The Open Agriculture Journal

Content list available at: https://openagriculturejournal.com

RESEARCH ARTICLE

Genetic Diversity of *Salvia Officinalis* L. (Lamiaceae) and its Related Species using TU-DAMD Analysis

Basel Saleh[1,*]

[1]*Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria*

Abstract:

Background:

Salvia tomentosa Mill., *Salvia fruticosa* Mill., and *Salvia officinalis* L. are Mediterranean species with different pharmaceutical and medicinal applications. However, genetic relationships among these species are still unclear.

Objective:

The study aimed to investigate the genetic polymorphism among *S. officinalis* L. (SO) and its related species *S. tomentosa* Mill. (ST) and *S. fruticosa* Mill. (SF) collected from different geographical regions in Syria.

Methods:

Touch-up directed amplification of minisatellite DNA (TU-DAMD) assay has been employed to assess genetic relationships among the studied *Salvia* species based on the estimated percent disagreement values (PDV).

Results:

Seventeen DAMD primers highlighted a mean of 90.419, 0.254, and 2.398% for polymorphism level (P%), polymorphic information content (PIC), and marker index (MI) values, respectively, across the three studied *Salvia* species. Unweighted Pair Group Mean Arithmetic average (UPGMA) analysis revealed that the studied *Salvia* samples were clustered into three main clusters; each species was split into one cluster. Overall, moderate P% of 72.662 and 70.374% was recorded for SO and ST species, respectively. Whereas, low P% of 51.429% was recorded for SF species.

Conclusion:

TU-DAMD marker is a potential tool for studying genetic relationships among the three studied *Salvia* species.

Keywords: *Salvia officinalis*, *Salvia tomentosa*, *Salvia fruticosa*, TU-DAMD marker, Genetic diversity, Minisatellite.

1. INTRODUCTION

Salvia is one of the largest plant genera. This genus includes approximately 1000 species [1] and belongs to the Lamiaceae family. Around 250 species of this genus are common in the Mediterranean regions, and the *Salvia officinalis* group consists of 11 species [2]. Among these 11 species, *S. officinalis* L., *S. tomentosa* Mill., and *S. fruticosa* Mill. species are the most three prominent species in the Mediterranean region [3].

According to Mouterde [4], *S. tomentosa* Mill (Tomentose sage) is native to areas ranging from South-East Europe to Transcaucasia, including Albania, Bulgaria, East Aegean Is., Greece, Krym, Lebanon, Syria, Transcaucasus, Turkey, and Yugoslavia [5]. Whereas, *S. fruticosa* Mill. (Greek sage) is a native species of the East Mediterranean basin and distributed from Italy, Sicily, and Cyrenaica through the South Balkan Peninsula (Albania and Greece) to West Syria [2]. Wild populations of these two species are widespread in Lebanon and Syria [4]. Conversely, *S. officinalis* L. (Common sage or Dalmatian sage) is native to the northern coast of Mediterranean regions and grows under wild type in the calcareous mountains of northern and central Spain, southern

---

* Address correspondence to this author at the Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria; E-mail: ascientific@aec.org.sy

2 The Open Agriculture Journal, 2023, Volume 17

France, and the western part of the Balkan Peninsula [2, 6], and also to the Middle East and Mediterranean areas [7].

Whereas, *S. tomentosa* Mill. and *S. fruticosa* Mill. are present as wild, endemic species in Syria; however, the occurrence of wild *S. officinalis* L. among the Syrian flora is not mentioned by Mouterde [4].

Among the different *Salvia* species, *Salvia officinalis* L. displays the most valuable importance as an ornamental and medicinal plant. Due to its richness in bioactive compounds, it has a broad spectrum of uses ranging from food, pharmacology, and medicine to cosmetic applications [7 - 9]. It has been demonstrated that *S. officinalis* as a Mediterranean plant is characterized by exhibiting a high level of genetic diversity at the plastid genome (HT = 0.695) (as reported in other native aromatic/medicinal plants) and at nuclear DNA levels [8]. However, the genetic diversity in the canter of its origin is still unclear [3].

It has been demonstrated that some *Salvia* species have different pharmaceutical, medicinal, and industrial applications due to the richness of the essential oil in their bioactive components [7]. In Syria, they were used in folk medicine against winter diseases.

In Syria, *S. tomentosa* Mill. and *S. fruticosa* Mill. are known as miramiya, meramiya, mariamiya, and mirimiyah; they have a common Arabic name with a minor difference. *S. officinalis* is named cultivated mirimiyah, whereas *S. fruticosa* and *S. tomentosa* are known as wild mirimiyah. This classification is mainly based on their aromatic compounds. Mouterde [4] reported inferior aromatic compounds in *S. fruticosa* compared to *S. officinalis*. In Lebanon, *S. fruticosa* (syn. *S. triloba* L.fil. or *S. libanotica* Boiss. et Gaill) is an endemic species and named as mirimiyah or kas'in in Arabic. Indeed, this label was extended to Palestine and Jordan.

The molecular characterization of a given plant species is a potent tool for its conservation and breeding programs. DNA genetic variation within each *Salvia* species has been assessed in many reports. In this regard, generic polymorphism in *S. officinalis* L. has been assessed using random amplified polymorphic DNA (RAPD) markers [3, 10, 11]; single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers [6, 9, 12, 13]; plastid DNA intergenic spacers [8]; inter simple sequence repeat (ISSR) markers [14], and recently, by amplified fragment length polymorphism (AFLP) markers [15]. As for *S. fruticosa*, RAPD markers [16] and microsatellites [17] have been used.

Different molecular marker systems have also been employed to study the genetic relationships within other *Salvia* species, for *e.g.*, in *S. hispanica* L., by using the RAPD markers [18]; in *S. lachnostachys*, by using the ISSR markers [19], sequence-related amplified polymorphism (SRAP) and ISSR [20], and ISSR markers [21]; in *S. lutescens* var.

intermedia, by using nuclear ribosomal DNA and plastid DNA sequences [22]; in *S. divinorum*, by using chloroplast simple sequence repeats (cpSSR's) [23]; in *S. japonica*, by using chloroplast and nuclear ribosomal DNA sequences and allozyme polymorphisms [24], and in *S. euphratica sensu lato* by using the internal transcribed spacer (ITS) and chloroplast DNA regions [trnT-trnL intergenic spacer (IGS)] markers [25].

