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Variability of essential oil in cultivated populations of *Rosmarinus officinalis L.* in Spain

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Abstract *Rosmarinus officinalis* L. (synonym *Salvia rosmarinus* Schleid) grows in the Mediterranean basin and is known to be a source of natural bioactive compounds and one of the most important aromatic species in terms of the marketing of the essential oil. However, wild collection and the lack of selection lead to the absence of standardized material that ensures the homogeneity and quality of the essential oils over time. In the present work, thirteen wild Spanish populations of rosemary were cultivated in two experimental fields and their essential oil composition monitored during two years. The main compounds present in the essential oils were camphor (21.9%), α-pinene (14.8%), 1,8-cineole (11.6%), β-pinene + myrcene (11.3%) and camphene (8.3%), although their proportions differ greatly among populations. Other terpenes as limonene had a significant presence in some populations, up to 10.2%. The results showed that the variability in the composition of essential oil was mainly controlled by genetics and little affected by soil and climate conditions. Statistical processing allowed to group populations into three different groups based on the geographical origin of the populations. In conclusion, the characterization of essential oils of these populations is a starting point for the development of breeding programmes aimed to commercialize standardized plants (varieties).

Keywords Essential oil · Rosmarinus officinalis · Chemotype, pinene · Cineole · Camphor

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Introduction

Rosemary is a species belonging to the *Lamiaceae* family distributed in a wide range of edaphoclimatic conditions of the Mediterranean area. Initially identified by Linnaeus as *Rosmarinus officinalis*, phylogenetic studies (Drew et al. 2017) and taxonomic, morphological and practical considerations have led to its inclusion in the genus *Salvia* as *Salvia rosmarinus* Schleid. although both scientific names are valid. It grows spontaneously in Spain, except in the more humid regions of the north and northwest (Morales 2004), and is cultivated mainly on the Mediterranean coast and Southern Spain although wild collection still accounts for an important contribution to its production. This plant is well known as an important source for the extraction of various natural bioactive compounds, and one of the most important aromatic species in terms of the marketing of essential oils.

Essential oils are complex mixtures with characteristic flavor and fragrance properties. Around 90% of global essential oil production is consumed by the flavor and fragrance industries to be used mainly in cosmetics, perfumes, soft drinks and food (Lubbe and Verpoorte 2011). In addition, essential oils present numerous biological activities, including antioxidant and antimicrobial properties, and enormous potential in human and animal health (Bozin et al. 2007; Burt 2004; Miguel 2010).

Rosemary essential oil has been used extensively in traditional medicine to heal wounds since ancient times, corroborated by scientific evidence (Abu-Al-Basal 2010). Wang et al. (2012) suggest a synergistic effect to explain the higher cytotoxic activity of rosemary essential oil against certain human cancer cells in comparison with the effect of its main individual compounds α-pinene, β-pinene and 1,8-cineole. A research with rats showed that a rosemary essential oil with high content in 1,8-cineole (43%), camphor (12.5%) and α-pinene (11.5%) had not only antioxidant activity but also a hepatoprotective effect through the activation of defense mechanisms (Raskovic et al. 2014). Synergistic effects are also common for the antimicrobial activities. A rosemary essential oil showed a higher antibacterial activity than its main compounds, α-pinene and 1,8-cineole (Jiang et al. 2011), and a study with fractions rich in rosemary essential oil obtained by supercritical CO_2 extraction confirmed greater antimicrobial activity than that of camphor, borneol and verbenone assayed separately (Santoyo et al. 2005). Isman et al. (2008) concluded that the toxicity of rosemary essential oil against some insects is a consequence of the combined (and probably synergistic) activity of some compounds. Studies of its activity against larvae of *Tichoplusia ni* have also focused on the synergies between 1,8-cineole and camphor (Tak et al. 2016). In the agri-food sector, the high antibacterial activity of rosemary essential oil makes it suitable for its incorporation in active films for food preservation (Abdollahi et al. 2012). However, in such a complex mixture of compounds, any change in composition is expected to affect to the efficacy of the essential oil, that is to say, the biological activities of the essential oil are clearly dependent on its terpene profile, particularly when synergisms and antagonisms may occur. This leads to the necessity of supplying a well characterised plant material that ensures as much as possible the homogeneity of the essential oils and hence, the biological activity that is intended.

Unfortunately, rosemary essential oil shows a high intraspecific chemical variability according to geographical origin (Angioni et al. 2004; Salido et al. 2003; Celiktas et al. 2007; Zaouali and Boussaid 2008; Zaouali et al. 2010), environmental and/or agronomic conditions, harvest time (Salido et al. 2003; Celiktas et al. 2007) or extraction method (Okoh et al. 2010). Several chemotypes of rosemary have been described in the literature based on the relative percentages of α-pinene, 1,8-cineole, camphor, borneol, verbenone, and bornyl acetate (Satyal et al. 2017). The cultivation of selected plant material seems to be an appropriate technique to obtain homogeneous productions in terms of quantity and quality (Herraiz-Peñalver et al. 2010). Problems such as adulteration or misidentification of material are minimized with the use of cultivated plants. It is also easier to fit the quality standards and have less batch-to-batch variation when the plants are grown under controlled conditions (Lubbe and Verpoorte 2011). Although some studies have been published on the variability in the chemical composition of the essential oils of wild populations of *Rosmarinus officinalis L.* in the Iberian Peninsula (Varela et al. 2009), researches on the essential oil composition of cultivated populations under homogeneous environmental conditions are required to differentiate among plant chemotypes (Abu-Al-Basal 2010).

