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Research paper

Morpho-Chemical Characteristics of *Salvia Sclarea L.* At Two Different Locations in Jammu and Kashmir

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Abstract

Salvia sclarea L. commonly known as clarysage, is an important medicinal herb of temperate region with high market demand. In order to assess the morpho-chemical and biochemical plasticity in *S. sclarea*, studies were conducted at two contrastingly different environmental regimes. Plants were grown at Jammu (305 m, subtropical), and Srinagar (1730 m, temperate) till harvesting. Essential oils from inflorescence were quantified along with morphological parameters at both the locations. Morphological parameters such as plant height and number of leaves, which depict physiological strength of plant performed best at temperate conditions. Leaf area however was highest at lower altitude of 305 m. Number of chemical compounds and percentage of linalool increased with respect to altitude. Amount of linalool at 1730 m increased by 37% than 305 m. These findings reflect that temperate conditions were best suited for commercial growth of *Salvia sclarea*.

Keywords: Altitude, Essential oil, Morpho-chemical adaptation, *Salvia sclarea* L., Yield parameter

Introduction

Salvia sclarea L. (Family Lamiaceae), known as Clary sage, is an important medicinal plant native to the Northern Mediterranean region. The plant has traditionally been used in folk medicine, pharmaceutical and cosmetic, chemical, food and many other industries (Pesic & Bankovic, 2003). *S. sclarea* is a herbaceous biennial and reaches 40 to 140 cm in height with square stems that are covered with hairs (Mossi et al., 2011). The flowers are in verticils with 2-6 flowers in each vertical, held in large colourful bracts that range in colour from white to pink. Commercial cultivation of this crop is done for its essential oils (Clebsch & Carol, 2003) that have several pharmacological activities such as antimicrobial, anti-inflammatory and analgesic (Abravesh et al., 2005; Peana and Moretti, 2002). Investigation on essential oil of *S. sclarea* and their composition at different places was carried out by various wokers and linalool was found to be major constituent (Souleles & Argyriadou, 1997; Pesic & Bankovic, 2003). Genus *Salvia*

consists of more than 900 species which are present widely in subtropical and temperate regions (Walker and Sytsma 2007).

Environmental conditions play a key role in defining quantitative and qualitative distribution of plants. Changing climate affect the environmental conditions and it is predicted that climate change will remain one of the major factors of biodiversity patterns in future (Sala *et al*. 2000; Pressey *et al*. 2007). There is already strong evidence that plant species are shifting their ranges in altitude and latitude as a response to changing regional climates (Parmesan & Yohe, 2003; Walther *et al*. 2002). Changes are expected in environmental factors including temperature, light intensity and partial pressure of gases that will define new physiological and metabolic status in plants. Climate change has led to major changes in phenology and timing of seasonal activities especially flowering (Willis *et al*. 2008). However, knowledge of how species adapt due to environmental changes is still relatively limited.

The present investigation, therefore, aims at understanding morphological and biochemical variability under contrastingly different environments in *Salvia sclarea* L. This acclimatization study would not only help to understand essential oil characteristics, but also help to recognize cultivation site for high potency and yield. It is hypothesized that alterations in biochemical processes as a consequence of adaptability will have definite bearing on qualitative and quantitative yield. It would also be interesting to follow the changes in essential oils at all the locations, as they have been reported to be influenced by environmental conditions. Base line data, thus, generated would be helpful in understanding survival and adaptation mechanisms of several high value temperate medicinal plants at different environments.

Materials and Methods

Plant material

Present investigations were carried out with *S. sclarea.* Planting material was raised under controlled conditions at IIIM experimental field station, Jammu. One month old seedlings at four leaf stage, were transplanted to selected cultivation sites at two different locations viz. Jammu (32°43′N, 74°54′E, 305 m asl) and Srinagar (34°50′N, 74°47′E, 1730 m asl) ranging from sub-tropical to temperate climate in the months suitable for plant growth i.e., January (Jammu) and March (Srinagar).

Plant Weight

Uprooted plants (20 Nos.) were washed carefully using distilled water to remove any dirt. Moisture was blotted off with the help of blotting paper. Fresh weight of individual plant was recorded and then dried in an electronic oven at 60°C for 48 h after which dry weight was recorded.

