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Efficiency of ISSR, SRAP and ISTR markers for the detection of polymorphisms and genetic relationships in (*Salvia* spp.)

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Abstract: *(Salvia spp.) this genus includes an enormous assemblage of nearly 1,000 species dispersed around the world. Due to possible threats to this genus, there is an urgent requirement to evaluate the diversity among the wild and cultivated species of the genus salvia. In the current investigation the set of ISSR, SRAP and ISTR marker molecular techniques were used to evaluate the genetic relationships among the two species Salvia aegyptiaca and Salvia officinalis. The genetic diversity analysis has been verified using 12 ISSR, 15 SRAP and 10 ISTR to assess the systematic knots between the wild and cultivated species. In order to detect the level of polymorphism and molecular phylogeny, a comparative analysis of the three markers was performed based on the exposure of efficiency, discriminating capacity and phylogenetic heatmap tree. Our results revealed that ISTR marker followed by ISSR and SRAP markers has superior discriminating capacity across the polymorphic information content (PIC), assay efficiency index, effective multiples ratio and marker index for identified the genetic diversity and is able to differentiate among the wild and cultivated species of the genus Salvia. Currently, our finding of the PCA and HCA data have drawn a positive annotation relationship among Salvia aegyptiaca and Salvia officinalis to assess whether the two species sort individual or overlap groups.*

Keywords: *marker molecular, Salvia aegyptiaca, Salvia officinalis, ISSR, SRAP, ISTR, Heatmap, PCA.*

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I. Introduction

Salvia represents one of the largest genera in the Lamiaceae, comprising nearly 1000 species throughout the old and New Worlds. Common sage (*Salvia officinalis* L.), also known as garden sage or Dalmatian sage is native to the northern coastalregion of the Mediterranean and grows wild in the calcareous mountains of northern and central Spain, southern France, and the western part of the Balkan Peninsula [1]. It is economically the most important species of the *Salvia* group, and, alongside *S. fruticosa* Mill.*; S. officinalis* has a very long tradition as a medicinal and aromatic herb with a wide range of applications [2]. Common sageis cultivated in the countries of the Balkan Peninsula, throughout the Mediterranean region, and in the United States. While *Saliva aegyptiaca* L. (Egyptian sage) is a green dwarf shrub that grows in various locations in the Arabian Peninsula, Egypt, Palestine, Iran and Afghanistan [3, 4]. It is commonly used in local folk medicine. The seeds of the plant are used as demulcent for diarrhea and for piles, and the whole plant is used in diarrhea, gonorrhea and haemorrhoids, eye diseases, and as an antiseptic, antispasmodic and stomachic [4]. The plant is also used in cases of nervous disorders, dizziness and trembling and stopping perspiration [3, 5].

Although knowledge and use of common sage can be dated back to ancient Greece, there is remarkable confusion concerning its taxonomy, distribution, and variability. Therefore, molecular classification represents a useful tool for genetic analysis and allows the linkage of heritage traits connected to genomic divergence. Presently, these genomic tools are useful basics for knowledge and developing resource for understanding and utilizing various frequent sequences approaches such as Inter simple sequence repeat (ISSR), sequence related amplified polymorphism (SRAP) and inverse sequence tagged repeat (ISTR).

ISSR marker became to be addressed development of minisatellite DNA for category of varieties or species and population genetic structure, with high effectiveness, low cost, constancy and simple process. This marker is involving PCR amplification of DNA by a single 16-18 bp long primer composed of a repeated sequence anchored at the 3' or 5' end of 2-4 arbitrary nucleotides [6]. ISSR has been used with high efficiency in several endangered and rare genus of medicinal plants, have elucidate the hypervariable type of microsatellite loci and their natural effectiveness in species identification and inhabitant's diversity [7].

However, SRAP marker system was developed by Li and Quiros, [8] to target overlapping coding and non-coding regions of the genome. Compared with other markers, the preferential amplification of open reading frames (ORF) by SRAP markers have the potential to strengthen the relationship between DNA polymorphisms and morphological traits that characterize different morphotypes [9]. Particularly, SRAP not only amplifies the

interval between genes and the non-coding flanking regions, but also closely links to actual genes, which would generate a fingerprint of the coding sequences and permit easy isolation of these bands for sequencing [10]. It is better than SSR as it requires comparatively little sequence information before it can be implemented in a new species with the universal primers.

SRAP has been recognized as a powerful marker system used in genetic map construction, genealogical classification, gene tagging and cloning, marker-assisted selection, germplasm resources evaluation and prediction of heterosis. The wide applicability of SRAP demonstrates that these markers are effective and reliable for investigating the degree of genetic polymorphism in different genomes, and thus could be adapted for avariety of usage on endangered medicinal plants species, rare flowers and other living organisms [10].

In view of ISTR marker, it is a retrotransposon based marker which has been presented in most shape of the organism, are common distribution, useful and abundant in eukaryotic genomes [11]**.** ISTR markers are co-dominant markers with profits from the abundant repeats that are characteristic of plants with large genomes. This marker has the ability to characterize wild species and genetic relationships at an individual level [12]. The properties of this marker and its usefulness for classifying genetic differences among plants of the *Agave* genus have been described [13, 14].

We put forward, the three molecular markers listed beyond are co-dominant or dominant inheritance, and the jointly-used could be further advantageous to identify diverse portions of the genome. Therefore, comparative is required to verify the marker sensitivity and suitable for the topic being studied [15].

The current study based an extensive sampling of the cultivated *Salvia officinalis* and the wild type of *Salvia aegyptiaca*, species measures the genetic diversity analysis and phylogeny. Through this research, the performance of the ISSR, SRAP and ISTR markers has been made to perform the discriminating power, efficiency and the status of genetic diversity and phylogeny of the individual marker and the combined data. To date, there has been no announcement regarding the performance and effectiveness of ISSR, SRAP, and ISTR markers among the wild species of *Salvia aegyptiaca* and the cultivated species *Salvia officinalis.*

II. Materials and methods

Plant materials

A total of six accessions of the wild type of *Salvia aegyptiaca* in addition to six accessions of cultivated species *Salvia officinalis* were collected from Western Coastal desert and Sinai Peninsula, respectively (Fig.1).

