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BREEDING FOR CONSERVATION OF *Salvia multicaulis* POPULATION IN SAINT KATHERINE PROTECTORATE

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ABSTRACT

A first step of the program of breeding medicinal plants of Genetic Resources Department, Desert Research Center (DRC), is not only producing new genotypes from wild relatives, adapted to climate change status, but also profoundly shaping conservation priorities. The present work was divided into two parts as follows: The first one was studying the status of bardakosh Salvia multicaulis population in the three largest locations (Enshial, Abu Twita and Farsh Messila) within Saint Katherine Protectorate (SKP). The state of wild plants was better at the third location (Farsh Messila). The field observation revealed that drought followed by over-collection especially collection of flowering parts are considered as the major threats of Salvia multicaulis. All these factors are pushing them to the brink of extinction. The second, trial was set up during 2016 until 2018 seasons. Crossing has been done between Salvia multicaulis (wild) × Salvia officinalis (cultivated) and evaluation of three populations (P_1, P_2 and F_1generation) was done at the community gardens. The experimental design was randomized complete block design, with three replicates. The results showed that P_1 (Salvia multicaulis) recorded the highest values of the mean calyx length (2.50 cm) and calyx width (2.00cm). The good flower shape reflects the importance of the wild plant to the local community. While, F_1 generation recorded the highest values of the mean plant inflorescence length (46.93cm) and no. of flowers per inflorescence (86.00). Also, F_1 generation gave good values of the mean calyx length (1.83 cm) and calyx width (0.80cm).This reflects the improvement in the flowers shape desired by the local community. By selection in advanced segregating generations, the characteristics of the flowers can be improved. Also, the F_1 flowers bloomed early and gave the highest number of flowers on the plant. It was a good plant for the beekeepers on medicinal plant. The estimate $\sigma^2 p$ was high for all traits, except stem thickness and percentage of oil. Heritability in broad-sense (H^2) estimates were high for all traits. The estimates of PCV and GCV for calyx width were the highest. Potence ratio (P) shower over dominance to high parent for inflorescence length and no.of flowers per inflorescence. Heterosis over mid-parents and better-parentwere highly significant and positive for inflorescence length andno. of flowers per inflorescence. Calyx length had highly significant and positive correlation with calyx width. The dendrogram and similarity matrix showed that the highest genetic similarity was detected between P_2 and F_1.

Key words: ***Salvia spp., Breeding, Conservation, Genetic parameters, Interspecific hybrids.***

INTRODUCTION

The family *Lamaiceae* (*Labiatae*) has worldwide distribution and includes over 250 genera and up to 7000 species (Thorne 1992). The family is known for its fine ornamental or culinary herbs like *Ocimum*, *Lavandula*, *Mentha*, *Origanum*, and *Salvia*, and is a rich source of essential oils for the flavoring and perfume industry (Wagstaff *et al*. 1998). The genus *Salvia* is a large genus with approximately 945 species; it is highly various in

distribution, ecology, life form, morphology, and karyology (Will 2013). Genus *Salvia* is represented in Egyptian flora by 9 species (Boulos 2009). Saint Katherine protectorate (SKP) hosted 6 of them, namely *S. aegyptiaca, S. deserti* Decne, *S. lanigra* Poir, *S. sclarea*, *S. spinosa*, *S. palaestina* Benth and *S. multicaulis* (El-banhawy *et al* 2016).

Bardakosh *(Salviamulticaulis)* isutilized as tonsillitis, hack, hypersensitivity, decreasing hypertension and alleviating teeth and chest torment treatment as traditional use in addition to its extracts and essential oils, which could be used as a possible source of antimicrobial and antioxidative agents as they have the capacity to scavenge free radicals and to inhibit the growth of microorganisms (Bektas *et al* 2004 and Orhan *et al* 2007).

Omar *et al* (2015) studied *Salvia multicaulis* distribution in SKP in addition to the threats on it; they concluded that (a) *Salvia multicaulis* is distributed mainly in wet mountain habitat with granite rocky ground of mountain areas, mainly in North aspect, followed by East and West aspect. (b) *Salvia multicaulis* is restricted in five localities viz Farsh Messila, Wadi Abu Twita, Enshil, Elsomra, and Baharia, the first three sites were the most important places within SKP; (c) they determined drought as the major threat affecting the distribution of *Salvia multicaulis*, with the overgrazing, over-collection and feral donkeys considered as human impacts, most of the *Salvia multicaulis* sub populations are very small, occurring sporadically. The number of mature plants has been observed to decline as a result of mentioned threats.

