

Article

Assessment of Production and Qualitative Characteristics of Different Populations of *Salvia sclarea* L. Found in Sicily (Italy)

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Abstract: *Salvia sclarea* L. is an important industrial crop, valued for its herbal-aromatic properties and high quality essential oils, that is used in food, pharmaceuticals and cosmetics. In this study, carried out from 2009 to 2010, the morphological and production characteristics and essential oil content and composition of three Sicilian populations were studied. In particular, the composition of essential oils extracted from primary and secondary inflorescences using steam distillation was assessed. Morphological, production and qualitative data from the three populations were subjected to analysis of variance and cluster analysis. Regarding the quality of the oils, only the most prevalent compounds were taken into consideration in this study. The three populations were linalyl acetate/linalool chemotypes. Highly significant variations were found for the effective local population and inflorescence type in the composition of the essential oil principal components. In particular, the primary inflorescences were found to be accumulation sites favoured by monoterpenes, and secondary inflorescences were favoured by sesquiterpenes and sclareol. Populations “S. Stefano Quisquina” and “Alcara Li Fusi” performed best on a morphological and production level, whereas populations “Prizzi” and “Alcara Li Fusi” performed best in terms of quality. Population “S. Stefano Quisquina” produced high levels of sclareol. Biotype selection from within the populations should be based on both morphological, production and quality analyses.

Keywords: *Salvia sclarea* L.; spike yield; primary and secondary inflorescences; local populations; essential oil principal components



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1. Introduction

Salvia sclarea L. is a medicinal and aromatic species from the Mediterranean belonging to the *Lamiaceae* family. Clary sage is a hardy plant that grows wild in temperate areas and is xerophyte in nature. The whole plant is highly aromatic [1]. However, during flowering, which occurs during the second year, the inflorescences are covered in a dense, exceedingly aromatic resin similar in fragrance to muscat wine; hence the name “moscatella” or muscat sage [2]. It is one of the most highly valued Mediterranean species as a result of these aromatic properties.

The dried inflorescences and leaves are used in the production of spirits, herbal medicines, extracts and teas; the floral heads are used chiefly for essential oil extraction. The essential oils (EOs), characterized by an intense floral aroma and the scent of fresh grass, are used in the food, pharmaceutical and cosmetics industries. Clary sage essential oil is used to aromatize beer, tonic water, spirits and even muscat wine and vermouth [3]. In the perfume industry, the essential oil is highly prized due both to the quality of its fragrance and the fact it is an excellent fixative. Furthermore, sclareol, one of the principal

components of the essential oil, is used as a base for the chemical synthesis of Ambrox. Ambrox is commonly used as an alternative to ambergris, a waxy substance produced in the digestive tract of the sperm whale and the source of one of the most prized animal-extract essences in the industry [3]. Within the perfume industry, *S. sclarea* provides the dry amber/tobacco note in oriental tobacco scents [4]. Recent studies on *S. sclarea* have shown it to have allelopathic and insecticidal properties, even acting as a biofilter in water treatment [5–8].

Scientific literature reports the traditional use of clary sage oil as an agent against gingivitis, stomatitis and mouth ulcers. Other scientific evidence demonstrates the analgesic, anti-inflammatory, antioxidant, antimicrobial, antiviral, antifungal and cytotoxic activities of its essential oils [9–20]. Clary sage is also used in aromatherapy as a highly effective relaxant for the treatment of stress, asthma, digestion and menstrual problems as well as an aid to induce childbirth [21,22]. Some authors have found that clary sage seeds are rich in polyunsaturated fatty acids, which make them ideal for use in nutraceuticals. They are also a good source of edible oils, having omega 3-linoleic acid [23,24].

Its hardy nature and essential oil profile mean that clary sage is widely grown for extraction purposes in France, Bulgaria, the post-Soviet states, the United States and Western China [25]. In Italy, however, although the species grows wild in a number of areas, it is one of many medicinal and aromatic species that are either not cultivated or are generally underused.

These aromatic species, especially Mediterranean species, have high phenotypical plasticity; they adapt well to a range of environments, such as the xerophytic conditions typical of the Mediterranean and, as a consequence, are able to change their chemical composition [26–28]. It is worth noting that percentage content and essential oil composition are important parameters in the evaluation of aromatic species, as they delineate numerous and varied properties (antioxidant, antimicrobial, etc.) that can be used to create innovative products [29].

It is widely known that the essential oils of a number of species belonging to the *Lamiaceae* family show a degree of chemical variability due to certain exogenous factors (climate, soil, altitude, latitude, agronomic techniques, post-harvest management, etc.) and endogenous factors (plant age, development stage, genetic properties, plant parts, etc.) [30–38]. There is also known to be a strict correlation between the formation of primary and secondary compounds. The latter can be affected by the amount of biomass and by the relationship between the organs of the plant and substance accumulation levels in its tissues [39,40].

For the species in this study, therefore, it is important to evaluate a number of factors that could lead to greater efficiency in terms of biomass yield, particularly with regard to inflorescence production, but also in terms of essential oil composition.

These aspects are fundamental for agronomic selection in the development of industrial crops.