Touch-down directed amplification of minisatellite DNA polymerase chain reaction (TD-DAMD-PCR) marker among various molecular markers available nowadays has been successfully employed for DNA genetic variability assessment in different plant crops, for *e.g.*, in common bean landraces [26], *Salvia* species [27], *Allium* sp [28], carnation cultivars [29], commercial cotton [30], and *S. tomentosa* [31].

TU-DAMD assay has been recently employed to study the genetic diversity in *Salvia judaica* and *Salvia palaestina* [32], and more recently in *Origanum syriacum* L [33].

The genetic structure of plant populations reflects various interaction processes involving various phenomena (long-term evolutionary history of the species (shift in distribution, habitat fragmentation, and population isolation), mutations, genetic drifts, mating system, gene flow, and selection).

DNA genetic variability within *S. officinalis* group has not been investigated neither in Syria nor worldwide yet. In particular, studies relative to the Mediterranean *S. officinalis* L., *S. tomentosa* Mill., and *S. fruticosa* Mill. species are still lacking. Therefore, the current study has been conducted to assess the genetic relationships of *S. officinalis* and its related species using the TU-DAMD molecular marker.

2. MATERIALS AND METHODS

2.1. Sampling

Samples were collected from different geographical regions in Syria (Table **1**). Cultivated accessions of *S. officinalis* L. (SO) (10 samples (2 from Lattakia, 2 from Jableh, 1 from Tartous, 2 from Hama, 2 from Damascus, and 1 from Darra), and natural populations of *S. tomentosa* Mill (ST) (5 samples (3 from Lattakia, 1 from Tartous, and 1 from Hama) and *S. fruticosa* Mill (SF) (4 samples (1 from Lattakia, 1 from Jableh, 1 from Banyas and 1 from Tartous) were collected from different location sites in Syria. Moreover, wild *Origanum syriacum* L. **(**Lamiaceae**)** species collected from Lattakia was used as the reference. Leaf sampling has been carried out during the blooming stage.

2.2. Genomic DNA Extraction

Leaf genomic DNA of the studied samples was isolated using CTAB (cetyltrimethylammonium bromide) as in the protocol described by Doyle and Doyle [34]. DNA fluorimeter instrument was used to determine DNA concentration. DNA was stored at −80°C until use.

Table 1. Collection sites description of the three studied *Salvia* species along with *O. syriacum*.

Species	Collection Site	Code	Altitude (m)	Annual Rainfall (mm)
S. officinalis	Lattakia	SOL1	20	800
	Lattakia	SOL2	60	800
	Jableh	SOJ3	20	1200
	Jableh	SOJ4	20	1200
	Tartous	SOT5	247	1400
	Hama	SOH6	170	750
	Hama	SOH7	500	1500
	Damascus	SOD8	950	260
	Damascus	SOD9	800	300
	Darra	SOD10	780	500
S. tomentosa	Lattakia	STL11	400	1100
	Lattakia	STL12	134	800
	Lattakia	STL13	540	1100
	Tartous	STT14	377	1500
	Hama	STH15	300	1400
S. fruticosa	Lattakia	SFL16	650	110
	Jableh	SFJ17	75	1200
	Banyas	SFB18	485	1400
	Tartous	SFT19	890	1400
O. syriacum	Lattakia	OS	80	800

Table 2. DAMD primers used in the current study.

Primer No.	Primer Name	Primer Sequence 5'-3'
1	URP1F	ATCCAAGGTCCGAGACAACC
2	URP2R	CCCAGCAACTGATCGCACAC
3	URP9F	ATGTGTGCGATCAGTTGCTG
4	URP30F	GGACAAGAAGAGGATGTGGA
5	URP38F	AAGAGGCATTCTACCACCAC
6	OGRB01	AGGGCTGGAGGAGGGC
7	FVIIex8	ATGCACACACACAGG
8	HBV3	GGTGAAGCACAGGTG
9	14C2	GGCAGGATTGAAGC
10	33.6	GGAGGTGGGCA
11	PM13	GAGGGTGGCGGCTCT
12	HBVb	GGTGTAGAGAGAGGGGT
13	HVR	GGAGGTTTTCA
14	URP6R	GGCAAGCTGGTGGGAGGTAC
15	URP17R	AATGTGGGCAAGCTGGTGGT
16	M13	GAGGGTGGCGGTTCCT
17	HVV	GGTGTAGAGAGGGGT

2.3. TU-DAMD Assay

DNA genetic variability among the Mediterranean *S. officinalis* L., *S. tomentosa* Mill., and *S. fruticosa* Mill. species has been investigated using seventeen DAMD primers (Table **2**). TU-DAMD assay was performed as more recently described by Saleh [32] in 25 µl total volume using a T-gradient thermal cycler (Bio-Rad, Hercules, USA) programmed as follows: 1 cycle for 4 min at 94 ºC, followed by ten cycles of pre-PCR involving 30 s at 94 °C for denaturation, 45 s at 55 °C for annealing, and 3 min at 72 °C for extension. The annealing temperature was increased by 0.5 °C/cycle for the first 10 cycles. Then, 30 cycles were carried out at a constant temperature of 55 °C as the annealing temperature, followed by a final extension at 72 °C for 10 min. Final PCR products were separated on a 2% ethidium bromide-stained agarose (Bio-Rad) in 0.5× Tris-borate-EDTA (TBE) buffer. Electrophoresis was carried out at 85 V for 2.5 h and visualized with a UV transilluminator. The molecular weight of TU-DAMD amplification products was estimated using a VC 100bp Plus DNA Ladder (Vivantis) standard.

2.4. TU-DAMD Data Analysis

Band scoring has been manually done as 0 or 1 for the absence or presence of each band size, respectively. The Unweighted Pair Group Mean Arithmetic average (UPGMA) analysis using the Statistica program [35] was constructed based on percent disagreement values (PDV). Genetic similarity among the three studied *Salvia* samples was determined [36]. Indeed, polymorphic information content (PIC) was determined [37] according to the following formula:

$$PIC = 1 - \Sigma(Pij)^2$$

Where, Pij is the frequency of the ith pattern revealed by the jth primer summed across all patterns revealed by the primers. Moreover, the marker index (MI) was also determined [38] according to the following formula:

$$MI = PIC \times \eta\beta$$

Where, PIC is the mean PIC value, η is the number of bands, and β is the proportion of polymorphic information.