For this aim, 13 populations of rosemary have been previously selected from a survey throughout the natural distribution area of the species in Spain according to the yield and the variability in the chemical composition of their essential oils. Subsequently, the populations were cultivated in two locations. The objective is to evaluate the influence of edaphological and climatic conditions on the composition of the essential oil of Spanish wild populations of *Rosmarinus officinalis L.* propagated vegetatively and cultivated.

Materials and methods

Plant material and hydro-distillation

Thirteen wild populations of *Rosmarinus officinalis L* from Spain were selected on the basis of their essential oil yield and composition (Table 1). Individual plants from each population were vegetatively propagated by cuttings and rooted under greenhouse conditions. The experimental plot consisted in 25 individual specimens in a block design of 5×5 plants, with separations of 1 m between rows and 1 m between plants and fixed in three replications per population. This trial was repeated in two different locations: Centro de Investigación Agroforestal-CIAF Albaladejito (Cuenca, Spain) and Centro de Evaluación de Variedades (Aranjuez, Spain).

Experimental fields were established under rainfed conditions in March 2010. Cultural practices were limited to weed control. The aerial parts of each plot (leaves, stems and flowers) were collected in full-bloom stage (April) for two seasons (2013 and 2014). After harvesting, the plant material was dried at room temperature and around 150 g of a representative sample of each plot were hydrodistilled for 3 h using a Clevenger type apparatus, according to the European Pharmacopoeia (Council of Europe 1996). The oils were collected and dried over anhydrous magnesium sulphate and stored at 4 ºC under dark conditions prior to analysis.

Essential oil analysis (GC)

The chemical analysis of the essential oils was determined by means of a gas chromatograph (GC-FID), using an Agilent Technologies 5890 Series II plus gas chromatograph (GC) equipped with a 30 m x 0.25 mm i.d. HP-5 (cross-linked phenyl-methyl siloxane) non-polar column with 0.25 mm film thickness supplied by Agilent Technologies (Palo Alto, CA, U.S.A.) with a FID detector. Essential oil was diluted in diethyl ether (1:10) and 0,5 µL injected in the equipment using a split ratio of 10:1. Helium was used as carrier gas with a constant flow through the column of 1 mL min^{-1}. The initial oven temperature was kept at 60 ºC for 4 min and then increased at a rate of 3 ºC/min to 250 ºC; the injection port was fixed at 250 ºC. Peak identification was carried out by comparison of their retention times with commercial standards (Across Organics BVBA/SPRL, Fisher Scientific S.A. and Sigma Aldrich Química A.) and the quantification was expressed as their relative peak areas (%).

Statistics

All statistical analyses were perfomed using the IBM® SPSS® Statistics ver. 22 (IBM corp.©, 2013) package. Analysis of variance (ANOVA) was performed setting as independent factors the parameters ''Year'', "Environment", ''Population'', and their interactions. The sum of squares were used to determine the proportion of the total variation explained by the regression model. Principal Component Analysis (PCA) and two-step cluster analysis were carried out with those compounds (15) higher than 1% in a significant number of samples. PCA was performed on the correlation matrix, and two-step clustering process was carried out with the automatic clustering method using the Schwarz Bayesian criterion (BIC); log-likehood criterion was applied in the distance calculation and Student t-test to measure the importance of variables in the formation of the clusters. One-way analysis of variance (ANOVA) was conducted with each compound as a dependent variable and the number of cluster as a categorical factor. Tukey´s test was performed when significant differences ($p < 0.05$) were detected among the different clusters.

Results

Chemical composition of the essential oils of populations of Rosmarinus officinalis

Fifteen main compounds (> 1%) were identified in the essential oil of populations of *R. officinalis* (Table 2). The highest mean percentages corresponded to camphor, α-pinene, 1,8-cineole, β-pinene + myrcene and camphene, although the composition showed a great variability among populations. Thus, in case of camphor contents ranged from 14.4−30.7%, α-pinene from 9.4−21.1%, 1,8-cineole from 7.5−17.1%, β-pinene + myrcene from 6.3 −35.1%.