Morphology

Main diagnostic morphological features viz. plant height, leaf characteristics, biomass production were recorded in the field from each site. For each character an average of 20 readings from as many plants was taken.

Floral Biology

Number of spikes/plant, spike length, inflorescence length, stamen length, anther length, carpel length, size of calyx and number of seeds/fruit were recorded for randomly selected 20 individual plants of uniform age at each site.

Isolation of Essential Oils

Plant material was collected from experimental plots at two locations during the flowering period in month of May & July, respectively for Jammu & Srinagar. Fresh inflorescence (500g) were harvested and trimmed to remove the stem below lowest flowering nodes and oil was extracted by hydrodistillation in a Clevenger type apparatus for 5 hours. Oils were dried

over anhydrous sodium sulphate and stored in sealed dark glass vials at low temperature (0 to -4°C) prior to analysis.

Gas Chromatography Mass Spectrometry and identification of compounds

GC-MS analysis of essential oils was performed on a Varian mass spectrometer-4000 series system fitted with a CP-SIL 8 CB column (30 x 0.32 mm ID, film thickness 1 µm). Temperature programming of column oven was performed at 60°C to 240°C with 3°C/min rising rate. Helium was used as carrier gas at flow rate of 1 ml/min. Mass spectra was recorded over 50-400 amu range at one scan per second with E.I. at 15-70 eV. Identification of peaks was carried out by comparison with authentic reference compounds and comparison of Kovat retention indices from C-9-C-21 alkanes with literature values and comparison of the mass spectra with those reported in the NIST and WILEY computer libraries and those of published in literature. Data were compared by a variance analysis by the ANOVA procedure and the t-test with the SAS statistical package (1998).

Results and Discussion

Vegetative phase ranges from January – March and March – June for Jammu and Srinagar, respectively, followed by flowering initiation. After full blooming (>90%), harvesting of inflorescence were done for estimation of oil content during first week of May (Jammu)and July (Srinagar). Based on planting location, age of the plants during harvest at different locations under study ranged from 5-6 months. This investigation was aimed at finding the extent of stability and flexibility in growth parameters and biochemical characteristics during acclimatization/ adaptation of *S. sclerea* under different locations with varied climates. Climatic condition at both the locations have been summarized in table 1.

Morphological Parameters

Mean performance of vegetative and floral characteristics is given in table 2. Results showed that the plant attained maximum height (105.18 cm) at Srinagar and minimum (44.8cm) at lower altitude. Number of leaves was also found to be maximum at Srinagar in comparison to Jammu. Leaf area was maximum at lower altitude (268.6cm^2) of 305 m and decreased with the increase in altitude. Similarly, inflorescence length, spike length, number of spikes and seed output per plant was maximum at higher altitudes and decreased progressively with decline in the altitude. Other floral characters like length of anthers stamens and carpels, number of seeds per fruit did not show any significant change with the change in altitude.

These results suggest that there is considerable effect of environment on all morphological traits. There was an overall positive correlation between altitude and biomass as is evident from the dry matter. At Jammu, percent flowering per plant is significantly low (66.8%) as compared to Srinagar (100%) which is reflected in low seed output/plant at this site (data not shown).

Environmental factors affect plant growth through their effects on assimilatory photosynthetic apparatus (Zhu *et al*., 2010). These changes are expressed morphologically in relative growth rates. Altitude also has a major effect on leaf morphology and physiology within a species (Prakash *et al.* 2011). High irradiance and low temperature at higher altitude result in photo-oxidation of photosynthetic pigments and hence, lower leaf area at higher altitudes. Leaves generally decrease in length, width and area in many species with relation to increasing altitude (Hovenden & Schoor, 2003; Caritat *et al.* 1997). However, we could not find any significant changes in leaf morphology suggesting a strong genetic control in this species of Salvia. Increase in number of

leaves at higher altitude seems logical as plants attained maximum height and secondary branching at that altitude (Table 2). For the same reason, dry weight per plant was more at higher altitudes (Srinagar) with respect to the lower altitude (Jammu).