3. RESULTS

TU-DAMD markers produced PCR products with sizes ranging from 100-3000 bp. TU-DAMD polymorphism among the studied species yielded by OGRB01, 14C2, and M13 DAMD primers is shown in Fig. (**1**). The different primers produced a total band number ranging from 4 (HVR) to 19 (14C2) with a mean average of 9.824 bands/primer (Table **3**). The number of polymorphic bands ranged between 2 (HVR) and 19 (14C2) with a mean average of 8.882 polymorphic bands/primer (Table **3**). Six DAMD (URP2R, URP30F, FVIIex8, HBV3, 14C2, and M13) primers among the 17 DAMD-tested primers successfully produced a polymorphism level of 100%. Whereas, for the remaining primers, this value ranged between 50% (HVR) and 92.308% (HVV) (Table **3**). Indeed, the PIC value ranged between 0.140 (URP9F) and 0.368 (M13) with a mean average of 0.254. As for MI, it ranged between 0.360 (HVR) and 6.175 (14C2) with a mean average of 2.398. In general, the TU-DAMD marker highlighted a mean average value of 90.419%, 0.254, and 2.398 for P%, PIC, and MI, respectively, across the three studied species (Table **3**).

Genotypic-specific markers ranged between 0 (SOJ3) and 10 (SOH7) (Table **4**). TU-DAMD assay highlighted 68 total genotypic-specific markers; they were 31, 19, and 18 for SO, ST, and SF species, respectively. Two DAMD primers (HVR and 33.6) did not reveal genotypic-specific markers among all the tested samples. Whereas, for the remaining 15 DAMD primers, this value varied from 1 (URP2R and M13) to 10 (HVV) with respect to genotypic-specific markers (Table **4**).
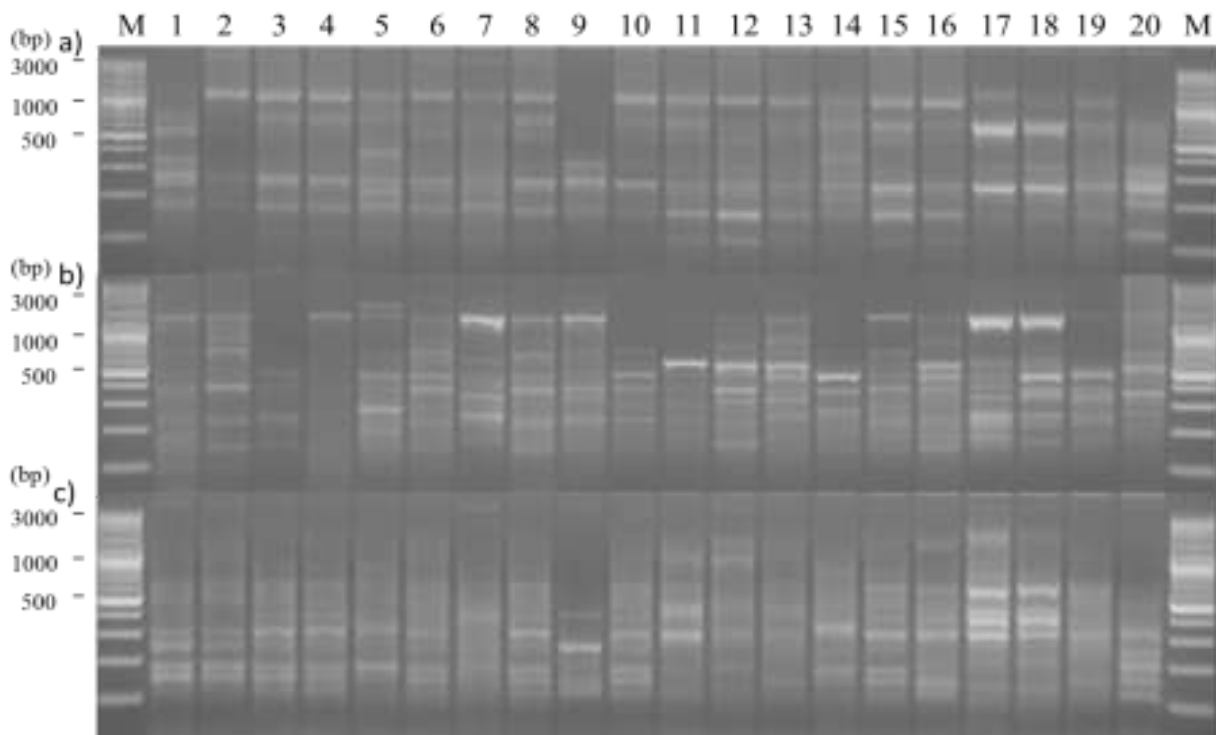

Fig. (1). TU-DAMD polymorphism pattern among the three studied Salvia species as yielded by OGRB01 (**a**), 14C2 (**b**), and M13 (**c**) DAMD primers. S. *officinalis* (lanes 1-10), S. *tomentosa* (lanes 11-15), S. *fruticosa* (lanes 16-19) and O. syriacum (lane 20). M: VC 100bp Plus DNA Ladder (Vivantis) standard.

Table 3. TU-DAMD data including total bands (TB), polymorphic bands (PB), polymorphic % (P %), polymorphic information content (PIC), and marker index (MI) values.