Table 1 Geographical data of the original collecting locations of cultivated populations of *Rosmarinus officinalis* L

Populations	Origin (Municipality/Province)	Latitude	Longitude	Altitude (m[a])
1	Ontígola (Toledo)	40º 00′51″ N	3º 35’ 14″ W	589
2	Almorox (Toledo)	40º 17′29’’ N	4º 21’ 54″ W	715
3	Alcaudete de la Jara (Toledo)	39º 49′57’’ N	4º 52’ 09″ W	477
4	Lorca (Murcia)	37º 52’ 22″ N	1º 53′ 20″ W	833
5	Moratalla (Murcia)	38º 08′ 41″ N	2º 13′ 43″ W	1161
6	Robledo (Albacete)	38º 47′ 04″ N	2º 27′ 05″ W	1002
7	Lliria (Valencia)	39º 45′ 21″ N	0º 41′ 04″ W	502
8	Alcocer (Guadalajara)	40º 28′ 09″ N	2º 32′ 52″ W	710
9	Huete (Cuenca)	40º 08′ 29″ N	2º 41′ 58″ W	847
10	Cifuentes (Guadalajara)	40º 48′ 37″ N	2º 40′ 22″ W	936
11	Pina de Ebro (Zaragoza)	41º 35′ 14″ N	0º 20′ 29″ W	407
12	Pontils (Tarragona)	41º 28′ 59″ N	1º 27′ 05″ E	704
13	Flix (Tarragona)	41º 16′ 15″ N	0º 35′ 14″ E	149
Centro Agrario Albaladejito	Cuenca	40º 04′ 19″N	2º 12′ 09″ W	995
Centro de Evaluación de Variedades	Aranjuez (Madrid)	40º 03′ 17″N	3º 31′ 53″ W	504

Table 2 Means of the essential oil composition by population of *R. officinalis L.* during two years in two locations. Quantification of compounds is expressed as their relative peak areas (%). Different letters indicate statistically significant differences at $p < 0.05$ in post-hoc Tukey's test

	RI*	Populations												
		1	2	3	4	5	6	7	8	9	10	11	12	13
α-pinene	926	10.72^{f}	10.89^{f}	9.42^{f}	21.09^{a}	15.34^{cd}	14.90^{cde}	16.04^{bcd}	12.61^{def}	16.26^{bc}	11.47^{ef}	17.96^{abc}	19.14^{ab}	16.95^{bc}
Camphene	942	7.79^{de}	5.47^{f}	5.77^{f}	10.70^{a}	8.61^{cd}	7.33^{e}	9.15^{bc}	7.72^{de}	7.96^{de}	7.99^{de}	9.62^{abc}	10.28^{ab}	9.83^{ab}
β-pinene + myrcene**	972	7.90^{d}	35.14^{a}	20.37^{b}	8.06^{d}	8.39^{d}	6.90^{d}	8.27^{d}	7.67^{d}	6.54^{d}	8.19^{d}	14.63^{c}	6.34^{d}	9.66^{d}
Limonene	1021	10.29^{a}	4.20^{d}	7.80^{b}	4.06^{d}	3.69^{d}	4.82^{cd}	3.63^{d}	6.68^{bc}	8.06^{b}	8.23^{ab}	3.77^{d}	4.05^{d}	4.09^{d}
1,8-cineole	1023	8.20^{ef}	7.48^{f}	8.51^{ef}	17.14^{a}	12.21^{bcd}	14.67^{ab}	15.70^{a}	10.92^{cdef}	10.30^{def}	10.74^{cdef}	10.43^{def}	11.21^{bcde}	14.13^{abc}
ϒ-terpinene	1053	1.31^{ab}	1.55^{a}	1.53^{a}	1.08^{bc}	0.85^{cd}	0.97^{bc}	0.76^{cd}	0.95^{bcd}	0.58^{d}	0.78^{cd}	0.89^{cd}	0.83^{cd}	0.96^{bcd}
Linalool	1065	1.13^{cdef}	1.86^{a}	1.40^{bcd}	0.88^{ef}	0.85^{ef}	0.71^{f}	1.00^{def}	1.29^{cde}	0.97^{def}	1.28^{cde}	1.30^{cde}	1.46^{abc}	1.80^{ab}
Camphor	1133	28.11^{ab}	14.75^{f}	21.19^{cde}	14.39^{f}	25.50^{abcd}	21.09^{de}	23.32^{bcd}	26.47^{abc}	23.62^{bcd}	30.71^{a}	16.83^{ef}	23.83^{bcd}	14.86^{f}
Borneol	1154	4.17^{bcd}	1.93^{f}	2.64^{ef}	5.05^{ab}	5.78^{a}	4.46^{abc}	4.99^{ab}	2.96^{def}	4.25^{bcd}	4.15^{bcd}	5.52^{ab}	3.54^{cde}	5.04^{ab}
Terpinen-4-ol	1160	1.53^{a}	1.38^{abc}	1.57^{a}	1.21^{bcd}	1.00^{d}	1.45^{ab}	1.08^{d}	1.37^{abc}	1.08^{d}	1.12^{cd}	1.22^{bcd}	1.09^{d}	1.55^{a}
α-terpineol	1177	1.95^{ab}	1.38^{de}	1.79^{abc}	1.86^{abc}	1.63^{bcde}	2.08^{a}	1.99^{ab}	1.85a^{bc}	1.76^{abcd}	1.89^{abc}	1.37^{e}	1.34^{e}	1.53^{cde}
Verbenone	1193	1.95^{cdef}	2.07^{bcdef}	1.21^{ef}	2.28^{abcd}	1.51^{def}	3.04^{ab}	1.51^{def}	2.89^{abc}	3.00^{ab}	1.07^{f}	3.16^{a}	2.16^{abcde}	2.53^{abcd}
Bornyl acetate	1274	2.34^{ab}	1.43^{bcd}	2.22^{abc}	1.24^{cd}	1.67^{bcd}	1.17^{d}	1.97^{abcd}	1.82^{bcd}	2.95^{a}	2.39^{ab}	1.94^{abcd}	1.19^{d}	1.58^{bcd}
Trans-caryophyllene	1407	1.11^{bcd}	0.86^{de}	1.43^{abc}	0.98^{cde}	1.15^{abcd}	0.93^{cde}	0.86^{de}	1.56^{ab}	1.69^{a}	0.90^{cde}	0.52^{e}	1.16^{abcd}	0.84^{de}