Although there is a few data on the interspecific hybridization of *Salvia* species, the application of interspecific crossing might have much more importance in the future based on the outstanding results of the first attempts. Mainly wild growing species have been crossed with *S. officinalis* and *S. sclarea*in order to bring useful characteristics into the cultivated species. The existence of interspecific hybridization was reported in the case of other *Salvia* species. Spontaneous hybridization of *S. officinalis* and *S. fruticosa* was described by Putievsky *et al* (1990). Mainly the intermediate heritability character of essential oil composition was proved also by Spanish experts (Sanchez-Gomez *et al* 1995) making hybrids between *S. officinalis* L. and *S. lavandulifolia* Vahl. ssp. *Vellera* (Cuatr.). Joseph and Warner (2011) studied the interspecific crossability of selected salvia species and potential use for crop improvement and found that the wide diversity in the genus *Salvia* represents an untapped genetic resource to

improve and diversify *Salvia* grown as floriculture crops. Interspecific hybrids have formed naturally or by chance hybridization of cultivated plants, but the degree to which species are cross-compatible is largely unknown. The cross ability of nine *Salvia* species selected to cover a wide range of the diversity in European and American species was evaluated in a full diallel mating scheme. Overall, cross ability of the selected species was low with only five of 72 interspecific cross combinations producing viable seed, whereas all nine species were self-fertile. Successful crosses were mostly within close phylogenetic groupings. The majority of successful crosses were between species with different chromosome numbers, suggesting that chromosome number differences alone are not a major barrier to hybridization in this genus. A *Salvia nemorosa* × *Salvia transslyvanica* F_2 population exhibited transgressive segregation for several horticulturally important traits, including flower size, plant height, and time to flower. Plant height was correlated positively with flower length, inflorescence branch number, and time to flower. Time to flower was correlated positively with flower length.

The growing demand through a rapidly increasing world population further accentuates the need for improved cultivars. Plant breeding plays a pivotal role in securing the production of healthy feed and food while at the same time optimizing resource utilization and minimizing environmental impacts. At the same time breeding objectives are constantly changing as a result of climate change, a scarcity of natural resources and the necessity to sustain biodiversity. Participatory and evolutionary plant breeding are able to increase crop biodiversity, promotes the use of landraces and wild relatives, and allows crops to continue to evolve and therefore are the most dynamic way to cope with climatic changes (Salvatore 2009).

ISSR-PCR is a genotyping technique based on variation found in the regions between microsatellites and widely used in studies on germplasm resource identification, phylogeny of species, plant taxonomy, evolution, and genetic diversity (Ansari *et al* 2012). The PCR amplification of these regions using a single primer based on SSRs anchored 5′ or 3′ with 2–4 purine or pyramidine residues, yields multiple amplification products that can be used as a dominant multilocus marker system for the study of genetic variation in various organisms. ISSRs are easy to use, low-cost, and methodologically less demanding compared to other dominant markers, making it an ideal

genetic marker for beginners and for organisms whose genetic information is lacking (Adawy *et al* 2002).

Conservation and evaluation of genotypes in arid and semi-arid regions comes in the first order for our department due to its importance in determining this adaptation. The objective of this study was conservation of wild plants genetic resources and maximizing their use to meet the needs of the local population through the introduction of *Salvia multicaulis* (local name Bardakosh) plants in breeding program to produce new genotypes with good production characteristics and environmental conditions that can be cultivated under the SKP cultivation terms; within local community farms to reduce the over collection of *Salvia* flowers, also to expand its cultivation under Egyptian conditions.

MATERIALS AND METHODS

A first step of the program of breeding medicinal plants of Genetic Resources Department, Desert Research Center (DRC), is not only producing new genotypes from wild relatives, especially in climate change status, but also profoundly shaping conservation priorities. Study area; Saint Katherine Protectorate was established in 1996, under the support of the Egyptian Environmental Affairs Agency (EEAA). It extends over virtually the entire mountain massif of southern Sinai, an area of 4350 km^2. It lies between 33° 55′ to 34° 30′E and 28° 30′ to 28° 35′N with an elevation range of 1300–2641 m above sea level.

The present work was divided into two parts as follows

The first part: Studying the status of *Salvia multicaulis* population in Saint Katherine protectorate. From previous studies, the three largest locations (sites) where *Salvia multicaulis* exists are Enshial, Abu Twita and Farsh Messila (Omar 2015).

Salvia multicaulis population was evaluated in a randomized complete blocks design with three replications of each site separately. Each sitewas surveyed during the5^{th} of April 2018 season dependent on the occurrence of *S. multicaulis.* Replicate (quadrat) area was 25 m^2 (5 × 5 m). Data recorded on five randomly selected plants were: plant height, the mean crown diameter, number of branches, stem thickness, leaf (length and width), inflorescence length, No. of flowers per inflorescence and calyx (length and width).The terminology used for morphological attributes follows Stearn (1973).The threats were determined depending on filed observation we record any sign that may be considered a threat to the target species in each site.