Primer Name	TB	PB	P%	PIC	MI
URP1F	11	10	90.909	0.249	2.490
URP2R	10	10	100.000	0.320	3.200
URP9F	5	3	60.000	0.140	0.420
URP30F	8	8	100.000	0.290	2.320
URP38F	11	10	90.909	0.246	2.460
OGRB01	11	8	72.727	0.177	1.416
FVIIex8	8	8	100.000	0.260	2.080
HBV3	9	9	100.000	0.260	2.340
14C2	19	19	100.000	0.325	6.175
33.6	8	6	75.000	0.260	1.560
PM13	11	10	90.909	0.368	3.680
HBVb	7	6	85.714	0.270	1.620
HVR	4	2	50.000	0.180	0.360
URP6R	7	6	85.714	0.210	1.260
URP17R	11	10	90.909	0.215	2.150
M13	14	14	100.000	0.284	3.976
HVV	13	12	92.308	0.271	3.252
Total	167	151	-	-	-
Average	9.824	8.882	87.359	0.254	2.398

Table 4. Genotypic-specific markers characterizing the three studied *Salvia* species based on TU-DAMD data.

Primer Name	SOL1	SOL2	SOJ3	SOJ4	SOT5	SOH6	SOH7	SOD8	SOD9	SOD10	STL11	STL12	STJ13	STT14	STH15	SFL16	SFJ17	SFB18	SFT19	Total
URP1F	1	1	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	6
URP2R	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
URP9F	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	3
URP30F	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
URP38F	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0	4
OGRB01	0	0	0	0	1	0	0	1	0	0	1	1	0	0	1	1	1	0	0	7
FVIIex8	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	4
HBV3	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	1	1	5
14C2	0	0	0	0	1	0	2	0	0	0	0	1	1	0	0	0	0	1	0	6
33.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PM13	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
HBVb	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
HVR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
URP6R	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	3
URP17R	1	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	1	6
M13	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	2	2	0	7
HVV	0	0	0	1	0	1	2	0	0	0	1	2	0	0	0	1	1	1	0	10
Total	4	5	0	1	5	2	10	1	2	1	6	8	3	1	1	3	6	5	4	68
SO Total	31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ST Total	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SF Total	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Clustering analysis has been conducted based on PDV (Fig. **2**). This analysis showed that *O. syriacum* L. is genetically distant from the studied *Salvia* samples, as expected. More than this, it showed that *Salvia* samples are clustered into three main groups. The first cluster included SO samples of which SOT5 & SOH7 were genetically distinct from the remaining studied SO samples (PDV of 0.22 and similarity of 0.71) (Tables **5** and **6**). Whereas, the second one included ST samples divided into two subclusters, with the first subcluster including STL11 and STL12 (PDV of 0.14 and similarity of 0.76) (Tables **5** and **6**) and the second subcluster including STT14, STH15, and STL13. While, the third cluster included studied SF samples and divided itself into two subclusters; the first subcluster included SFL16 and SFT19 (PDV of 0.13 and similarity of 0.76), whereas the second one included SFJ17 and SFB18 (PDV of 0.06 and similarity of

0.91) (Tables **5** and **6**). Tables **5** and **6** showed that the closest samples were SFJ17 and SFB18, exhibiting the lowest PDV of 0.06 and the highest similarity of 0.91. Whereas, the most distant samples were SOD8 and SFJ17 (PDV of 0.38 and similarity of 0.48) (Tables **5** and **6**).

Table 5. Percent disagreement values (PDV) among the three studied *Salvia* species based on TU-DAMD data.

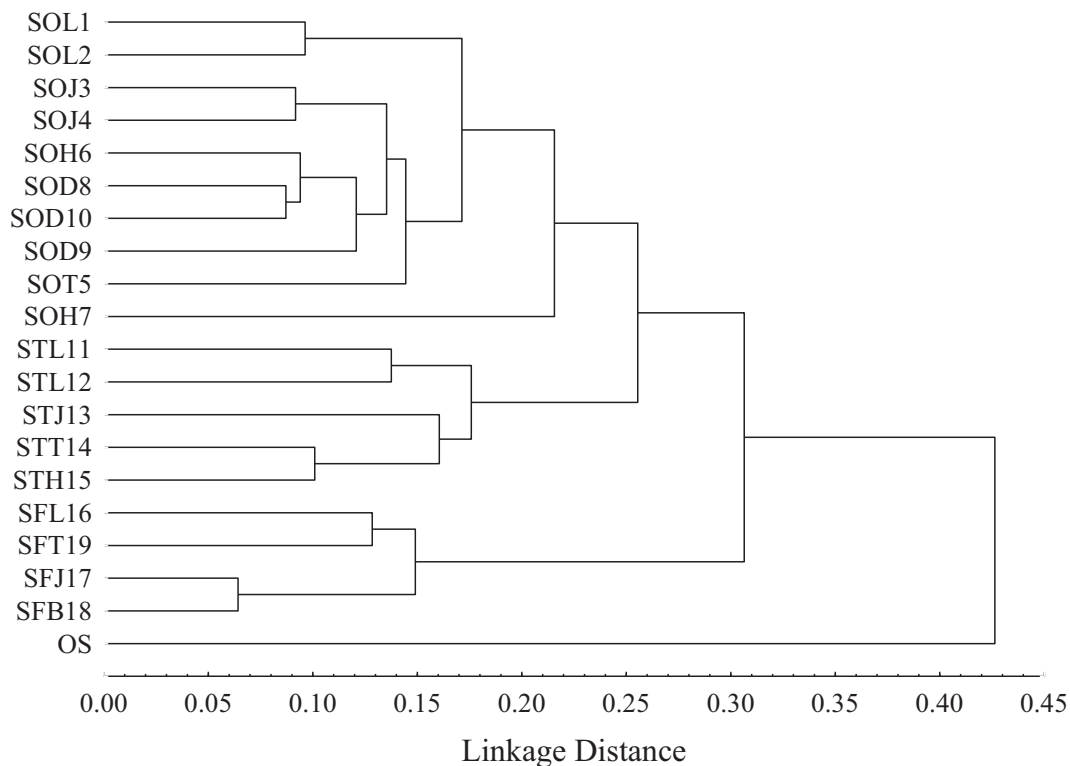| Genotype	SOL1	SOL2	SOJ3	SOJ4	SOT5	SOH6	SOH7	SOD8	SOD9	SOD10	STL11	STL12	STJ13	STT14	STH15	SFL16	SFJ17	SFB18	SFT19	OS
SOL1	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOL2	0.10	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOJ3	0.14	0.11	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOJ4	0.16	0.13	0.09	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOT5	0.21	0.18	0.14	0.16	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOH6	0.18	0.12	0.09	0.13	0.11	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOH7	0.27	0.27	0.21	0.23	0.22	0.19	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-
SOD8	0.22	0.18	0.13	0.15	0.17	0.10	0.19	0.00	-	-	-	-	-	-	-	-	-	-	-	-
SOD9	0.21	0.19	0.17	0.17	0.17	0.13	0.16	0.11	0.00	-	-	-	-	-	-	-	-	-	-	-
SOD10	0.20	0.15	0.10	0.14	0.12	0.09	0.21	0.09	0.12	0.00	-	-	-	-	-	-	-	-	-	-
STL11	0.28	0.26	0.22	0.25	0.21	0.22	0.31	0.26	0.27	0.22	0.00	-	-	-	-	-	-	-	-	-
STL12	0.30	0.28	0.25	0.26	0.24	0.24	0.32	0.30	0.26	0.26	0.14	0.00	-	-	-	-	-	-	-	-
STJ13	0.27	0.25	0.22	0.22	0.23	0.24	0.34	0.26	0.26	0.22	0.18	0.16	0.00	-	-	-	-	-	-	-
STT14	0.27	0.27	0.26	0.24	0.28	0.28	0.33	0.30	0.33	0.25	0.18	0.22	0.18	0.00	-	-	-	-	-	-
STH15	0.23	0.22	0.17	0.19	0.21	0.21	0.28	0.24	0.28	0.20	0.12	0.19	0.14	0.10	0.00	-	-	-	-	-
SFL16	0.30	0.32	0.28	0.28	0.30	0.29	0.35	0.31	0.32	0.28	0.25	0.29	0.24	0.24	0.20	0.00	-	-	-	-
SFJ17	0.33	0.36	0.31	0.33	0.36	0.35	0.37	0.38	0.36	0.35	0.32	0.33	0.32	0.28	0.26	0.17	0.00	-	-	-
SFB18	0.32	0.33	0.29	0.30	0.31	0.33	0.37	0.35	0.36	0.30	0.29	0.32	0.29	0.26	0.24	0.14	0.06	0.00	-	-
SFT19	0.30	0.31	0.28	0.28	0.31	0.32	0.36	0.33	0.36	0.29	0.26	0.33	0.25	0.24	0.23	0.13	0.17	0.13	0.00	-
OSL	0.41	0.43	0.41	0.44	0.46	0.45	0.47	0.46	0.46	0.43	0.41	0.44	0.40	0.39	0.34	0.42	0.44	0.43	0.39	0.00