*Kovats retention index relative to n-alkanes on non-polar column HP-5MS

**Co-eluted

Influence of "Population", "Year" and "Environment" parameters on the chemical composition of the essential oils of Rosmarinus officinalis

The data of the analysis of variance (ANOVA) performed to evaluate the influence of parameters "Population", "Year", "Environment" and their interactions on the main essential oil compounds are shown in Table 3. The model was highly significant ($p < 0.001$) for all terpenes, particularly high in β-pinene + myrcene (89.9%) and camphene (86.9%) whereas bornyl acetate (59.6%), terpinen-4-ol (66.8%) and borneol (69.6%) showed the lowest values. "Population" was the most explicative parameter and highly significant ($p < 0.001$) for all compounds, especially for β-pinene + myrcene (97.1%) and limonene (91.5%). The variable "Year" showed moderate influence over α-terpineol (24.0%), verbenone (28.0%) and *trans*-cariophyllene (16.3%), and "Environment" over *trans*-cariophyllene (27.3%) and bornyl acetate (15.0%). The interaction "Population x Year" (P x Y) was only significant ($p < 0.05$) for camphene although with scarce influence (3.8%). "Population x Environment" (P x E) had some influence on terpinen-4-ol (19.1%) and bornyl acetate (18.4%) whilst "Year x Environment" (Y x E) slightly influenced on α-terpineol (7.3%) and camphene (6.1%). Finally, the interaction "Population x Year x Environment" (P x Y x E) showed a slight influence ($p < 0.05$) on α-pinene (8.7%), camphor (6.4%), and ϒ-terpinene (6.2%).

Table 4 Matrix of components of the principal component analysis (PCA)

	Component			
	1	2	3	4
α-pinene	0.770	− 0.371	0.069	0.312
Camphene	0.825	− 0.237	− 0.125	0.197
β-pinene + myrcene	− 0.699	− 0.483	− 0.034	0.239
Limonene	− 0.397	0.617	− 0.098	0.124
1,8-cineole	0.567	− 0.122	0.329	− 0.552
ϒ-terpinene	− 0.656	− 0.292	0.371	0.165
Linalool	− 0.424	− 0.473	− 0.206	− 0.177
Camphor	− 0.079	0.561	− 0.518	− 0.519
Borneol	0.690	0.157	0.193	0.027
Terpinen-4-ol	− 0.540	− 0.066	0.575	− 0.212
α-terpineol	0.007	0.612	0.578	− 0.329
Verbenone	0.130	0.129	0.697	0.305
Bornyl acetate	− 0.032	0.525	− 0.204	0.556
Trans-caryophyllene	− 0.074	0.524	0.101	0.460
% Variability explained	26.06	17.26	13.06	11.49
% Variability accumulated	26.06	43.33	56.39	67.88

Table 3 Percentages of the sum of squares obtained in the analysis of variance of the essential oil compounds

	Model (R^2)	Population (P)	Year (Y)	Environment (E)	P × Y	P × E	Y × E	P × Y × E
α-pinene	79.14***	74.86***	2.85**	2.73**	4.08	3.25	3.54***	8.70**
Camphene	86.98***	79.00***	1.10**	4.93***	3.83*	2.27	6.14***	2.73
β-pinene + myrcene	89.94***	97.18***	0.04	0.29	0.45	1.10	0.07	0.88
Limonene	76.39***	91.51***	0.02	0.14	2.79	2.61	0.15	2.78
1,8-cineole	71.81***	75.66***	0.64	0.08	5.89	8.32	2.09*	7.31
ϒ-terpinene	68.79***	70.21***	0.02	4.53**	6.02	12.34*	0.65	6.23***
Linalool	69.59***	70.70***	8.86***	3.31**	3.17	5.75*	0.83	7.39
Camphor	77.17***	78,35***	0.40	4.76***	3.72	5.33	1.00	6.44*
Borneol	69.62***	79.93***	5.00**	0.16	5.72	4.70	2.19*	2.29
Terpinen-4-ol	66.88***	68.23***	0.24	5.20**	4.65	19.13***	0.02	2.54
α-terpineol	71.47***	45.38***	24.01***	2.37*	8.38	6.55	7.33***	5.98
Verbenone	74.82***	43.71***	28.01***	6.18***	5.61	10.18**	0.40	5.91
Bornyl acetate	59.67***	50.49***	7.19**	15.07***	2.74	18.42*	0.51	5.58
Trans-caryophyllene	71.14***	36.88***	16.33***	27.34***	6.64	10.35*	0.45	2.02

