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Genetic Diversity of the Endemic Species *Phlomis aurea* Decne. in Southern Sinai, Egypt

Laila M. EL-Sadek[1], Ghada E. El-Badan[1#] and Hatem A. Shabana[2]
[1]Botany & Microbiology Department, Faculty of Science, Alexandria University, Alexandria;[2]Nature Conservation Sector, Egyptian Environmental Affairs Agency, Cairo, Egypt and [2]Sharjah Research Academy, Sharjah University Sharjah, UAE.

PHLOMIS AUREA Decne. (*Lamiaceae*), which have future antidiabetic drug yielding potentials, is an endemic species, restricted to the high altitudes in five main habitats in southern Sinai. The genetic characterization of individuals from different populations is necessary to construct proper conservation programs. Thus, the present study was conducted to achieve two main goals; **1**) Finding the genetic diversity among *Phlomis aurea* populations through variation in seed storage proteins electrophoretic pattern and Inter Simple Sequence Repeats (ISSR) finger printing as a dominant DNA molecular marker. **2**) Perceive the relationship among these biochemical and molecular parameters with the different habitats in Saint Katherine Protectorate (SKP) are in South Sinai. Mature dry seeds from 30 individuals representing different populations were used for protein analysis using SDS-PAGE technique, 12 individuals of them were chosen for ISSR analysis. The former test produced 20 protein bands, three of them were common to all plants (species specific) and could be used as finger prints for *Phlomis aurea*. It was suggested that seed storage protein patterns are affected by aspect direction, altitude and slightly affected by habitats. ISSR analysis showed that decreasing genetic variation of *Phlomis aurea* individuals from different habitats in the following order: Wadibed> Slope> Gorge>Basin. This open area habitat contributes to high percent of hybridization and consequently results in high genetic polymorphism.

Keywords: *Phlomis aurea*, Seed storage protein, ISSR markers, Genetic diversity.

Introduction

The genus *Phlomis* (family Lamiaceae) comprises approximately 100 species distributed throughout Euro-Asia and North Africa continents (Amor et al., 2009). It has various medicinal uses that differ from one country to another. In Egypt, the genus includes two species: *Phlomis aurea* Decene and *Phlomis floccosa* D. Don (Boulos, 2002). *Phlomis aurea* is endemic to the high altitudes in Southern Sinai especially Saint Katherine Protectorate (SKP). It is medicinally used as antidiabetic (Khafagi & Dewedar, 2000 and Mohamed et al., 2000). Climate change (drought) and unmanaged human activities caused this species to become endangered (Boulous, 2008 and Shaltout et al., 2004).

The Saint Katherine Protectorate (SKP) is one of Egypt's largest protected areas and includes the country's highest mountains. Five land form types were identified by Kheder (2007) in SKP, which support the growth of *Phlomis aurea*, these are: (1) Wadi beds, are drainage systems collecting water from catchment areas and form favorable habitats, occur at high elevations ranging from altitudes 1290 to 1900 m ASL (above **s**ea level). (2) Terraces**,** are platforms of bedrock mantled with a sheet of gravel and sand or rocky surface, occur at higher elevations ranging from 1453 to 1928 m.ASL. (3) Slopes, appears at different elevations ranging from 1634 to 2300 m.ASL. (4) Gorges,originate from joints or faults, occur at higher elevations ranging from 1594 to 2037 m.ASL. (5) Basins, occur as depressions between the peaks of high mountains and restricted to higher elevations of > 2000 m.ASL.

Seed storage protein electrophoretic profiles had been used to characterize wild and cultivated varieties of a number of plant species (El-Shazly et al., 2006; Mirali et al*.,* 2007 and Sharawy & Badr, 2008) because they are stable, uniform, reliable, reproducible and largely independent of age and environmental fluctuations (Sammour, 2014). It is also used in the investigation of genetic diversity within and among populations thus provides valid evidence for genetic relatedness (Crawford,

*Corresponding author email: ghadaelbadan@hotmail.com
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1990). Yuzbasioglu et al. (2009) studied seed storage protein pattern of 39 *Phlomis* taxa collected from different parts of Turkey; *Phlomis aurea* was not one of them. Thus, relationships among populations of *Phlomis aurea* based on seed protein electrophoresis and DNA genetic diversity studies of this species have not been found in the literature so far.

The DNA molecular markers are not influenced by the external environmental factors unlike that of morphological and biochemical markers, which had its own limitations as they were always, do not completely represent the genetic structure (Noli et al., 1997) and hence accurately testify the genetic relationship between and among individual groups(Lawrence et al., 1992). Inter-simple sequence repeats (ISSR)has been used regularly for genetic diversity assessment as a thorough knowledge of the level and distribution of genetic variation (of length of DNA fragments between simple sequence repeats), which was essential for conservation (Dreisigacker et al., 2005; Sharma et al., 2008; Naik et al., 2010 and Das et al., 2011). This PCR-based DNA finger printing technique was proven to be very informative and cost-effective technique in many individual species as their primers do not require prior knowledge of a species genome (Williams et al., 1990; Zietkiewicz et al., 1994; Kharazian et al., 2015 and Badr et al., 2014; 2017).

This study was conducted to achieve three main goals; 1- Assess the genetic diversity of soluble seed storage proteins patterns among *Phlomis aurea* populations in four of the fore-mentioned land form types (habitats). 2-Finding the genetic diversity among those populations by ISSR molecular marker. 3- Determination of the relationship between biochemical and molecular markers with both morphological characters and environmental factors carried out in a previous study by Shaltout et al.(2015).

