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TAXONOMIC VARIATION AMONG *SCHINUS MOLLE* L. PLANTS ASSOCIATED WITH A SLIGHT CHANGE IN ELEVATION

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Abstract

This study examined the degree of variations in DNA fingerprints associated with slight altitudinal change of *Schinus molle* grown in Abha region, Saudi Arabia. Seven populations from *Schinus molle* plants located at 2193.0, 2246.0, 2197.7, 2441.0, 2372.0, 2250.6 and 2175.0 meters had been investigated. The degree of genetic variability was evaluated using random amplified polymorphic DNA (RAPD), mixed RAPD and inter-simple sequence repeat markers (ISSR). The genetic similarity coefficients from RAPD analysis revealed the maximum similarity value (89.9%) was between population at 2250.6 m and population at 2175.0 m. The genetic similarity coefficients from mixed RAPD primers displayed the highest similarity value (87.6%) between population at 2246.0 m and population at 2197.7 m. Similarity coefficients from ISSR analysis revealed the highest similarity value (86.2%) among populations at 2193.0 m, 2246.0 m, 2441.0 m and at 2250.6 m. Super tree analysis (RAPD + mixed RAPD + ISSR) showed the highest similarity value (85.5%) between population at 2441.0 m and population at 2250.6 m. In conclusion, marker systems including RAPD, mixed RAPD and ISSR, alone or combined can be effectively used in determining the genetic relationship among *Schinus molle* plants even at very close populations.

Introduction

Abha region has a specialized environmental condition among all other areas in the Kingdom of Saudi Arabia which have an indirect effect on the weed plants growth. *S. molle* plants (family, Anacardiaceae) are among the most common weed in Saudi Arabia especially in Tharawat Mountains. The tree of *S. molle* plant is an evergreen, dioecious, grows up to 20 meters in height. Flowers are small, with yellowish white petals and all plant parts especially fruits having strong aroma (Lim, 2012). *S. molle* is common weed in South America and recently into many tropical and subtropical countries (Olafsson *et al*., 1997). In Abha region, *S. molle* tree has been planted in many areas as in valleys, public gardens and for house decorations. After that, the plant became a common weed in many areas of Abha city growing beside road and next the wall of houses as it is reproduced by seeds. *S. molle* plant showed to be resistant to the harsh environmental condition such as high temperature, cold and increasing soil salinity (Lim, 2012). In addition, *S. molle* plants usually used for the restoration of degraded areas and showed tolerance to heavy metals (Doganlar *et al*., 2012; Pereira *et al*., 2016). Toward this approach examine the genetic diversity of *S. molle* plant is highly needed as no reports available.

In recent years, a number of randomly amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR) and inter-simple sequence repeat–polymerase chain reaction (ISSR-PCR) markers had been used to study genetic diversity among plant species. For example, RAPD

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technique was successfully applied genetically to distinguish among *Ocimum* spp. (Vieria *et al*., 2003), to study the genetic diversity in *Monodora myristica* (Uyoh *et al*., 2014) and various population of *Ziziphus spina-christi* L. (Moustafa *et al*., 2016). ISSR technique was used to study genetic diversity of the *Lens* spp. (Fikiru *et al*., 2007), and genetic relationships of *Chukrasia* spp. (Wu *et al*., 2014).

Therefore, the aim of this research is to study genetic diversity of *S. molle* plants growing at close locations in Abha region, KSA. To the best of our knowledge, there are few reports indicating the use of mix primer to estimate the genetic diversity among plant /or to study plant DNA fingerprint. Therefore, this study also aimed to check the status of DAN fingerprints using mixed primers.

Materials and Methods

Plant material

Seven locations at various elevations in Abha region, KSA,include 2193.0, 2246.0, 2197.7, 2441.0, 2372.0, 2250.6 and 2175.0 meters have been selected (Fig. 1). At each site, random samples of young fresh leaves from *S. molle* trees having a height 1500 cm had been collected.