Biochemical Parameters

Total percentage yield of essential oil obtained from hydro-distillation of flower inflorescence at Jammu and Srinagar were found to be 0.088%, and 0.12%, respectively on a fresh weight basis. This showed direct proportionality with altitudinal gradient showing maximum oil percent (0.12%) present at Srinagar (Table 2). The composition of the recovered oil, as analyzed by GC and GC-MS showed the presence of 93 compounds of which 39 were identified, accounting for 41.9% of the oil (Table 3). Data showed that the oil contained mainly oxygenated monoterpenoids and monoterpenoids hydrocarbons and small amount of oxygenated sesquiterpernoids and sesquiterpenoid hydrocarbons. Major constituents of (30.50%, and 41.83%), ocimenyl acetate (32.29% and 32.11%), (-) (1s, 2r, 4r)-β-fenchol (13.84%, and 5.78%), geraniol acetate (5.34% and 4.94%) and neryl acetate (2.99% and 2.47%), respectively for Jammu and Srinagar. Out of 39 compounds identified, 18 constituents namely linalool oxide, 2-(4'-methylphenyl)-propanal, 4-terpineol, verbenol, citral, trans-geraniol, α-cubebene, benzenebutanal, epoxysalvial, (-)-spathulenol, caryophyllene oxide, 1H-indene, ambrox, sclareoloxide, retinol acetate, cedren-13-ol, manoyl oxide and biformene were absent at lower altitude (Jammu) whereas, three compounds octatriene, α-farnasene and β-germacrene were presented only in Jammu. Epoxysalvial was present only at Srinagar (Table 3). 6 compounds namely levo- β -pinene, limonene, linalool, β-bourbonene, α-eudesmol and sclareol were found to be present in ascending order and 10 compounds namely α-terpinol, (-)-(1s, 2r, 4r)-β- fenchol, cis-geraniol, neryl acetate, geraniol acetate, copaene, β-bourbonene, α-caryophyllene, β-germacrene and δ-cadinene in descending orders with the gradual increase in altitudinal gradients. Ocimenyl acetate did not show any significant change with change in location.

Data in table 3 shows that essential oils of *S. sclarea* is dominated by two monoterpenes, linalool and ocimenyl acetate in which linalool gradually increases with increase in altitude where as there was no effect of altitude observed in ocimenyl acetate content. It is reported that sesquiterpenes are mainly produced in plants grown at lower altitude (Azevedo *et al*., 2002) but in contrary, our results showed that monoterpene are dominant at both locations. During previous work on oil composition of *Zanthoxyulum alatum*, it was reported that linalool found to be higher concentration at lower altitude and essential oil of both male and female plants have quantity varied with altitude (Gupta *et al.* 2011).

It has been earlier suggested that soil, climate and altitude may have affect on components of essential oil bearing plant (Jamshidi *et al.* 2009). Comparing the composition of oils of *S. sclarea* produced at different altitude, it seems that the oil from the higher altitude produced a greater number of compounds. Our data agree with the literature on the composition of the oils of *Senecio nulans* (Feo *et al.* 2003). Altitudinal gradients play an important role on the content and composition of essential oil in *Zanthoxylum* (Gupta *et al*. 2011).

Conclusion

Overall studies revealed that altitudinal variation differentially influenced morphological and biochemical responses in *S. sclarea* L. Two major compounds (linalool and sclareol) increased substantially at higher altitudes. These findings reflect that altitude of 1730 m and temperate conditions were best suited for plant growth. Higher altitude also shows better floral response and higher oil percentage. Salvia sclareaL., therefore,

seems better suited to temperate regions of Himalayas.

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Table 1. Climatic Conditions at two different experimental locations.

			Jammu (Subtropical)	Srinagar (Temperate)
Altitude (meter above sea level)			305	1730
Longitude			32°43′N	34°50′N
Latitude			74°54′E	74°47′E
Mean Temperature (°C)	Vegetative	Max.	25.6	21.5
		Min.	9.6	8.7
	Flowering	Max.	38.2	31.0
		Min.	21.1	18.0
Mean Relative Humidity (%)	Vegetative		66.6	64.0
	Flowering		29.5	64.5
Mean Precipitation (mm)	Vegetative		56.7	70.5
	Flowering		28.0	59.5

Table 2. Morphometric and floral characteristics of *Salvia sclarea* cultivated at different locations.

