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Research Article
Genetic Variation Analysis for Breeding Purposes in Wild and Cultivated Sunflower Germplasm (*Helianthus annuus* Sp.)

[1,2]Maissa M. Morsi, [3,4]Hala M. Abdelmgid and [5]Doaa M. Hammad

[1]Department of Biology, Faculty of Science, Taif University, Taif, Zip code 21944, Kingdom of Saudi Arabia
[2]Department of Botany, Faculty of Women for Art, Science and Education, Ain Shams University, Egypt
[3]Department of Botany, Faculty of Science, Mansoura University, Egypt
[4]Central Laboratory for Environmental Quality Monitoring, National Water Research
[5]Center, El-Kanater, Qalubiya, P.O. Box 13621/6, Cairo, Egypt

Abstract

Background and Objective: Molecular genetic methods show conclusive turn in phylogeny investigations and therefore, understanding the allocation and extension of genetic diversity inside and among species. The current study aims to evaluate genetic diversity using molecular markers (5 SSR and 5 ISSR) on 13 sunflowers (*Helianthus annuus*) genotypes from different regions. **Materials and Methods:** The data acquired with the highest degree of polymorphism were confirmed. Polymorphic bands (24 and 55) were demonstrated totally through SSR and ISSR, respectively. Nevertheless, ISSR was superior in exhibiting polymorphism rate (97.37%) compared to SSR markers (90.83%). A range from 2-21 of amplified fragments, with molecular weights varying from 34-1331 bp, was noticeable. The genetic similarity coefficient was employed to set UPGMA dendrogram. **Results:** Dissimilarity was found in genotypes clustering within groups, whilst the manner of clustering in genotypes kept adjacent in ISSR and aggregated results of SSR and ISSR. Dice similarity ranged from 0.058-0.792, 0.168-0.770 and 0.154-0.847 with SSR, ISSR and the combined phylogeny dendrogram, respectively. The genetic variance of sunflower accessions of wild and cultivated cultivars from different geographic regions was established. **Conclusion:** Knowledge of these results could be applied to confirm a wide genetic basis for outlook sunflower and manage germplasm breeding programs.

Key words: Molecular marker, phylogeny, polymorphism, genotypes, clustering, dice similarity

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Corresponding Author: Maissa M. Morsi, Department of Biology, Faculty of Science, Taif University, Taif, Zip code 21944, Kingdom of Saudi Arabia

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Investigating the levels and patterns of genetic diversity of invasive plant species is crucial to understand the ecological factors promoting invasions and for better pre-breeding strategies in breeding field crops. Sunflower (*Helianthus annuus* L.), is one of the most important crops in the world grown for edible oil, belongs to the family Asteraceae family. Funk *et al.*[1] reported that species of Asteraceae family exist in a wide range of environments including deserts, forests, wetlands, salt marshlands and cultivated meadows[2]. Currently, cultivated sunflower (*H. annuus* L.) is ranked as the fourth most important vegetable oil crop in the world[3]. The plant is extremely cross-pollinated, annual and diploid (2n = 2x = 34). Variability of speciation mechanisms makes it a perfect model system for molecular genetic studies on speciation and adaptation[4-7].

Common sunflower is a widely distributed herb whose native geographic range is centered in the central United States and Canada[8]. Sunflower is presumed to has been domesticated by Native Americans who primarily used it as a source of edible seed. It has been introduced to Europe in the early 16th century[9], where it was grown as an ornamental plant. Then, progressive use of sunflowers as a vegetable oil source arisen, which led to its cultivation on a large scale in Eastern Europe. Even though cultivated sunflower has undergone varied evolutionary history, it showed a narrow genetic diversity compared to wild sunflower. Increasing genetic diversity is a special interest of sunflower breeders for developing genetic resources with high oil and seed yield as well as resistance to biotic and abiotic stresses[10]. Evaluating genetic variance and elucidating the phylogenetic relations in germplasm sets promotes the genetic improvement and effective germplasm management[11-14].