The second part: *Salvia multicaulis*, a perennial low shrub found mainly in high altitude region of Saint Katherine protectorate, the effect of over collection of flowersis the most harmful and may lead to reduction of the population size with time. Heterosis was in specific crosses of *salvia* spp. to produce new genotypes with good production characteristics for the flowers.

The trial was set up during 2016 until 2018 seasons. Crossing has been done between *Salvia multicaulis* (wild) × *Salvia officinalis* (cultivated) and evaluation of three populations (P_1, P_2 and F_1generation) was at the community gardens in Saint Catherine. The experimental design was randomized complete block design (RCBD), with three replicates. Each replicate consisted of 3 plants, one plant for each generation (P1, P2 and F_1). Seed drilling was done in the 15th of September 2016. Normal agricultural treatments were applied. Harvesting was occurred in 15th of March 2018. Data were recorded on: plant height, number of branches, stem thickness, leaf (length and width), inflorescence length, no. of flowers per inflorescence, calyx (length and width) and percentage of oil. The essential oil was separated from fresh collected material (15th of March 2018) using hydro distillation in Ginsberg apparatus and the oil composition was recalculate to dry matter.

Statistical analysis was performed using analysis of variance technique by means of "MSTAT" computer software package. The treatment means were compared using Duncan's multiple range test (Duncan 1955). The phenotypic and genotypic coefficients of variation were estimated by using the formulae developed by Burton (1952).Heritability estimates were obtained as described by Burton and Devan (1953).Dominance degree (Potence ratio), this parameter was calculated according to Mather and Jinks (1971). Heterosis: the amount of heterosis was expressed as the percentage deviation of the F_1 hybrids mean ($\overline{F}_1$) from the average of the two parents (MP) and better parent (B.P.) (Sinha and Khanna1975).

Inter simple sequence repeat (ISSR) technique

DNA extraction: Genomic DNA was extracted from young leaves of each genotype using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). The quantity and quality of the extracted DNA were determined using spectrophotometric measurement of UV absorbance at 260 nm and 280 nm in a Thermo Scientific Nano Drop 2000™ spectrophotometer.

ISSR reaction and amplification: The reaction conditions were optimized and the following reagents were mixed in a final volume of 25 µl: 1 X of green GoTaq® Flexi buffer, 1.5mM of $MgCl_2$, 200 µM of dNTPs, 30pM of primer, 1 U of GoTaq® Flexi DNA Polymerase (Promega), 40ng of template DNA and up to 25 µl distilled H_2O. Amplification was carried out in a Gene Amp® PCR System 9700 thermal cycler (Applied Biosystems) programmed as follows: 94°C/5min (1 cycle); [94°C/45 sec, 45°C/50 sec, 72°C/1.5 min] (40 cycles); 72°C/7 min (1 cycle) and 4°C (infinitive). A volume of 10µl of the ISSR-PCR product was resolved using (1.5%) agarose gel electrophoresis containing ethidium bromide. A 100bp DNA marker (Fermentas) was used as a DNA molecular weight standard. Results were visualized on a UV transilluminator and photographed by Molecular Imager® Gel Doc™ System with Image Lab™ Software, Bio-Rad.

ISSR data analysis: The generated/ amplified bands were visually scored as either present (1) or absent (0) to create the binary data sets included both polymorphic and monomorphic bands. Polymorphism percentage was calculated by dividing number of amplified polymorphic bands by the total number of amplified bands by the same primer or primer combination. Then, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated between varieties using the unweighted pair group method with arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

The first part: The analysis of variance for all traits is presented in Table (1). Locations mean squares were highly significant for all traits. This was due to the nature of the three locations. By observing the threats to each location, we found that the first location (Enshial) was the most affected by the drought. While, the second (Abotwita) and the third (Farsh Messila) locations were more affected by over collection of flowers. The collection of flowers before the maturity of seeds and spread leads to the absence of new generations of *Salvia multicaulis.* Our filed observation leads to that over collection especially collection of flowering parts is the main human threats followed by over grazing and feral donkeys is in partial agreement with Omar *et al* (2015) and Assi (2007); who repeated the same threats with different priorities. As a result of flowering parts over-collection, *Salvia multicaulis* become seedless, viz there are no new generations to be formed.

Table 1. Analysis of variance (ANOVA) for plant height, crown diameter, number of branches, stem thickness, leaf (length and width), inflorescence length, No. of flowers per inflorescence and calyx (length and width) of *Salvia multicaulis* under three location in 2018 season.