Materials and Methods

The present study is a part of an overall ecological study on the variability among *Phlomis aurea* populations in SKP at South Sinai (Fig. 1). The ecological part is discussed in Shaltout et al. (2015). A total of 65 sampling stands distributed in 20 sites represented by 882 individuals were surveyed for the ecological study; they were selected to represent the utmost variations among *Phlomis aurea* Decne populations in South Sinai. Thirty individuals of them were chosen for the genetic diversity of seed total soluble proteins representing the 14 vegetation groups that resulted from the multivariate analysis"two-way indicator species analysis" (TWINSPAN) as a classification technique (Hill, 1979a) depending on morphological characters, habitats, location, altitude and aspect direction at level 2 (Table 1) (Shaltout et al., 2015). These individuals represented five major types of habitats (wadi beds, gorges, slopes, terraces and basins) and the following topographic variables: 1- Elevation gradient of 1374 to 2019 m ASL (meters above sea level), 2- Seven aspect directions: East (E), North (N), South (S), North East (NE), North West (NW), South East (SE), South West (SW).

Polyacrylamide gel electrophoresis (PAGE) was used for separation of the soluble seed storage proteins as described by Hames (1981). Twelve wild *Phlomis aurea* individuals from the previous 30 individuals, representing the different groups, altitude, aspects and habitats of protein pattern (Fig. 2) were chosen for ISSR analysis. The studied individuals were numbered as; 1, 7, 10, 12, 13, 17, 20, 23, 25, 27, 29, 30. Genomic DNA was extracted from seeds according to manufacturer protocol of Omega Bio-tek's gel extraction kit. A set of 10 primers obtained from BIOSEARCH Technologies, USA were used to screen the genomes of the 12 individuals for polymorphism. However, only six of the primers produced clear scorable bands with good, reproducibility and amplification patterns. The nucleotide sequences and GC content of these primers are presented in Table 2. Annealing temperatures were optimized and it was found to be 55°C for all the primers.

The ISSR technique followed the manufacturer protocol of DreamTaq Green PCR Master Mix (2X). Amplification was done using GeneAmp Polymerase Chain Reaction (PCR) system cycler and the reaction consisted of 35 cycles; each cycle consisted of denaturation at 93°C for 30 sec followed by annealing at 55°C for 30 sec and extension at 72°C for 30 sec. There was an initial delay for 15 min at 95°C at the beginning of the first cycle and 10 min delay at 72°C at the end of the last cycle as a post extension step. The amplified PCR products were electrophoresed in 1.5 % agarose gels and visualized on the UV trans-illuminator and photographed by using gel documentation system (Geldoc-it, UVP, England). DNA ladder (Thermo scientific co.) of different fragment sizes ranging from 100 bp to 1 kbp were used as a DNA fragment size marker.

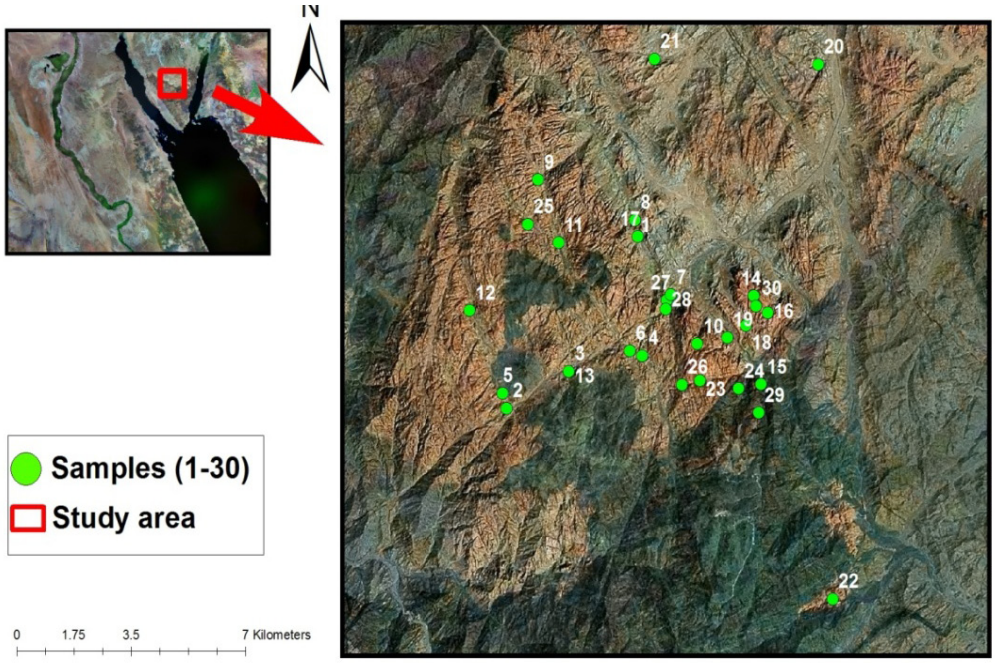


Fig. 1. Map of the study area for the 30 samples (individuals) at different sites in Saint Katherine prot

TABLE 1. Distribution of the 30 individuals of Phlomis aurea representing their population in South Sinai according to habitat type, site, altitude and aspect direction.Sa No: Sample number, Al: Altitude, As: Aspect direction.