DNA Extraction

Genomic DNA of Salvia species was extracted from the fresh leaves following the procedure as previously described elsewhere [16]. The quality and concentration of the DNA samples were tested using Quawell Q5000 UV-Vis spectrophotometer (V2.1.4, USA) and a sub-aliquot of the DNA was consequently diluted to50 ng/µl. Both the stock and diluted portions were stored at − 20 °C.

ISSR analysis

The ISSR-PCR amplification was prepared according to the earlier method demonstrated by Sankar and Moore, [17]. PCR-amplification was carried out in 25 µl reaction (Thermo Fisher Scientific), 0.2 mM primer and 25–30 ng genomic DNA. The amplification was performed in Thermal Cyclers from (Bio-Rad). PCR buffer, 1 µM of each primer, 0.5 µM of dNTPs (10mM each) (Thermo Fisher Scientific), 1 unit of Taq DNA polymerase (5 U/µl) (Thermo Fisher Scientific), and 40 ng DNA template. The PCR program was as follows, denaturation (one cycle) in 94 °C for 2 min, followed by 40 cycles as follows: 94 °C for 30 s, 44 °C for 45 s, 72 °C for 1 min and 30 s, and lastly one cycle extension at 72 °C for 10 min, and 4 °C (infinitive). The amplified products were separated on 1.2% agarose gel by electrophoresis. A 100 bp DNA ladder (Thermo Scientific) was used as the molecular standard to verify the competent ISSR markers. The gels were stained in ethidium bromide (0.5 µg/ml), and the amplicons were pictured below UV light using the Gel Doc XR system (Bio-rad, America).

SRAP analysis

A set of primers was designed following the description of Li and Quiros, [8] (Table 3)., All PCRs were performed in 10µl reaction volume containing 25 µl PCR buffer, 3 mM Mg+2, 200µM dNTP, 0.5 U Taq DNA polymerase (Thermo Fisher Scientific), 10 µM of each primer, and 25 ng template DNA. The amplification regime followed the recommendation of Li and Quiros, [8] as follows: an initial denaturing step was performed at 94°C for 5 min followed by 5 cycles at 94°C for 1 min, 35°C for 1 min and 72°C for 1 min, subsequently followed by 35 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 1 min with a final extension step at 72°C for 7 min. PCR products were electrophorese through 8% non-denaturing polyacrylamide gels run at 300 V for 2.5 h in 0.59 TBE buffer, and visualized by silver staining by Bassam et al., [18].

ISTR analysis

ISTR assessment was conducted following the description of Aga et al., [19]. ISTR primer combinations were primarily examined using a total of 35 primer combinations from five reverse and seven forward primers. Within all primers screened, only 10 ISTR combinations were picked for advanced analysis (Table 3). Each PCR included a reaction mix of 3.5 µl of green PCR buffer, 200 µM of dNTPs, 0.3 µM of each primer, 50 ng of genomic DNA, 1 unit of Taq DNA polymerase, and finally deionized water up to 25 µl. PCR amplification performed involved of 1 cycle at 95 °C, 3 min; 40 cycles of 94 °C, 30 s; 45 °C, 30 s; 72 °C, 2 min; 1 cycle at 72 °C, 10 min; and 4 °C for infinitive. However, separated and visualized in the same procedure described for ISSR.

Data scoring and analysis

Each band generated by ISSR, SRAP and ISTR primers was considered as an independent character or locus. Only polymorphic bands, which were clearly resolved and consistently appeared on the gels, were scored as present (1) or absent (0). To evaluate the discriminatory power of molecular markers, polymorphic information content (PIC) and marker index (MI) were calculated. The PIC value was determined by applying the simplified provided by Roldàn-Ruiz et al., [20] as follows:

PIC i = 2fi (1 – fi), where fi is the percentage of the amplified allele (band present) and (1 – fi) is the frequency of the null allele (band absent) of marker. Polymorphism information content (PIC) values were calculated according to Smith et al., [21], using the algorithm for all primer combinations as follows: $PIC=1-\sum fi^2$ where fi is the frequency of the j^{th} allele and the summation extends over *n* alleles. Discriminatory power of a locus by considering not only the number of alleles that are expressed but also bands was scored manually.

To gain accurate perspectives on genetic diversity analyses among the wild type of *Salvia aegyptiaca* and the cultivated *Salvia officinalis* germplasm, a graphic demonstration of principal component analysis (PCA) and the heatmap cluster analysis tree (HCA) was provided a description to demonstrate the multidimensional genetic relationship and its split among species using ClustVis web tool for visualizing clustering of multivariate data.

III. Results

Comparison of polymorphic levels and informativeness obtained with ISTR, SRAP and ISSR markers

In this study, we used a total of twelve genotypes of Salvia, to investigate either ISSR, SRAP, or ISTR markers were polymorphic enough to be appropriate for genotype discrimination of the cultivated *Salvia officinalis* and the wild type of *Salvia aegyptiaca.*