Based on the above, this study compared three local Sicilian populations (LP) of *Salvia sclarea* to evaluate both quality and production aspects. Furthermore, the effects of two types of inflorescence—primary and secondary—on the principal components of the essential oils of the three populations were evaluated.

2. Materials and Methods

2.1. Site of Experiments and Treatments

The tests were carried out in the two years 2009 and 2010 at the Orleans Experimental Station, University of Palermo (Italy) (Table 1).

Table 1. Test site information.

Test Site	Province	Geographical Coordinates	Altitude (m a.s.l.)	Average Annual Rainfall (mm)	Average Annual Temperature (°C)
“Orleans” experimental station	Palermo	38°06′26.2″ N 13°20′56.0″ E	34	605	18.40

Soils in the test area were sandy clay loam (Aric Regosol, 54% sand, 23% clay, and 21% silt), with a pH of 7.6, 14 g kg⁻¹ organic matter, 3.70% active carbonates, 1.32% total nitrogen, 18.1 ppm available phosphorus and 320 ppm exchangeable potassium. The hot, temperate climate is characterized by humid winters and dry, hot summers, typical of the Mediterranean. August is the hottest month of the year, with an average temperature of 26.2 °C, and January is the coldest at 12.1 °C.

Three populations of clary sage sourced from the wild from different sites in Sicily (Italy) were compared (Table 2).

Table 2. Provenance and local *Salvia sclarea* L. population code.

Provenance	Province	LP Code
S. Stefano Quisquina	Agrigento	SS
Prizzi	Palermo	PR
Alcara Li Fusi	Messina	AF

The main climatic and environmental characteristics of the test site are shown in Table 1.

The plants used to create the experimental plot were obtained from seeds taken from each population located in the plant collection field at the test site. As shown in Table 2, the plants were identified using initials linked to their provenance: SS, PR and AF. In order to assess their quantitative and qualitative characteristics, the three populations were planted in the field using a plant density of 2 plants per m². The plants were grown using the same organic cropping techniques in both years. Weed control was carried out mechanically without the use of herbicides or chemical fertilizers, and irrigation was not used.

2.2. Plant Measurements

Observations were carried out at the full flowering stage, which occurred the year following planting in the open field, as the species is a biennial.

The following parameters were recorded during harvesting: plant height, plant fresh weight, plant dry weight, number of branches, number of stems and inflorescence length. In addition, inflorescence as well as leaf and stem ratios (as % of total dry weight of the plant) were also measured.

Dry matter weight was calculated when constant sample weight was reached (dried in a shaded and well-aerated environment at a temperature of approximately 30 °C). Spike yield per hectare was also estimated.

2.3. Essential Oil Extraction and Oil Yield Calculation

For a sample of 500 g of dried inflorescences, the total essential oil (EO) content was determined (expressed as a % *v/w*: oil volume/sample weight in g) following steam distillation extraction. Oil yields were calculated by multiplying inflorescence yields by oil content and 0.90 (approximate specific gravity of oil) [41]. Furthermore, both the content and composition of the essential oils were determined for two types of inflorescence: primary inflorescence stem “ISP” and secondary inflorescences stem “ISS”. The length of the ISP inflorescences and the ISS inflorescences was also measured.

2.4. GC and GC/MS Analyses of Essential Oils

In accordance with international guidelines [42], gas chromatographic (GC) analyses were run on a Shimadzu gas chromatograph, Model 17-A, equipped with a flame ionization detector (FID) and an operating software Class VP Chromatography Data System, version 4.3 (Shimadzu Corporation, Duisburg, Germany). Analytical conditions were as follows: SPB-5 capillary column (15 m × 0.10 mm × 0.15 µm), helium as carrier gas (1 mL min⁻¹), injection in split mode (1:200), injected volume 1 µL (4% essential oil/CH₂Cl₂ v/v), injector and detector temperature 250–280 °C, linear velocity in column 19 cm s⁻¹. The oven temperature was held at 60 °C for 1 min, then programmed from 60 to 280 °C at 10 °C min⁻¹, then 280 °C for 1 min. Percentages of compounds were determined from their peak areas in the GC/FID profiles. Gas chromatography mass spectrometry (GC/MS) was carried out in the fast mode on a Shimadzu GC/MS mod. GCMS-QP5050A, with the same column and the same operative conditions as used for analytical GC/FID, using operating software GC/MS solution, version 1.02 (Shimadzu). The ionization voltage was 70 eV, the electron multiplier was 900 V, and the ion source temperature was 180 °C. Mass spectra data were acquired in the scan mode in an m/z range of 40–400. The same oil solutions (1 µL) were injected with the split mode (1:96).

2.5. Identification of Components of Essential Oils

The identity of components was based on their GC retention index (relative to C₉–C₂₂ n-alkanes on the SPB-5 column), computer matching of spectral MS data with those from NIST MS libraries [43], the comparison of the fragmentation patterns with those reported in the literature [40] and, whenever possible, co-injections with authentic samples.

For each sage population three samples of essential oils were subjected to GC. The values shown in the tables are the result of the average of the 3 replicates.