Sa. No.	Habitats	Sites	Al. (asl)	As.	Sa. No.	Habitats	Sites	Al. (asl)	As.
1	Wadi bed	WadiItlah	1501	NE	16	Gorge	Farsh El-Losa	2009	N
2	Wadi bed	WadiGebal	1842	N	17	Wadi bed	Wadi El-Tala'a	1545	E
3	Slope	WadiGebal	1903	N	18	Wadi bed	Wadi El-Fara'a	1852	NE
4	Wadi bed	Wadi El-Shak	1829	N	19	Terraces	Wadi El-Arbae'en	1680	NE
5	Wadi bed	Farsh El-Rommana	1825	E	20	Gorge	Tala'a EL-Kaleb	1413	NE
6	Wadi bed	Wadi EL-Zwateen	1832	NE	21	Wadi bed	EL-Ferea	1573	NE
7	Wadi bed	EL-Maserdy	1604	E	22	Wadi bed	Wadi El-Rotk	1786	NE
8	Wadi bed	WadiItlah	1501	NE	23	Basin	Gebel El-Ahmar	2110	NW
9	Wadi bed	WadiTiniya	1757	NE	24	Gorge	Musa's Gorge	1910	NE
10	Basin	Abo-Hamman	2050	S	25	Wadi bed	Wadi Abu-Tuweita	1850	E
11	Wadi bed	WadiTiniya	1819	NE	26	Gorge	Gebel El-Ahmar	2032	NW
12	Slope	Farsh El-Rommana	1839	E	27	Gorge	EL-Maserdy	1627	E
13	Slope	WadiGebal	1901	N	28	Slope	EL-Maserdy	1702	NE
14	Gorge	FarshShoeib	1978	NE	29	Gorge	WadiGraginya	1910	NE
15	Gorge	WadiGraginya	1842	NE	30	Gorge	Farsh El-Losa	2010	SE

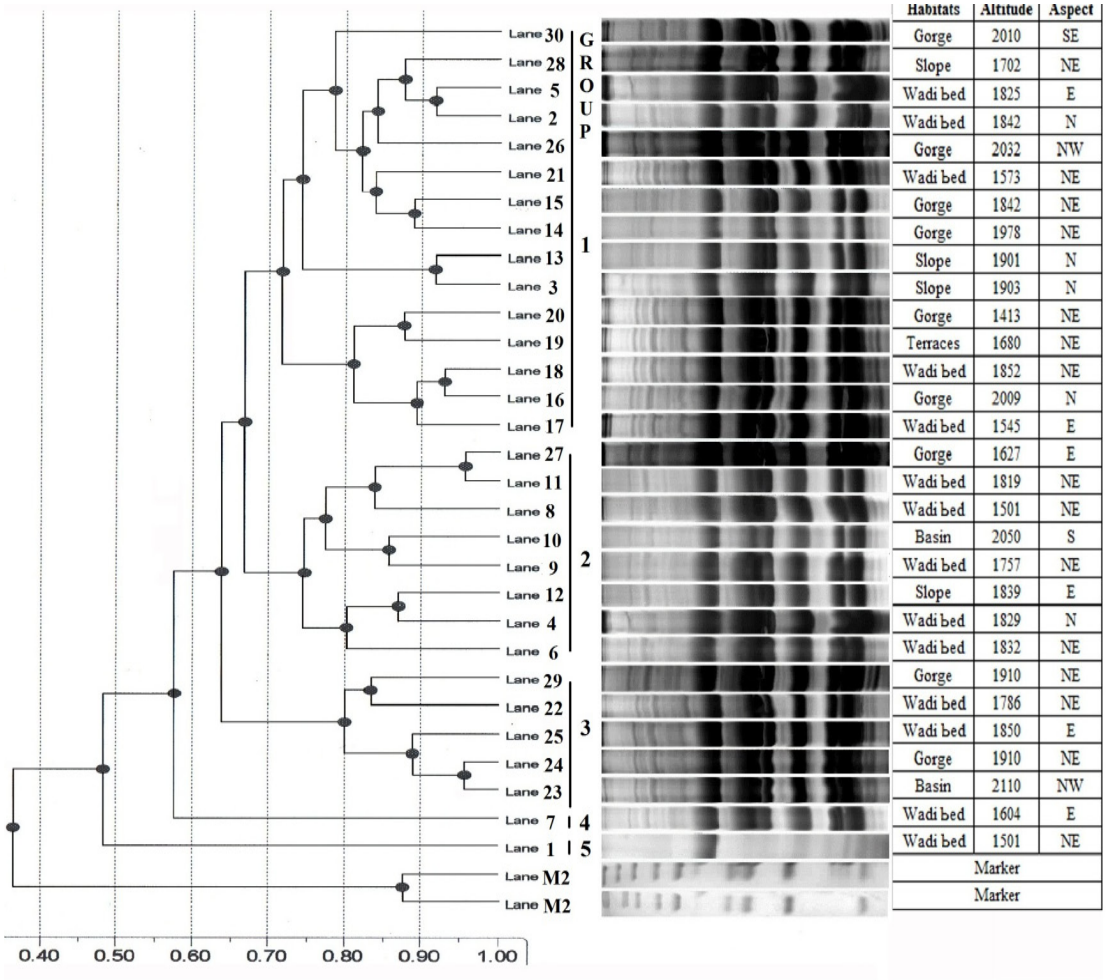


Fig. 2. UPGMA dendrogrambased on protein electrophoresis of 30 individuals of *Phlomis aurea* populations in South Sinai.