*** Statistical difference at $p < 0.001$; ** Statistical difference at $p < 0.01$; * Statistical difference at $p < 0.05$

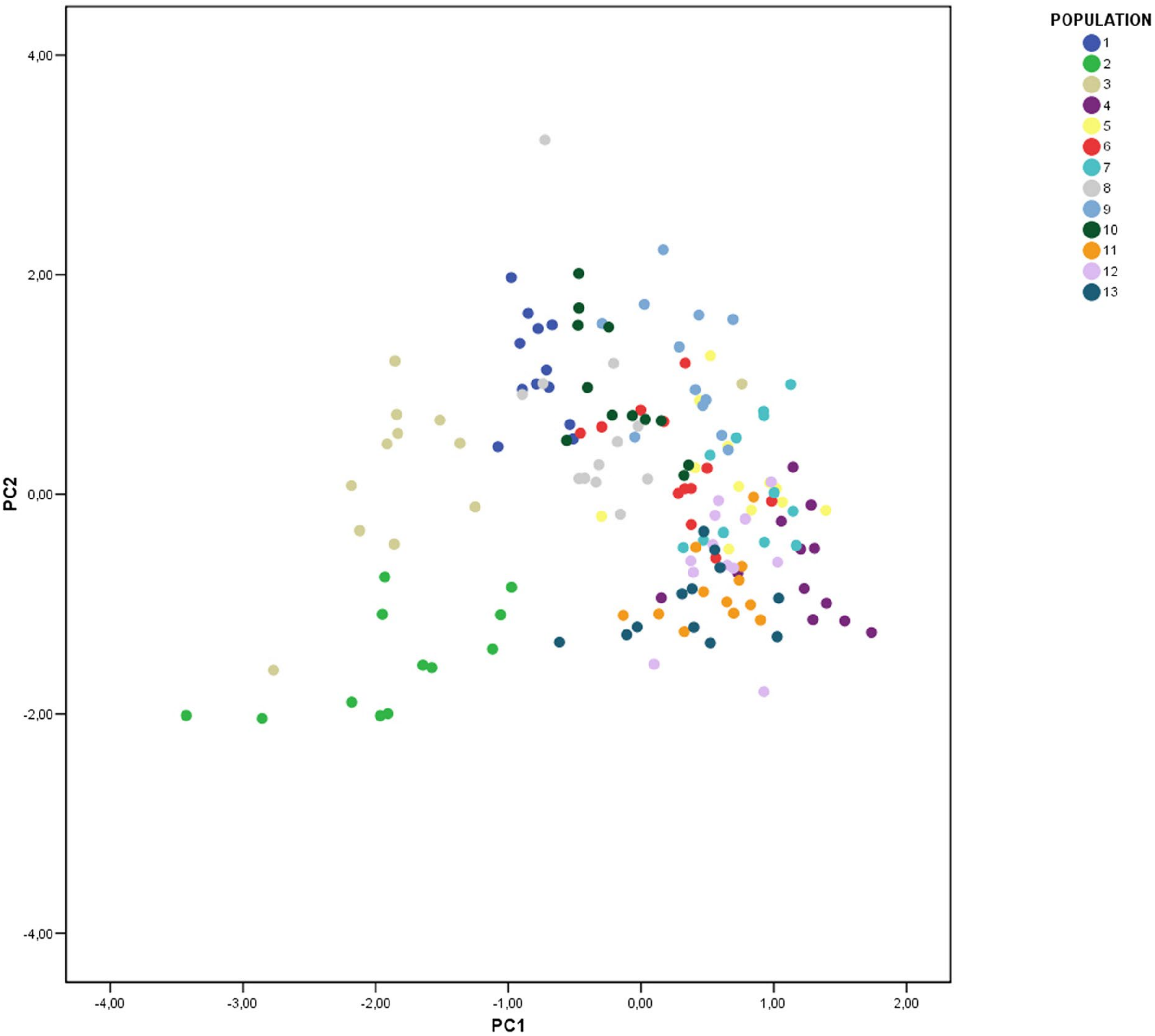

Fig. 1 Scatterplot of the 2 first principal components extracted with the PCA of samples labelled by population

Principal component analysis (PCA)

A principal component analysis (PCA) was performed in order to explain the variability of samples, resulting in 4 components that accounted for 67.8% of the total variability (Table 4). PC1 (26.0% of variability) was positively correlated with α-pinene (0.770) and camphene (0.825) and inversely with β-pinene + myrcene (− 0.699). PC2 (17.2%) was positively correlated with α-terpineol (0.612), limonene (0.617) and camphor (0.561) and inversely with linalool (− 0.473) and β-pinene + myrcene (− 0.483). PC3 (13.0%) was positively correlated with verbenone (0.697) and α-terpineol (0.578) and inversely with camphor (− 0.518). PC4 (11.4%) was positively correlated with bornyl acetate (0.556) and inversely with 1,8-cineole (− 0.552) and camphor (− 0.519).