2.6. Statistical Analysis

Data (two-year averages) relating to the morphology and production of the three populations were subjected to analysis of variance (one-way ANOVA) followed by cluster analysis (UPGMA).

Analysis of variance (two-way ANOVA) and cluster analysis (UPGMA) were also carried out on the essential oil compounds to assess the effects of the local populations and the type of inflorescence (TIPS)—primary (IPS) and secondary (ISS). Arcsine transformation was performed on all data percentages prior to elaboration. Variations between treatments were compared using Tukey's test, with a 5% probability level. A randomized plot design with three replications was used, and statistical analysis was conducted using the software PAST 3.

3. Results

Following analysis of variance (Table 3), the three local populations of clary sage in the study showed significant differences for most of the characteristics under examination. Statistical differences were not found for plant height, percentage incidence of stems and the number of stems per plant.

As Table 3 clearly demonstrates, populations SS and AF obtained greater spike yields at 2.76 and 2.10 Mg ha⁻¹, respectively, both statistically differing from PR, which produced 1.8 Mg ha⁻¹. Similar trends were also found regarding plant fresh weight and dry weight, percentage incidence of leaves and number of branches. Fresh weight was found to be approximately 1200 g in both population AF (1232 g) and SS (1114 g), while PR was found to be considerably lower (796 g).

Table 3. Morphological and production characteristics of three LPs (Local Populations) of *Salvia sclarea* L.

Characteristic	AF	SS	PR	Significance
spike yield (Mg ha ⁻¹)	2.10 a	2.77 a	1.8 b	*
plant fresh weight (g)	1232 a	1114 a	768.9 b	*
plant dry weight (g)	337.2 a	356.1 a	214.5 b	*
plant height (cm)	137.9	142	139.8	n.s
inflorescence (%)	34.00 b	40.02 a	42.52 a	**
leaves (%)	25.63 a	19.49 a,b	19.18 b	**
stems (%)	40.37	40.48	37.62	n.s
no. stems	4.63	4.28	3.88	n.s
no. branches	13.18 a	13.93 a	9.8 b	**
inflorescence length (cm)	42.01 b	46.10 a,b	49.81 a	*
EO content (%)	1.29 a	0.68 c	0.91 b	**
EO yield (kg ha ⁻¹)	24.20 a	15.98 b	14.05 b	*
ISP length (cm)	54.42 b	58.25 a,b	61.63 a	*
ISS length (cm)	45.96 b	52.33 a	47.47 a	**
EO yield % ISP	0.98 a	0.58 b	0.98 a	**
EO yield % ISS	1.61 a	0.78 b	0.83 b	**

Local population code: PR = Prizzi; AF = Alcara Li Fusi; SS = S. Stefano Quisquina. EO = essential oil; ISP = primary inflorescences; ISS = secondary inflorescences. ** = significant at $p < 0.01$; * = significant = $p < 0.05$; n.s. = not significant. Within the same row, means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey's test.

In a similar fashion, plant dry weights for AF (337.20 g) and SS (356.10 g) were found to be considerably higher than for PR (214.50 g). This was also true for number of branches, which was recorded at 13 for both AF and SS and 10 for PR. The AF population (25.60%) was shown to have a leaf percentage incidence significantly higher than PR (19.20%; the lowest value), and SS maintained an intermediate position (19.50%), with no statistical differences between the other two populations.

The PR population produced greater average inflorescence length (49.81 cm), ISP length (61.63 cm) and ISS length (47.47 cm) as well as inflorescence percentage incidence (42.52%) compared to the other two populations, although statistical differences were not found with SS.

Differences were only found compared to AF, which recorded the lowest values for the abovementioned parameters. The population with the greatest essential oil content compared to the others was the AF population (1.29%), followed by PR (0.91%) and finally SS (0.68%).

AF performed the best for both essential oil yields (24.20 kg ha⁻¹ vs. PR and SS averages of 15 kg ha⁻¹) and oil percentage content of the primary inflorescences (ISP) (0.98%) and secondary inflorescences (ISS) (1.61%). It is also worth noting that PR obtained similar results to AF regarding essential oil % content of the ISP. The dendrogram (Figure 1), based on cluster analysis using morphological and production characteristics, shows the two main clusters. The first cluster grouped the two populations AF and SS, and the second cluster was constituted only by the population PR. These results are in accordance with data from ANOVA analysis.

Table 4 shows average values for the population characteristics in each cluster. The values are purely descriptive and are shown only to highlight the distinctive features of each cluster, as grouped by the analysis.

The two populations SS and AF located in the first cluster (Table 4) recorded greater plant fresh (1173 g vs. 760 g for PR) and dry (347 g vs. 215 g for PR) biomass production, greater inflorescence yield (2.43 vs. 1.80 Mg ha⁻¹ for PR), number of branches (14 vs. 10 for PR), and percentage incidence of leaves (23 vs. 19% for PR) and stems (40 vs. 38% for PR).