Fig. (2). TU-DAMD clustering analysis constructed based on PDV value among the three studied *Salvia* species.

Table 6. Nei and Li genetic similarity (GS) index among the three studied *Salvia* species based on TU-DAMD data.

Genotype	SOL1	SOL2	SOJ3	SOJ4	SOT5	SOH6	SOH7	SOD8	SOD9	SOD10	STL11	STL12	STJ13	STT14	STH15	SFL16	SFJ17	SFB18	SFT19	OS
SOL1	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOL2	0.84	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOJ3	0.78	0.83	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOJ4	0.75	0.79	0.86	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOT5	0.67	0.73	0.80	0.76	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOH6	0.72	0.82	0.87	0.81	0.84	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOH7	0.61	0.63	0.72	0.68	0.71	0.75	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
SOD8	0.67	0.74	0.82	0.78	0.77	0.87	0.76	1.00	-	-	-	-	-	-	-	-	-	-	-	-
SOD9	0.68	0.73	0.77	0.75	0.76	0.83	0.79	0.85	1.00	-	-	-	-	-	-	-	-	-	-	-
SOD10	0.69	0.78	0.86	0.80	0.83	0.87	0.73	0.88	0.84	1.00	-	-	-	-	-	-	-	-	-	-
STL11	0.50	0.56	0.63	0.57	0.66	0.66	0.53	0.61	0.58	0.64	1.00	-	-	-	-	-	-	-	-	-
STL12	0.51	0.57	0.61	0.58	0.63	0.64	0.55	0.57	0.62	0.61	0.76	1.00	-	-	-	-	-	-	-	-
STJ13	0.54	0.59	0.65	0.63	0.63	0.62	0.51	0.62	0.60	0.65	0.67	0.74	1.00	-	-	-	-	-	-	-
STT14	0.51	0.52	0.56	0.57	0.53	0.53	0.50	0.53	0.46	0.59	0.65	0.60	0.66	1.00	-	-	-	-	-	-
STH15	0.58	0.61	0.70	0.66	0.65	0.66	0.57	0.62	0.56	0.67	0.76	0.67	0.74	0.80	1.00	-	-	-	-	-
SFL16	0.52	0.52	0.58	0.58	0.55	0.58	0.52	0.57	0.55	0.61	0.58	0.55	0.61	0.59	0.66	1.00	-	-	-	-
SFJ17	0.49	0.46	0.55	0.51	0.49	0.51	0.51	0.48	0.51	0.51	0.49	0.51	0.50	0.52	0.58	0.76	1.00	-	-	-
SFB18	0.49	0.48	0.56	0.53	0.53	0.52	0.49	0.50	0.49	0.56	0.51	0.50	0.53	0.55	0.59	0.79	0.91	1.00	-	-
SFT19	0.48	0.49	0.54	0.52	0.50	0.50	0.47	0.51	0.45	0.54	0.52	0.45	0.55	0.54	0.57	0.79	0.74	0.79	1.00	-
OSL	0.33	0.33	0.37	0.30	0.31	0.34	0.34	0.34	0.33	0.36	0.30	0.29	0.34	0.30	0.40	0.35	0.34	0.33	0.34	1.00

4. DISCUSSION

DNA genetic variation among *Salvia officinalis* L. (SO), and its related species of *S. tomentosa* Mill. (ST) and *S. fruticosa* Mill. (SF), collected from different geographical regions in Syria, has been assessed using the TU-DAMD assay.