When the two main principal components (PC1 and PC2) of Table 4 were used as the axis of a 2D scatter− plot, samples labelled with the same original locations ("Population") placed together in the graphic regardless the season ("Year") or location of cultivation ("Environment") (Fig. 1).

Table 5 Cluster mean values (two-step cluster analysis). Different letters means statistical differences in Tukey´s test groups ($p<0.05$)

Populations	Cluster 1	Cluster 2	Cluster 3
	1, 8, 9, 10	4, 5, 6, 7, 11, 12, 13	2, 3
	Mean	Mean	Mean
α-pinene	12.77[b]	17.34[a]	10.16[c]
camphene	7.86[b]	9.36[a]	5.62[c]
β-pinene + myrcene	7.57[b]	8.89[b]	27.76[a]
limonene	8.31[a]	4.02[c]	6.00[b]
1,8-cineole	10.04[b]	13.64[a]	7.99[c]
ϒ-terpinene	0.91[b]	0.91[b]	1.54[a]
linalool	1.17[b]	1.14[b]	1.63[a]
camphor	27.23[a]	19.97[b]	17.97[b]
borneol	3.88[b]	4.91[a]	2.28[c]
terpinen-4-ol	1.27[b]	1.23[b]	1.47[a]
α-terpineol	1.86[a]	1.69[ab]	1.59[b]
verbenone	2.23[ab]	2.31[a]	1.64[b]
bornyl acetate	2.37[a]	1.54[b]	1.82[b]
trans-caryophyllene	1.31[a]	0.92[b]	1.15[ab]

Grouping of samples

The elevate number of samples (156) of this study allowed the utilization of the exploratory tool two-step clustering analysis in order to identify natural groupings (or clusters) of populations. A one-way ANOVA was performed to compare mean values of clusters, which resulted in three different clusters (Table 5; Fig. 2). C1 included three populations from a homogeneous geographical area called "Alcarria" in the province of Guadalajara and one from the eastern part of the province of Toledo, characterized by a higher content in camphor and limonene. C2 comprised seven populations from the eastern part of the Iberian Peninsula, with a higher content in α-pinene, camphene, 1,8-cineole and borneol. C3 was formed by two populations with origin in the silicean central part of the Iberian Peninsula (more to the west than C1), characterized by a higher content in β-pinene + myrcene and a lower content in α-pinene and 1,8-cineole (Fig. 3).

Discussion

The phenotype of an individual plant, the essential oil composition in this case, 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 favors the selection and propagation of plants with desirable characteristics (Falconer and MacKay 1996). The main compounds identified in the essential oils of the populations of *R. officinalis* of this study (Table 2) agree with those found in other studies throughout the Mediterranean area. Thus, camphor, 1,8-cineole and α-pinene are predominant in the essential oil of populations of rosemary in distant areas such as Tunisia (Ben Jemia et al. 2015), Spain (Jordán et al. 2011) or Iran (Bajalan et al. 2018). However, the percentages of some terpenes like α-pinene, camphene, limonene, 1,8-cineole, camphor and borneol differed 2–3 times between some populations, a variability that is comparable with that of wild populations of this species in Spain (Varela et al. 2009). Nonetheless, all populations included in this study are aligned with the characteristics of "Spanish essential oil" market type, that is, with a higher percentage of camphor and verbenone and a lower content of 1,8-.cineole in comparison with "Morocco essential oil".

It seems that the composition of the essential oils was scarcely ruled by environmental conditions of cultivation, which is consistent with the genetic control of the chemical composition of the essential oil observed in most of species (Usano-Alemany et al. 2016). Actually, "Population" was the parameter that mainly explained the composition of the essential oil, especially for the contents of the main compounds like camphor, α-pinene, 1,8-cineole, β-pinene + myrcene and camphene, with percentages of the sums of squares in our model over 75% for each one (Table 3). Location of trials and year of cultivation only had some importance in minor compounds as verbenone, α-terpineol, *trans*-cariophyllene and bornyl acetate while the interaction among parameters had only a limited influence in the content of terpinen-4-ol (19.1%). This confirms that the composition of essential oil in rosemary was determined mainly by genetics rather than environmental factors. Similarly, Li et al. (2016), from an investigation