TABLE 2. Primers used in the ISSR-PCR technique, their sequencesand GCcontent.

Primers	Sequences (5' → 3')	GC %
ISSR-1	$GAG(CAA)_5$	48
ISSR-2	$(GA)_8T$	48
ISSR-3	$(AG)_8T$	48
ISSR-4	$(AC)_8C$	50
ISSR-5	$(AGC)_6G$	62
ISSR-6	$(TG)_8A$	48

Data analysis

Both SDS-PAGE profiles and ISSR finger printing were documented as photos and these were analyzed using Total lab software (www.totallab.com, Ver.1.0.1) by which molecular size and intensity of bands were calibrated and the UPGMA dendrogram

was constructed, based on the presence and absence of bands (Sneath & Sokal, 1973), to indicate the hypothetical relationships among the studied populations. The polymorphic bands percentage was calculated as: Total bands in each sample – common bands / total bands of all samples) X 100. Correlation between protein patterns and each of morphological, edaphic and geographic characters of the 30 accessions (extracted from Shaltout et al., 2015) was carried out. The percentage of maximum and minimum number of protein band intensity, which gave the highest (or lowest) values in the 5 habitats, 6 aspect directions and 8 altitude classes were calculated as follows:

Percentage of characters having the maximum(or minimum) value=

Number of characters having maximum (or minimum) values/ total number of characters) X 100. The final result is written as: Max % and Min%.

Results and Discussion

Atotal of 20 protein bands were recorded for the 30 individuals (Table 3, Fig. 2).The number of total bands per individual ranged from six in individual 1 to 15 bands in individuals 16 and 17. Three common bands were found at 45.1-39.9, 22-20 and 16.8-14.9 kDa. This characterizes *Phlomis aurea* as species specific bands. Three different unique bands, individuals, one (17.4-17 kD), seven (75.8 kD) and seventeen (95 kD). Each of these bands characterizes its individual. The cluster analysis extracted from the total soluble protein indicated the discrimination of the 30 studied individuals into five clusters at 0.7 similarity distance (Fig. 2). Two of these clusters (4&5) represented by one individual each from Wadi bed habitat which expressed the utmost variation of protein patterns. The other three clusters contained 5, 4 and 2 individuals in cluster 1, 2 and 3, respectively. Individuals of other habitats showed polymorphism and were found in different clusters which indicate that *Plomis aurea* is a high polymorphic species.

When the character of mean % of band intensity was compared with habitat, altitude and aspect direction, it was found that maximum values were of those individuals of gorge habitat (30.4%, Table 4-a), altitude class of <1500 (21.7%, Table4-b) and SE aspect direction (30.4%, Table 4-c). Thus, when these results were compared with those of morphological and environmental characters of Shaltoutet al.(2015), it could be concluded that the highest mean% of storage protein band intensity, morphological and environmental characters are always found at gorge habitat and at SE direction. However, low altitude (<1500 m ASL.) expressed the highest mean protein characters, while higher altitudes expressed the highest mean morphological and environmental characters.

In addition,correlation analysis of pairs of variables showed that significantly high positive correlations ($r= 1$, $P \leq 0.01$) occurred between number of total bands and polymorphic band percent, while highly negative correlation ($r= -0.5$, $P \leq 0.01$) occurred between the number of specific bands and altitude. The pairs of variables that gave slightly negative correlation ($r = -0.4$, $P \leq 0.05$) were the number of specific bands with the highest lamina length and between total number of bands and polymorphic band percent each with Clay percent (data extracted from Shaltout et al. 2015).

The genomic DNA analysis of the 12 chosen individuals, using ISSR technique showed that one common band was amplified with primer ISSR-5 (Fig. 3, Table 5). This could be used for finger printing *Phlomis aurea* species. Each of the six primers amplified large number of bands from the 12 studied individuals ranged from 74 for primer 6 to 90 bands for primers 3 and 4. Moreover, individuals from Basin habitat amplified the highest mean number of bands (43) while those of Gorge and Wadi bed habitats amplified the lowest mean number (40). ISSR-2 showed the highest number of specific bands (18) and ISSR-1 showed the lowest number (6). Those specific bands characterized these individuals. The highest mean percentage of polymorphic bands was 94 % for ISSR-5, while the lowest was 76 % for ISSR-2. Accordingly, primer ISSR-5 could be considered as an efficient primer to analyze *Phlomis aurea* species. Herein, individuals of Wadi bed habitats displayed the highest genome variation (mean % of polymorphism = 86.4%) than those of other habitats. In this context ISSR findings supported those of protein pattern analysis. This indicates the important role of habitat causing high levels of cross fertilization and/or hybridization leading to high level of polymorphism (heterozygosity) (Maquet et al., 1996). Accordingly, each individual is characterized by its active genes giving its specific protein and DNA patterns as a result of overall interactions. Clustering of the genomic DNA analysis data showed a similar pattern of the distribution of individuals from different habitats, altitude and aspect direction as those resulted from protein data analysis indicating high polymorphism. (Fig. 4).

TABLE 3. Comparative analysis of relative concentration (Band %) and molecular weight (M.wt.) of seed storage protein bands of 30 populations of *Phlomis aurea* in different habitats in South Sinai.