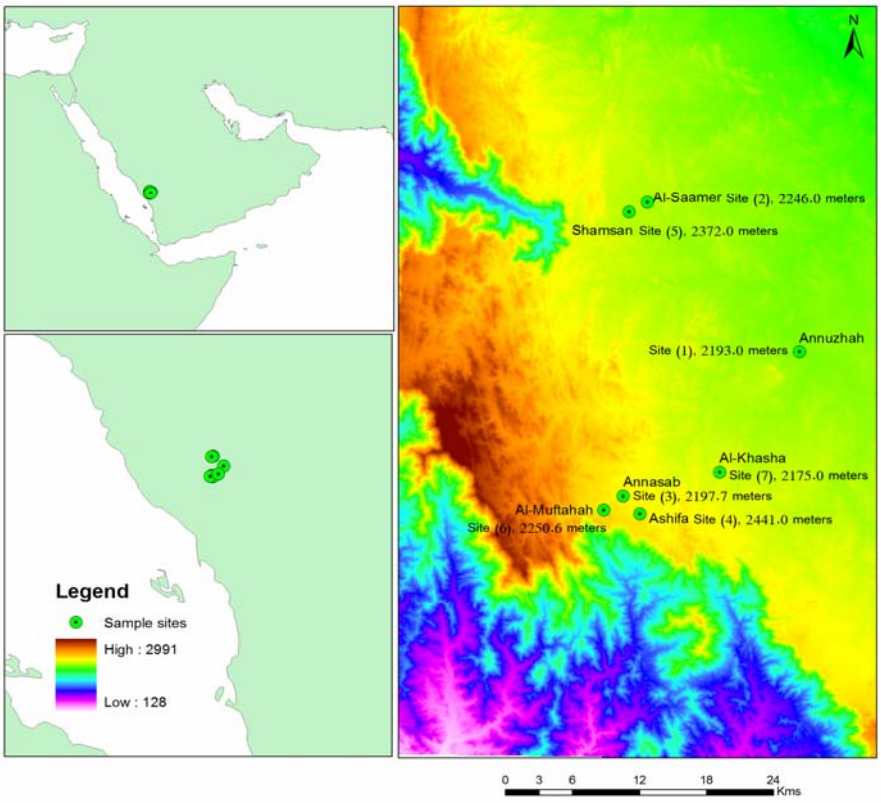

Fig. 1.Sampling sites in Abha region**,** KSA. Site (1), (2193.0); Site (2), (2246.0); Site (3), (2197.7); Site (4), (2441.0), Site (5), (2372.0), Site (6), (2250.6) and Site (7), (2175.0 m).

Extraction the genomic DNA from leaves of S. molle plants

Genomic DNA was extracted from fresh young leaves of *S. molle* plants by using DNeasy plant mini kit. DNA concentration was estimated by a Thermo Scientific™ BioMate 3S UV-Visible at 260 nm.

PCR amplification

Eight RAPD, nine ISSR and eight mixed RAPD markers were used in this study (Table 1). PCR reaction consists from 1 X GoTaq Green Master Mix, 4 µl from each primer, 20 ng of genomic DNA and nuclease-free water to get a final 25 µl volume. PTC 200 Peltier Thermal Cycler (MJ Research - USA) adjusted as follows: Initial degree at 94°C for 5 minutes followed by forty nine cycles at 92°C for 1 minute, primer annealing temperature at 29°C for 1 minute, extension at 72°C for 2 minutes and final process for primer extension at 72°C for 7 minutes. An equal amount of each amplified product of 20 ul was separated by electrophoresis using 1.3 % agarose gels in 0.5X TBE buffer. Stained gel with ethidium bromide was photographed by gel documentation system using UV transilluminator at 365 nm (Hashemi *et al*., 2009). Each experiment was repeated three times and molecular weight of RAPD-PCR, mixed RAPD-PCR and ISSR-PCR fragments were estimated using marker 1 kb DNA ladder between 250 to 10,000 bp.

Table 1. RAPD, mixed RAPD and ISSR primers.

