).

14350653, 2022, 2, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.1002/csc2.20691 by Universidad De Valladolid, Wiley Online Library on [10/01/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

5 | CONCLUSIONS

Salvia lavandulifolia Vahl. is a valuable aromatic and medicinal plant with an important market demand in flavor and food industry, aromatherapy, cosmetics, and pharmacology. All these applications require plants with a well-defined and stable composition of essential oil that ensures a homogeneous supply of material over time. Accordingly, gaining insight into the variability and stability of essential oil composition is a prerequisite for obtaining a standardized material that enables a rational exploitation of this crop. This study shows a wide variability in the composition of essential oils of populations of *S. lavandulifolia* that was strongly dependent on the geographical origin of the populations. This variability was preserved in a large extent under the cultivation conditions. In contrast, environmental factors affecting the cultivation like soil or climatic conditions had a minor influence, that is, the composition of essential oil seems regulated mainly by genetics. These findings favor the selection and breeding of individual plants (clones) with a favorable composition for a specific application with a homogeneous quality from year to year. In contrast, agronomical traits as dry biomass production and essential oil yield were highly influenced by environment, and they should be evaluated in multi-environment trials.

ACKNOWLEDGMENTS

This work was supported by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, Spain, grants RTA2012-00057 and RTA2017-00031). We thank to the European Social Fund and to Fundación Parque Científico y Tecnológico de Castilla-La Mancha for additional financing, and to Orencio Sánchez Ruiz and Brígido De Benito López for their technical assistance.

AUTHOR CONTRIBUTIONS

Raúl Sánchez-Vioque: Funding acquisition, Writing-original draft. David Herraiz-Peñalver: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing-review & editing. Enrique Melero Bravo: Formal analysis; Validation; Visualization; Writing – review & editing. Gonzalo Ortiz de Elguea-Culebras: Validation, Writing-review & editing. Baudilio Herrero: Conceptualization; Funding acquisition; Writing – review & editing. Yolanda Santiago: Funding acquisition; Investigation; Resources. Marta Bueno: Funding acquisition; Investigation; Resources. Silvia Pérez-Magariño: Funding acquisition; Investigation; Resources. María del Carmen Asensio-Sánchez-S.-Manzanera: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing-review & editing.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

ORCID

Raúl Sánchez-Vioque https://orcid.org/0000-0003-1048-7505
Silvia Pérez-Magarino https://orcid.org/0000-0002-0880-7154

REFERENCES

Boland, D. J., Brophy, J. J., & House, A. P. N. (1991). *Eucalyptus leaf oils: Use, chemistry, distillation and marketing*. Inkata Press. https://doi.org/10.1002/ffj.2730070209

Corell, M., García, M. C., & Cermeño, P. (2008). Water use efficiency in two species of the genus Salvia. *Acta Horticulturae*, *52*, 295–299.

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.). Addison Wesley Longman.

Guillén, M. D., & Ibargoitia, M. L. (1995). Wild growing Spanish sage as a raw material in the food industry. Study by gas chromatography/mass spectrometry of its essential oil and extracts in several organic solvents. *Chemie, Mikrobiologie, Technologie der Lebensmittel*, *17*, 118–121.

Hedge, I. C. (1972). *Salvia* L. In T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walter, & D. A. Webb (Eds.), *Flora Europaea* (Vol. 3, pp. 188–192). Cambridge University Press.

Herraiz-Peñalver, D., Usano-Alemany, J., Cuadrado, J., Jordan, M. J., Lax, V., Sotomayor, J. A., & Palá-Paúl, J. (2010). Essential oil composition of wild populations of Salvia lavandulifolia Vahl. from Castilla-La Mancha (Spain). *Biochemical Systematics and Ecology*, *38*, 1224–1230. https://doi/10.1016/j.bse.2010.10.015

Herraiz-Peñalver, D., Ortiz de Elguea-Culebras, G., Sánchez-Vioque, R., & Santana-Méridas, O. (2015). Identification of a hybrid species of sage (*Salvia officinalis* L. x *S. lavandulifolia* subsp. lavandulifolia) through the study of the essential oil. *Journal of Essential Oil Research*, *27*, 1–10. https://doi.org/10.1080/10412905.2015.1031918

Herraiz-Peñalver, D., Asensio-Manzanera, M. C., Herrero, B., Martin, H., Santiago, Y., Zalacaín, A., Berruga, M. I., & Sánchez-Vioque, R. (2017). Evaluation of the morphological variability in Iberian *Salvia lavandulifolia* Vahl accessions. *Genetic Resources and Crop Evolution*, *64*, 1797–1806. https://doi.org/10.1007/s10722-017-0545-x

Herraiz-Peñaver, D., Fernández-Sestelo, M., Asensio-Sánchez Manzanera, M. C., Herrero, B., & Sánchez-Vioque, R. (2017). Variability in the essential oil composition of cultivated populations of *Rosmarinus officinalis* L. *48th International Symposium on Essential Oils*, *4*(3), 71.