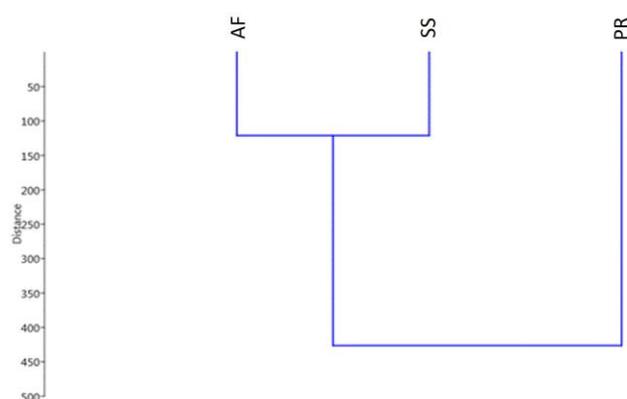


Figure 1. Cluster analysis of local populations in the study base on morphological and production data. Local population code: PR = Prizzi; AF = Alcara Li Fusi; SS = S. Stefano Quisquina.

Table 4. Average values regarding morphological and production characteristics of the populations in each cluster.

Characteristic	First Cluster (SS, AF)	Second Cluster (PR)
	Average	Average
inflor. yield (Mg ha ⁻¹)	2.43	1.80
EO content (%)	0.99	0.91
EO yield (kg ha ⁻¹)	20.10	14.05
plant height (cm)	139.96	139.83
plant fresh weight (g)	1173.05	768.86
plant dry weight (g)	346.62	214.51
inflorescence (%)	37.01	43.19
leaves (%)	22.57	19.18
stems (%)	40.42	37.62
no. branches	13.55	9.80
no. stems	4.45	3.88
inflor. length (cm)	44.05	49.81
ISP length (cm)	56.33	61.63
ISS length (cm)	49.14	47.47
EO yield % ISP	0.78	0.98
EO yield % ISS	1.20	0.83

Local population code: PR = Prizzi; AF = Alcara Li Fusi; SS = S. Stefano Quisquina. EO = essential oil; ISP = primary inflorescences; ISS = secondary inflorescences.

These same populations (SS and AF) on average also produced the greatest EO yields (20 vs. 14 kg ha⁻¹ for PR) and % content (0.99 vs. 0.90% for PR), with greater incidence in ISS (1.2 vs. 0.83% for PR).

In contrast, the greatest % incidence of inflorescences per plant (43% vs. 37% for SS and AF) was found in the population PR, in addition to the greatest average inflorescence length (50 cm vs. 44 cm for SS and AF). This greater length was linked to ISP length (62 cm vs. 56 cm for SS and AF) in particular, which was further found to have a higher EO % incidence (0.98% vs. 0.78% for SS and AF) than the other two populations.

Seventy-six components emerged from GC analysis, constituting approximately 98% of the chemical profile. Regarding the compound classes, the monoterpenes were the most abundant class. Oxygenated monoterpenes, in particular (73 ÷ 79%), were far more abundant than hydrocarbons (5 ÷ 6.0%). Sesquiterpenes oscillated between 11 and 14% and diterpenes were also worthy of note, ranging between 4 and 7.0%. Finally, the content of the class named “others” was negligible, being far below 1.0% (Figure 2).

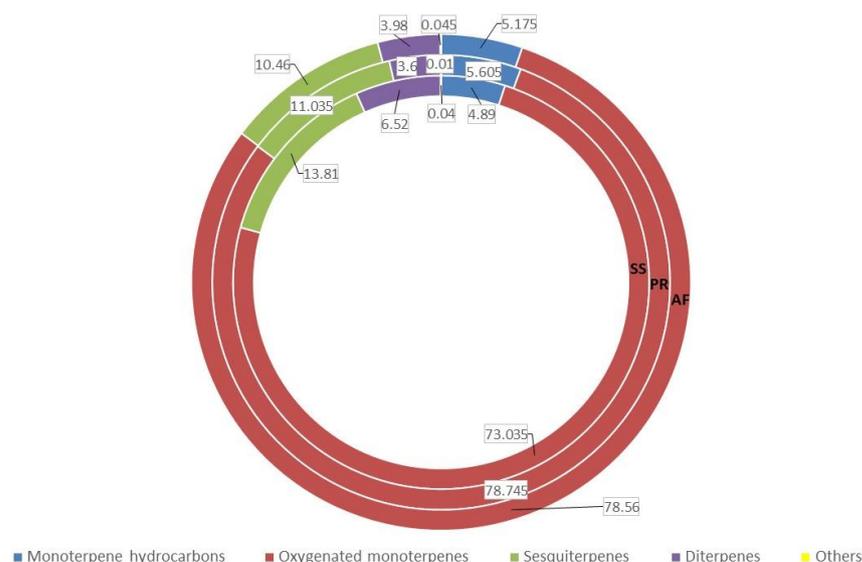


Figure 2. Class of compounds of the essential oils of the local populations in the study. Local population code: PR = Prizzi; AF = Alcara Li Fusi; SS = S. Stefano Quisquina.