For *S. officinalis*, the application of the TU-DAMD marker showed a polymorphism level (P%) of 72.662% among the ten studied samples. Whereas, other reports revealed that this value for the same species varied between 32.03%-90%. Our data were compared with those reported by other investigations. In this regard, it was recorded to be 90% [14], 80.681% [3], 63.54% [13], 59.5% [11], 57.2% [10], and 32.03% [39], respectively. This difference could be attributed to the following factors: I. Studied population type and size, wherein the current study, samples were introduced, cultivated, and domesticated under different climatic conditions varied from dry to wet; while, for the other investigations, genetic diversity was carried out on natural populations. II. Marker system employed and primers number, wherein the current study, TU-DAMD marker was employed, whereas in the other investigations, RAPD [3, 10, 11] and ISSR [14, 39] have been employed. It has been demonstrated that species, geographical distribution, selection, and cross-pollination are considered the main factors affecting genetic diversity in *Salvia* species [40]. Genetic diversity observed in *S. officinalis* species in the current study compared to other ones has been summarized in Table **7**.

Overall, the current study revealed P% to be 72.662, 70.374, and 51.429% for SO, ST, and SF species, respectively. This observation has been reported to be consistent with previous and recent published reports on the *Salvia* genetic diversity at the species level. Regarding this index, our data was found to be between 32.03% in *S. officinalis* [39] and

95.6% in *S. lachnostachys* [19]. reported in the related literature.

Table 7. A comparative study between the genetic diversity of *S. officinalis* species in the current study and other ones.

Molecular Marker	Results	P%	References
17 TU-DAMD	-	72.66	Current study
RAPD	88 TB and 71 PB (ranged between 4-13 bands) with an average of 8.9	80.68	[3]
8 SSR	165 total alleles (ranging from 13-30) and PIC ranging between 0.63-0.94, with an average of 0.81	-	[9]
39 RAPD	TB ranging between 2-13 bands and PB ranging between 0-12 bands	57.20	[10]
RAPD	-	59.50	[11]
9 SSR	125 TB (ranging between 8-21 loci) and PIC ranging between 0.70-0.92, with an average of 0.841	63.54	[13]
ISSR	-	90.00	[14]
4 AFLP PCs	-	63.54	[15]
16 ISSR	128 TB (ranging between 3-16 bands) and 41 PB (ranging between 1-5 bands)	32.03	[39]

Echeverrigaray and Agostini [11] reported P% to be 59.5% within *S. officinalis* only and to be 73% when *S. officinalis* and *S. sclarea* were introduced together in genetic analysis, using the RAPD marker. Whereas, Boszrmenyi *et al*. [10] reported P% to be 57.2% in *S. officinalis* and 83.6% when *S. judaica*

was introduced in the genetic analysis using the RAPD marker. Moreover, Mader *et al.* [12] reported genetic variability among 19 accessions of *S. officinalis* using SNP and SSR markers. They reported that the AMOVA test revealed 51% of the variance between the populations and 49% within the principal component analysis and that the samples were clustered in the main cluster. Whereas, Radosavljević *et al.* [6] reported a mean PIC of 0.802 in wild and cultivated populations of common sage *S. officinalis* L. using SSR loci. Indeed, Radosavljević *et al.* [13] reported a mean PIC of 0.841 in *Salvia officinalis* L. natural population using SSR loci.

Liber *et al.* [3] reported a P% of 80.681% using the RAPD marker among ten natural populations of *S. officinalis*. They reported that molecular variance analysis (AMOVA) showed most DNA genetic variation to be related to differences among the studied samples within populations, and also that genetic differences were recorded among the populations. Whereas, Rešetnik *et al.* [9] reported a PIC average of 0.81 within *S. officinalis* population using the SSR loci. They reported clear genetic DNA polymorphism between wild and cultivated *S. officinalis* populations restricted from one geographical region using SSR markers.

Moreover, Sarrou *et al.* [14] reported a P% of 90% in 7 *S. officinalis* populations using the ISSR marker. Whereas, Altindal [39] reported a P% of 32.03% among 8 *S. officinalis* samples using the ISSR marker. Recently, Jug-Dujaković1 *et al.* [15] reported a P% average of 63.54% in *S. officinalis* using the AFLP marker.

Previously, Skoula *et al.* [16] reported 155 total bands among 48 *S. fruticosa* clones using the RAPD marker. Whereas, Leontaritou *et al.* [17] reported high genetic diversity within the same species using the microsatellites marker.

Tychonievich and Warner [41] reported that spontaneous hybrids occurred either in the wild or in cultivated type due to intentional crosses between *S. officinalis* and *S. lavandulifolia*, and also between *S. fruticosa* and *S. tomentosa*.

Genetic diversity in other *Salvia* species has also been investigated. In this regard, Cahill [18] reported DNA genetic diversity in *S. hispanica* L. using the RAPD marker as higher among wild types compared to domesticated and commercial types. Whereas, Song *et al.* [20] reported high genetic similarity reflecting low genetic diversity in *S. miltiorrhiza* using SRAP and ISSR markers. Indeed, Zhang *et al.* [21] reported the high importance of DNA genetic diversity of *S. miltiorrhiza* using ISSR marker in plant breeding programs. Moreover, Saleh [31] reported a P% of 82.911% combined with 0.264 and 2.269 for PIC and MI average values, respectively, in *S. tomentosa* using the TD-DAMD analysis.

Recently, Saleh [32] reported low genetic diversity (P%) in *S. judaica* (40.45%) and *S. palaestina* (42.31%) species, whereas a high genetic diversity of 90.00% has been recorded between the two mentioned species using the TU-DAMD marker.

Based upon TU-DAMD data presented herein, the current study revealed moderate genetic diversity (P%) of 72.662 and 70.374% for SO and ST species, respectively. Whereas, low genetic diversity of 51.429% has been recorded for SF species.

While high genetic diversity of 90.419% has been recorded across the three studied *Salvia* species.

Genetic diversity observed across the three studied *Salvia* species in the current study could be attributed to outcrossing process, as similarly reported for *S. officinalis* [8], or variation between wild or cultivated *S. officinalis* species [9], or to spontaneous hybrids occurring either in the wild or cultivated *Salvia* species [41]; it can also be due to an interspecific hybrid, as similarly reported in *S. divinorum* [23], or reproductive biology, gene flow, seed dispersal, and nature selection [19]. These events have encouraged efficient gene flow, leading finally to heterozygosity and genetic diversity expansion.