SOV	df	Plant height (cm)	Crown diameter (cm)	Number of branches	Stem thickness (cm)	Leaf length (cm)
Rep.	2	14.17	11.97	4.00	0.0002	0.013
Loc.	2	404.92**	1262.67**	2005.46**	0.019**	0.49**
Error	4	2.753	5.268	1.507	0.0001	0.0083
SOV	df	Leaf width (cm)	Inflorescence length (cm)	No. of flowers per inflorescence	Calyx length (cm)	Calyx width (cm)
Rep.	2	0.007*	0.443	3.576	0.045	0.011
Loc.	2	0.381**	93.08**	173.268**	0.758**	0.723**
Error	4	0.0011	0.347	1.749	0.0184	0.0039

***and **: Significant at the 0.05 and 0.01 levels of probability, respectively. Rep = Replications Loc= Locations**

Therefore, the over collection of flowers was the most threatening of *Salvia multicaulis* in the wild natural after the drought. All these factors are pushing them to the brink of extinction.

The results presented in Table (2) showed that all traits were indication to the status of *Salvia multicaulis* in locations. The second location (Abo twita) recorded the highest means of crown diameter (62.34 cm), leaf length (3.13 cm), leaf width (1.867 cm), inflorescence length (19.76cm), calyx length (2.16 cm) and calyx width (1.76 cm). As well, the third location (Farsh Messila) gave the highest means of plant height (40.27 cm), number of branches per of plant (65.0), stem thickness (0.437 cm) and No. of flowers per inflorescence (24.62). While, the first location (Enshial) recorded the lowest means for all traits under this study. In general, the state of wild plant was better at the third location.

The second part: The analysis of variance showed highly significant differences among parents and f_1 hybrid for all traits, except percentage of oil which was significant (Table 3). Figs are (1, 2 and 3) illustrate the variations between P1 (*Salvia multicaulis),* P2 (*Salvia officinalis*) and F_1 generation for inflorescence, flower shape and leaf shape, respectively.

Table 2. Mean performance for plant height, crown diameter, number of branches, stem thickness, leaf (length and width), inflorescence length, No. of flowers per inflorescence and calyx (length and width) of *Salvia multicaulis* under three locations in 2018 season.

Genotypes	Plant height (cm)	Crown diameter (cm)	Number of branches	Stem thickness (cm)	Leaf length (cm)
Enshial	19.33 b	26.77 b	14.47 c	0.287 c	2.33 c
Abu Twita	38.53 a	62.43 a	30.23 b	0.407 b	3.13 a
FarshMessila	40.27 a	62.17 a	65.0 a	0.437 a	2.83 b
Grand Mean	32.71	50.46	36.57	0.377	2.767
CV%	5.072	4.549	3.357	2.423	3.299
Location	**Leaf width (cm)**	**Inflorescence length (cm)**	**No. of flowers per inflorescence**	**Calyx length (cm)**	**Calyx width (cm)**
Enshial	1.167 c	8.93 b	10.14 c	1.15 c	0.79 c
Abu Twita	1.867 a	19.76 a	21.40 b	2.16 a	1.76 a
FarshMessila	1.633 b	16.60 b	24.62 a	1.7 b	1.17 b
Grand Mean	1.556	15.09	18.720	1.674	1.242
CV%	2.143	3.899	7.064	8.111	5.087

Means in the same column followed by different letter are significantly different at 0.05 probably level.

Table 3. Analysis of variance (ANOVA) of plant height, number of branches, stem thickness, leaf (length and width), inflorescence length, No. of flowers per inflorescence, calyx (length and width) and percentage of oil of three populations (P1, P2 and F_1 generation) of the cross *Salvia multicaulis* × *Salvia officinalis* in 2018 season.

SOV	df	Plant height (cm)	Number of branches	Stem thickness (cm)	Leaf length (cm)	Leaf width (cm)
Rep.	2	1.204	2.333	0.002	0.003	0.008
Gen.	2	154.75**	37.233**	0.185**	2.093**	0.634**
Error	4	1.003	0.667	0.005	0.017	0.008
SOV	**df**	**Inflorescence length (cm)**	**No. of flowers per inflorescence**	**Calyx length (cm)**	**Calyx width (cm)**	**Oil%**
Rep.	2	0.234	1.00	0.013	0.018	0.0008
Gen.	2	300.688**	804.333**	2.583**	2.217**	0.0075*
Error	4	1.538	8.333	0.002	0.003	0.0009

*** and **: Significant at the 0.05 and 0.01 levels of probability, respectively. Rep=replications Gen= genotypes.**

Fig. 1. Inflorescence for P1 (*Salvia multicaulis*), P2(*Salvia officinalis*)and their F_1cross.

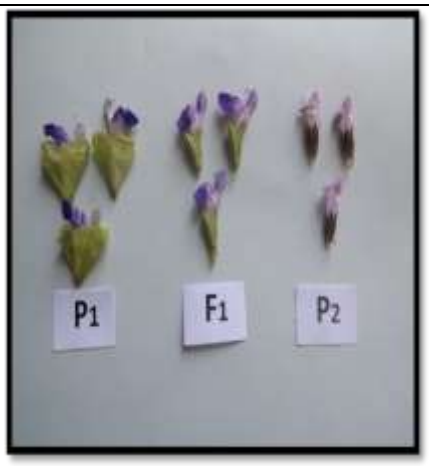

Fig. 2. Flower shape for P1 (*Salvia multicaulis*), P2 (*Salvia officinalis*) and their F_1cross.