All marker systems examined turned out to be useful tools for detecting polymorphism and assessing genetic diversity in Salvia germplasm, while the degree of resolution differed on the applied technique. We primarily tested 30 ISSR, 25 SRAP, and 35 ISTR primers between the cultivated species *Salvia officinalis* and the wild type of *Salvia aegyptiaca*. Among all, 12 ISSR SSR, 15 SRAP and 10 ISTR primers presented high levels of polymorphism. Within the three markers system, ISTR followed by ISSR and SRAP performed diverse polymorphism levels as shown in (Table 2 and Fig. 3). However, the total number of bands recorded for ISTR was almost high followed by SRAP and ISSR markers, respectively. However, the total numbers of polymorphic bands (*p*) were ranged from (6 to 6.7) for ISSR, (5.1 to 6.1) for SRAP and for (15.1 to 14.8) for ISTR markers. On behalf of the total number of effective alleles (*Ne*), it was correlated significantly with the total number of bands (*L*) and the total numbers of polymorphic bands (*p*). Additionally, the average number of polymorphic bands/assay unit (*np/U*) was relatively high for ISTR being 15.1 and 14.8 for *Salvia officinalis* and Salvia *aegyptiaca*, respectively, with an intermediate value for ISSR and SRAP, respectively. Meanwhile, the PIC value for ISTR was relatively high (0.98) compare to ISSR and SRAP markers system. Here, the present result showed that the ISTR marker was the most powerful marker in several detected parameters and PIC values.

A comparative summary of the discriminating capacity of ISSR, SRAP and ISTR, markers are summarized in (Table 2) and (Fig. 4). On average, the four factors, Fraction of polymorphic loci (β), assay efficiency index (*Ai*), effective multiples ratio (*E*), and marker index (*MI*) presented higher value in ISTR marker, highlight the notable characteristics of this marker compared to SRAP and ISSR (2.9× and 2.4×, respectively).This certainly due to the highest value of the assay efficiency index for the ISTR marker, supposing that ISTR has a higher discriminating capacity for counting the genetic diversity and can simultaneously determine several polymorphic markers per reaction. These outcomes reveal that ISTR following by ISSR markers can be utilized to evaluate the level of polymorphism among *Salvia aegyptiaca* and *Salvia officinalis*, respectively.

Species diversity and genetic phylogeny

Principal component analysis (PCA)

To further determine the genetic relationships among the Salvia species and the resolution of the individual markers, a graphic demonstration of the principal component analysis (PCA) was constructed to express the results based on data obtained from the ISSR, SRAP and ISTR markers. The two-dimensional PCA plot separated the studied species within the standard four quadrates. The PCA plot for the combined markers data revealed 10.5% and 55.8% of the total molecular variation (Fig. 5). Cluster I compressed all accessions of *Salvia aegyptiaca*, while cluster II assembled together all *Salvia officinalis* accessions in a particular group.

Similarity matrix analysis

The results of similarity matrix among the twelve accessions within *Salvia aegyptiaca* and *Salvia officinalis* are presented in table (4). Overall, the coefficient similarity indicated that 77% similarity was observed between site three and site four, as well as between site four and site five and in addition to between site nine and site ten, while the lowest value was recorded between site six and site nine, being 51% of similarity.

Heatmap cluster analysis (HCA)

To further determine the genetic diversity, HCA tree exhibits the abundance of the relationships between the twelve accessions of *Salvia aegyptiaca* and *Salvia officinalis*. The distribution of hot points indicates significant variations between the two major groups of the Salvia species and able to cluster in two sub-clade. As a result, the HCA tree was constructed based on the three sets of ISSR, SRAP and ISTR markers (Fig. 6). The results were similar to each other with a tiny difference in the placement of the two species, where the ISTR-HCA tree was the most consistent with the combined data of Salvia Sp. Overall, two confirmed clades were identified which have the ability to clearly distinguish among the twelve accessions. In detail, the first clade assembled together all accessions of *Salvia aegyptiaca* in a particular monophyletic clade. In the framework, the second clade occupies all accessions of *Salvia officinalis* with a high proportion of close relationships. Collectively, we found both PCA and HCA tree data have drawn a successful annotation relationship in *Salvia aegyptiaca* and *Salvia officinalis* species to evaluate whether the specific group sort individual or overlap groups.

IV. Discussion

Several molecular marker techniques are presently offered for genetic analysis in medicinal plant species. Although there has yet no individual molecular method that can answer problems facing gene banks management. A better considerate of the effectiveness of the diverse DNA-based markers is a vital step towards plant germplasm classification and characterization, and the requirement for the effective application of marker techniques with in breeding programmers [22]. The optimal of the most suitable technique for the exact study is not clear and depends principally on purpose of the research as well as the biology and genetic structure of the species. One of the challenges of the present investigation, is the use of several molecular markers to discover the genetic relationships among the Egyptian *Salvia aegyptiaca* and *Salvia officinalis* species grown within the Egyptian desert. Therefore, evaluations are required in order to choose which technique most appropriate for the topic being examined [22]. The development of high-throughput methods for the detection of SRAP and ISTR has led to a revolution in their use as molecular markers [23]. The use of ISSR, SRAP and ISTR marker is powerfully suggested by numerous studies talking the efficiency of such markers for investigating Salvia diversity. Song et al., [24] reported that both ISSR and SRAP markers were successful and consistent for evaluating the degree of genetic variation in the traditional Chinese medicinal herb of *Salvia miltiorrhiza*.

In this article, the three markers recorded above have the ability to identified deferent portions of the genome, they have a dominant or co-dominant inheritance and the usage together may be more effectiveness. Therefore, the relatively high values of the effective number of alleles for markers were used to offer suggestion of their discrimination capacity when study a huge number of plants. This trend is essential for the germplasm bank's documentation when many species need to be correctly distinguished and classified [25]. In this revised, the effective number of alleles following the method: ISTR > ISSR > SRAP. This result recommends that the ISTR and ISSR is more useful evidence for *Salvia aegyptiaca* and *Salvia officinalis* classification and certification. Indeed, our results are in accord with the recent finding among Iranian Salvia ecotypes/species, recommended that several molecular markers such as TRAP and CoRAP are beneficial for Salvia germplasm fingerprinting, and thus improving germplasm management and helping germplasm utilization [26].