Only the most abundant components were taken into consideration in this study, in decreasing order from linalyl acetate (38.73–41.63%), linalool (22.42–25.55%), α -terpineol (5.76–7.14%), germacrene D (3.61–4.50%), sclareol (2.93–5.50%), geranyl acetate (2.64–3.04%), β -caryophyllene (2.00–2.66%), valencene (1.77–2.56%), β -myrcene (1.56–1.91%), neryl acetate (1.54–1.71%), trans-ocimene (1.19–1.46%), and nerol (1.19–1.40%), constituting a little over 90% of the oil composition.

In all three populations, the only chemotype found was “linalyl acetate/linalool”, as the two compounds together accounted for the highest percentage of the total, with values of 61.15% (SS), 63.50% (PR) and 65.60% (AF) (Table 5).

Table 5. Effect of the local population (LP) on the composition of the principal components of *Salvia sclarea* L. essential oils.

Component	LP			Significance
	SS	PR	AF	
β -myrcene	1.56 c	1.91 a	1.70 b	**
trans-Ocimene	1.19 c	1.46 a	1.29 b	**
linalool	22.42 c	25.55 a	23.97 b	**
α -Terpineol	5.76 c	7.14 a	6.48 b	**
nerol	1.19 c	1.40 a	1.25 b	**
linalyl acetate	38.73 c	38.95 b	41.63 a	**
neryl acetate	1.54 b	1.71 a	1.54 b	**
geranyl acetate	2.64 b	3.04 a	2.74 b	**
β -caryophyllene	2.66 a	2.00 c	2.23 b	**
germacrene D	4.42 b	4.49 a	3.61 c	**
valencene	2.56 a	1.77 c	1.80 b	**
sclareol	5.50 a	2.93 c	3.21 b	**

Local population code: PR = Prizzi; AF = Alcara Li Fusi; SS = S. Stefano Quisquina. ** = significant at $p < 0.01$; n.s. = not significant. Within the same row, means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey's test.

As illustrated in Table 5, highly significant variations were found for the effect of local populations on the composition of the essential oil principal components. Population AF had the highest percentage of linalyl acetate (41.63%), followed by PR (38.95%) and SS (38.73%), while linalool varied from 25.55% in PR to 22.42% in SS, with the intermediate value of 23.97% obtained by AF.

Furthermore, following comparison of the three populations, results showed that the chemical profile of PR had a greater content of β -myrcene, trans-Ocimene, linalool, α -Terpineol, nerol, neryl acetate, geranyl acetate and germacrene D compared to AF and SS, which followed in decreasing order. It is worth noting that AF produced intermediate quantities of nearly all the components examined, although it excelled in the production of linalyl acetate and produced the lowest levels of germacrene D (3.61%). SS, though lagging behind the other two populations regarding most of the chemical components examined, pulled ahead in β -caryophyllene, valencene and, in particular, sclareol (5.50%) production; for the latter component, it obtained three percentage points more than AF and PR. As the dendrogram (Figure 3) shows, cluster analysis of the percentage composition of the essential oil principal components highlighted 2 groups. The first group included populations PR and AF, and the other group comprised only SS.

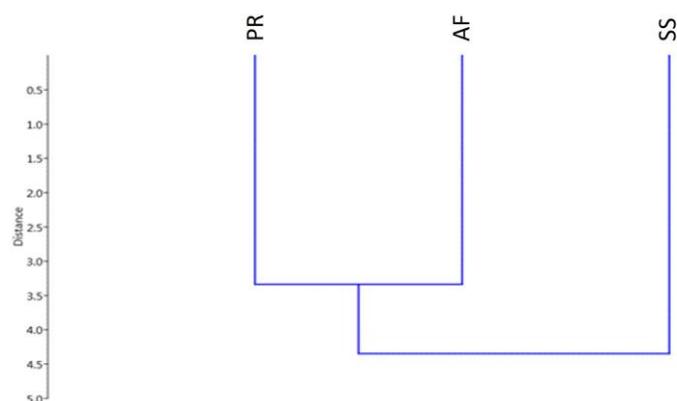


Figure 3. Cluster analysis of local populations in the study based on qualitative data. Local population code: PR = Prizzi; AF = Alcara Li Fusi; SS = S. Stefano Quisquina.

ANOVA analysis showed highly significant variations for the effect of inflorescence type on all of the principal components of the essential oils tested (Table 6, Figure 4).

Table 6. Effect of inflorescence type (TIPS) on the composition of the essential oil principal components.

Component	Inflorescence		Significance
	ISP	ISS	
β -myrcene	1.59 b	1.86 a	**
trans-Ocimene	1.23 b	1.41 a	**
linalool	23.20 b	24.78 a	**
α -Terpineol	5.87 b	7.06 a	**
nerol	1.19 b	1.37 a	**
linalyl acetate	39.78 b	39.86 a	**
neryl acetate	1.53 b	1.68 a	**
geranyl acetate	2.55 b	3.06 a	**
β -caryophyllene	2.57 a	2.02 b	**
germacrene D	4.53 a	3.82 b	**
valencene	2.33 a	1.75 b	**
sclareol	4.59 a	3.18 b	**

ISP = primary inflorescences; ISS = secondary inflorescences. ** = significant at $p < 0.01$; n.s. = not significant. Within the same row, means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey's test.