RAPD primers	Sequence of primer (5' – 3')
Oligo 342	GAGATCCCTC
Oligo 345	GCGTGACCCG
Oligo 349	GGAGCCCCCT
Oligo 33	CCGGCTGGAA
OPK-8	GAACACTGGG
OPJ-1	CCCGGCATAA
Oligo 214	CATGTGCTTG
Oligo 213	CAGCGAACTA
Mixed RAPD primers	**Sequence of primer (5' – 3')**
Oligo 203+Oligo 342	CACGGCGAGT+GAGATCCCTC
Oligo 203+ Oligo 345	CACGGCGAGT+GCGTGACCCG
Oligo 203+Oligo 42	CACGGCGAGT+TTAACCCGGC
Oligo 203+Oligo 349	CACGGCGAGT+GGAGCCCCCT
Oligo 203+Oligo 214	CACGGCGAGT+CATGTGCTTG
Oligo 203+Oligo 213	CACGGCGAGT+CAGCGAACTA
Oligo 203+Oligo 33	CACGGCGAGT+CCGGCTGGAA
Oligo 203+OPK-8	CACGGCGAGT+GAACACTGGG
ISSR primers	**Sequence of primer (5' – 3')**
Primer (3)	TGGATGGATGGATGGA
Primer (4)	CACACACA CACACA AG
UBC 823	TCT CTC TCT CTC TCC
UBC 824	TCT CTC TCT CTC TCG
UBC 826	ACA CAC ACA CAC ACC
HB 14	CTC CTCCTC GC
Primer (1)	GAGAGAGAGAGAGAGAC
Primer (2)	GAGAGAGAGAGAGAGAGAGAG
HB 11	GTG TGT GT GTGTCC

Data analysis

All scored fragments gained from RAPD-PCR, mixed RAPD-PCR and ISSR-PCR were manually recorded as present (1) or absent (0). Matrix of similarity based on binary-double zeros-S3, and squared Euclidean distance was used to calculate the distances and to generate dendrogram (Sneath and Sokal, 1973). Polymorphism percentage was estimated by calculating polymorphic bands/total number of bands.

Results

RAPD analysis

RAPD primers produced a total of 109 scorable bands from genotypes of *S. molle*, out of which 23.0 (21.1%) were found to be polymorphic, 1.00 (0.91%) to be monomorphic bands and 85.0 (77.9%) to be unique bands. Primer Oligo 345, yielded the maximum number of bands (20.0 bands) while the lowest number of bands (3.00 bands) obtained from Primer Oligo 214. The percentage of polymorphism ranged from 0.00% (Primer Oligo 33 and Primer Oligo 214) to 57.1% (Primer Oligo 342). The maximum number of unique bands (17.0 bands) was recorded from Primer Oligo 33, while the lowest number of unique bands (3.00 bands) from the Primer Oligo 342 and Primer Oligo 214 (Table 2 and Fig. 2 Panel A).

The genetic similarity coefficients (Table 3) revealed that the maximum similarity value (89.9%) was between population at 2250.6 m and population at 2175.0 m, while the least similarity value (72.5%) between population at 2246.0 m and population at 2372.0 m.

Dendrogram analysis (Fig. 3 Panel A) showed that population at 2193.0, 2197.7 and 2441.0 m found to be forming one cluster whereas population at 2246.0 m separated from them in a single cluster while population at 2372.0, 2250.6 and 2175.0 m found to be forming another one cluster.

Table 2. Polymorphism of eight RAPD primers.

Primer ID	Total no. of bands per primer	No. of polymorphic bands	No. of monomorphic bands	No. of unique bands	Polymorphism %
Oligo342	7.00	4.00	0.00	3.00	57.1
Oligo 345	20.0	7.00	0.00	13.0	35.0
Oligo 349	17.0	5.00	1.00	11.0	29.4
Oligo 33	17.0	0.00	0.00	17.0	0.00
OPK-8	19.0	3.00	0.00	16.0	15.7
OPJ-1	13.0	1.00	0.00	12.0	7.69
Oligo 214	3.00	0.00	0.00	3.00	0.00
Oligo 213	13.0	3.00	0.00	10.0	23.0
Total	109	23.0	1.00	85.0	20.9

Mixed RAPD analysis

Mixed RAPD primers generated a total of 100 reproducible bands of which (19.0%) were polymorphic bands, (81.0%) unique bands, and no any monomorphic bands (Table 4 and Fig. 2 Panel B). Primer OPK-8 produced the highest number of bands (21.0) while primer Oligo 42 gave the minimum number of bands (3.00). Primer Oligo 33 showed the highest percentage value of polymorphism of 50.0% and the zero polymorphism rate gained from the primer Oligo 42 and

primer Oligo 214. The maximum number of unique bands were (18.0 bands*)* gained from primer OPK-8, while the minimum numbers were (3.00) gained from primer Oligo 42.

