Egypt. J. Bot. **Vol. 59,** No.3, pp. 581-594 (2019)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Authentication of Ecological, Biochemical and Molecular Features for Some Lamiaceae Species from Saudi Arabia

Shawkat M. Ahmed[(1)#], Yassin M. Al-Sodany[(2)]
[(1)]Biology Department, Faculty of Education, Ain Shams University, Cairo, Egypt; [(2)]Botany Department, Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt.

THE FAMILY Lamiaceae (Labiatae) contains several genera, such as sage (*Salvia*), mint (*Mentha*) and basil (*Ocimum*) with a rich diversity of ethno botanical uses. It has an important role as a source of medicinal and aromatic compounds of commercial importance. Medicinal plants represent an important health and economic component of biodiversity. It is essential to make the complete inventory of the medicinal component of the flora of any country for conservation and sustainable use. The conservation of the threatened and endangered medicinal species in the wild is indispensable. Therefore, protein, isozymes, RAPD and ISSR markers were employed for molecular characterization, variability evaluation and genetic relationships of 12 Lamiaceae species collected from various areas Taif region, Saudi Arabia. All biochemical and molecular markers revealed high polymorphism percentages among the studied genotypes. The UPGMA clusters result indicated that all genotypes could be distinguished by these markers. The polymorphism information obtained through biochemical and molecular analyses may also help for further studies of other Lamiaceae genotypes in Saudi Arabia.

Keywords: Biodiversity, Ecology, ISSR, Lamiaceae, Molecular marker, RAPD.

Introduction

The diversity of plant life plays an important role in maintaining the region's environmental stability and balance. Saudi Arabia contains one of the diverse floras of the Asian continent. The influences of the surrounding floristic regions can be seen in many parts of the plant diversity areas of Saudi Arabia (Schultz & Whitney, 1986; Miller & Nyberg, 1991). According to Collenette (1998) the greatest species diversity has been observed in Asir (Sarawatt) and Hijaz mountains, the western mountainous area, of which 76 species belong to family Lamiaceae. Most members of the family are perennial or annual herbs with significance to humans as fragrance, flavour, or exhibit medicinal properties.

The biochemical identification is useful to ensure the genetic purity of plant species, discriminating between their parental and hybrids, analyzing phylogenetic relationships, assessing the geographical origin of germplasm and describing a new species. Seed proteins and isozymes were investigated by several authors to measure genetic variation between Lamiaceae plants (Lopez-Pujol et al., 2004; Ali et al., 2011; Hnia et al., 2013). On the other hand, molecular approaches such as RAPD and ISSR, have revolutionized the field of DNA fingerprinting of plant genomes (Cervera et al., 1998) and in genetic diversity researches (Isshiki et al., 2008). Many authors used these techniques for initial evaluation of genetic variation in *Rosmarinus tomentosus* (Martiân & Bermejo, 2000), *Ocimum* spp. (Vieira et al., 2003), *Salvia deserti* (Echeverrigaray & Agostini , 2006), *Teucrium polium* (Boulila et al., 2010), *Mentha spicata* and *Mentha longifolia* (Al-Rawashdeh, 2011) and *Thymus kotschyanus* (Khoshsokhan et al., 2014).

#Corresponding author email: shamahmoh@gmail.com
Received 8/11/2018; Accepted 12/4/2019
DOI: 10.21608/ejbo.2019.6144.1246
Edited by: Prof. Dr. Adel El-Gazzar, Faculty of Science, El-Arish University, El-Arish, N. Sinai, Egypt.

Limited work has been done on the nature of genetic diversity and characterization of Lamiaceae species from Saudi Arabia. Therefore our present work aims to study the ecological characters of different habitats, the genetic variations and phylogenetic relationships between some Lamiaceae species to establish species database for the vegetable and related wild species scattered in Taif highlands.

Materials and Methods

Study area

Saudi Arabia extends over approximately 16º degrees of latitude, from 16º 22' at the borders with Yemen in the south; to 32º 14' at the Jordanian border in the north, and between 34º 29'E and 55º 40` E. Longitude. (Fig. 1). Taif region is located in the central foothills of the western mountains at an altitude of up to 2500m above sea level. It is an important place for the people due to its scenic views and fertile valleys which support the growth of a favorable fruits and vegetables. Over the years, vast areas of virgin lands have turned into agricultural lands, which resulted in the disappearance of many wild species including medicinal plants.

Climate of the area is tropical and arid. The monthly record according to Taif meteorological station (1997–2009) indicated that the monthly average of minimum and maximum ambient temperatures ranged from 7.9±1.2 to 23.4±0.8°C and 22.9±1.1 to 36.3±0.8°C, respectively with a total monthly mean of 23.2±5.1°C (Al-Sodany et al., 2016). The mean maximum temperature during 2006 to 2008 was 36.33±1.15°C, while average values from 1991 to 2005 were 33.60±3.03°C. During the same period, mean monthly humidity ranged from approximately 19.6±4.2 to 60.0±6.0%. The data from last 10 years shows considerable inter-annual variation in the monthly amount (range 4.3±5.7-294.1 ±383.8mm mo^{-1}) and timing of rainfall. The yearly amount of rainfall ranges from 83.3mm yr^{-1} in 2007 to 3312mm yr^{-1} in 2001.

Plant materials

Fresh leaves of 38 individuals of 4 cultivated species and 8 wild species (Table 1), varying from 2 to 4 per species belonging to family Lamiaceae were collected from Taif highlands of Saudi Arabia. The collected wild materials were identified according to Collenette (1998) as shown in Table 1 and Fig. 1.

Soil analysis

At each site, three soil samples randomly distributed were collected close to sampling species (rhizosphere) as a profile of 20cm depth and mixed as a composite sample. The soil sample was air dried and passed through 2mm sieve to separate gravel and debris. Soil-water extracts at 1:5 were prepared for the determination of carbonates, bicarbonates, Ca, Mg, EC (as a measure of salinity), pH, Cl and SO_4. Determination of carbonates and bicarbonates (as a measure of alkalinity) was carried out by the titration with 0.01N HCL, using phenol phethalein as indicator to carbonates and methyl orange as indicator to bicarbonates. EC and pH was measured with conductivity (mS cm^{-1}) and pH- meters. Chlorides were estimated by direct titration against silver nitrate using 5% potassium chromate as indicator. Sulphates were determined using the "gravimetric with ignition of residue method", where sulphates were precipitated in 1% HCl solution as barium sulphate by adding of barium chloride (10%), filtered, washed with hot distilled water, ignited at 800°C for two hours, then weighted as barium sulphate. Soil texture analysis was carried out using the Bouyoucos hydrometer method, whereby the percentage of gravel, sand, silt and clay were calculated. Total organic matter was determined by loss-on-ignition at 550°C for two hours. DTPA solution (diethylenetriaminepenta acetic acid) was used for the extraction of available heavy metals (Zn, Mn, Fe and Cu). Sodium Bicarbonate solution was used for the extraction of available P. Potassium sulphate solution was used for the extraction of available N. Atomic absorption was used for the determination of Zn, Mn, Fe and Cu. Estimation of Ca, Na and K was carried out by flame photometer. Molybdenum blue and indo-phenol blue methods were applied for the determination of P and N, respectively, using a spectrophotometer (Bear, 1975; Allen et al., 1986).

Protein electrophoresis

Leaves of each sample (1g) were homogenized with 1M Tris-HCl buffer to extract proteins using a mortar and pestle. Electrophoresis was carried using DISC SDS-PAGE method (Laemmli, 1970). Gels were stained overnight, destained and photographed.