Fig. 3. Leaf shape for P1 (*Salvia multicaulis*), P2 (*Salvia officinalis*)and their F_1cross.

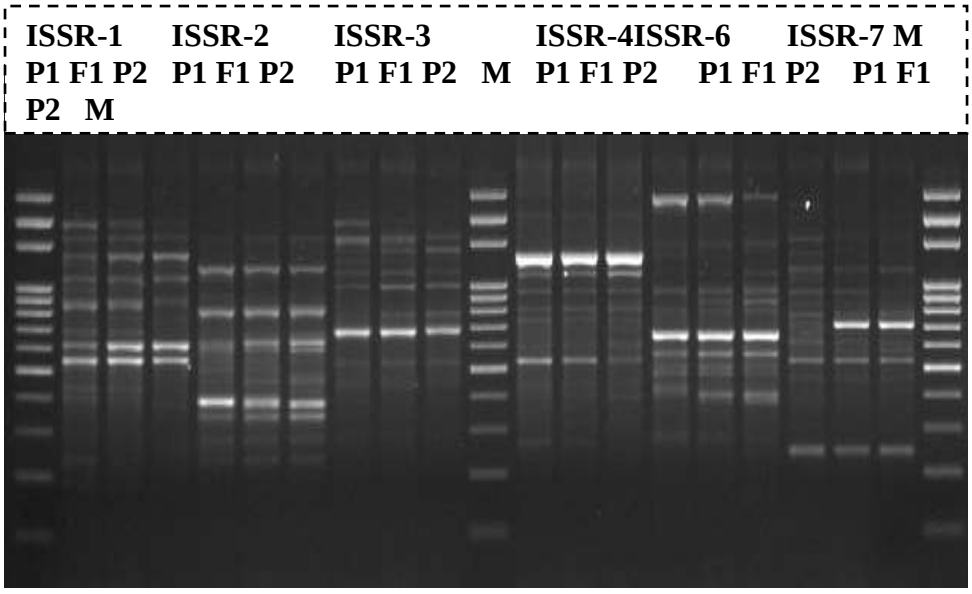

Fig. 4. ISSR profiles of the P1, P2 and F1 generation using primers (ISSR-1, ISSR-2, ISSR3, ISSR-4, ISSR-6 and ISSR-7) M: DNA molecular weight marker (100bp ladder).

The results presented in Table (4) showed that P1 (*Salvia multicaulis)* recorded the highest means of leaf width (3.30cm), calyx length (2.50 cm) and calyx width (2.00cm). The good flower shape reflects the importance of the wild plant to the local community. While, P2 (*Salvia officinalis*) recorded the highest means of number of branches (26.00), stem thickness (0.980cm), leaf length (7.00 cm) and percentage of oil (0.529%). the F_1 generation recorded the highest means of plant height (52.33 cm), leaf width (3.47 cm), inflorescence length (46.93 cm)and no. of flowers per inflorescence (86.00). Also, F_1 generation showed good values of the mean calyx length (1.83 cm) and calyx width (0.80 cm). Fig were (1, 2 and 3) showed that variations between P1 (*Salvia multicaulis)*, P2 (*Salvia officinalis*) and F1 generation for inflorescence, flower shape and leaf shape, respectively.

Table 4. Mean performance of plant height, number of branches, stem thickness, leaf (length and width), inflorescence length, No. of flowers per inflorescence, calyx (length and width) and percentage of oil of three populations (P1, P2 and F_1 generation) of the cross *Salvia multicaulis*× *Salvia officinalis* in 2018 season.

Genotypes	Plant height (cm)	Number of branches	Stem thickness (cm)	Leaf length (cm)	Leaf width (cm)
P_1	39.67 b	19.33 b	0.483 c	5.33 c	3.30 a
P_2	51.87 a	26.00 a	0.980 a	7.00 a	2.60 b
F_1	52.33 a	24.67 a	0.730 b	6.07 b	3.47 a
Grand mean	47.96	23.33	0.731	6.13	3.12
CV%	2.088	3.499	10.061	2.104	2.824
Location	Inflorescence length (cm)	No. of flowers per inflorescence	Calyx length (cm)	Calyx width (cm)	Oil%
P1	42.37 b	53.33 c	2.50 a	2.00 a	0.498 ab
P2	27.77 c	67.67 b	0.67 c	0.33 c	0.529 a
F1	46.93 a	86.00 a	1.83 b	0.80 b	0.431 b
Grand mean	39.02	69.00	1.67	1.04	0.486
CV%	3.177	4.184	2.449	5.049	6.193

P1 = *Salvia multicaulis* P2 = *Salvia officinalis*

Means in the same column followed by different letter are significantly different at 0.05 probably level.