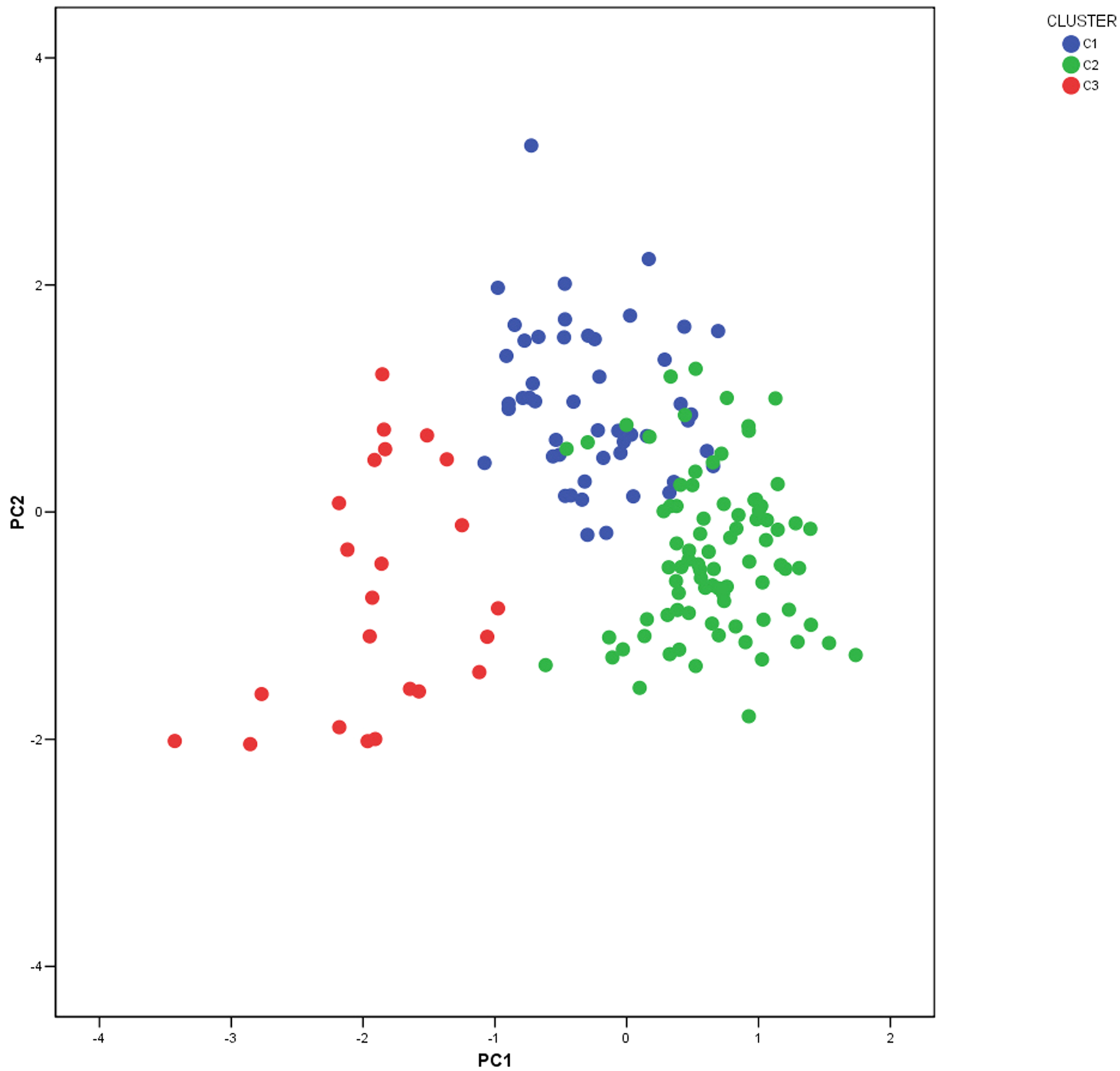

Fig. 2 Scatterplot of the 2 first principal components extracted with the PCA of samples labelled by clusters generated in the two-step cluster analysis

of the variability of volatiles in populations from the vicinity of the Tyrrhenian Sea cultivated under homogeneous environmental conditions, concluded that genetics and origin are the key factors in the chemotype variation.

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 2 first principal components generated from the PCA analysis (Fig. 1). This analysis showed a clear 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. The contents of camphor, α-pinene, and 1,8-cineole have been mainly used to identify the chemotypes of Spanish wild populations of rosemary although some plants have important amounts of other compounds