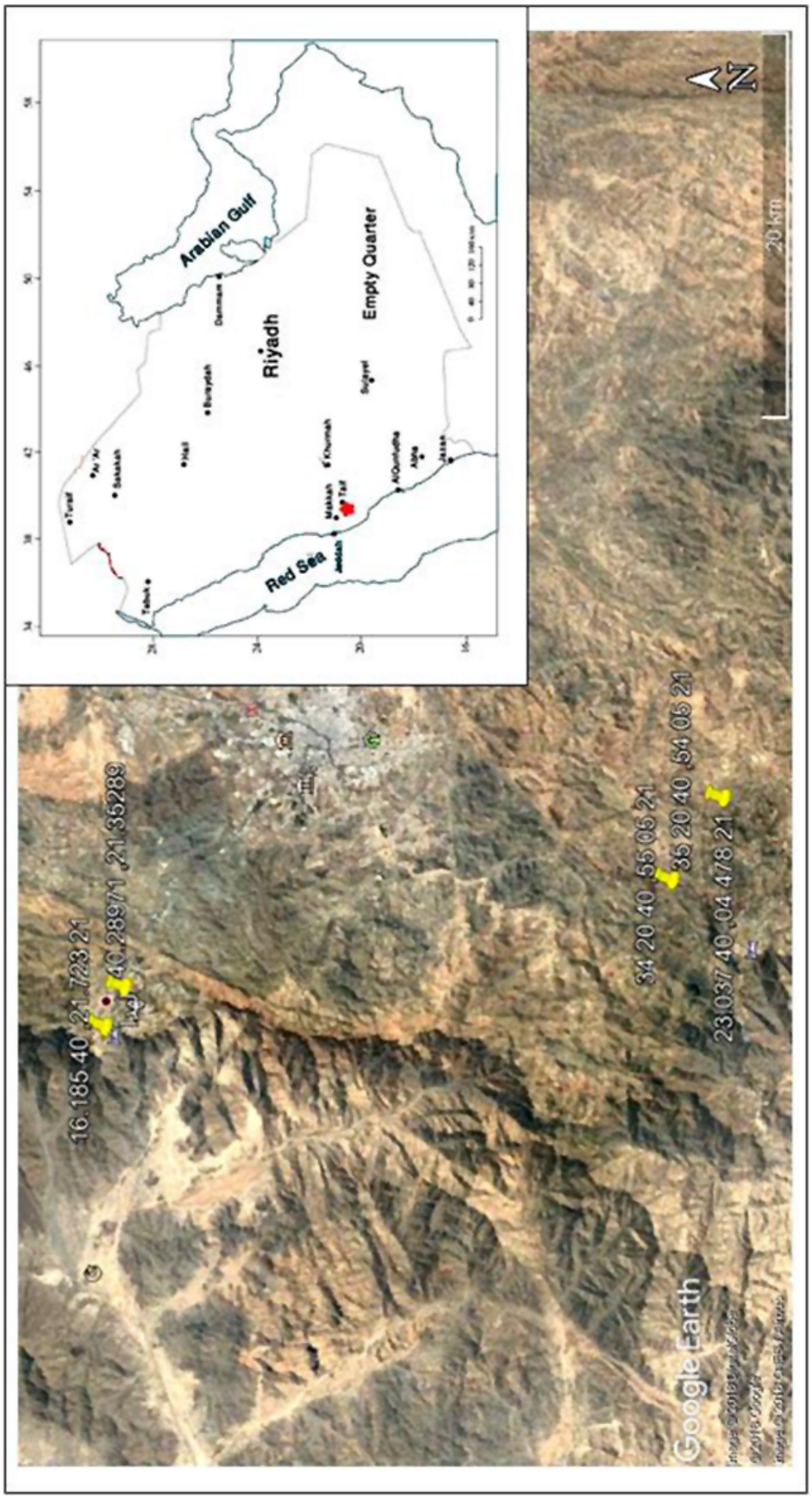

Fig. 1. Map showing the study area and sampled sites.

TABLE 1. Names and sources of twelve species of family Lamiaceae.

No.	Species	No. of samples	Type	Coordinates	
				Latitude (N)	Longitude (E)
1	*Lavandula pubescens* Decne.	1-3	wild	21 05 55	40 20 34
2	*Lavandula dentata* L..	4-7	wild	21 05 55	40 20 34
3	*Otostegia fruticosa* (Forssk. Schweinf. ex Penzig)	8-10	wild	21 05 54	40 20 35
4	*Mentha longifolia* L.	11-14	wild	21 04.478	40 23.037
5	*Marrubium vulgare* (Tourn.) L.	15-17	wild	21.35289	40.28971
6	*Teucrium polium* (L.) Tausch	18-20	wild	21 21.723	40 16.185
7	*Micromeria imbricata* (Forssk.)	21-23	wild	21 21.723	40 16.185
8	*Salvia deserti* Decne.	24-26	wild	21 21.723	40 16.185
9	*Mentha viridis* L.	27-29	cultivated	21 25.9366	40 29.5855
10	*Ocimum basilicum* L.	30-32	cultivated	21 25.9366	40 29.5855
11	*Origanum majorana* L.	33-35	cultivated	21 25.9366	40 29.5855
12	*Plectaranthus comosus* Sims.	36-38	cultivated	21 25.9366	40 29.5855

Isozyme electrophoresis

The investigated isozymes were: Acid phosphatase (ACP); (EC 3.1.3.2), alcohol dehydrogenase (ADH); (E.C. 1.1.1.1), aldehyde oxidase (AO); (E.C. 1.2.3.1), α-and β-esterases (EST); (E.C.3.1.1.1), malate dehydrogenase (MDH); (E.C.1.1.1.37) and peroxidase (PRX); (E.C.1.11.1.7). According to Stegemann et al. (1986), the supernatant of each sample was separated by 10% Native-polyacrylamide gel electrophoresis method. ACP, AO, ADH, MDH, PRX and α- & β-EST gels were stained according to protocols of Wendel & Weeden (1989), Weeden & Wendel (1990), Jonathan & Wendell (1990), Heldt (1997) and Scandalios (1964), respectively.

DNA isolation and amplification

Fresh leaves of Lamiaceae species were used for DNA isolation using CTAB method (Doyle & Doyle, 1987). To obtain different DNA markers, each species was analyzed with random (RAPD) and specific ISSR primers (Table 2). A PCR amplification for each sample was done with a 25µl total reaction/sample that included 10µl Taq Master Mix, 1µl each, forward and reverse primers, and 1µl DNA. Thermal cycling was done with the following program: 105°C heated lid, initial denaturation of 94°C for 5min, and 35 cycles (1min at 94°C), annealing (45sec at different temperatures) and extension (1min at 72°C) and a final extension at 72°C for 8min. Primers details are provided in Table 2.

TABLE 2. List of the investigated primers of RAPD and ISSR.

Parameter	Primer	Sequences
RAPD	OPO-08	5-CCTCCAGTGT-3
	OPO-09	5-TCCCACGCAA-3
	OPO-10	5-TCAGAGCGCC-3
	OPO-11	5-GACAGGAGGT-3
	OPO-13	5-GTCAGAGTCC-3
ISSR	HB 11	$(GT)_6CC$
	HB 12	$(CAC)_3GC$
	HB 13	$(GAG)_3GC$
	HB 14	$(CTC)_3GC$
	HB 15	$(GTG)_3GC$

Statistical analysis

The levels of polymorphism were estimated by dividing the polymorphic bands by the total number of scored bands for biochemical and molecular analyses. The differences in intensities of bands were not considered. The presence or absence of each biochemical and molecular band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to construct dendrograms between the studied samples using unweighted pair group method with arithmetic average (UPGMA) using SAHN and TREE modules, respectively depending on NTSYS-pc 2.2 program (Rohlf, 1998).