Ref Band	MW range	Population 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Band %														
1	95.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	75.8	0.0	0.0	0.0	0.0	0.0	0.0	**0.2**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	74.8 - 72.0	0.0	0.8	0.2	1.4	0.6	0.7	0.0	1.4	0.9	1.1	1.8	1.9	1.6	1.4	2.9
4	72.0 - 70.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.6	0.0	0.0
5	70.0 - 68.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	2.4
6	66.0 - 64.2	0.0	2.0	0.7	0.0	1.5	2.4	0.6	3.1	2.3	1.4	1.7	2.1	2.4	2.6	3.8
7	63.7 - 59.7	0.0	0.9	0.0	3.6	0.6	0.7	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0
8	59.0 - 56.9	0.0	1.0	0.0	1.0	0.2	0.0	0.0	0.0	0.0	0.0	1.0	0.8	1.2	1.2	2.2
9	56.0 - 53.9	0.0	0.0	0.0	2.9	0.0	2.1	0.9	2.8	1.8	0.9	1.3	1.2	0.0	0.0	0.0
10	53.0 - 50.8	0.0	3.0	1.8	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.3	2.9
11	45.1 - 39.9	41.4	17.7	17.0	19.7	18.8	18.6	17.7	15.7	16.6	16.2	16.4	16.6	15.2	15.0	14.4
12	37.8 - 35.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	1.2	1.2	2.3
13	34.0 - 30.0	0.0	13.7	13.8	13.0	15.4	14.0	17.9	18.2	23.4	17.7	14.9	14.9	12.7	12.7	11.3
14	28.0 - 26.0	0.0	13.4	17.2	15.3	15.2	16.7	11.9	11.1	9.8	11.9	15.2	15.5	15.3	15.9	12.4
15	25.0 - 24.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	22.0 - 20.0	10.4	19.9	18.1	17.6	17.9	18.5	19.4	18.5	17.9	18.9	18.4	17.5	17.9	18.2	17.9
17	17.4 - 17.0	**5.1**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	16.8 - 14.9	8.4	10.3	14.4	9.5	10.7	10.5	13.5	13.1	12.8	13.2	13.1	13.3	10.8	10.3	11.8
19	14.6 - 12.7	20.8	13.2	15.9	11.2	16.5	13.8	17.7	0.0	14.5	17.7	15.9	13.4	16.5	16.0	15.0
20	12.8 - 10.0	13.8	4.2	0.7	4.7	1.1	2.0	0.0	1.5	0.0	0.2	0.1	1.4	2.2	2.6	0.6
Total bands		6	12	11	11	12	11	9	9	9	12	12	12	13	13	13
Common band		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
No. of specific bands		1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Polymorphic bands %		15	45	40	40	45	40	30	30	30	45	45	45	50	50	50

Common bands are highlighted

Table 3. Cont.

Ref Band	MW range	Population 16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
		Band %														
1	95.0	0.0	**11.1**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	75.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	74.8 - 72.0	1.8	1.6	1.5	1.1	1.2	1.4	0.0	0.0	0.0	0.0	4.8	4.0	4.6	0.0	2.8
4	72.0 - 70.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	5.2	4.0	0.0	0.0	0.0	4.0	0.0
5	70.0 - 68.0	2.1	1.9	0.0	1.3	1.6	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	66.0 - 64.2	3.3	0.0	3.3	2.9	3.0	3.1	0.0	0.0	0.0	0.0	5.3	5.9	4.3	0.0	3.8
7	63.7 - 59.7	1.6	3.2	0.0	1.3	1.7	0.0	7.5	3.0	3.0	3.8	0.0	0.0	0.0	4.2	0.0
8	59.0 - 56.9	2.2	2.2	2.7	2.0	2.3	2.2	0.0	0.0	0.0	2.8	3.7	4.1	3.8	3.8	0.0
9	56.0 - 53.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7	0.0	0.0	3.9
10	53.0 - 50.8	3.2	3.4	3.0	2.8	3.2	5.2	5.7	4.1	3.7	3.7	3.2	0.0	4.1	3.2	2.7
11	45.1 - 39.9	13.2	17.5	14.5	15.3	15.6	15.6	14.1	14.6	13.9	14.9	12.9	12.8	14.0	12.9	13.1
12	37.8 - 35.7	3.8	1.6	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	2.6
13	34.0 - 30.0	10.5	13.3	13.8	12.8	14.4	13.4	14.2	14.4	13.7	13.4	13.4	12.0	11.9	12.8	12.2
14	28.0 - 26.0	12.3	10.7	13.1	12.6	11.0	10.0	11.0	10.4	11.1	11.3	11.9	11.5	10.4	8.6	8.2
15	25.0 - 24.0	2.2	2.2	1.6	0.8	1.6	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6
16	22.0 - 20.0	13.4	13.6	13.8	13.6	13.7	14.2	13.6	15.4	15.2	15.5	13.7	13.5	15.0	14.1	15.0
17	17.4 - 17.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	16.8 - 14.9	7.6	9.4	10.5	9.7	9.0	9.8	10.0	10.7	8.8	9.3	12.1	11.4	10.8	10.1	9.2
19	14.6 - 12.7	17.9	13.8	14.5	15.7	13.3	16.1	17.2	17.1	15.6	15.9	11.1	11.6	14.5	16.2	16.5
20	12.8 - 10.0	5.1	5.7	5.5	3.2	7.4	4.2	5.9	8.2	9.7	5.5	7.9	9.5	6.6	7.4	7.4
Total bands		15	15	13	14	14	13	9	10	10	11	11	11	11	12	13
No. of specific bands		0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Common bands		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Polymorphic bands %		60	60	50	55	55	50	30	35	35	40	40	40	40	45	50