It is well known that the marker index (MI) may probably be a suitable value for marker effectiveness [25]. By this criterion, a comparison of the overall efficiency of the three marker systems was provided by the marker index (MI). Almost three fold and 2.4 fold higher MI calculated for ISTR in comparison to SRAP and ISSR, highlights the unique nature of the ISTR assay, this is defiantly due to the high effective multiplex ratio and assay efficiency index which returns to high levels of detected polymorphism, which agrees with previous

reports in many plant species [25-29]. The Mantel matrix test was used to compare the similarity matrix, however, our evidence suggested that ISTR and ISSR were recorded the highest correlations comparing the SRAP markers. This due to the associated with a large number of pair-wise genetic similarity coefficients with intermediate values, which allow a number of similar variants for dendrogram branching [30, 31].

Recent evidence subjected that the retrotransposon marker, e.g., ISTR had a higher discrimination capacity and have the flexibility to distinguish several polymorphic loci per single reaction [7, 32]. Recently**,** Du et al., [33] suggested that retrotransposons (RT) excuse for 28.1 Mb of the genome sequence, accounting for 9.74% of the whole genome. These results showed that ISTR had an abundant presence of *Ty-1* Copia retrotransposons, which offers beneficial polymorphism, and numerous unique private loci that would allow diversity within among the tested genotypes of *Salvia aegyptiaca* together with *Salvia officinalis* germplasm, which is in concurrence with earlier reports markers analysis [11, 34].

In view of the previous revisions, our PCA and heatmap phylogenetic tree analysis clear evidence supporting the undisputed viewpoint that *Salvia aegyptiaca* together with *Salvia officinalis* are very closely associated in individual clade and seem to be distinguished. Recently, Resetnik, et al., [35] revealed a clear different between the indigenous and cultivated/naturalized groups. Following the phylogeny analysis of Erbano and Santos, [36] the principal component analysis (PCA) and the phylogenetic tree were consistent, and all three populations of the Salvia genus appear distinct as in structure analysis, which is congregant with our results of PCA and heatmap analysis tree. Certainly, our PCA and HCA tree are able to tentatively imply forward this theory as *Salvia aegyptiaca* and *Salvia officinalis* species formed a particular clade. These results might be applied in systematics and evolutionary biology studies within the Egyptian species of Salvia clarify the complex interactions among species, as demonstrated in previous studies [37, 38].

It is well know that, the close wild relatives of domesticated medicinal plant species and a wild relatives are a rich source of readily accessible diversity for development medicinal plant breeding and improvement. Hence, several molecular markers analysis systems have improved identify the available diversity within domesticated gene pools and wild medicinal plant relatives that support plant breeding for agro-biodiversity [39]. Indeed, the advent of molecular markers overcame the majority of the challenges linked to developing morphological markers through which main phenotype-changing genes were theoretical as genetic markers**.**

V. Conclusion

In the present investigation, we highlight that the results of the genetics classification based on ISTR, ISSR and SRAP, can capture the taxonomy and systematics of the Salvia genus recovered with a good performance in clarifying genetic diversity. Our results indicated that ISTR following by ISSR had the superior discrimination capacity to evaluate the level of polymorphism among *Salvia officinalis* and *Salvia aegyptiaca*, respectively. Indeed, our results of PCA and HCA tree data have drawn an effective annotation relationship in *Salvia aegyptiaca* and *Salvia officinalis* species to assess whether the overlap groups or the specific group sort separately. Collectively our results not only help within the classification within domesticated gene pools and a wild relatives medicinal plant but may recognize species limitations, flagging of modern species, genus definition, and support plant breeding and improvement.

Abbreviations

ISSRs: Inter-simple sequence repeats; SRAP: Sequence-related amplified polymorphism; ISTR: Inverse sequence-tagged repeat; PIC: Polymorphic information content; MI: Marker index; PCA: Principal coordinate's analysis; HCA: Heatmap cluster analysis tree.

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Table 1. A list of selected *Salvia aegyptiaca* and *Salvia officinalis* accessions used in this study with ISSR, SRAP and ISTR marker.

No	Sites	Type	Location	No	Sites	Type	Location
1	Site 1	*Salvia aegyptiaca*	Egypt - North Sinai-ElArish	7	Site 7	*Salvia officinalis*	Egypt - Matrouh -Elsallum
2	Site 2			8	Site 8		
3	Site 3			9	Site 9		
4	Site 4			10	Site 10		
5	Site 5			11	Site 11		
6	Site 6			12	Site 12		

Table (2). Relative efficiency of molecular markers in determining polymorphism among *Salvia officinalis* and *Salvia aegyptiaca* accessions.

Parameters for marker efficiency	**ISSR**		**SRAP**		**ISTR**	
	Salvia officinalis	***Salvia aegyptiaca***	***Salvia officinalis***	***Salvia aegyptiaca***	***Salvia officinalis***	***Salvia aegyptiaca***
Number of markers	12	12	15	15	10	10
Number of loci (L)	134	136	129	137	159	154
Polymorphic bands (p)	61	81	77	92	151	148
Number of loci/assay unit (nu)	11.1	11.3	8.6	9.1	15.9	15.4
Total number of effective alleles (Ne)	243.1	232.6	232.8	340.3	1111.4	823
Average number of polymorphic bands/assay unit(np/U)	6	6.7	5.1	6.1	15.1	14.8
Polymorphic information content (PIC)	0.94	0.91	0.89	0.90	0.98	0.98
Fraction of polymorphic loci (β)	0.54	0.59	0.59	0.67	0.94	0.96
Assay efficiency index (Ai)	20.2	19.3	15.5	22.6	111.1	82.3
Effective multiples ratio (E)	6	6,7	5.1	6.1	15.1	14.8
Total Banding pattern (Bp)	79	81	84	95	119	113
Effective number of patterns/ assay unit (p)	6.5	6.7	5.6	6.3	11.9	11.3
Marker index (MI) = Hav × MR	5.7	6.1	4.6	5.5	14.9	14.5

Table (3). List of ISSR, SRAP and ISTR primer combinations obtained from the current investigation.