Results

Ecological features

Soil analysis

As indicated in Table 3, soil of *Mentha viridis* had the highest values of organic matter (6.0±1.5%), chlorides (21.8±21.9m.eq/L), Ca^{++} (6.3±3.8m.eq/L), Mg++ (3.9±2.3m.eq/L), Na^{+} (16.5±16.5m.eq/L), P (62.4±47.9mg kg soil) and K (410.5±364.2mg kg soil). The soil of *Teucrium polium, Micromeria imbricata*, *Salvia deserti* and *Marrubium vulgare* had the highest value of sulphate (5.0m.eq/L), K^{+} (1.2m.eq/L) and Mn (33.6mg/kg soil) but the lowest of chlorides, Na^{+}, Fe, Cu, gravel, EC and organic matter (4.1, 3.6m. eq/L, 10.1, 1.1mg/kg soil, 27.0, 0.9 and 0.3%, respectively). Soil of *Origanum majorana* had the highest bicarbonates (1.2±0.3m.eq/L) and gravel (38.4±3.0%), but the lowest of silt and clay (14.6±4.5 and 5.4±2.4%, respectively). Soil of *Ocimum basilicum* had the highest of Zn, Fe and N (9.1±5.3, 57.2±61.7 and 292.3±265.3mg/kg soil, respectively) and the lowest of sulphate, Ca^{++} and Mg^{++} (3.2±1.0, 3.1±1.0 and 1.6±0.4m. eq./L, respectively). Soil of *Otostegia fruticosa* had the highest of clay (10.1±4.4%) and the lowest of pH (7.4±0.2), while that of *Mentha longifolia* had the highest of Cu (7.0±9.1mg/kg soil) and silt (23.5±1.1%) and the lowest of bicarbonates, K^{+}, Mn, K , N and sand (0.8±0.4, 0.4±0.1m. eq./L, 5.3±4.5, 69.4±69.4, 82.3±83.1mg/kg soil and 36.8±3.7%, respectively).

Biochemical features

SDS-protein analysis

Figure 2 demonstrates the SDS-protein profile of Lamiaceae genotypes. A maximum number of 34 bands, which were not necessarily present in all the studied genotypes, were detected at approximately molecular weights ranging between 125.22 to 10.17kDa. The resulted profile comprised one monomorphic band at 66.88kDa and could be used as a common band for this family. The band at about 22.47kDa was recorded only in *Otostegia fruticosa*, while bands with apparent molecular weights 116.33, 95.60, 37.01, 30.16, 26.25, 18.96, 18.37 and 10.97kDa were scored in all individuals of each of *Mentha longifolia, Lavendula dentata, Otostegia fruticosa and Marrubium vulgare,* respectively and could be considered as specific bands for these genotypes. High polymorphism percentage (97%) was scored among the studied genotypes (Table 4).

Isozyme analysis

The seven isozymes (Fig. 3) produced polymorphism percentages ranging between 83 to 100% (Table 4). Three bands at Rf 0.29, 0.54 and 0.76 were specific for *Mentha longifolia* (lanes 11-14) in acid phosphatase profile. Alcohol dehydrogenase scored the lowest number of bands (3). Only one band at Rf 0.14 characterized *Otostegia fruticosa* (lanes 8-10) in aldehyde oxidase pattern. The resulted α-esterase pattern recorded the highest number of bands (13). The band at Rf 0.05 distinguished the cultivated genotype; *Mentha viridis* (lanes 27-29), whereas bands at Rf 0.18 and 0.33 characterized *Lavendula dentata* (lanes 4-7). Three bands discriminated *Otostegia fruticosa* from other genotypes at Rf 0.59, 0.67 and 0.71. In β-esterase pattern, two bands at Rf 0.64 and 0.93 were recognized as unique bands in *Otostegia fruticosa* and *Plectranthus comosus* (lanes 36-38), respectively. As in α-esterase pattern, *Lavendula dentata* was also distinguished by two specific bands at Rf 0.18 and 0.30.

Malate dehydrogenase pattern revealed one monomorphic band and *Plectranthus comosus* was discriminated from other genotypes by the band at Rf 0.07. Peroxidase enzyme identified three polymorphic bands with polymorphism percentage (100%). Although alcohol dehydrogenase, aldehyde oxidase and peroxidase isozymes detected 100% polymorphism, they are considered less efficient than both α- and β-esterases isozymes (Table 4).

UPGMA tree

The cluster (Fig. 4) indicated that the genotypes could be distinguished by SDS-PAGE and isozymes markers. A dendrogram divided the 12 genotypes into 3 main clusters at similarity coefficient (1.17). The first category (I) included *Otostegia fruticosa* and *Marrubium vulgare*. The second category (II) were further divided into two subclusters (IIa and IIb) at similarity coefficient (1.08). Subcluster (IIa) comprised *Teucrium polium, Micromeria imbricata* and *Salvia deserti*. *Plectranthus comosus* was found as a separate clade (IIb). The third group (III) was also divided into two subclusters (IIIa and IIIb), the first grouped *Mentha viridis Origanum majorana* and *Ocimum basilicum,* whereas, the other included *Lavendula pubescens* and *Lavendula dentata. Mentha longifolia* was separated in an independent clade at coefficient 1.30.

TABLE 3. Mean±standard deviation of some anions and cations of soil of studied species of family Lamiaceae.

Species		*Lavandula pubescens*	*Lavandula dentata*	*Otostegia fruticosa*	*Mentha longifolia*	*Teucrium polium*	*Micromeria imbricata*	*Salvia deserti*	*Marrubium vulgare*	*Mentha viridis*	*Ocimum basilicum*	*Origanum majorana*	Total
Gravel		29.3±5.3	29.3±5.3	30.6±4.4	31.0±4.2	27	27	27	27	32.3±6.0	36.8±5.3	38.4±3.0	32.0±5.3
Sand		38.1±2.3	38.1±2.3	37.0±5.7	36.8±3.7	45.3	45.3	45.3	45.3	41.3±1.9	38.9±3.5	41.6±4.4	39.8±4.4
Silt	%	23.1±1.6	23.1±1.6	22.4±3.5	23.5±1.1	20.9	20.9	20.9	20.9	18.6±1.3	17.3±5.3	14.6±4.5	20.2±4.2
Clay		9.6±1.4	9.6±1.4	10.1±4.4	8.8±4.3	6.8	6.8	6.8	6.8	8.0±2.8	7.0±3.4	5.4±2.4	8.0±3.1
O.M		3.9±5.2	3.9±5.2	4.2±5.3	2.1±0.9	0.3	0.3	0.3	0.3	6.0±1.5	3.6±4.5	3.4±2.7	3.2±3.5
pH		7.5±0.1	7.5±0.1	7.4±0.2	7.7±0.0	7.5	7.5	7.5	7.5	7.9±0.1	7.7±0.1	7.7±0.0	7.6±0.2
EC	dS m^{-1}	1.8±0.7	1.8±0.7	1.0±0.2	1.3±0.1	0.9	0.9	0.9	0.9	2.8±2.3	1.1±0.4	1.4±0.2	1.4±0.8
HCO_3^-		0.9±0.1	0.9±0.1	0.9±0.3	0.8±0.4	0.9	0.9	0.9	0.9	1.0±0.4	0.9±0.2	1.2±0.3	0.9±0.2
Cl^-		12.6±7.2	12.6±7.2	5.7±2.0	8.2±0.8	4.1	4.1	4.1	4.1	21.8±21.9	6.5±3.1	8.6±1.6	8.7±7.0
SO_4^{--}		4.6±0.1	4.6±0.1	3.7±1.3	4.0±0.7	5	5	5	5	4.7±1.8	3.2±1.0	4.5±1.1	4.2±1.0
Ca^{++}	m.eq L^{-1}	4.5±0.7	4.5±0.7	3.3±0.9	3.9±0.4	3.2	3.2	3.2	3.2	6.3±3.8	3.1±1.0	3.8±0.5	3.8±1.3
Mg^{++}		2.2±0.5	2.2±0.5	1.6±0.5	1.9±0.2	2	2	2	2	3.9±2.3	1.6±0.4	2.2±0.4	2.1±0.8
Na^+		9.5±7.4	9.5±7.4	4.0±1.0	6.9±0.8	3.6	3.6	3.6	3.6	16.5±16.5	5.0±2.6	7.5±1.7	6.8±5.5
K^+		0.9±0.1	0.9±0.1	0.9±0.4	0.4±0.1	1.2	1.2	1.2	1.2	0.9±0.6	0.9±0.1	0.8±0.2	0.8±0.3
P		15.0±4.4	15.0±4.4	23.7±12.3	24.0±5.7	29.3	29.3	29.3	29.3	62.4±47.9	40.6±19.2	50.3±34.2	32.6±22.7
K		97.0±2.5	97.0±2.5	143.2±114.5	69.4±69.4	127	127	127	127	410.5±364.2	129.8±113.5	258.8±152.9	162.6±144.9
N		99.8±22.3	99.8±22.3	255.2±180.8	82.3±83.1	420	420	420	420	252.0±96.5	292.3±265.3	269.1±66.5	242.3±158.7
Zn	mg kg^{-1}	1.2±1.5	1.2±1.5	3.6±2.9	5.6±6.8	2.9	2.9	2.9	2.9	2.6±0.7	9.1±5.3	1.8±1.0	3.6±3.7
Mn		11.1±6.8	11.1±6.8	28.6±25.9	5.3±4.5	33.6	33.6	33.6	33.6	13.1±3.1	10.0±8.1	6.1±2.7	16.7±15.7
Fe		29.3±19.4	29.3±19.4	31.6±45.0	39.1±56.4	10.1	10.1	10.1	10.1	23.3±20.5	57.2±61.7	42.0±39.0	32.8±36.7
Cu		4.2±3.9	4.2±3.9	2.9±3.1	7.0±9.1	1.1	1.1	1.1	1.1	1.2±0.9	3.2±0.7	1.3±1.0	2.9±3.7