Common bands are highlighted

Table 4. Percentage of maximum and minimum mean numbers of *Phlomis aurea* protein bands in each a- habitat, b- altitude class, c- aspect direction.

a-Habitat:

No. of protein bands%	Habitats				
	Basin	Gorge	Slope	Terraces	Wadi bed
Max %	17.4	30.4	17.4	21.7	21.7
Min %	4.3	13	8.7	8.7	43.5

b- Altitude class:

No. of protein bands%	Classes							
	< 1500	1500-1600	1600-1700	1700-1800	1800-1900	1900-2000	2000-2100	> 2100
Max %	21.7	17.4	13	4.3	8.7	4.3	13	17.4
Min %	8.7	21.7	13	13	17.4	13	4.3	0.0

c- Aspect direction:

No. of protein bands%	Aspect					
	E	N	NE	NW	S	SE
Max %	13	4.3	13	17.4	17.4	30.4
Min %	8.7	8.7	8.7	17.4	21.7	17.4

Elevation along with aspect direction and slope was found in many respects to determine the microclimate and hence large-scale spatial distribution and pattern of vegetation (Allen & Peet, 1990 and Busing et al*.,* 1992). In the mean time, sinceseed storage proteins are regulated by an integrated genetic and physiological network (Golombek etal*.,* 2001; Elke et al., 2005 and Fait et al., 2006), the tight genetic control of seed maturation and development is modified within a certain range by maternal nutrition and environmental effect (microenvironment) (Weber et al., 2005). Accordingly, the composition of seed proteins can be broadly defined as a developmental genetic program that is modified by nutrient source availability and the demands of forming storage substance sink (Schmidt et al*.*, 2011).

Genetic variation is fundamentally brought about by random mutations. It is commonly measured as the percentage of gene loci that are polymorphic (heterozygous). Mutations are likely to be rare and most mutations are neutral or deleterious, but in some instances the new alleles can be favored by natural selection and/ or genetic drift leading to differences in the genetic structure of populations of different habitats. Natural selection can also maintain genetic variation in balanced polymorphisms which may occur when heterozygotes are favored or when selection is frequency dependent on certain habitat (Delph & Kelly, 2014).Accordingly, it can be suggested that the genetic diversity of *Phlomis aurea* which is endemic and endangered species, should be conserved in SKP through *in situ* or/and *ex situ* technologies. Storing seeds or any type of available germ plasm for conservation from different maternal plants separately in seed banks is a must to restore all variations of the gene structure of this endemic endangered species.