This reflects the improvement in the flower shape desired by the local communityand proves that by selection in advanced generations, the characteristics of the flowers can be improved. By observing the flowers blooming date of the P1, P2 and F_1 generation, it was found that P1 (*Salvia*

multicaulis) bloomed on 1st March, while, P2 (*Salvia officinalis*) was on 10th March and F_1 generation was early on 25th February. This reflects the observation of the honey bees' number on the F_1generation plant, where, the F_1 bloomed early and had the highest number of flower on the plant. It was a good plant for the beekeepers on medicinal plant.

Genetic parameters: Estimates of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), phenotypic variance (σ^2p), genotypic variance (σ^2g), environmental variance (σ^2e), heritability in broad-sense (H^2), potence ratio (P) and heterosis (H) are presented in Table (5). The estimates σ^2p was high for all traits, except stem thickness and percentage of oil. The σ^2p and σ^2g estimates for No. of flowers per inflorescence were the highest followed by inflorescence length and plant height traits. The σ^2g estimates were found greater than the σ^2 e estimates for all traits in this study. The higher proportion of σ^2p observed on all traits was due to the larger proportion of σ^2g. Heritability in broad-sense (H^2) estimates were high for all traits. The PCV and GCV were convergent for all the traits. The estimates of PCV and GCV for calyx width gave the highest values, while percentage of oil recorded the lowest values. Potence ratio (P) indicated over dominance to high parent for leaf width, inflorescence length and No.of flowers per inflorescence, while indicated over dominance to lowest parent for percentage of oil. It indicated partial dominanceto high parent for number of branches and calyx length, while indicated partial dominance to least parent for stem thickness, leaf length and calyx width. Also it was near complete dominance to high parent for plant height. Heterosis as a percentage over mid-parents and better-parent, is given in Table 5. Heterosis over mid-parents was highly significant and positive for plant height, number of branches, leaf width, inflorescence length, No.of flowers per inflorescence and calyx length. While, better-parentsheterosis was highly significant and positive for leaf width, inflorescence length and No.of flowers per inflorescence. Joseph and Warner (2011) studied the interspecific crossability of selected *Salvia* species and potential use for crop improvement and found that in most cases, hybrid traits were intermediate between the two parents, although in the crosses S. transsylvanica × S. involucrate and S. transsylvanica × S. nemorosa, leaf length and width of the F_1 were significantly larger than either parent plant. Mainly the intermediate heritability character of essential oil composition wasalso proved by Spanish experts (Sanchez-

Gomez *et al* 1995) when making hybrids between *S. officinalis* L. and *S. lavandulifolia*Vahl. ssp. *vellera*(Cuatr.).

Table 5. Genetic parameters of plant height, number of branches, stem thickness, leaf (length and width), inflorescence length, No. of flowers per inflorescence, calyx (length and width) and percentage of oil of three populations (P1, P2 and F_1 generation) of the cross *Salvia multicaulis* × *Salvia officinalis* in 2018 season.

Parameters	Plant height (cm)	Number of branches	Stem thickness (cm)	Leaf length (cm)	Leaf width (cm)
σ^2 g	51.25	12.19	0.061	0.692	0.208
σ^2 p	51.58	12.41	0.076	0.698	0.211
σ^2 e	0.33	0.22	0.015	0.006	0.003
h^2 (b.s.) %	99.36	98.23	78.95	99.14	98.58
G.C.V. %	14.93	14.97	33.51	13.56	14.61
P.C.V.%	14.98	15.09	37.71	13.62	14.71
Potence %	1.08	0.59	-0.002	-0.12	1.49
H.(M.P.) %	14.33 **	8.82 **	-0.27 **	-0.154 **	17.63 **
H. (B.P.) %	0.89 ns	-5.12 **	-25.51 **	-13.29 **	5.15 **
Parameters	**Inflorescence length (cm)**	**No. of flowers per inflorescence**	**Calyx length (cm)**	**Calyx width (cm)**	**Oil%**
σ2 g	99.716	265.33	0.860	0.738	0.0023
σ2 p	100.229	268.11	0.861	0.739	0.0026
σ2 e	0.513	2.78	0.001	0.001	0.0003
h2 (b.s.) %	99.48	98.96	99.88	99.86	88.46
G.C.V. %	25.59	23.61	55.53	82.29	9.86
P.C.V.%	25.66	23.73	55.56	82.34	10.49
Potence %	1.62	3.56	0.27	-0.44	-5.35
H.(M.P.) %	33.82 **	42.15 **	15.82 **	-31.62 **	-16.15 **
H. (B.P.) %	10.76 **	27.09 **	-26.80 **	-60.00 **	-18.53 **

ns and **: Insignificant and significant at 0.01 level of probability, respectively.