Jordán, M. J., Martínez, C., Moñino, M. I., Lax, V., Quílez, M., & Sotomayor, J. A. (2009). Chemical characterization of Salvia lavandulifolia subsp. vellerea in South-Eastern Spain. *Acta Horticulturae*, *826*, 317–324.

Lal, R. K., Gupta, P., Gupta, V., Sarkar, S., & Singh, S. (2013). Genetic variability and character associations in vetiver (*Vetiveria zizanioides* L. Nash). *Industrial Crops and Products*, *49*, 73–277. https://doi.org/10.1016/j.indcrop.2013.05.005

Méndez-Tovar, I., Novak, J., Sponza, S., Herrero, B., & Asensio-S-Manzanera, M. C. (2016). Variability in essential oil composition of wild populations of Labiatae species collected in Spain. *Industrial*

14350653, 2022, 2, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.1002/csc2.20691 by Universidad De Valladolid, Wiley Online Library on [10/01/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Crops and Products, *79*, 18–28. https://doi.org/10.1016/j.indcrop.2015.10.009

Porres-Martínez, M., Carretero-Accame, M. E., & Gómez-Serranillos, M. P. (2013). Pharmacological activity of Salvia lavandulifolia and chemical components of its essential oil. A review. *Lazaroa*, *34*, 237–254. https://doi/10.5209/rev_LAZA.2013.v34.n1.43298

Rajabi, Z., Ebrahimi, M., Farajpour, M., Mirza, M., & Ramshini, H. (2014). Compositions and yield variation of essential oils among and within nine Salvia species from various areas of Iran. *Industrial Crops and Products*, *61*, 233–239. https://doi.org/10.1016/j.indcrop.2014.06.038

Rzepa, J., Wojtal, L., Staszek, D., Grygierczyk, G., Labe, K., Hajnos, M., Kowalska, T., & Waksmundzka-Hajnos, M. (2009). Fingerprint of selected Salvia species by HS-GC-MS analysis of their volatile fraction. *Journal of Chromatographic Science*, *47*, 575–580. https://doi.org/10.1093/chromsci/47.7.575

Sáez, L. (2010). Salvia L. In R. Morales, A. Quintanar, F. Cabezas, A. J. Pujadas, & S. Cirujano (Eds.), *Flora Ibérica* (Vol. 12, pp. 301–306). Real Jardín Botánico, CSIC.

Usano-Alemany, J., Herraiz-Peñalver, D., Cuadrado, J., Díaz, S., Santa-Cruz, M., & Palá-Paúl, J. (2012). Seasonal variation of the essential oils of *Salvia lavandulifolia*: Antibacterial Activity. *Journal of Essential Oil Bearing Plants*, *15*, 195–203. https://doi.org/10.1080/0972060X.2012.10644036

Usano-Alemany, J., Palá-Paul, J., Rodríguez, M. S., & Herraiz-Peñalver, D. (2014). Chemical description and essential oil yield variability of different accessions of Salvia lavandulifolia. *Natural Product Communications*, *9*, 273–276. https://doi.org/10.1177/1934578X1400900236

Usano-Alemany, J., Palá-Paúl, J., & Herraiz-Peñalver, D. (2016). Phenological changes in the biosynthesis and chemical composition of the essential oils. In M. Peters (Ed.), *Essential oils: Historical significance, chemical composition and medicinal uses and benefits* (pp. 19–50). Nova Publisher.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Sánchez-Vioque, R., Herraiz-Peñalver, D., Melero Bravo, E., Ortiz de Elguea-Culebras, G., Herrero, B., Santiago, Y., Bueno, M., Pérez-Magariño, S., & del Carmen Asensio S. Manzanera, M. (2022). Variability of the essential oil composition of cultivated populations of *Salvia lavandulifolia* Vahl. *Crop Science*, *62*, 744–752. https://doi.org/10.1002/csc2.20691

14350653, 2022, 2, Downloaded from https://access.onlinelibrary.wiley.com/doi/10.1002/csc2.20691 by Universidad De Valladolid, Wiley Online Library on [10/01/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License