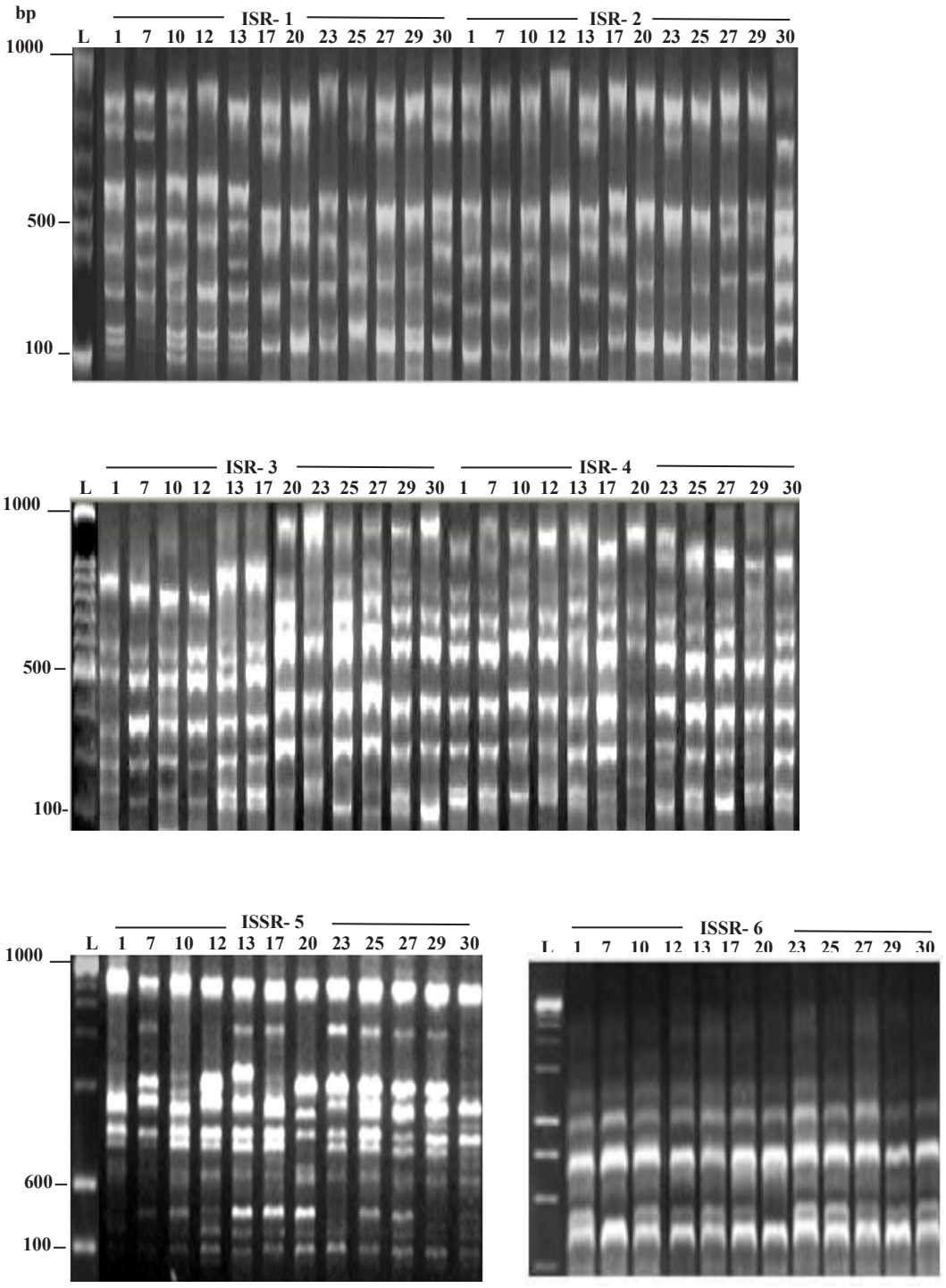
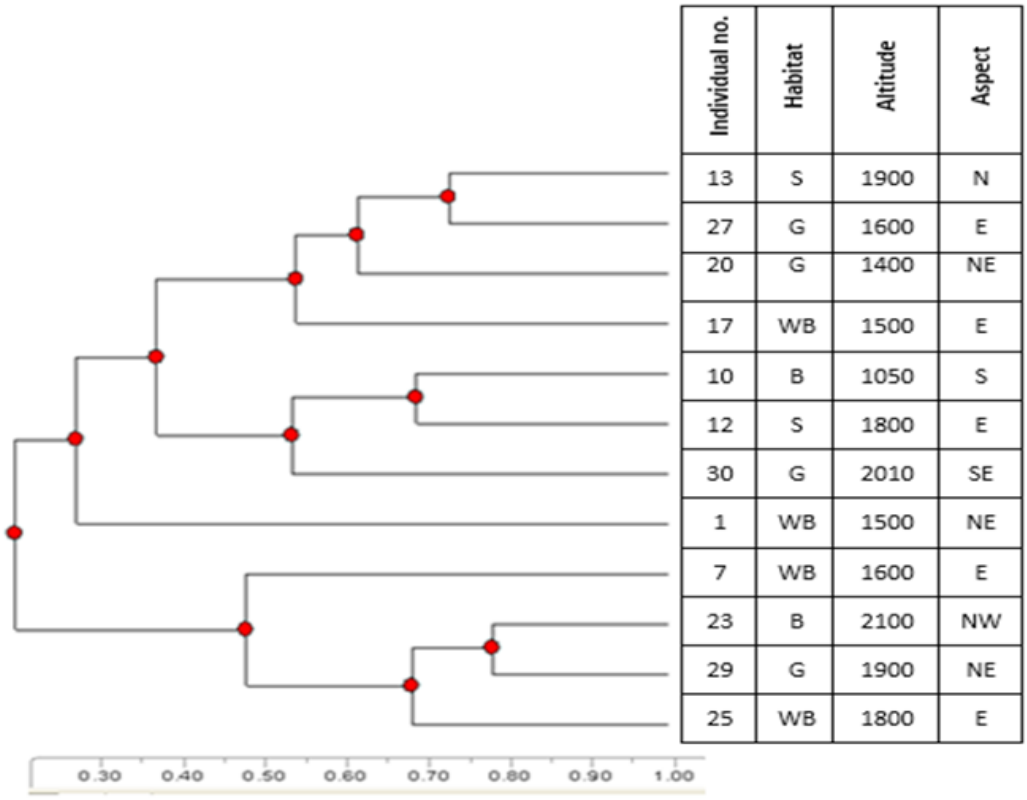


Fig 3. ISSR-PCR pattern for the twelve *Phlomis aurea* individuals using six ISSR primers; L: 1 kbp DNA ladder (The lane numbers refer to Table 1).

TABLE 5. DNA banding parameters obtained from 6 ISSR primers for the twelve wild *Phlomis aurea* individuals according to primer efficiency and habitats.∑b: Total bands, % of Pb: Percentage of polymorphic bands, S.b: Number of specific bands.