	No	Primer Name	Sequence	NO	Primer Name	Sequence
ISSR Marker	1	807	(AG)8 T	7	HB2	CAG) 5
	2	814	(CT) 8TG	8	HB4	(GACA) 4
	3	844A	(CT)8 AC	9	HB8	(GA)6 GG
	4	844B	(CT) 8 GC	10	HB10	(GA) 6 CC
	5	17898A	(CA) 6 AC	11	HB12	(CAC)3 GC
	6	HB1	(CAA)5	12	HB15	(GTG) 3 GC

	No	Primer Name	Forward primer	Reverse primer
SRAP Marker	1	Em 1 R/DN 7 F	TGAGTCCAAACCGGTCC	GACTGCGTACGAATTAAT
	2	Em 7 R/me 4 F	TGAGTCCAAACCGGACC	GACTGCGTACGAATTCAA
	3	Em 7 R/me 5 F	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCAA
	4	Em 7 R/me 9 F	TGAGTCCAAACCGGAGG	GACTGCGTACGAATTCAA
	5	Em 7 R/me 12 F	TGAGTCCAAACCGGAGA	GACTGCGTACGAATTCAA
	6	Em 8 R / DN 9 F	TGAGTCCAAACCGGTCA	GACTGCGTACGAATTCTG
	7	Em 9 R / me 5 F	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCAG
	8	Em 12 R/me 2 F	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTCTC
	9	Em 16 R/me 5 F	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCGA
	10	Em 16 R/me 9 F	TGAGTCCAAACCGGAGG	GACTGCGTACGAATTCGA
	11	Em17 R/DN11 F	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTAGC
	12	Em 18R/me 6 F	TGAGTCCAAACCGGACA	GACTGCGTACGAATTGAG
	13	Em 19 R/me 4 F	TGAGTCCAAACCGGACC	GACTGCGTACGAATTGCC
	14	Em 19 R/me 9 F	TGAGTCCAAACCGGAGG	GACTGCGTACGAATTGCC
	15	Em 19 R/me 9 F	TGAGTCCAAACCGGAGG	GACTGCGTACGAATTGCC

	No	Primer Name	Forward primer	Reverse primer
ISTR Marker	1	F 1 / B 2	AGGAGGTGAATACCTTAG	GGATATCCTATGAATCAAGC
	2	F 1 / B 3	AGGAGGTGAATACCTTAG	ATTCCCATCTGCACCAAT

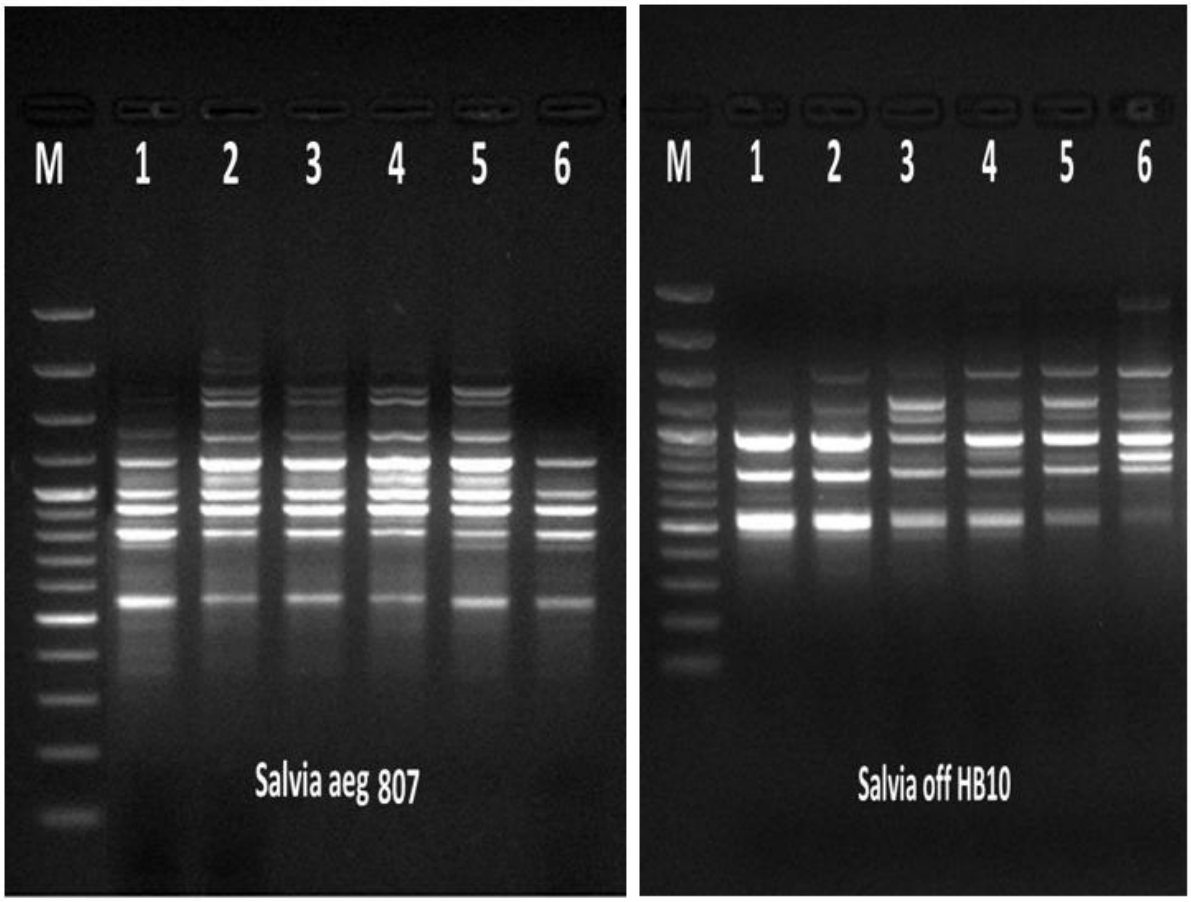
3	F 1 / B 8	AGGAGGTGAATACCTTAG	CCTCCTTATTGGGAATGATAT
4	F 3 / B 5	GTCGACATGCCATCTTTC	CTTCTGTGAAAGTCCTAG
5	F 3 / B 8	GTCGACATGCCATCTTTC	CCTCCTTATTGGGAATGATAT
6	F 3 / B 10	GTCGACATGCCATCTTTC	GACCCTTTTGAAAACACATG
7	F 5 / B 7	ATATATGGACTTAAGCAAG	GGAATATCATTCCCAATAAG
8	F 5 / B 8	ATATATGGACTTAAGCAAG	CCTCCTTATTGGGAATGATA
9	F 9 / B 3	ATATGGACTTAAGCAAGCCA	ATTCCCATCTGCACCAAT
10	F 10 / B 6	GATCAAAAAGTTTGGTTTCAT	ATATATGGACTTAAGCAAGCA