The results presented in Table (6) showed that plant height had a significant or highly significant and positive correlation with each of number of branches; stem thickness, leaf length and No. of flowers per inflorescence. In the contrary, a significant or high significant and negative correlation was found between plant height and each of calyx length and calyx width. A significant or highly significant and positive correlation was

observed between number of branches and each of stem thick, leaf length and No. of flowers per inflorescence. On the other hand, calyx length and calyx width showed a highly significant and negative correlation with number of branches/plant. Stem thickness had a highly significant and positive correlation with leaf length. However, a significant or high significant negative correlation was found between stem thickness and each of leaf width, inflorescence length, calyx length and calyx width. Leaf length had a significant or highly significant and negative correlation with each of leaf width, inflorescence length, calyx length and calyx width. Highly significant and positive correlation was observed between leaf width and each of inflorescence length and calyx length. On the other hand, a significant and negative correlation was found between leaf width and oil content. Inflorescence length had a highly significant and positive correlation with calyx length. However, a high significant and negative correlation was found between inflorescence length and oil content .Calyx length had highly significant and positive correlation with calyx width. Joseph and Warner (2011), found that plant height was correlated positively with flower length, inflorescence branch number, and time to flower. Time to flower was correlated positively with flower length.

Table 6. Pearson's correlation coefficient among agronomic characters of three populations (P1, P2 and F1generation) of the cross Salvia multicaulis× Salvia officinalis in 2018 season.

Characters	1	2	3	4	5	6	7	8	9	10
1-Plant height (cm)	1.000	0.943**	0.800**	0.796*	-0.290	-0.249	0.840**	-0.742*	-0.934**	-0.195
2-Number of branches		1.000	0.838**	0.883**	-0.459	-0.442	0.686*	-0.823**	-0.930**	0.078
3-Stem thickness (cm)			1.000	0.938**	-0.694*	-0.708*	0.399	-0.945**	-0.931**	0.271
4-Leaf length (cm)				1.000	-0.796*	-0.769*	0.366	-0.986**	-0.934**	0.329
5-Leaf width (cm)					1.000	0.979**	0.249	0.847**	0.568	-0.699*
6-Inflorescence length (cm)						1.000	0.304	0.819**	0.525	-0.779*
7-No.of flowers per inflorescence							1.000	-0.285	-0.639	-0.640
8- Calyx length (cm)								1.000	0.916**	-0.370
9- Calyx width (cm)									1.000	-0.027
10- Oil content										1.000

*** and **: Significant at the 0.05 and 0.01 levels of probability, respectively**.

Genetic diversity and cluster analysis among P1, P2 and F_1based on ISSR markers

Genetic diversity among the P1 (*Salvia multicaulis),* P2 (*Salvia officinalis*) and their F_1 generation was further analyzed by ISSR markers. The amplification products produced from 10 ISSR primers are listed in Table (7) and Fig. (4), in terms of the percentage of PCR products appeared in the studied genotypes. Ten primers produced 112 DNA fragments with an average of 11.2 bands per primer. Out of the total of 112 amplified fragments, 23 were polymorphic, with an average of 2.3 polymorphic bands per primer. This represented a level of polymorphism of 20% from these ten primers. The dendrogram (Fig. 5) among the P1 (*Salvia multicaulis),* P2 (*Salvia officinalis*) and F_1 generation based on ISSR markers using UPGMA and similarity matrix (Table.8) was computed according to Dice coefficient. The highest similarity (91%) was detected between the F1 and P2. This was followed by 85% of similarity between F_1 and P1, while the lowest similarity (80%) was detected between P1 and P2.

Table 7. Primer, Sequence, Total number of amplicons(TM), monomorphic amplicons (MP), polymorphic amplicons (PP) and percentage of polymorphism (%P) as revealed by ISSR markers among the P1, P2 and F_1.

Primer	Sequence	TM	MP	PP	P %
ISSR-1	5'-AGAGAGAGAGAGAGAGC-3'	11	9	2	18
ISSR-2	5'-AGAGAGAGAGAGAGAGG-3'	10	8	2	20
ISSR-3	5'-ACACACACACACACACT-3'	11	8	3	27
ISSR-4	5'-ACACACACACACACACG-3'	12	9	3	25
ISSR-6	5'-CGCGATAGATAGATAGAT-3'	12	10	2	17
ISSR-7	5'-GACGATAGATAGATAGATA-3'	10	8	2	20
ISSR-8	5'-AGACAGACAGACAGACGC-3'	11	9	2	18
ISSR-9	5'-GATAGATAGATAGATAGC-3'	10	8	2	20
ISSR-10	5'-GACAGACAGACAGACAAT-3'	12	10	2	17
ISSR-11	5'-ACACACACACACACACYA-3'	13	10	3	23
Total		112	89	23	
Average		11.2	8.9	2.3	20%

Table 8. Genetic similarity (GS) as revealed by ISSR data.