CONCLUSION

Genetic diversity across the three studied *Salvia* species (*S. officinalis* L., *S. tomentosa* Mill., and *S. fruticosa* Mill.) grown in different geographical regions in Syria has been investigated through the TU-DAMD marker. This marker successfully discriminates among the three studied *Salvia* species. Cluster analysis revealed that the three studied *Salvia* species were clustered in three main clusters, with each cluster associated separately with each species. Due to the recent success of the TU-DAMD marker for the assessment of the genetic diversity of *S. judaica, S. palaestina,* and *O. syriacum* L. species, it is worth noting to expand its employment in molecular studies in order to discover its effectiveness in genetic diversity assessment of other plants species. Over all, the current investigation could be considered as the first report highlighting the genetic relationships among the three prominent *Salvia* species grown in Mediterranean regions. The genetic diversity observed among the three studied *Salvia* species could be considered as a potential tool to be exploited in *Salvia* breeding programs. The current study provides a useful tool to be integrated with the genetic and phytochemical diversity of these species, providing a potential benefit to plant breeding programs.

LIST OF ABBREVIATIONS

AFLP	=	Amplified fragment length polymorphism
cpSSR's	=	Chloroplast simple sequence repeats
CTAB	=	Cetyltrimethylammonium bromide
DAMD	=	Directed amplification of minisatellite-region DNA
ISSR	=	Inter simple sequence repeats
MI	=	Marker index
P%	=	Percentage polymorphism
PB	=	Polymorphic bands
PIC	=	Polymorphic information content
PDV	=	Percent disagreement values
RAPD	=	Random amplified polymorphic DNA
SNP	=	Single nucleotide polymorphism
SRAP	=	Sequence-related amplified polymorphism
SSRs	=	Simple sequence repeats
TB	=	Total bands
TD-DAMD	=	Touch-down directed amplification of minisatellite DNA

TU-DAMD = Touch-up directed amplification of minisatellite DNA

UPGMA = Unweighted pair group mean arithmetic average

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article will be available from the author [B.S] upon request.

CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

[1]   Walker JB, Sytsma KJ, Treutlein J, Wink M. *Salvia* (Lamiaceae) is not monophyletic: Implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. Am J Bot 2004; 91(7): 1115-25.
[http://dx.doi.org/10.3732/ajb.91.7.1115] [PMID: 21653467]

[2]   Hedge IC. *Salvia* L. In: Davis PH, Ed. Flora of Turkey and the East Aegean islands 7. Edinburgh 1982; pp. 188-92.

[3]   Liber Z, Židovec V, Bogdanović S, *et al.* Genetic diversity of Dalmatian Sage (*Salvia officinalis* L.) as assessed by RAPD markers. ACS Agric Conspec Sci 2014; 79(2): 77-84.

[4]   Mouterde PSJ. Nouvelle Flore du Liban et de la Syria 1983; 3: 159-70.

[5]   Govaerts R. World Checklist of Selected Plant Families Database in ACCESS. Kew: The Board of Trustees of the Royal Botanic Gardens 2003; pp. 1-216203.

[6]   Radosavljević I, Jakse J, Javornik B, Satovic Z, Liber Z. New microsatellite markers for *Salvia officinalis* (Lamiaceae) and cross-amplification in closely related species. Am J Bot 2011; 98(11): e316-8.
[http://dx.doi.org/10.3732/ajb.1000462] [PMID: 22003176]

[7]   Ghorbani A, Esmaeilizadeh M. Pharmacological properties of *Salvia officinalis* and its components. J Tradit Complement Med 2017; 7(4): 433-40.
[http://dx.doi.org/10.1016/j.jtcme.2016.12.014] [PMID: 29034191]

[8]   Stojanović D, Aleksić JM, Jančić I, Jančić R. A Mediterranean medicinal plant in the continental Balkans: A plastid DNA-based phylogeographic survey of *Salvia officinalis* (*Lamiaceae*) and its conservation implications. Willdenowia 2015; 45(1): 103-18.
[http://dx.doi.org/10.3372/wi.45.45112]

[9]   Rešetnik I, Baričevič D, Batîr Rusu D, *et al.* Genetic diversity and demographic history of wild and cultivated/naturalised plant populations: Evidence from Dalmatian Sage (*Salvia officinalis* L., Lamiaceae). PLoS One 2016; 11(7): e0159545.
[http://dx.doi.org/10.1371/journal.pone.0159545] [PMID: 27441834]

[10]   Böszörményi A, Héthelyi É, Farkas Á, *et al.* Chemical and genetic relationships among sage (*Salvia officinalis* L.) cultivars and Judean sage (*Salvia judaica* Boiss.). J Agric Food Chem 2009; 57(11): 4663-7.
[http://dx.doi.org/10.1021/jf9005092] [PMID: 19449812]

[11]   Echeverrigaray S, Agostini G. Genetic relationships between commercial cultivars and Brazilian accessions of *Salvia officinalis* L. based on RAPD markers. Rev Bra de Plantas Medicinais, Botucatu 2006; 8: 13-7.

[12]   Mader E, Lohwasser U, Börner A, Novak J. Population structures of genebank accessions of *Salvia officinalis* L. (Lamiaceae) revealed by high resolution melting analysis. Biochem Syst Ecol 2010; 38(2): 178-86.
[http://dx.doi.org/10.1016/j.bse.2010.01.001]

[13]   Radosavljević I, Šatović Z, Jakse J, *et al.* Development of new microsatellite markers for *Salvia officinalis* L. and its potential use in conservation-genetic studies of narrow endemic *Salvia brachyodon* Vandas. Int J Mol Sci 2012; 13(12): 12082-93.
[http://dx.doi.org/10.3390/ijms130912082] [PMID: 23109901]

[14]   Sarrou E, Ganopoulos I, Xanthopoulou A, *et al.* Genetic diversity and metabolic profile of *Salvia officinalis* populations: Implications for advanced breeding strategies. Planta 2017; 246(2): 201-15.
[http://dx.doi.org/10.1007/s00425-017-2666-z] [PMID: 28314999]