Table (4). Similarity matrixes among the twelve accessions on Salvia species based on ISSR, SRAP and ISTR marker.

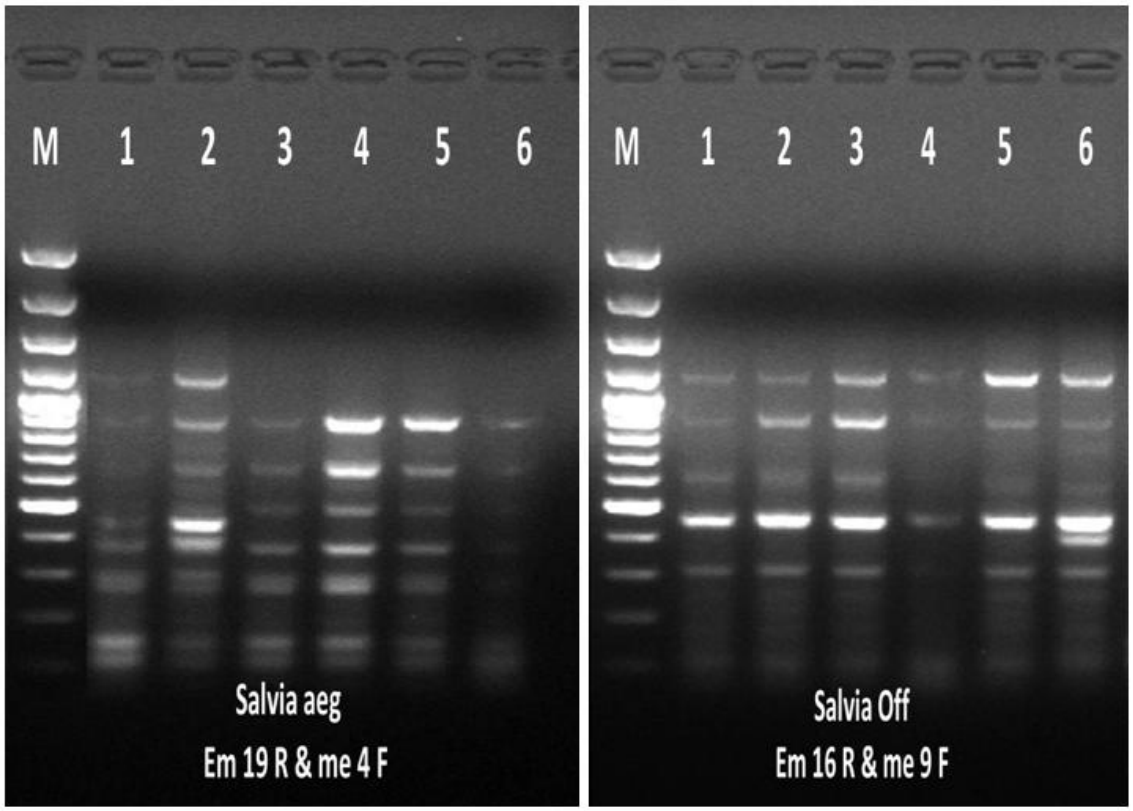
Case	Proximity Matrix Matrix File Input										
Site	**Site1**	**Site2**	**Site3**	**Site4**	**Site5**	**Site6**	**Site7**	**Site8**	**Site9**	**Site10**	**Site11**
Site1											
Site2	.68										
Site3	.68	.74									
Site4	.65	.67	**.77**								
Site5	.65	.64	.72	**.77**							
Site6	.62	.65	.68	.73	.76						
Site7	.58	.62	.57	.52	.53	.53					
Site8	.57	.65	.57	.53	.56	.55	.75				
Site9	.57	.60	.54	.54	.54	.51	.69	.70			
Site10	.58	.65	.61	.57	.57	.57	.68	.71	**.77**		
Site11	.60	.64	.59	.59	.58	.57	.71	.69	.75	.76	
Site12	.58	.64	.57	.58	.59	.59	.65	.72	.70	.74	.75

Figure (1): Plant shape in the natural native used in our study.