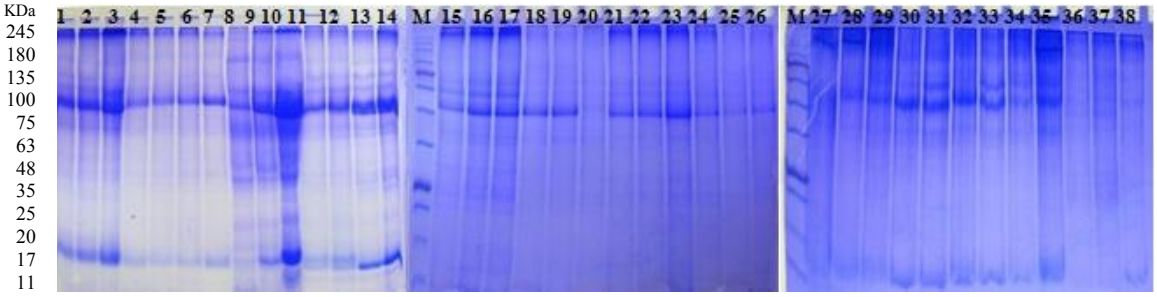

Fig. 2. SDS-protein banding pattern of Lamiaceae species. M:Marker.

TABLE 4. Number and types of bands as well as the percentage of the total polymorphism generated by the biochemical and molecular approaches for Lamiaceae species.

Approach	Parameter	Monomorphic bands	Polymorphic bands		Total bands	Polymorphism %
			Non-unique bands	Unique bands		
Protein	SDS-PAGE	1	32	1	34	97
Isozyme	ACP	0	7	0	7	100
	ADH	0	3	0	3	100
	AO	0	4	0	4	100
	α-EST	0	11	2	13	100
	β-EST	0	8	2	10	100
	MDH	1	5	0	6	83
	PX	0	3	0	3	100
RAPD	OPO-08	3	8	2	13	77
	OPO-09	3	7	0	10	70
	OPO-10	5	5	1	11	54.5
	OPO-11	0	12	9	21	100
	OPO-13	2	3	1	6	75
ISSR	HB-11	1	12	9	22	95
	HB-12	0	9	2	11	100
	HB-13	2	5	0	7	71
	HB-14	0	12	10	22	100
	HB-15	0	8	5	13	100

Molecular features

RAPD analysis

Different levels of polymorphism were observed among the genotypes (Fig. 5). The maximum value of 100% was recorded by primer OPO-11, while the lowest value was 54.5% and scored in primer OPO-10. 13 monomorphic bands were detected. A sum of 48 polymorphic bands were generated by these primers, from them 13 unique bands were identified and could be used to discriminate among the studied genotypes (Table 4). The size of the amplified fragments ranged from about 887.7bp (OPO-09) to 237.8 bp (OPO-11) across the profiles generated by the five primers. The pattern of OPO-08 scored two unique ones distinguishing *Salvia deserti* at 421.1 and 403.2 bp. OPO-10 primer recorded one unique band in *Mentha longifolia* at 398.2bp, whereas, primer OPO-11 generated 7 unique bands; two in *Origanum majorana* at about molecular sizes of 631.3 and 515.3bp; two in *Origanum majorana* and *Lavendula dentata* at 569.2 and 391.2bp respectively, the later 3 in *Plectranthus comosus* at 276.9, 251.6 and 237.8bp. Only one unique band was detected in *Otostegia fruticosa* at 716.7bp by primer OPO-13.

ISSR analysis

High levels of polymorphism were observed in profiles (Table 4 and Fig. 6). The values ranged from 71 to 100% detecting the importance of ISSR as a molecular marker technique. A

maximum of 22, 11, 7, 22 and 13 DNA bands were scored in the ISSR profiles generated by the primers HB-11, 12, 13, 14 and 15, respectively. Only, three monomorphic bands were scored. The size of the amplified fragments ranged from about 1173.1bp (HB-12) to 95.6bp (HB-14) across the profiles generated by the five primers. A sum of 72 polymorphic bands was generated, of them 26 unique bands were identified. All genotypes were discriminated by one or more unique bands.

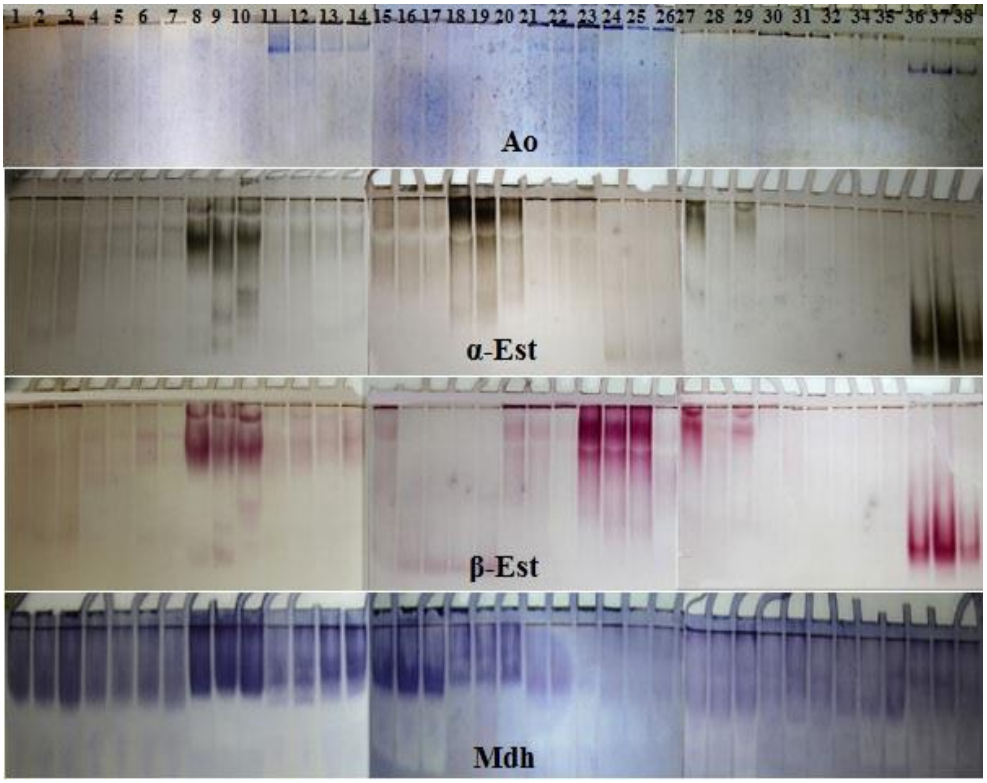

Fig. 3. Isozyme banding patterns of Ao, α-est, β-est and Mdh for Lamiaceae species.