Evaluation of genetic diversity of sunflower germplasm via various molecular markers is very important in sunflower breeding programs[15-16]. DNA markers, which are available throughout the genome are unlimited in number and do not influence by environments or epistasis[15,17,18]. Genetic characterization of wild and cultivated sunflower based on DNA markers would be helpful to examine variance patterns within the species[19-23].

Simple sequence repeats (SSRs) or microsatellites exist in the eukaryotic genome, distinguished by broad allocation, high polymorphism, co-dominance and repeatability have been considerably used for genetic tests all over the world[24-27]. SSR markers have been proved as suitable markers for genetic variation analysis of sunflower hybrids as well as populations from diverse geographical areas[28-30].

Inter-Simple Sequence Repeat (ISSR) markers have been extremely utilized to assess the genetic variation of diverse plant species. ISSR markers survey numerous positions simultaneously all over the genome, providing an extremely beneficial marker for diversity analysis and fingerprinting[31]. Drine *et al.*[32] and Shehata *et al.*[33] studied different barley accessions and concluded that ISSR revealed more genetic diversity, compared to RAPD. ISSR was one of the preliminary progressing molecular methods applied to estimate the genetic variance of the plant because it is practically convenient and technically easy[34].

The current research focused on genetic diversity and inhabitance composition in wild and cultivated accessions of sunflowers from distant three geographical locations; North America, Egypt and Tunisia. All varieties were genotyped using a genome-broad collection molecular marker derived from (SSR) and (ISSR) genomic regions. Specific objectives of this study were: (i) Estimating genetic variation within 13 sunflower genotypes employing ISSR and SSR markers (ii) Phylogenetic analysis of those genotypes based on a combination of molecular profiles and (iii) Evaluation of the genetic diversity assessed by molecular marker system to study the genetic patterns with providing data applicable to conservation and breeding uses.

MATERIALS AND METHODS

Plant materials: The study was carried out at the Biology Department, College of Science, Taif University from Jan, 2018-April, 2019). Thirteen varieties of healthful seeds of *Helianthus annuus* L. taken from different sources were investigated, representing different regions including wild and cultivated members. The genotypes included are shown in Table 1.

Germination: Dry mature seeds of *Helianthus* were soaked in distilled water at 4°C for 24 h. and transferred to Petri dish

Table 1: List of sunflower cultivars used in this study, their origin and pedigree information

Numbers	Code	Location
1	Red sun (c)	North America
2	Velvet queen (c)	
3	Wild (w)	
4	Autumn beauty (c)	
5	Fall teddy (c)	
6	Lemon queen (w)	
7	Sakha (53) (c)	Egypt
8	Sidi nsir (c)	Tunisia
9	Zriba (c)	
10	Zahret median (c)	
11	Arcade de lancien train (c)	
12	Nagachya (c)	
13	Rumbosol (c)	

c: Cultivated, w: Wild cultivar

containing 11 cm diameter filter paper at a temperature of 15°C in the dark until the emergence of 2 mm primary roots. Symmetrical germinated seeds were chosen and taken to plastic pots containing 1 kg of sterilized soil at 2.0 cm depth and were incubated at a temperature of 23±1°C and light/dark photoperiod of 15 h light/ 8h dark. Leaf materials were harvested for further DNA analyses at the 4-6-leaf stage.

Genomic DNA extraction: DNA extraction was achieved by 40-100 mg of plant tissues. A small-scale DNA isolation technique by Wizard®. Genomic DNA Purification Kit (Promega) was carried out for the extraction following the manufacturer guidelines. To assess whether extracted DNA is degraded and to confirm the quantification, an aliquot of 1-2.5 µL DNA samples and 10 µL loading buffer were loaded on 1% agarose gel and visualized by UV transilluminator (Biometra UV star 15).