[15]   Jug-Dujaković M, Ninčević T, Liber Z, Grdiša M, Šatović Z. *Salvia officinalis* survived *in situ* Pleistocene glaciation in 'refugia within refugia' as inferred from AFLP markers. Plant Syst Evol 2020; 306(2): 38.
[http://dx.doi.org/10.1007/s00606-020-01665-9]

[16]   Skoula M, Hilali IE, Makris AM. Evaluation of the genetic diversity of *Salvia fruticosa* Mill. clones using RAPD markers and comparison with the essential oil profiles. Biochem Syst Ecol 1999; 27(6): 559-68.
[http://dx.doi.org/10.1016/S0305-1978(98)00122-7]

[17]   Leontaritou P, Lamari FN, Papasotiropoulos V, Iatrou G. Morphological, genetic and essential oil variation of Greek sage (*Salvia fruticosa* Mill.) populations from Greece. Ind Crops Prod 2020; 150: 112346.
[http://dx.doi.org/10.1016/j.indcrop.2020.112346]

[18]   Joseph P C. Genetic diversity among varieties of Chia (Salvia hispanica L.). Genet Resour Crop Evol 2004; 51(7): 773-81.
[http://dx.doi.org/10.1023/B:GRES.0000034583.20407.80]

[19]   Erbano M, Schühli G, Santos É. Genetic variability and population structure of *Salvia lachnostachys*: Implications for breeding and conservation programs. Int J Mol Sci 2015; 16(12): 7839-50.
[http://dx.doi.org/10.3390/ijms16047839] [PMID: 25856679]

[20]   Song Z, Li X, Wang H, Wang J. Genetic diversity and population structure of *Salvia miltiorrhiza* Bge in China revealed by ISSR and SRAP. Genetica 2010; 138(2): 241-9.
[http://dx.doi.org/10.1007/s10709-009-9416-5] [PMID: 19844793]

[21]   Zhang Y, Li X, Wang Z. Diversity evaluation of *Salvia miltiorrhiza* using ISSR markers. Biochem Genet 2013; 51(9-10): 707-21.
[http://dx.doi.org/10.1007/s10528-013-9600-2] [PMID: 23712760]

[22]   Takano A. Taxonomic study on Japanese *Salvia* (Lamiaceae): Phylogenetic position of *S. akiensis*, and polyphyletic nature of S. lutescens var. intermedia. PhytoKeys 2017; 80(80): 87-104.
[http://dx.doi.org/10.3897/phytokeys.80.11611] [PMID: 28781560]

[23]   Casselman I. Genetics and phytochemistry of Salvia divinorum. Lismore, NSW: PhD thesis, Southern Cross University. 2016.

[24]   Sudarmono , Okada H. Genetic differentiations among the populations of *Salvia japonica* (Lamiaceae) and its related species. Hayati J Biosci 2008; 15(1): 18-26.
[http://dx.doi.org/10.4308/hjb.15.1.18]

[25]   Dizkirici A, Celep F, Kansu C, Kahraman A, Dogan M, Kaya Z. A molecular phylogeny of *Salvia euphratica sensu lato* (*Salvia* L., Lamiaceae) and its closely related species with a focus on the section Hymenosphace. Plant Syst Evol 2015; 301(10): 2313-23.
[http://dx.doi.org/10.1007/s00606-015-1230-1]

[26]   Ince AG, Karaca M. Genetic variation in common bean landraces efficiently revealed by Td-DAMD-PCR markers. Plant Omics 2011; 4(3): 220-7.

[27]   Ince AG, Karaca M. Species-specific touch-down DAMD-PCR markers for *Salvia* species. J Med Plants Res 2012; 6(9): 1590-5.

[28]   Deniz İG, Genç İ, Ince AG, *et al.* Taxonomic data supporting differences between *Allium elmaliense* and *Allium cyrilli.* Biologia 2013; 68(3): 373-83.
[http://dx.doi.org/10.2478/s11756-013-0163-9]

[29]   İnce AG, Karaca M. Td-DAMD-PCR assays for fingerprinting of commercial carnations. Turk J Biol 2015; 39: 290-8.
[http://dx.doi.org/10.3906/biy-1407-66]

[30]   Gocer EU, Karaca M. Genetic characterization of some commercial cotton varieties using Td-DAMD-PCR markers. J Sci Eng Res 2016; 3(4): 487-94.

[31]   Saleh B. Genetic diversity of *Salvia tomentosa* Miller (Lamiaceae) species assessment using Td-DAMD molecular marker. Acta Biol Szeged 2019; 63(2): 135-41.
[http://dx.doi.org/10.14232/abs.2019.2.135-141]

[32]   Saleh B. TU-DAMD employment for molecular characterization of *Salvia judaica* and *Salvia palaestina* species. Acta Biol Szeged 2021; 65(1): 11-6.
[http://dx.doi.org/10.14232/abs.2021.1.11-16]

[33]   Saleh B. Genetic diversity of *Origanum syriacum* L. (Lamiaceae) species through Touch-Up Direct Amplification of Minisatellite-region DNA (TU-DAMD) marker. Not Sci Biol 2022; 14(2): 11174.
[http://dx.doi.org/10.55779/nsb14211174]

[34]   Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 1987; 19: 11-5.

[35]   Statsoft 2003. Statistica (Data analysis software system), version 6. Statsoft Inc 2003.

[36]   Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci 1979; 76(10): 5269-73.
[http://dx.doi.org/10.1073/pnas.76.10.5269] [PMID: 291943]

[37]   Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 1980; 32(3): 314-31.
[PMID: 6247908]

[38]   Powell W, Morgante M, Andre C, *et al.* The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 1996; 2(3): 225-38.
[http://dx.doi.org/10.1007/BF00564200]

[39]   Altindal D. Determination of genetic diversity of natural sage populations in Muğla region of Turkey. Int J Environ Sci Technol 2019; 16(2): 1-6.

[40]   Sepehary-Javan Z, Rahmani F, Heidari R. Assessment of genetic variation of genus *Salvia* by RAPD and ISSR markers. Aust J Crop Sci 2012; 6: 1068-73.

[41]   Tychonievich J, Warner RM. Interspecific crossability of selected *Salvia* species and potential use for crop improvement. J Am Soc Hortic Sci 2011; 136(1): 41-7.
[http://dx.doi.org/10.21273/JASHS.136.1.41]