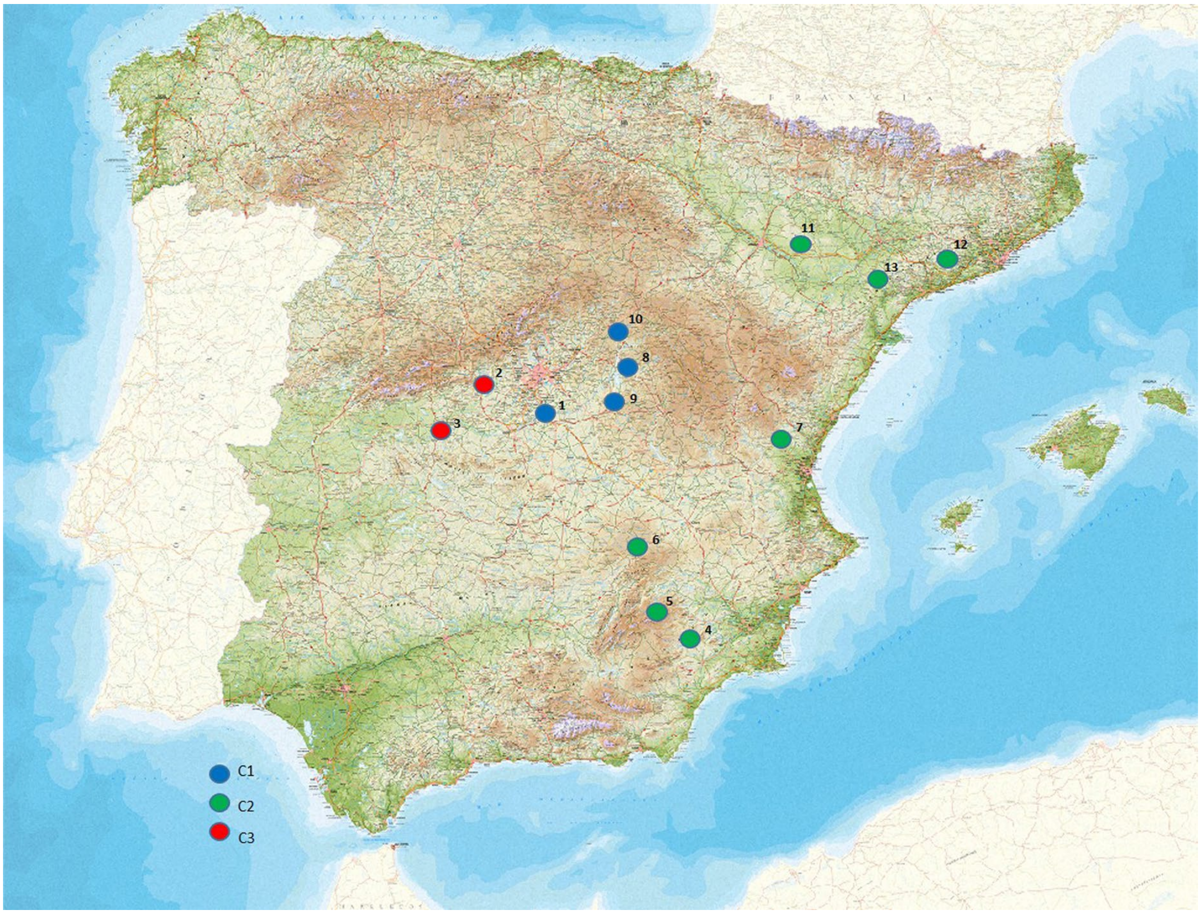

Fig. 3 Geographical distribution of the original populations and clusters (C)

like verbenone, camphene or borneol (Varela et al. 2009; Jordan et al. 2010). Overall, these same terpenes have been the responsible for the cluster formation of this study despite β-pinene + myrcene was predominant (27.7%) in C3 (Table 5). Myrcene has been demonstrated as a discriminating compound in a cluster formation of 78 rosemary populations from all over the world (Satyal et al. 2017), and may well lead to a new chemotype in Spanish populations. Populations included in clusters C1, C2 and C3 are linked to a determined geographical origin although some differences have been found within each cluster (Fig. 3). C1 and C3 were restricted to a smaller area and were more uniform than C2, whose samples are original from a vast area from Northeast to Southeast of the Iberian Peninsula, and showed a considerable variability in the characteristics of its populations. Accordingly, in a survey of 87 wild populations of rosemary form different regions of Spain collected in their habitat, Varela et al. (2009) found that samples were grouped in four eco-regions but the composition of essential oil was not uniform within them. Even in smaller geographic areas, as the Region of Murcia in the Southeast part of the Iberian Peninsula, high intraspecific variability was detected (Jordan et al. 2010).

The chemical variation of essential oil is a limiting factor that directly affects the properties and uses of aromatic plants including rosemary. The prevalence (or absence) of specific components can be crucial in evaluating its commercial success in fields like pharmacology, food and aromas, perfumery or agriculture. Consequently, gaining insight into the variability and stability of essential oil composition is crucial for obtaining a homogeneous material that enables a rational exploitation of rosemary. Given that genetics seem to be the prevailing factor, the characterization of the essential oils of these thirteen Spanish populations of *R. officinalis* is a promising starting point for the development of breeding programs in this species to obtain standardized plant materials (commercial varieties). However, multi-environment trials are still necessary to study the response to agronomic techniques, as fertilization or irrigation.

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Declarations

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

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