Table 3. Genetic similarity among *S. molle* plants based on RAPD markers.

	2193.0 m	2246.0 m	2197.7 m	2441.0 m	2372.0 m	2250.6 m	2175.0 m
2193.0 m	1.00						
2246.0 m	0.7614	1.00					
2197.7 m	0.8216	0.7821	1.00				
2441.0 m	0.7956	0.7684	0.828	1.00			
2372.0 m	0.7399	0.7251	0.7753	0.8022	1.00		
2250.6 m	0.8404	0.828	0.8705	0.8705	0.8821	1.00	
2175.0 m	0.7471	0.7326	0.7821	0.7956	0.7821	0.899	1.00

The genetic similarity coefficients displayed the highest similarity value (87.6%) between population at 2246.0 m and population at 2197.7 m, while the least similarity value (72.6%) was recorded between population at 2372.0 m and population at 2175.0 m (Table 5).

Resulted dendrogram showed that populations at 2193.0, 2246.0 and 2197.7 m found to be forming one cluster whereas population at 2441.0 m and population at 2372.0 m clustered together as well as population at 2250.6 m and population at 2175.0 m (Fig. 3 Panel B).

Table 4. Polymorphism of eight mixed RAPD primers.

Primer ID	Total no. of bands per primer	No. of polymorphic bands	No. of monomorphic bands	No. of unique bands	Polymorphism %
Oligo 342	17.0	3.00	0.00	14.0	17.6
Oligo 345	16.0	6.00	0.00	10.0	37.5
Oligo 42	3.00	0.00	0.00	3.00	0.00
Oligo 349	15.0	1.00	0.00	14.0	6.66
Oligo 214	10.0	0.00	0.00	10.0	0.00
Oligo 213	8.00	1.00	0.00	7.00	12.5
Oligo 33	10.0	5.00	0.00	5.00	50.0
OPK 8	21.0	3.00	0.00	18.0	14.2
Total	100	19.0	0.00	81.0	17.3

ISSR analysis

A total of 231 counted bands were generated by using the nine ISSR primers from *S. molle* genetic materials (Table 6 and Fig. 2 Panel C). Sixty-two polymorphic bands (26.8%), 1.00 (0.43%) monomorphic bands, 168 (72.7%) unique bands with polymorphism rate 23.2% were recorded. Primer UBC 826 generated the maximum number of bands (63.0), while primer UBC 824 showed the minimum number of bands (6.00). Primer (1) showed the highest rate of polymorphism (51.4%) and Primer (3) showed the least rate numbers (4.54%). The highest number of unique bands (46.0) was recorded from primer UBC 826, while the least number of unique bands (5.00) resulted from primer UBC 824.

Resulted genetic similarity coefficients exhibited the highest similarity value among populations at 2193.0 m, 2246.0 m, 2441.0 m and population at 2250.6 m recording 86.2%, while the least similarity value between population at 2246.0 m and population at 2175.0 m with value of 69.1% (Table 7).

A dendrogram pattern revealed that population at 2193.0 m and population at 2246.0 m formed one cluster whereas the populations at 2197.7 m, 2441.0 m, 2250.6 m and 2372.0 m found to be in another cluster and population at 2175.0 m formed out-group from the in-group including populations at 2193.0 m, 2246.0 m, 2197.7 m, 2441.0 m, 2250.6 m and 2372.0 m (Fig. 3 Panel C).

Table 5. Genetic similarity among *S. molle* plants based on mixed RAPD markers.