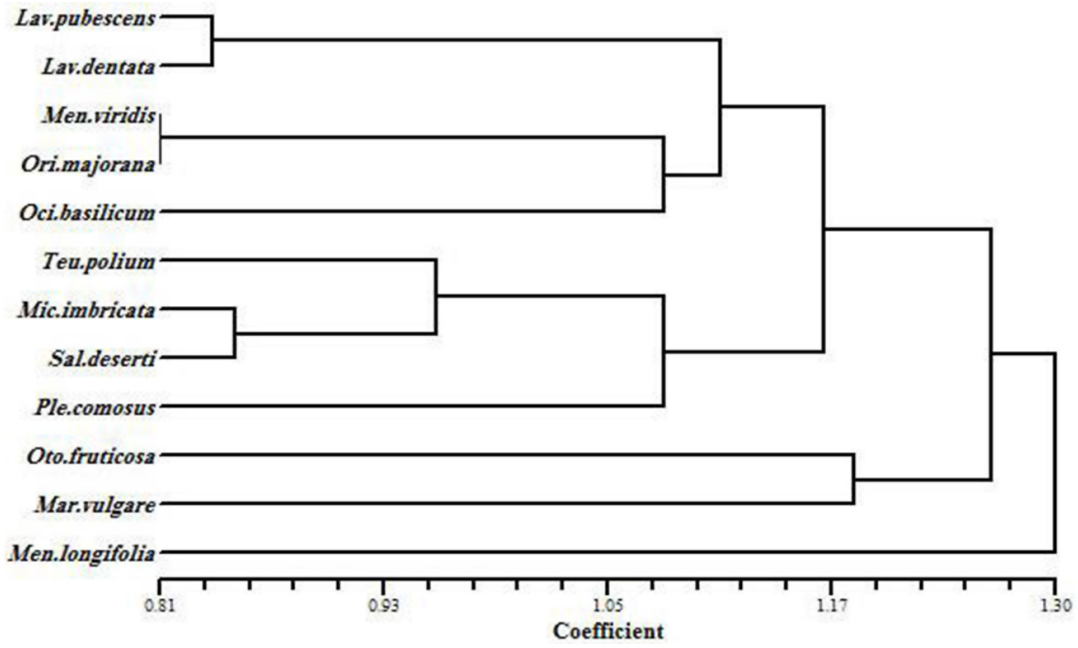

Fig. 4. UPGMA- phenogram based on SDS-PAGE and isozymes data of 12 Lamiaceae species.

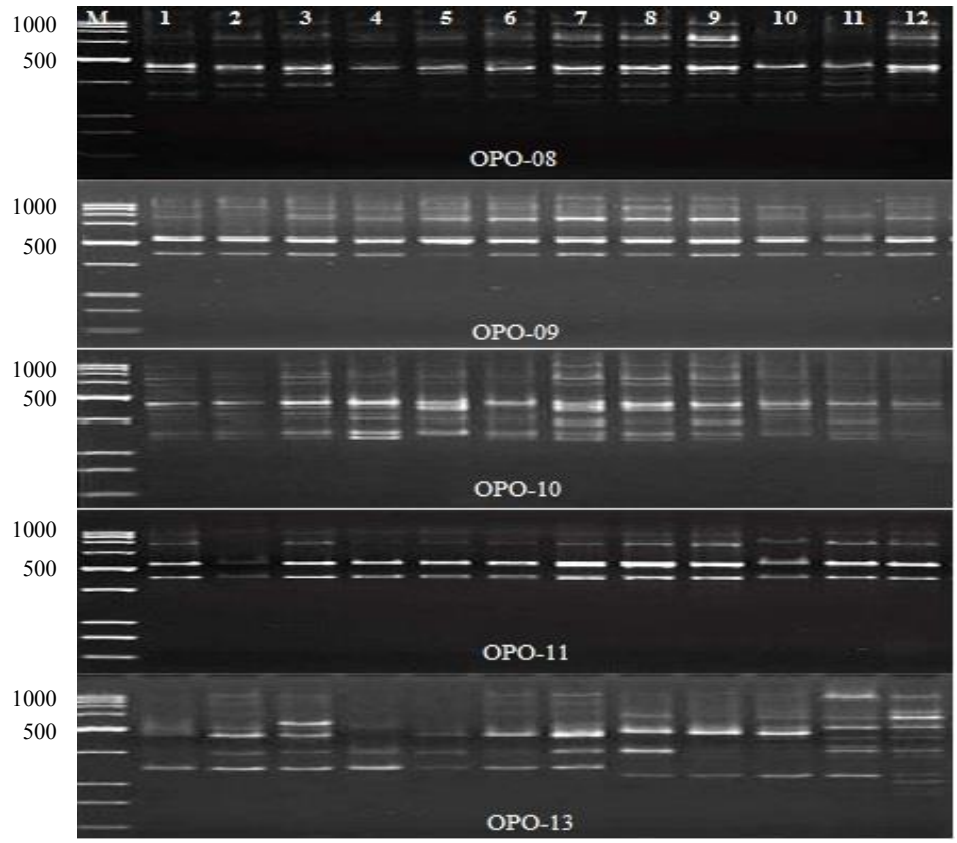

Fig. 5. RAPD fingerprints of 12 Lamiaceae species.

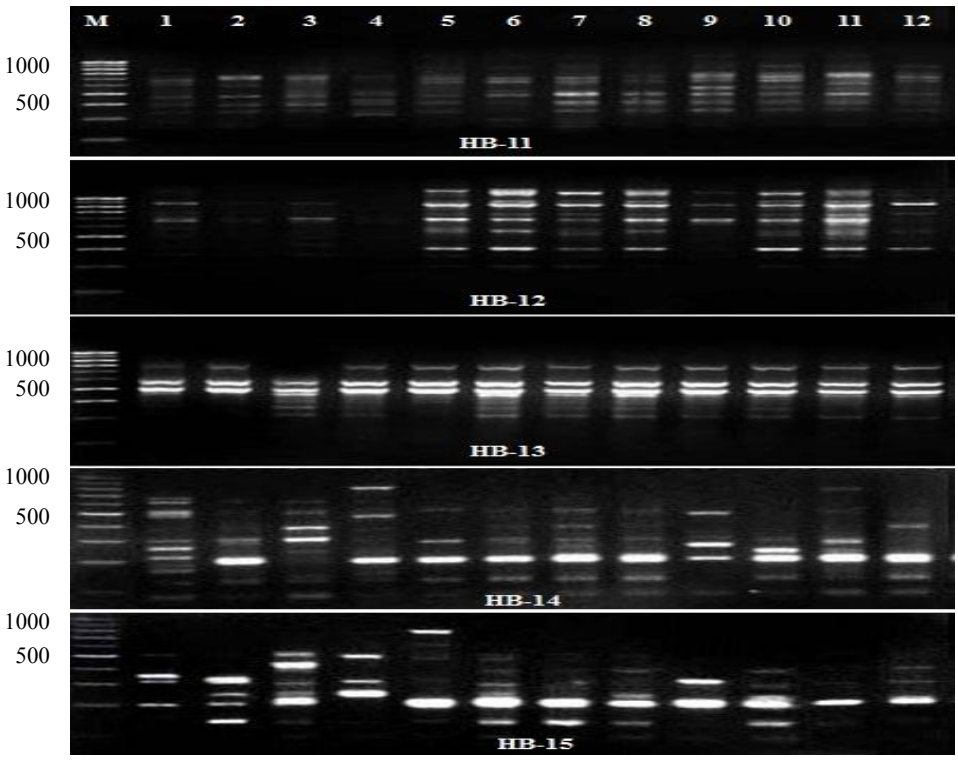

Fig. 6. ISSR fingerprints of 12 Lamiaceae species.