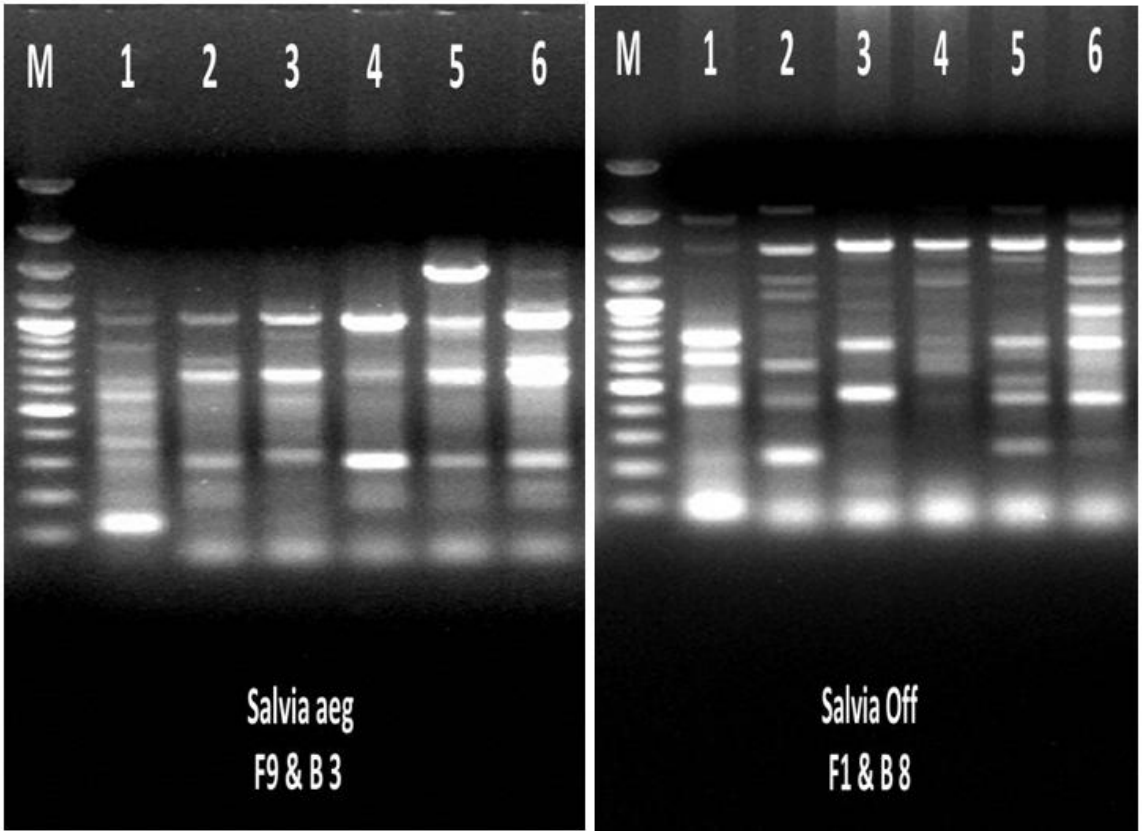
Figure (2). Map showing the distributions and locations of twelve accessions of *Salvia aegyptiaca* and *Salvia officinalis* accessions collected from Western Coastal desert and Sinai Peninsula in Egypt, respectively.



(A): ISSR Marker



(B): SRAP Marker



(C): ISTR Marker

Figure (3). (A) ISSR, (B) SRAP and (C) ISTR profiles of *Salvia aegyptiaca* and *Salvia officinalis* accessions.

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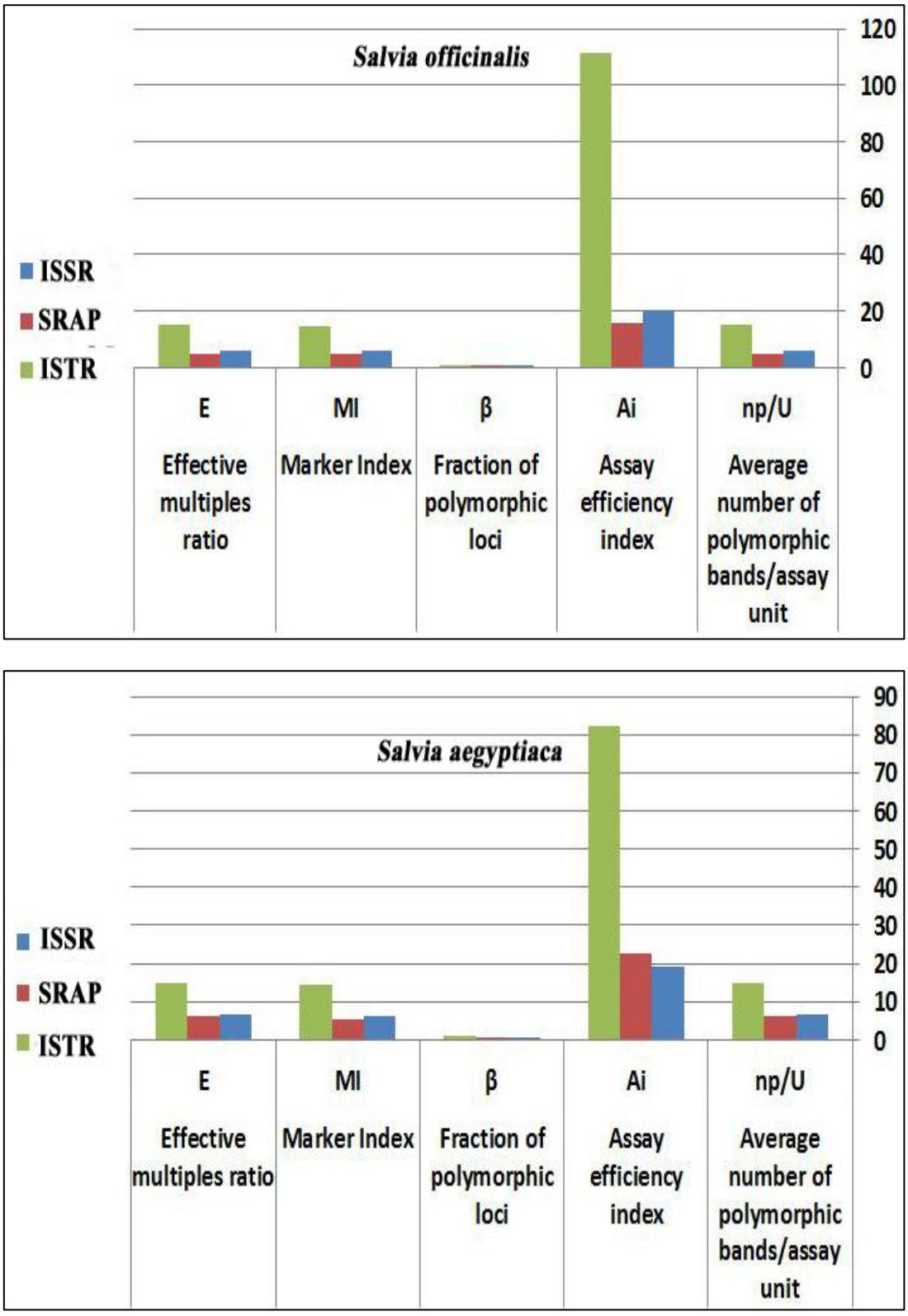