Habitats		Primer number						Total	Mean
		1	2	3	4	5	6		
Basin (individual 10)	∑b	9	5	8	7	7	8	44	
	S.B	2	1	2	2	1	1	9	
	%Pb	77.78	80.00	75.00	71.43	85.71	87.50	477.42	79.57
Basin (individual 23)	∑b	5	5	7	9	8	7	41	
	S.b	1	0	1	3	0	0	5	
	%Pb	80.00	100.00	85.71	66.67	100.00	100.00	532.38	88.73
Gorge (individual 20)	∑b	5	4	7	6	7	4	33	
	S.b	0	0	1	2	0	0	3	
	%Pb	100.00	100.00	85.71	66.67	100.00	100.00	552.38	92.06
Gorge (individual 27)	∑b	5	6	8	8	13	6	46	
	S.b	0	1	2	0	2	2	7	
	%Pb	100.00	83.33	75.00	100.00	84.62	66.67	509.62	84.94
Gorge (individual 29)	∑b	5	6	9	8	7	4	39	
	S.b	0	2	1	1	1	0	5	
	%Pb	100.00	66.67	88.89	87.50	85.71	100.00	528.77	88.13
Gorge (individual 30)	∑b	6	9	7	8	6	4	40	
	S.B	1	5	0	1	1	1	9	
	%Pb	83.33	44.44	100.00	87.50	83.33	75.00	473.61	78.94
Slope (individual 12)	∑b	7	4	6	7	8	7	39	
	S.b	1	1	1	1	1	1	6	
	%Pb	85.71	75.00	83.33	85.71	87.50	85.71	502.98	83.83
Slope (individual 13)	∑b	7	6	9	7	9	6	44	
	S.b	0	1	2	1	0	1	5	
	%Pb	100.00	83.33	77.78	85.71	100.00	83.33	530.16	88.36
Wadibed (individual 1)	∑b	8	7	8	8	3	9	43	
	S.b	0	3	3	1	0	4	11	
	%Pb	100.00	57.14	62.50	87.50	100.00	55.56	462.70	77.12
Wadibed (individual 7)	∑b	7	5	6	8	6	7	39	
	S.b	0	0	0	0	0	1	1	
	%Pb	100.00	100.00	100.00	100.00	100.00	85.71	585.71	97.62
Wadibed (individual 17)	∑b	6	5	8	7	7	6	39	
	S.b	0	4	3	0	0	0	7	
	%Pb	100.00	20.00	62.50	100.00	100.00	100.00	482.50	80.42
Wadibed (individual 25)	∑b	6	4	7	7	8	6	38	
	S.b	1	0	0	3	1	0	5	
	%Pb	83.33	100.00	57.14	85.71	87.5	100.00	542.86	90.48
Mean % of Pb / primer		92.51	75.83	79.46	85.37	93.91	86.62		



Individual no.	Habitat	Altitude	Aspect
13	S	1900	N
27	G	1600	E
20	G	1400	NE
17	WB	1500	E
10	B	1050	S
12	S	1800	E
30	G	2010	SE
1	WB	1500	NE
7	WB	1600	E
23	B	2100	NW
29	G	1900	NE
25	WB	1800	E

Fig. 4. Phylogenetic tree for twelve *Phlomis aurea* individuals based on six primers of inter simple sequence repeats (ISSR) technique.

Conclusion

1- The differences in protein and ISSR banding patterns describing individuals of the same and different habitats could be due to the effect of microclimate causing either epigenetic changes or mutations.
2- The present study added information about soluble seed storage protein and genomic DNA ISSR pattern data to *Phlomis aurea* passport.
3- Storing seeds for conservation from different maternal plants separately in seed banks is necessary to restore all variations of the genes of endemic endangered species. This to grant further protection for *in situ* and *ex situ* conservation in SKP.

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التنوع الجيني للأنواع المستوطنة من نبات العورور "*Phlomis aurea* Decne" فى جنوب سيناء، مصر

ليلي محمد الصادق[1]، غادة السيد البدن[1] و حاتم أحمد شبانة[2]

قسم النبات والميكروبيولوجى - كلية العلوم - جامعة الأسكندرية - الأسكندرية - مصر ، [2]قطاع الحفاظ على الطبيعة - وكالة شئون البيئة المصرية - القاهرة - مصر و[2]أكاديمية الشارقة للبحوث - جامعة الشارقة - الشارقة - الإمارات العربية المتحدة.

ينتمي نبات العورور للعائلة الشفوية وهو نوع متوطن ويقتصر على الارتفاعات العالية في خمسة بيئات رئيسية في جنوب سيناء و له امكانيات مستقبلية كعقار مضاد السكري، فالتوصيف الجيني لنباتات مجمعة من عشائر مختلفة أمر هام لبناء برامج للمحافظة علي هذه النباتات و تم إجراء هذه الدراسة بغرض تحقيق الأتي:1) تحديد التنوع الجيني بين هذه العشائر من خلال تنوع البروتينات المخزنة في البذور، والدراسة الجزيئية للمادة الوراثية باستخدام تقنية ISSR . 2) فهم العلاقة بين القياسات البيوكيميائية والجزيئية مع البيئات المختلفة في محمية سانت كاترين. تم استخدام بروتين بذور جافة ناضجة من 30 نبات تمثل عشائر مختلفة للفصل الكهربي وفقا لتقنية (SDS-PAGE)، وقد تم اختيار 12 منهم لتحليل المادة الوراثية وفقا لتقنية (ISSR)

أنتج الاختبار السابق 20 شريط بروتيني، ثلاثة منهم شرايط مشتركة وتعتبر محددة للنوع، والتي يمكن أن تستخدم كبصمات لهذا النبات. وقد تبين أن أنماط البروتين تتأثر بالأتجاه الجغرافي والأرتفاع عن سطح البحر و قليلا ما تتأثر بالبيئات ومواقعها. وقد أظهرت تقنية ISSR أن الترتيب التنازلي لمدي الاختلاف الجيني لنبات العورور من حيث البيئات المختلفة هو: الوادي > الحوض > المنحدر > الجورج. وهذا يشير إلى أن البيئات المفتوحة تساهم بنسبة عالية للتهجين و بالتالي تعدد الأشكال الجينية.