UPGMA tree

A dendrogram based on UPGMA analysis grouped Lamiaceae genotypes into one main cluster and single genotype; *Lavendula pubescens,* formed a separate operational taxonomic units (OTU) in cluster at coefficient 1.18 (Fig. 7). Genotypes within main cluster were further divided into 4 subclusters at coefficient 1.00. The first comprised *Ocimum basilicum* with *Otostegia fruticosa.* The second included *Mentha viridis* and *Plectranthus comosus.* The third grouped *Micromeria imbricata, Mentha longifolia, Origanum majorana* and *Salvia deserti together,* whereas, the fourth included *Lavendula dentate, Teucrium polium* and *Marrubium vulgare.*

Discussion

The soil of *Mentha viridis* had the highest values of organic matter, chlorides, Ca^{++}, Mg^{++}, Na^{+}, P and K may be due to the fertilizers added to soil in cultivated lands. This confirmed by the study of Weisskopf et al. (2010) who reported that the soil compactness is an important component of land degradation syndrome and is a significant challenge facing advanced agriculture that adversely affects nearly all soil properties:physical, chemical and biological. When soil is compacted, its structure is altered by crushing aggregate units, reducing the size of pore spaces between the soil particles, reduction in soil volume and total porosity that leads to increase in soil bulk density and penetration resistance. Soil compaction refers to the formation of dense layers of well filled that occurs on cultivated layer, even more, the compressive forces are applied to compressible soil from wheels (Hamza & Anderson, 2005; Massah & Azadagan, 2016). In case of *Mentha viridis*, Stanev & Zheljazkov (2004) indicated that K is the element that is found in the greatest concentration in mint plant tissue followed by N and P. In this study, the highest concentration was N, followed by K and P, due to the accumulation of nutrients which have a direct relationship with the ionic concentration present in the nutrient solution (Garlet & Santos, 2008). This may be due to the response of the plant to the extraction of N that depends on the supply thereof in the nutrient solution (Xu et al., 2012). In this regard, Baranauskiene et al. (2003) indicated that nitrogen fertilization increases fresh biomass yield when the N dose is doubled. On the other hand, the soil of *Teucrium polium, Micromeria imbricata*, *Salvia deserti* and *Marrubium vulgare* had the lowest of chlorides, Na^{+}, Fe, Cu, gravel, EC and organic matter because it is very shallow soil due to its rocky habitats in the slope of mountains. The soil of *Ocimum basilicum* had the highest of Zn, Fe and N. This indicated that this species may be a good accumulator of heavy metals. The plant response to heavy metals in soil depends on the plant species, the total soil metal concentration, and on the bioavailability of the metal itself depending on physico-chemical properties of soils (Boularbah et al., 2006).

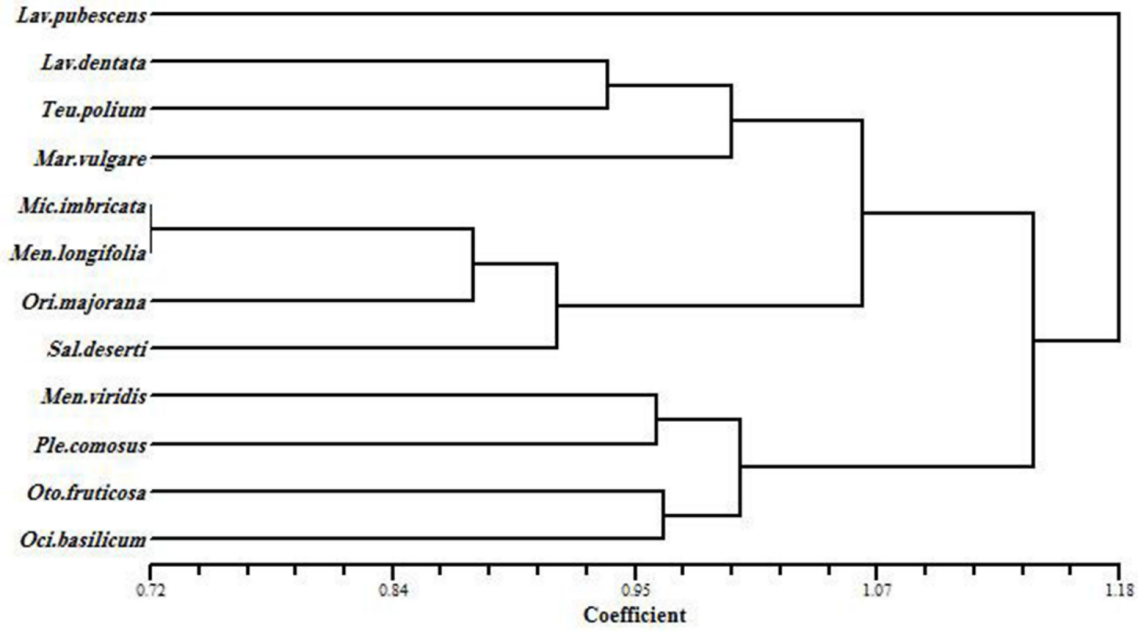

Fig. 7. UPGMA- phenogram based on RAPD and ISSR data of 12 Lamiaceae species.

The electrophoretic profile of SDS-protein detected apparent variations among the genotypes that were enough to differentiate among them. The high level of protein polymorphism could be attributed to the non conservative nature of the leaf protein that it easily affected by the environmental conditions. This conclusion was in accordance with those of Erum et al. (2011) and Ibrahim et al. (2011). On the other hand, due to higher percentages of polymorphism, it was obvious that isozymes provided sufficient polymorphic expressions to distinguish Lamiaceae genotypes. Furthermore, the combination of the obtained data of the utilized isozyme systems seemed to be sufficient for their complete identification. The same conclusion was reached by Lopez-Pujol et al. (2004) and Hnia et al. (2013). The current study is the first to report the use of seven enzymes for the identification of different species of Lamiaceaein Taif of Saudi Arabia, several studies have also demonstrated the usefulness of isoenzyme phenotypes to support and extend taxonomic characterization (Medina et al., 2004; Collet et al., 2005).