Figure (4) .Observed the comparison information obtained and the discriminating capacity of ISSR, SRAP and ISTR profile among *Salvia aegyptiaca* and *Salvia officinalis* accessions.

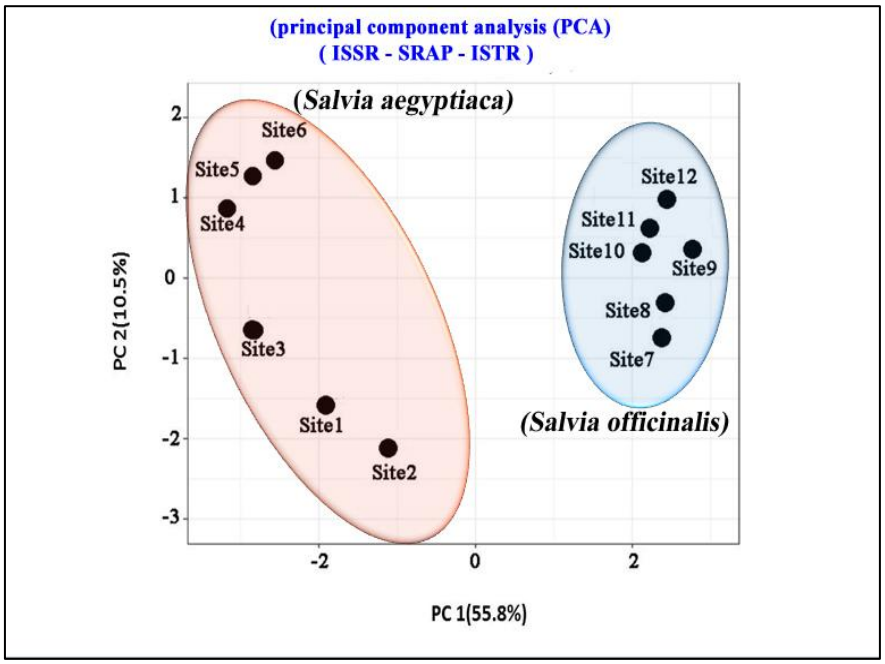

Figure (5). Schematic representation the principal component analysis of *Salvia aegyptiaca* and *Salvia officinalis* accessions based on ISSR, SRAP and ISTR markers. PC1 and PC2 refer to the first and second principal component, respectively.

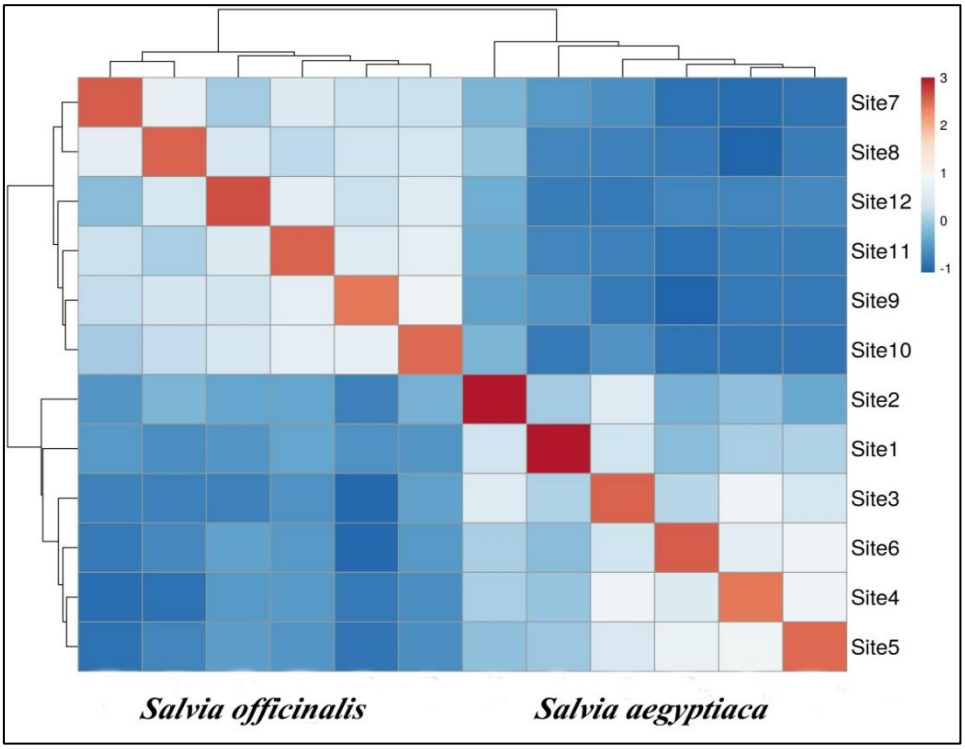

Figure (6). Heatmap phylogeny tree generated from ISSR, SRAP and ISTR data of *Salvia aegyptiaca* and *Salvia officinalis* accessions.

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