Characters	**Jammu**	**Srinagar**
Plant height (cm)	$44.80^{a} \pm 2.06$	$105.18^{b} \pm 3.15$
No. of branches	$2.05^{a} \pm 0.33$	$3.66^{b} \pm 0.49$
No. of leaves	$15.18^{a} \pm 1.06$	$44.21^{c} \pm 2.14$
Leaf area (cm^2)	$268.60^{a} \pm 7.13$	$244.00^{a} \pm 8.13$
No. of spikes /plant	$4.66^{a} \pm 0.76$	$15.33^{b} \pm 2.12$
Spike length (cm)	$12.03^{a} \pm 2.32$	$34.63^{b} \pm 3.83$
Inflorescence length (cm)	$13.34^{a} \pm 3.14$	$50.00^{b} \pm 6.31$
Length of stamen (cm)	$2.42^{a} \pm 0.07$	$2.52^{a} \pm 0.23$
Length of anther (mm)	$2.75^{a} \pm 0.14$	$2.40^{a} \pm 0.08$
Length of carpel (cm)	$3.27^{a} \pm 0.21$	$3.50^{a} \pm 0.09$
No. of seeds/ fruit	$3.81^{a} \pm 0.08$	$3.79^{a} \pm 0.02$
Seep output/ plant (g)	$18.00^{a} \pm 0.94$	$56.34^{b} \pm 4.58$
Dry weight/plant (g)	$21.50^{a} \pm 1.33$	$30.00^{b} \pm 2.12$

Each value is a mean of 20 individual replications. Significant differences (t-test $p<0.05$) between different altitude for each character are indicated by different superscript letters. Numbers followed by equal letters within each row are not significantly different.

Table 3. Chemical analysis of essential oil of *Salvia sclarea* L. at different altitudes.

RT (min.)	Peak Name	Amount %	
		Jammu	Srinagar
15.430	Levo-β-Pinene	0.266	0.703
17.675	Limonene	0.112	0.264
18.409	1,3,7-Octatriene, 3,7-Dimethyl, (e) -	0.619	--
19.785	Linalool oxide	--	0.062
20.576	α – Terpinol	0.259	0.049
21.162	Linalool	30.504	41.836
21.798	2-(4'-Meythylphenyl)-Propanal	--	0.094
25.460	4-Terpineol	--	0.058
26.150	(-) – (1s, 2r, 4r) –β – Fenchol	13.842	5.783
27.368	cis-Geraniol	1.414	0.861
28.089	Verbenol	--	0.063
28.428	Ocimenyl acetate	32.290	32.119
29.428	Citral	--	0.444
30.723	trans-Geraniol	--	0.038
33.160	α-Cubebene	--	0.023
33.375	Neryl acetate	2.998	2.476
34.231	Geraniol acetate	5.343	4.940
34.541	Copaene	0.861	0.738
34.950	β-Bourbonene	0.161	0.400
35.590	Benzenebutanal, 4-ethoxy-α-methyl – γ – oxo	--	0.310
36.555	β-Caryophyllene	2.172	0.810
38.066	α-Caryophyllene	0.154	0.099
39.083	Germacrene D	5.894	0.839
39.612	α-Farnesene	0.145	--
39.695	β-Germacrene	0.263	--
40.459	δ-Cadinene	0.096	0.095
42.692	1, 5-Epoxysalvial-4(14) – ene	--	0.011
42.990	(-)-Spathulenol	--	0.576
43.250	Caryophyllene oxide	--	0.572
44.092	1H-Indene, 1-ethylideneoctahydro-7a-meth	--	0.122
45.998	α-Eudesmol	0.358	0.898
50.150	Ambrox	--	0.115
54.285	Sclareoloxide (Cis-A/B)	--	0.317

RT (min.)	Peak Name	Amount %	
		Jammu	Srinagar
55.396	Retinol acetate	--	0.266
55.862	Cedren-13-ol, 8-	--	1.274
57.108	Phenol, 4- (3, 7- dimethyl 1- 3 -ethenylocata – 1,)	1.454	0.126
57.773	Manoyl oxide	--	0.092
59.526	Biformene	--	0.245
64.456	Sclareol	0.112	2.037