Furthermore, RAPD and ISSR-PCR approaches succeeded to generate polymorphic and reproducible amplification products. This result was in accordance with Shinwari et al. (2011) who detected that RAPD was efficient in detecting polymorphism and genetic variation within and between *Mentha spicata* and *Mentha royleana*. Also, these high levels of variability were detected in *Thymus* ssp. (Ali et al., 2012), *Ocimum* spp. (Lal et al., 2012) and *Salvia lachnostachys* (Erbano et al., 2015). The high percentages of differences obtained by each of the ten primers could be interpreted on the bases of variations that may be present in the repetitive DNA more than in the expressed DNA within the studied genotypes. Also, these authors reported that RAPD markers tend to reside in regions with many repeated sequences and therefore in non-coding regions, which are more susceptible to mutations than the coding ones (Irwin et al., 1998). Both RAPD and ISSR molecular markers were proved to be efficient to provide molecular data for Lamiaceae species (Al-Rawashdeh, 2011; Khoshsokhan et al., 2014). Furthermore, the ISSR method has been reported to be more reproducible than the RAPD approach. The previous result was in agreement with Goulao & Oliveira (2001), Chowdhury et al. (2002) and Abdelmigid (2012) and could be explained through different mechanisms generating genetic variation (Lalhruaitluanga & Prasad, 2009) and the higher mutation rate in the loci of ISSR (Moghaddam et al., 2010). For this reason, ISSR markers are suitable for discriminating similar genotypes, although, little information indicated that ISSR markers are functionally important (Esselman et al., 1999). These differences may also be attributed to marker sampling errors and/or the percent of polymorphism detected by different markers (Gajera et al., 2010).

Using cluster analyses depending on biochemical, molecular data matrices, the Lamiaceae genotypes were categorized into different clusters. These results showed the extensive genetic diversity existed in these markers used during this study. Al-Rawashdeh (2011) mentioned that *Mentha spicata* and *Mentha longifolia* were genetically different from *Ziziphora tenuior*. Moreover, Javan et al. (2012) obtained a dendrogram that produced five and four groups among eight species of *Salvia* based on RAPD and ISSR analyses, detecting high genetic distance at inter-species level. The inconsistence of the resulted dendrograms with the realistic taxonomic grouping of the studied taxa may be interpreted on the bases of high differences recorded in our genotypes.

Conclusions

The results indicated that SDS-protein, isozymes, RAPD and ISSR approaches were useful in the establishment of the genetic fingerprinting and preliminary database for the cultivated and the related wild species of family Lamiaceae scattered in Taif, Saudi Arabia. These techniques detected enough polymorphism in the studied genotypes and distinguished each genotype from all others by at least one unique band or a group of combined class patterns. The ISSR method has been reported to be more reproducible than the RAPD approach. The polymorphism information obtained through biochemical and molecular analyses may also help for further studies of other Lamiaceae genotypes in Saudi Arabia.

References

Abdelmigid, H.M. (2012) Efficiency of random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers for genotype fingerprinting and genetic diversity

studies in canola (*Brassica napus*). *African Journal of Biotechnology,* **11**, 6409-6419.

Ali, I.B., Guetat, A., Boussaid, M. (2011) Genetic diversity and structure of wild Tunisian *Thymus capitatus* (L.) Hoffm. et Link. (Lamiaceae) assessed using isozyme markers. *African Journal of Ecology,* **50**, 140-151.

Ali, I.B., Guetat, A., Boussaid, M. (2012) Inter-specific relationships among two Tunisian *Thymus* taxa: *Thymus capitatus* Hoffm. et Link. and *Thymus algeriensis* Boiss. et Reut. using molecular markers. *African Journal of Biotechnology,* **11**(36), 8810-8819.

Allen, D.O., Ahmed, B., Naseer, K. (1986) Relationships between cyclic AMP levels and lipolysis in fat cells after isoproterenol and forskolin stimulation. *Journal of Pharmacology and Experimental Therapeutics,* **238**, 659-664.

Al-Rawashdeh, I.M. (2011) Molecular taxonomy among *Mentha spicata*, *Mentha longifolia* and *Ziziphora tenuior* populations using the RAPD technique. *Jordan Journal of Biological Sciences,* **4**(2), 63-70.

Al-Sodany, Y.M., Al-Juaid, N.S., Kahil, A.A. (2016) Ecology of invasive species *Calotropis procera* (Ait) R.Br. in Saudi Arabia. *International Journal of Ecotoxicology and Ecobiology*, **1**(3), 127-140.

Baranauskiene, R., Venskutonis, P.R., Vikellis, P., Drambrauskien, E. (2003) Influence of nitrogen fertilizer on yield and composition of thyme (*Thymus vulgaris*). *Journal of Agricultural and Food Chemistry,* **51**(26), 7751-7758.

Bear, F.E. (1975) "*Chemistry of the Soil*". Oxford & IBH Publishing Co., New Delhi, India, 515p.

Boularbah, A., Schwartz, C., Bitton, G., Aboudrar, W., Ouhammou, A., Morel, J.L. (2006) Heavy metal contamination from mining sites in South Morocco: 2. Assessment of metal accumulation and toxicity in plants. *Chemosphere,* **63**, 811-817.

Boulila, A., Be´jaoui, A., Messaoud, C., Boussaid, M. (2010) Genetic diversity and population structure of *Teucrium polium* (Lamiaceae) in Tunisia. *Biochemical Genetics,* **48**, 57-70.

Cervera, M.T., Cabezas, J.A., Sancha, J.C., Martínez de Toda, F., Martínez- Zapater, J.M. (1998) Application of AFLPs to the characterization of grapevine *Vitis vinifera* L. genetic resources. A case study with accessions from Rioja (Spain). *Theoretical and Applied Genetics,* **97**, 51-59.

Chowdhury, M.A., Vandenberg, B., Warkentin, T. (2002) Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L). *Euphytica,* **127**, 317-325.

Collenette, S (1998) A checklist of botanical species in Saudi Arabia. International Asclepiad Society, Burgess Hill, England.

Collet, S.A.O., Collet, M.A., Machado, M.D.F.P.S. (2005) Differential gene experession for isoenzymes mutants of *Vitis vinifera* L. (Vitaceae). *Biochemical Systematics and Ecology,* **33**, 691-703.

Doyle, J.J., Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin,* **19**, 11-15.

Echeverrigaray, S., Agostini,. G (2006) Genetic relationships between commercial cultivars and Brazilian accessions of *Salvia officinalis* L. based on RAPD markers. *A Revista Brasileira de Plantas Medicinalis,* **8**, 13-17.

Erbano, M., Schühli, G.S., dos Santos, É.P. (2015) Genetic variability and population structure of *Salvia lachnostachys*: Implications for breeding and conservation programs. *International Journal of Molecular Sciences,* **16**, 7839-7850.

Erum, S., Naeemullah, M., Masood, S., Khan, M.I. (2011) Genetic variation in the living repository of *Ocimum* germplasm. *Pakistan Journal of Agricultural Research,* **24**, 1-4.

Esselman, E.J., Li, J.Q., Crawford, D., Winduss, J.L., Wolfe, A.D. (1999) Clonal diversity in the rare *Calamagrostis porteri* ssp. *Insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. *Molecular Ecology,* **8**, 443-451.

Gajera, B.B., Kumar, N., Singh, A.S., Punvar, B.S., Ravikiran, R., Subhash, N., Jadeja, G.C. (2010) Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers.

Industrial Crops and Products, **32**, 491-498.

Garlet, T.M.B., Santos, O.S. (2008) Nutrient solution and mineral composition of three mint species grown in hydroponic system. *Ciência Rural,* **38** (5), 1233-1239.

Goulao, L., Oliveira, C.M. (2001) Molecular characterization of cultivars of apple (*Malus domestica* Borkh.) using microsatellite (SSR and ISSR) markers. *Euphytica,* **122**, 81-89.

Hamza, M.A., Anderson, W.K. (2005) Soil compaction in cropping systems: a review of the nature, causes, and possible solutions. *Soil & Tillage Research,* **82**, 121-145.

Heldt, W.H. (1997) A leaf cell consists of several metabolic compartments. "*Plant Biochemistry and Molecular Biology*". Oxford Univ. Press, Oxford, UK.

Hnia, C., Yosr, Z., Mohamed, B. (2013) Genetic diversity of natural Tunisian *Lavandula multifida* L. (Lamiaceae) populations assessed by allozymes and random amplification of polymorphic DNA (RAPD). *African Journal of Biotechnology,* **12**(7), 648-657.