	2193.0-m	2246.0-m	2197.7-m	2441.0-m	2372.0-m	2250.6-m	2175.0-m
2193.0 m	1.00						
2246.0 m	0.8701	1.00					
2197.7 m	0.8439	0.8764	1.00				
2441.0 m	0.8235	0.8439	0.8571	1.00			
2372.0 m	0.7654	0.7879	0.8024	0.8372	1.00		
2250.6 m	0.7952	0.7879	0.8166	0.8235	0.75	1.00	
2175.0 m	0.7578	0.7654	0.8095	0.7879	0.7261	0.8166	1.00

Table 6. Polymorphism of nine ISSR primers.

Primer ID	Total no. of bands per primer	No. of polymorphic bands	No. of monomorphic bands	No. of unique bands	Polymorphism %
Primer (3)	22.0	1.00	0.00	21.0	4.54
Primer (4)	26.0	5.00	0.00	21.0	19.2
UBC 823	18.0	5.00	0.00	13.0	27.7
UBC 824	6.00	1.00	0.00	5.00	16.6
UBC 826	63.0	16.0	1.00	46.0	25.3
HB 14	30.0	12.0	0.00	18.0	40.0
Primer (1)	35.0	18.0	0.00	17.0	51.4
Primer (2)	20.0	3.00	0.00	17.0	15.0
HB 11	11.0	1.00	0.00	10.0	9.09
Total	231	62.0	1.00	168	23.2

Table 7.Genetic similarity among *S. molle* plants based on ISSR markers.

	2193.0 m	2246.0 m	2197.7 m	2441.0 m	2372.0 m	2250.6 m	2175.0 m
2193.0 m	1.00						
2246.0 m	0.8621	1.00					
2197.7 m	0.8123	0.8123	1.00				
2441.0 m	0.8093	0.7906	0.8304	1.00			
2372.0 m	0.7969	0.7713	0.7874	0.8392	1.00		
2250.6 m	0.8093	0.7713	0.8304	0.8621	0.8333	1.00	
2175.0 m	0.7273	0.6912	0.7514	0.7411	0.7131	0.7874	1.00

Super tree analysis (RAPD + mixed RAPD + ISSR)

A combined analysis using pooled RAPD, mixed RAPD and ISSR data showed that there are 20.5 % polymorphism among studied population growing at various height. The highest similarity values (85.5%) was found between populations at 2441.0 m and population at 2250.6 m and the lowest similarity values between population at 2246.0 m and population at 2175.0 m (71.9%) Table (8). Resulted dendrogram revealed that population at 2193.0-m and population at 2246.0-m clustered together whereas populations at 2197.7, 2441.0, 2250.6 and 2372.0 m found to be forming one cluster while population at 2175.0 m separated from them in a single cluster (Fig. 3 Panel D).

Table 8. Genetic similarity among *S. molle* plants based on combined analysis.

	2193 m	2246 m	2197.7 m	2441 m	2372 m	2250.6 m	2175 m
2193 m	1.00						
2246 m	0.8406	1.00					
2197.7 m	0.822	0.8204	1.00				
2441 m	0.8092	0.7978	0.836	1.00			
2372 m	0.7761	0.764	0.7879	0.8298	1.00		
2250.6 m	0.814	0.7895	0.8375	0.8557	0.8282	1.00	
2175 m	0.7393	0.7191	0.7727	0.7658	0.7338	0.8235	1.00