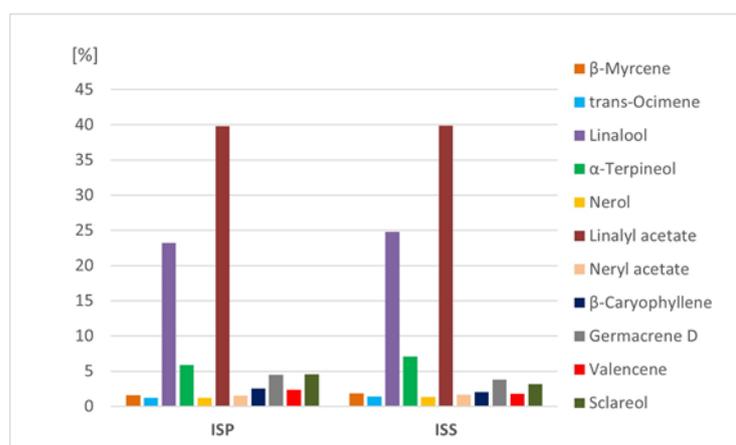


Figure 4. Principal components of the essential oils of primary inflorescences (ISP) and secondary inflorescences (ISS).

Relative to the components that characterize the chemotypes of the three populations (linalyl acetate and linalool), the most abundant contents were found in the secondary inflorescences (ISS) together with β -myrcene, trans-Ocimene, α -Terpineol, nerol, neryl acetate and geranyl acetate, therefore showing a prevalence of the monoterpene fraction. In contrast, primary inflorescences (ISP) had greater abundance of β -caryophyllene, germacrene D, valencene and sclareol—the sesquiterpene and diterpene classes.

Due to the combined effect of the two factors (Table 7, Figure 5), the primary inflorescences of population SS were statistically more abundant in germacrene D (5.08%), β -caryophyllene (3.28%), valencene (3.17%) and sclareol (7.76%), while the remaining components were found to be lower compared to the other treatments.

Table 7. Composition of the essential oils of the ISP and ISS of local *Salvia sclarea* populations—interaction of LP*TIPS factors.

Component							Significance
	SSP	PRP	AFP	SSS	PRS	AFS	LP*TIPS
β -myrcene	1.39 e	1.72 c	1.67 d	1.74 b	2.10 a	1.73 b	**
trans-Ocimene	1.07 e	1.35 b	1.28 d	1.33 c	1.59 a	1.32 c,d	**
linalool	21.42 f	23.79 c	24.40 b	23.43 e	27.31 a	23.59 d	**
α -Terpineol	4.83 e	6.40 d	6.38 d	6.69 b	7.90 a	6.58 c	**
nerol	1.07 f	1.27 d	1.23 e	1.32 b	1.52 a	1.28 c	**
linalyl acetate	35.80 e	42.46 a	40.85 d	41.71 c	35.46 f	42.42 b	**
neryl acetate	1.43 f	1.62 c	1.53 e	1.66 b	1.81 a	1.56 d	**
geranyl acetate	2.26 f	2.76 d	2.62 e	3.02 b	3.32 a	2.85 c	**
β -caryophyllene	3.28 a	2.03 d	2.39 b	2.03 d	1.97 e	2.07 c	**
germacrene D	5.08 a	4.55 b	3.95 d	3.76 e	4.42 c	3.27 f	**
valencene	3.17 a	1.93 c	1.88 d	1.94 b	1.60 f	1.72 e	**
sclareol	7.76 a	2.71 d	3.29 b	3.25 c	3.15 d	3.14 d	**

SSP = S. Stefano Quisquina Primary inflorescences; PRP = Prizzi Primary inflorescences; AFP = Alcara Li Fusi Primary inflorescences; SSS = S. Stefano Quisquina Secondary inflorescences; PRS = Prizzi Secondary inflorescences; AFS = Alcara Li Fusi Secondary inflorescences. ** = significant at $p < 0.01$; n.s. = not significant. Within the same row, means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey's test.

In population SS, the components most frequently found in the primary inflorescences were equally as prominent in the secondary inflorescences, except for β -caryophyllene and germacrene D, which were lower. All the other components were greater in value. In the PR primary inflorescences, the highest levels of linalyl acetate (42.46%) and germacrene D (4.55%) were found, although the latter was still lower than the levels found in the SS primary inflorescences. In the PR secondary inflorescences, the statistically highest levels of linalool (27.31%), α -Terpineol (7.90%), β -myrcene (2.10%), trans-Ocimene (1.59%), nerol (1.52%), neryl acetate (1.81%) and geranyl acetate (3.32%) were found. With the exception

of germacrene D (4.42%) (levels of which were among the highest but below those found in the primary inflorescences (PR)), the remaining components were among the lowest. In population AF, (except for linalool (24.40%), β -caryophyllene (2.39%) and sclareol (3.29%) from the primary inflorescences and β -myrcene (1.73%) and linalyl acetate (42.42%) from the secondary inflorescences (AF)—all of which were in a sub-apical position) all the components in both types of inflorescence were found to have medium-low values.