	P1	F1	P2
P1	100		
F1	85	100	
P2	80	91	100

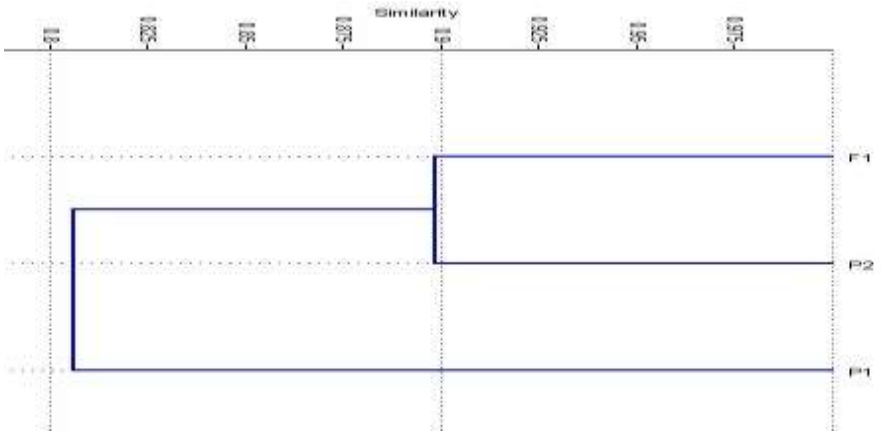

Fig. 5. Dendrogram for the P1, P2 and F1 constructed from ISSR data using UPGMA and similarity matrix computed according to Dice coefficient.

CONCLUSIONS

An obvious the good flower shape reflected the importance of the wild plant to the local community. The F_1 generation recorded the highest values of the means of inflorescence lengthand no. of flowers per inflorescence. Also, F_1 generation should good means of calyx length and width. The F_1 flowers bloomed early and had the highest number of flowers on the plant, it was a good plant for the beekeepers on medicinal plant. This reflects the improvement in the flower shape desired by the local community. By selection in advanced generations, the characteristics of the flowers can be improved. The F_1 generation needs many advanced studies to indentify the production and adapt ability under different environmental conditions and to study the quality of oil. So that it can be adopted in Egyptian agriculture and the expansion of its cultivation in different areas of Sinai for the beekeepers on medicinal plants, thus reducing pressure on the wild plant.

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التربية لصون عشائرالبردقوش *Salvia multicaulis* بمحمية سانت كاترين

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خطوة أولى فى برنامج تر بية وصون الاصول الوراثية من النباتات الطبية بمركز بحوث الصحراء والذى لاينتج فقط تراكيب وراثية جديدة للاقارب البرية وخاصة فى حالة تغير المناخ ولكن يشكل عمق اولويات الصون.تم دراسة حالة نبات Salvia multicaulis فى أكبر ثلاث مواقع يتواجد بها النبات بمحمية سانت كاترين وهم إنشيل ووادى ابوتويتة وفرش مسلة. وكانت حالة النبات البرى أفضل فى الموقع الثالث (فرش مسلة) وتم رصد الجمع الجائر للأجزاء الزهرية كأهم المهددات بعد الجفاف. تم إجراء التهجين بين النوع البرى Salvia multicaulis و النوع المنزرع Salviaofficinalis وتقييم الأبوين والجيل الأول خلال الفترة من ٢٠١٦ وحتى ٢٠١٨ بحدائق المجتمع المحلى فى تصميم القطاعات الكاملة العشوائية من ثلاث مكررات وكانت أهم النتائج تشير الى أن الأب البرى سجل أعلى قيم لمتوسطى طول وعرض الكأس (٢,٥سم) و(٢سم)على التوالى، وهذا يعكس اقبال المجتمع المحلى على جمع الأفرع الزهرية . سجل الجيل الأول أعلى قيم لمتوسط طول الفرع الزهرى (٤٦,٩٣ سم) ولعدد الأزهار على الفرع الزهرى (٨٦).وأيضا سجل قيم وسطية لطول وعرض الكأس وكان مبكراً فى الإزهار مما يجعلة نبات جيد لمربى النحل .كان التباين المظهرى مرتفع لجميع الصفات ماعدا سمك الساق ومحتوى الزيت. كفاءة التوريث كانت مرتفعه لجميع الصفات .وجدت سيادة فائقة للأب الأعلى لطول الفرع الزهرى وعدد الأزهار على الفرع الزهرى .كانت قوة الهجين لمتوسط الابوين والأب الأعلى موجبة وعالية المعنوية لصفتى طول الفرع الزهرى وعدد الأزهار على الفرع الزهرى.وجد ارتباط موجب ومعنوى بين طول وعرض الكأس. هناك تشابة وراثى عالى باستخدام احد المعلمات الجزيئية (ISSR) بين الاب المنزرع والجيل الاول .