Ibrahim, S.K., Ibrahim, L., Ismail, A., Basal, A., Kayal, M., Ghanem, H., Rammel, S. (2011) Differentiation of different species of *Origanum* and *Thymus* using proteins and isoenzymes profile. *International Journal of Botany,* **7**(4), 283-288.

Irwin, S.V., Kaufusi, P., Banks, K., de la Pena, R., Cho, J.J. (1998) Molecular characterization of taro (*Colocasia esculenta*) using RAPD markers. *Euphytica,* **99**, 183-189.

Isshiki, S., Iwataa, N., Khana, M.M.R. (2008) ISSR variations in eggplant (*Solanum melongena* L.) and related *Solanum* species. *Science Horticulture,* **117**, 186-190.

Javan, Z.S., Rahmani, F., Heidari, R. (2012) Assessment of genetic variation of genus *Salvia* by RAPD and ISSR markers. *Australian Journal of Crop Science* **6**(6), 1068-1073.

Jonathan, F.W., Wendell, N.F. (1990) Visualization and interpretation of plant allozyme. In: "*Allozymes in Plant Biology*", DE Soltis and PS Sottis (Eds.), pp. 5-45. Champan and Hall Press, London, UK.

Khoshsokhan, F., Babalar, M., Fatahi, M., Poormeidani, A. (2014) Assessment of genetic diversity of some wild populations of *Thymus kotschyanus* using RAPD molecular markers. *Cercetări Agronomiceîn Moldova,* **17**(3), 71-81.

Lal, S., Mistry, K.N., Thaker, R., Shah, S.D., Vaidya, P.B. (2012) Genetic diversity assessment in six medicinally important species of *Ocimum* from central gujarat (india) utilizing RAPD, ISSR and SSR markers. *International Journal of Advanced Biological Research,* **2**(2), 279-288.

Lalhruaitluanga, H., Prasad, M.N.V. (2009) Comparative results of RAPD and ISSR markers for genetic diversity assessment in *Melocanna baccifera* Roxb. growing in Mizoram state of India. *African Journal Biotechnology,* **8**(22), 6053-6062.

Laemmli, U. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680-685.

Lopez-Pujol, J., Bosch, M., Simon, J., Blanche, C. (2004) Allozyme diversity in the tetraploid endemic *Thymus loscosii* (Lamiaceae). *Annals of Botany,* **93**, 323-332.

Martiân, J.P., Bermejo, E.H.N. (2000) Genetic variation in the endemic and endangered *Rosmarinus tomentosus* Huber-Morath & Maire (Lamiaceae) using RAPD markers. *Heredity,* **85**, 434-443.

Massah, J., Azadagan, B. (2016) Effect of chemical fertilizers on soil compaction and degradation. *Agricultural Mechanization in Asia, Africa and Latin America,* **47**(1), 44-50.

Medina, R.B., Katz, M.B., Gonzalez, S. (2004) Differentiation of lactic acid bacteria strains by post electrophoretic detection of esterases. *Methods in Molecular Biology*, **268**, 459-463.

Miller, A.G., Nyberg, J.A. (1991) Patterns of endemism in Arabia. *Flora et Vegetatio Mundi,* **9**, 263-279.

Moghaddam, M., Mohammadi, M.S., Mohebalipour, N., Toorchi1, M., Aharizad, S., Javidfar, F. (2010) Assessment of genetic diversity in rapeseed cultivars as revealed by RAPD and microsatellite markers. *African Journal of Biotechnology,* **8**(14), 3160-3167.

Rohlf, F.J. (1998) NTSYSpc: Numerical Taxonomy

and Multivariate Analysis Systems, version 2.02. Exeter Software, New York, USA.

Scandalios, J.C. (1964) Tissue-specific allozyme variations in maize. *Journal of Heredity,* **55**, 281-285.

Schultz, E., Whitney, J.W. (1986) Vegetation in north-central Saudi Arabia. *Journal of Aird Environment*, **10**, 175-186.

Shinwari, Z.K., Sultan, S., Mahmood, T. (2011) Molecular and morphological characterization of selected *Mentha* species. *Pakistan Journal of Botany,* **43**(3), 1433-1436.

Stanev, S., Zheljazkov, V.D. (2004) Study on essential oil and free menthol accumulation in 19 cultivars populations and clones of peppermint (*Mentha* x *piperita*). *Acta Horticulturae,* **629**, 149-152.

Stegemann, H., Afifiy, A.M.R., Hussein, K.R.F. (1986) Identification of date (*Phoenix dectylifera*) cultivars by protein patterns. *Phytochemistry,* **26**(1), 149-153.

Vieira, R.F., Goldsbrough, P., Simon, J.E. (2003) Genetic diversity of basil (*Ocimum* spp.) based on RAPD markers. *American Society for Horticultural Science*, **128**(1), 94-99.

Weeden, N.F., Wendel, J.F. (1990) Genetics of plant isozymes. In: "*Isozymes in Plant Biology*", D.E. Soltis, P.S. Soltis (Eds.), pp. 46-72. Chapman and Hall Press, London, UK.

Weisskopf, P., Reiser, R., Rek, J., Oberholzer, H.R. (2010) Effect of different compaction impacts and varying subsequent management practices on soil structure, air regime and microbiological parameters. *Soil & Tillage Research,* **111**(1), 65-74.

Wendel, J.F., Weeden, N.F. (1989) Visualization and interpretation of plant allozymes. In: "*Allozymes in Plant Biology, Advances in Plant Sciences*", Soltis, D.E., Soltis, P.S. (Eds.), series 4, pp. 5–45. Dioscorides Press, Portland, OR, USA.

Xu, G., Fan, X., Miller, A.J. (2012) Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology,* **63**, 153-182.

توثيق الصفات البيئية والبيوكيماوية والجزيئية لبعض أنواع العائلة الشفوية من المملكة العربية السعودية

شوكت محمود أحمد[1]، يس محمد السودانى[2]

[1]قسم العلوم البيولوجية والجيولوجية - كلية التربية- جامعة عين شمس - القاهرة - مصر، [2]قسم النبات - كلية العلوم - جامعة كفر الشيخ - كفر الشيخ - مصر.

تضم العائلة الشفوية العديد من الأجناس النباتية مثل نباتات السالفيا والنعناع والريحان....الخ والتى تمتاز بالكثير من الإستخدامات الإقتصادية الهامة، فهى تمثل مصدر أساسى لكثير من الصناعات الدوائية والكيميائية. ولذا كان من الضروري عمل قائمة تشمل النباتات ذات الأهمية الطبية في أي بلد لحفظ تلك الأصول النباتية من الإختفاء ولضمان الإستخدام المستدام لها. ومن ثم استخدمنا فى ذلك البحث كاشفات البروتين والمشابهات الإنزيمية وقطع الدنا العشوائية والمتكررة للتوصيف الجزيئي وتقييم الإختلافات والعلاقات الوراثية لبعض أنواع العائلة الشفوية التى تم تجميعها من مناطق مختلفة فى المملكة العربية السعودية. وأظهرت نتائج الكاشفات البيوكيماوية والجزيئية نسب عالية من التباينات بين الأنواع موضع الدراسة. وأشارت علاقات القرابة إلى إمكانية التمييز بين الأنواع من خلال الكاشفات المستخدمة بما يعكس أهميتها وقدرتها على التمييز بين الأنواع داخل العائلة الشفوية مدعما مجالات تصنيف وتوثيق هذه الأنواع. وتساعد معلومات تعدد الأشكال التي تم الحصول عليها من خلال التحليلات البيوكيماوية والجزيئية في إجراء المزيد من الدراسات المتقدمة لهذه الأنواع مع ضم أنواع أخرى تابعة للعائلة الشفوية في المملكة العربية السعودية.