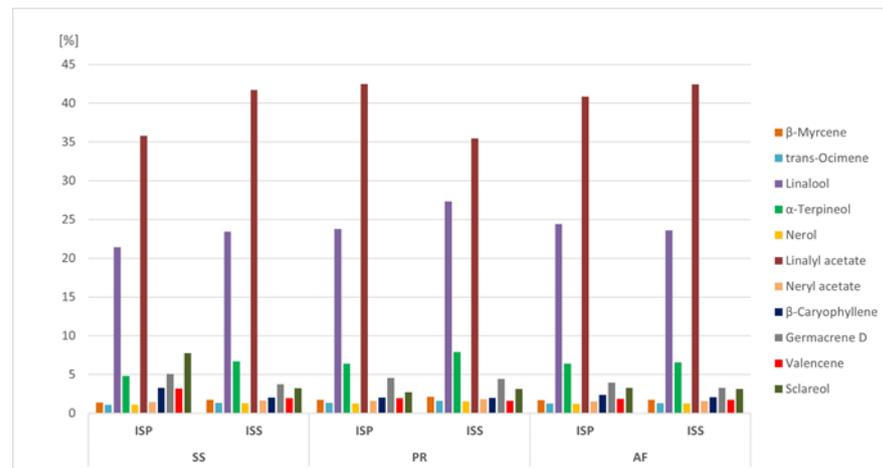


Figure 5. Principal components of the essential oils of the two inflorescence types (TIPS) of the three LPs. Local population code: PR = Prizzi; AF= Alcara Li Fusi; SS = S. Stefano Quisquina. ISP = Primary inflorescences steam; ISS = Secondary inflorescences steam.

4. Discussion

The morphological and production study provided an initial insight into the different populations in the study. Populations SS and AF demonstrated very similar characteristics, especially in terms of plant biomass production (both fresh and dry), inflorescence yields and essential oil yields. These populations performed the best in production terms, and plants were vigorous with dense leaves and inflorescence. In contrast, PR lagged in performance from this point of view. Despite the high incidence of inflorescences in the total plant weight produced by PR, both inflorescence and essential oil yields were not as high as the other two populations, as PR plants were smaller.

Population PR was different from the other two populations, with thinner plants and longer inflorescences. An analysis of the morphological characteristics is the first step towards crop improvement [44–46]. Morphological differences can be used to classify the plant material into various groups. As reported by Yaseen et al. [41], the populations in our study were classified as medium size (100–150 cm), as all the plants were a little under 150 cm in height. However, in studies carried out by Tibaldi et al. [1] in Piemonte (north Italy), tall biotypes were identified (>150 cm) and in Sicily small sizes (<100 cm) were identified [47]. Other studies on the species have shown that plant height can become an important distinguishing feature in the selection of accessions, as there is a positive correlation with a number of important production parameters, such as no. of inflorescences/plant, inflorescence length and oil yields [41]. In agreement with our study, Balmus et al. [48], while researching promising varieties in Moldova, also noted that encouraging results in terms of quality were shown by tall plants with a height of approximately 140 cm and with an inflorescence length approximately 20 cm longer than ours. In addition to confirming a number of Yaseen’s results [41], Tuttolomondo et al. [49] added that the length of the inflorescence (both primary (ISP) and secondary (ISS)), appears to be equally as interesting in terms of classifying accessions, deemed a reliable characteristic for selecting high EO-content biotypes. Furthermore, the same authors found longer ISS inflorescences were produced in the year with lower rainfall levels (compared to an exceptionally rainy year) together with a slightly higher EO content than the ISP. It emerged, therefore, that the

accessions that were able to employ this production feature as an adaptation strategy in reaction to difficult environmental conditions were also those that performed best in terms of biomass production. This suggests that the length of the inflorescence, in particular the ISS, should be given particular consideration when selecting accessions for production purposes, especially in Mediterranean areas. Regarding differences in EO % content, our study recorded statistical differences between populations: the EO % content for population AF was higher than the other two populations in the study, consistent with results from the best variety identified by Balmus et al. [48]. Referring once again to Yaseen's classification [41] regarding oil yields, population AF can be classified as high yielding ($>20 \text{ kg ha}^{-1}$), while populations SS and PR were medium yielding ($10\text{--}15 \text{ kg ha}^{-1}$). Although a high EO-yielding variety was identified, better yields were found in the varieties studied in Moldavia by Balmus et al. [48]. However, the study of wild populations is the first step towards identifying good biotypes to be used in the development of *S. sclarea* high EO-content varieties for the Mediterranean.

Cluster analysis results regarding the morphological and production data showed, as illustrated above, that two of the three populations (SS and AF), although originating in different areas, were grouped together in the same cluster due to similarity of characteristics. The populations seem to have been little affected by differences in geographic origin, when affected at all. Furthermore, agronomic characteristics are known to be easily influenced by environmental conditions and cropping practices [50].

Population PR, however, formed a separate cluster from the other two populations, even though the test environment and cropping practices were identical. In addition to highlighting a different phenotype, this could also indicate genetic differences based on different geographical locations of origin.