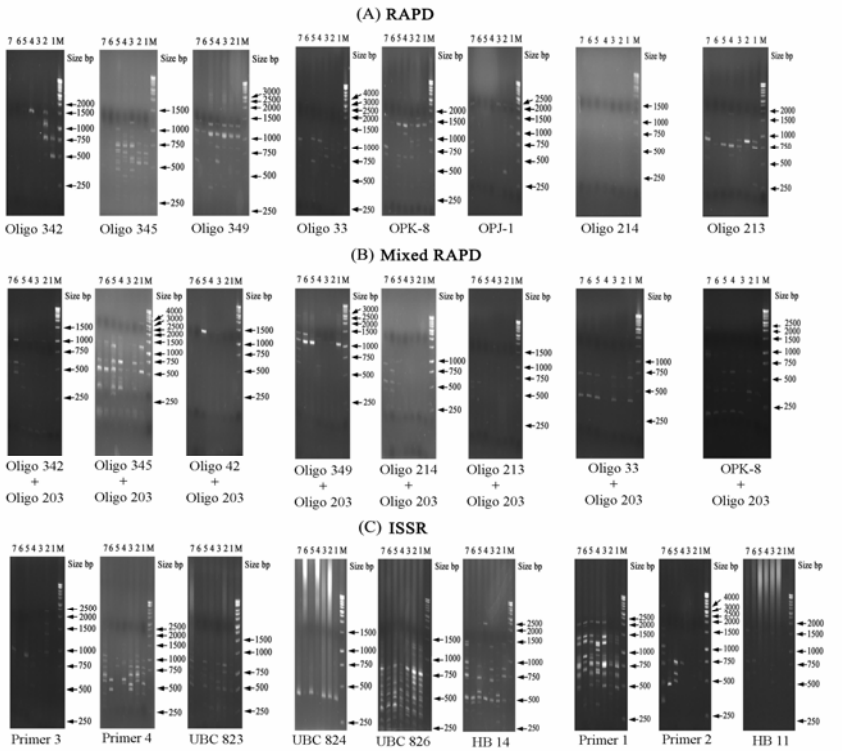

Fig. 2. RAPD, mixed RAPD and ISSR profiles of *S. molle* plants. Lane 1, 2193.0; Lane 2, 2246.0; Lane 3, 2197.7; Lane 4, 2441.0; Lane 5, 2372.0; Lane 6, 2250.6; Lane 7, 2175.0; M-1kb DNA Ladder.