Compared to the many studies in the scientific literature regarding the composition and biological activity of *S. sclarea* essential oils, there are relatively few that assess production aspects and essential oil quality together. A few studies have shown (for the most part) only differences in the chemical composition of *S. sclarea* essential oil in relation to geographical location of origin [13,16,17,19] and to different cropping and harvesting conditions. Field tests carried out in the Ukraine showed that agronomic-technical factors modify essential oil yields of *Salvia sclarea*; sowing in December rather than April, and harvesting in the cooler hours of the day produced higher yields [51]. Other studies underlined different yields from different plant parts (flowers and leaves in particular) [16]. Other variations in the production of the chemical components are linked to different ecotypes or chemotypes. It is worth noting that the cultivars produced greater yield stability in terms of chemical composition, while the effect of the environment was greater for ecotypes.

Regarding the chemical composition of *S. sclarea* essential oils, various authors have demonstrated that, in most cases, the principal volatile components belong to the terpenoids group [43,52], among which are linalyl acetate and linalool (which characterize good quality oils suited to aromatizing) [2,12,14,53]. No studies on the composition of the essential oils, in relation to the two types of inflorescence examined in this study, were found in the scientific literature. A small number of studies were found that reported that linalool, linalyl acetate and sclareol are essential oil components typical of the flowers, whereas germacrene D was found in higher proportions in the leaves [39,41,54].

These results reinforce data found in literature on possible quantitative and/or qualitative differences in essential oil components from local populations/ecotypes and different plant parts.

Regarding the qualitative aspects, population PR performed the best of the three populations as it obtained higher values in approximately 60.00% the chemical components examined. AF was found to have the greatest linalyl acetate content and the lowest germacrene D content while maintaining intermediate values for the other components. SS lagged behind the other two populations for most of the components, although it excelled in sclareol, β -caryophyllene and valencene content. These differences were highlighted by the cluster analysis, with the grouping of populations PR and AF and the separation of SS

from the other two populations. Similar results regarding essential oil composition between populations in different areas within the same region were reported by Pitarokili et al. [13] in Greece. Two populations were studied, one more abundant in linalyl acetate, linalool and α -terpinol, and the other with higher sclareol content, comparable to our population SS.

We would like to underline the difference in groupings of the populations in the clusters following analysis of the morphological and production characteristics and analysis of the essential oil components. The first dataset showed similarity between AF and SS (the most productive in biomass terms); the second dataset grouped PR and AF (based on affinity of the essential oil composition). The selection of ecotype from the populations should, therefore, include a morphological, production and quality analysis.

The inflorescences, divided into primary and secondary inflorescences, are characterized by the same predominant components but in differing ratios. Primary inflorescences were found to be accumulation sites favoured by sesquiterpenes and sclareol, and secondary inflorescences by monoterpenes. This differentiation was not shown by the interaction effect of LP*TIPS, as the levels of the different components were found to be relatively heterogeneous between the inflorescences of the three populations.

However, a closer examination of the results showed that most of the components followed the same trend. This is likely due to the fact that the populations, particularly abundant in monoterpene components or fraction (or other fractions), maintained high levels. These levels, however, were lower in one of the two inflorescence types and frequently higher than the corresponding inflorescence fractions in the other populations, thereby obscuring the general accumulation trend. This principle does not hold for linalool in AF and linalyl acetate in PR.

Finally, due to considerable interest regarding this species, further studies could be conducted on more efficient propagation methods, using in vitro technologies already adopted for other typical Mediterranean species, such as capers and hops [55–57].

5. Conclusions

The three populations in this study demonstrated significant production differences in terms of both biomass and essential oil yields. Quality analysis of the essential oils of the three populations produced only one chemotype, the “linalyl acetate/linalool” type. Primary inflorescences were found to be a preferred accumulation site of sesquiterpenes and sclareol, whereas secondary inflorescences hosted monoterpenes. Cluster analysis of the morphological, production and quality characteristics, each illustrated on a dendrogram, revealed two clusters for each.

Regarding the first dataset, populations SS and AF performed the best, while PR and AF excelled in the results of the quality analysis. SS was found to have the highest levels of sclareol, particularly in the ISP. These differences in characteristics within the populations can be of interest in terms of end use. AF, for example, successfully combined morphological and production parameters with quality characteristics, proving to be of interest for a number of final uses. Population SS showed good production levels but trailed in quality, particularly concerning sclareol content, one of the most valued components of essential oils for the perfume industry. PR produced high quality oils, chiefly with regard to monoterpene fractions; however, it was the least productive of the populations.

This considerable diversity among the three populations regarding most of the characteristics examined assumes different levels of importance depending on use. Knowledge of this kind is precious when selecting biotypes based on their morphological, production and/or qualitative performance as well as the intended final use.

Based on our results, this study of Sicilian *S. sclarea* populations is the first step toward identifying biotypes within populations and can contribute to the development of this crop. It is a crop of considerable importance to the area, both in economic and agronomic terms, and may provide producers with the opportunity to grow quality crops with local plant materials.

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