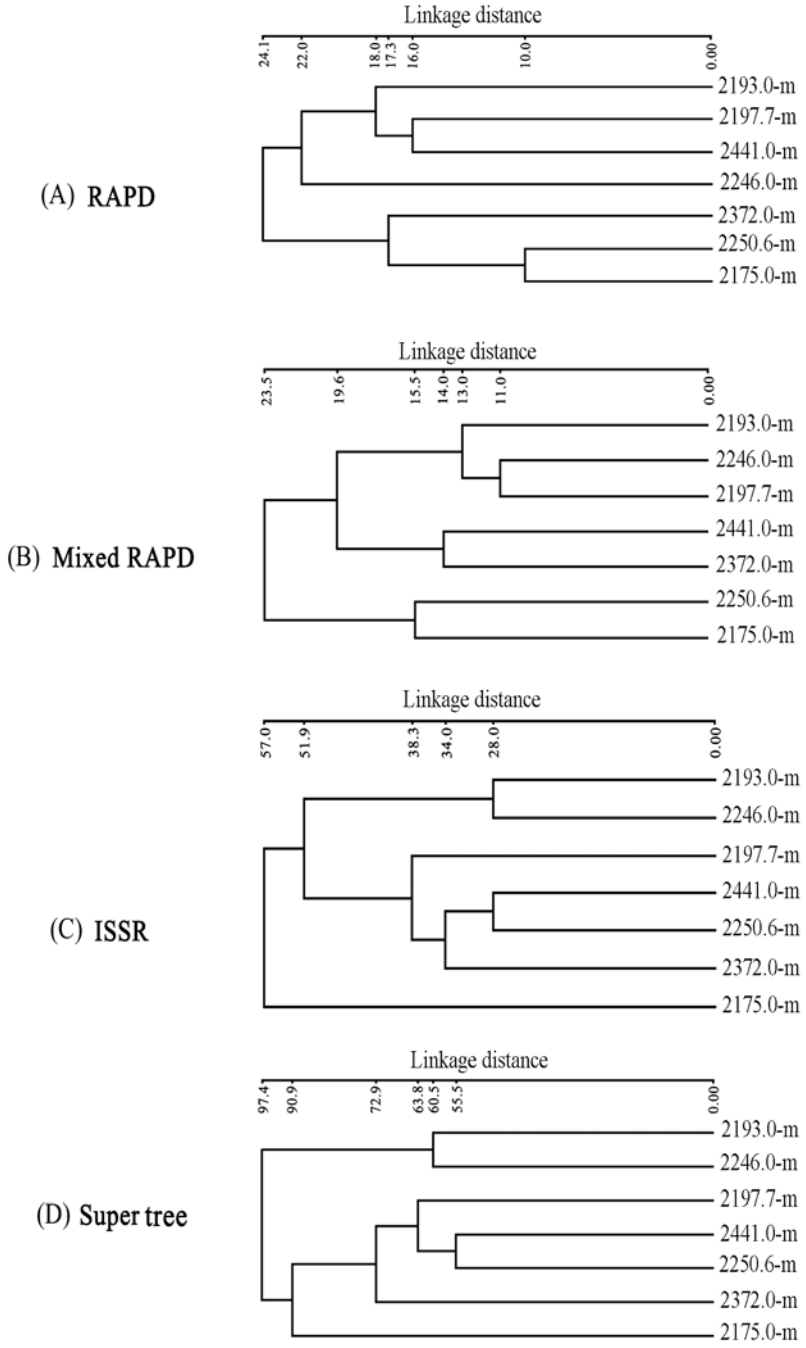

Fig. 3.Dendrogram based on RAPD, mixed RAPD, ISSR and super tree data of *S. molle*.

Discussion

This research article reports the use of the RAPD, mixed RAPD and ISSR makers to the *S. molle* plant and revealed its efficiency to determinate the DNA fingerprints. Also it revealed that RAPD, mixed RAPD and ISSR markers could be used alone or in combination to estimate the genetic diversifications of *S. molle* plants. Polymorphism rate obtained either from RAPD, mixed RAPD, ISSR or from combined analysis all showed that there was high genetic variability among *S. molle* at a very close distance populations. The percentage of polymorphism almost same to that detected in other examined plants that they have a wide genetic variability. For example, Adawy *et al*. (2004) and Hussein *et al*. (2005) found that RAPD polymorphism rate in various Egyptian date palm cultivars (*Phoniex dactylifera* L.) is in the range of 25.2% and for ISSR technique in the range of 28.6%. Among the studied *Pistacia vera* (L.) various cultivars polymorphism rate based on ISSR markers was 46.4% and 100% among *Mangifera indica* (L.) based on ISSR markers (Noroozi *et al*., 2009; Souza *et al*., 2011). RADP, mixed RAPD and ISSR showed various degrees in their ability to detect the diversifications among populations of *S. molle* plants. This variation may be due to that the genome *S. molle* plants having a considerable number of alleles per locus/or loci that vary in their distribution. Izzatullayeva *et al*. (2014) reported that such difference between RAPD and ISSR markers due to the fact of abundant nature of microsatellites that results from slippage in DNA replication. This explains why this plant can be found in various habitats vary from salinity soil to alkalinity soil and in different temperature condition ranging from very low to very high (Lim, 2012). In this study, total number of unique bands obtained from ISSR-PCR of *S. molle* plant more than that of RAPD-PCR and mixed RAPD-PCR. The results also showed that ISSR fingerprinting had a high number of scored bands and high polymorphic percentage rate. This in agreement with earlier studies showed that ISSR fingerprinting was more efficient than the RAPD assay in assessing genetic variation in *Arthrocnemum macrostachyum* (Saleh, 2011). Again this variation among RAPD, mixed RAPD and ISSR probably due to that amplified profiles of PCR of RAPD, ISSR, or mixed RAPD originated from different variable numbers of repetitive and non-repetitive sequence on the genomes of *S. molle* plant (Thormann *et al*., 1994). The presence of monomorphic bands from RAPD-PCR or from ISSR-PCR indication to the sharing characters based on the DNA fragment in genomic *S. molle* plants. Cluster analysis based on RAPD, mixed RAPD and ISSR markers individually or combined showed that the three markers differ from each other in the manner of distributing *S. molle* populations.

In our study, the amount of genetic similarity among various populations of *S. molle* plants based on RAPD markers were in range between 72.5% to 89.9% and for mixed RAPD between 72.6% to 87.6% and for ISSR 69.1% to 86.2% and for the sum of all data between 71.9% to 85.5%. These values to some extent are in accordance with the basis proofed by Weier *et al*. (1982) that operational taxonomic units between 85 to 100% among the same plant species and more than 65% between the same plant genus. In conclusion, our study confirms that there were a wide genetic diversity among *S. molle* plants that can be evaluated by using RAPD, mixed RAPD and ISSR markers.

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