SSR assay: Five primers were selected for generating SSR markers in the different genotypes of *Helianthus*, depending on the uniformity and number of amplified fragments. SSR primers were synthesized by Macrogen Co. Ltd. (Korea) (Table 2). The protocol for SSR analysis was carried out following[35]. PCR reactions were performed using 5 X FIREPol Master mixes in a 25 µL volume, following manufacturer's guidelines in PXE Thermal Cycler according to the following profile: an initial step of 5 min at 94°C, 35 cycles of 45 sec at 94°C, 45 s at 53°C and 1 min at 72°C and a final step of 10 min at 72°C. 10 µL PCR products in samples were analyzed on 2% Metaphor® agarose gels in TBE buffer running at 100 V. DNA molecular size ladder (100 bp marker; RTU) was used for each agarose gel. The gels were stained by ethidium bromide and visualized using UV transilluminator (Biometra UV star 15); after which the gel was photographed and documented using the GelPro32 software. To confirm the reproducibility of all polymorphic bands, all reactions were accomplished twice.

ISSR assay: Five primers based on dinucleotide repeats were selected from 15 primers (Macrogen Co. Ltd. (Korea), for the generation of ISSR markers, based on quality, polymorphism and the reproducibility of the amplification. The ISSR marker amplification technique was carried out[36] with some modifications. The reactions were performed using 5 X FIREPol Master mixes in a 25 µL volume, following the manufacturer's instructions in PXE Thermal Cycler with the following program: 94 °C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 54 or 56°C for 30 sec and an extension at 72 °C for 1 min; a final extension was performed at 72°C for 3 min. The amplified products were separated on 1.5% (w/v) agarose gels in a 1XTBE buffer[37]. DNA molecular size ladder (100 bp marker; RTU) was used for each agarose gel. The gels were stained by ethidium bromide and visualized using UV transilluminator (Biometra UV star 15); after which the gel was photographed and documented using the GelPro32 software. To confirm the reproducibility of all polymorphic bands, all reactions were accomplished twice.

Data analysis: The successfully amplified primers resulted in SSR and ISSR bands that distinguished from the agarose gel were recorded as diallelic characters: present = 1, absent = 0 at each position, with missing data scored as "9". Jaccard similarity coefficient for pairwise comparisons has been utilized to assess the genetic relations between genotypes depending on the proportion of shared bands produced by the primers. A dendrogram was produced by an unweighted pair group method for an arithmetic mean (UPGMA), which resulted from a similarity matrix when exposed to cluster analysis. The calculations were achieved using the program NTSYS-PC version 2.01 [38]. The principal component analysis was applied to Jaccard's similarity matrix.

RESULTS

Polymorphisms observed by SSR and ISSR markers: The studied genotypes of *H. annuus* revealed a high level of polymorphism (Table 2 and 3). All the SSR primers created reproducible and clear bands (Fig. 1). 26 fragments totally, ranged in size from 34-889 bp were scored in PCR profile of the tested plants, most of them show evident polymorphism. A single primer produced polymorphic bands ranged from 2 (ORS-818 and ORS-844) to 8 (ORS-718), with a mean of 4.8 of polymorphic bands per primer (Table 4). The percentage of polymorphism varied between 66.66% (ORS-818) and 100% (ORS-844, ORS-899, ORS-718) with an average of 90.83%.

ISSR primers revealed genetic variations across the 13 genotypes (Fig. 2). 57 bands produced by ISSR primers, 55 of which were polymorphic. The band's number ranged from 6 (UBC-857) to 20 (UBC-808) and the amplicon size varied from 103-1331 bp. 11 was the average number of polymorphic bands per primer (Table 4). The polymorphism percentage varied between 91.66% (ISSR-2) and 100% (ISSR-5, UBC-857, 811) with a mean of 97.37%. Both SSR and ISSR bands were listed (1) for the existence or (0) for disappearance through the genotypes and then applied for UPGMA study.

Table 2: List of SSR primers, the number of amplified products, the number of polymorphic and monomorphic bands and percentage of polymorphism obtained by analyzing 13 cultivars of sunflower

S/N	Primers	Primer sequence (5'-3')	MW (bp)	TB	PB	MB	P (%)
1	ORS-878	F: TGCAAGGTATCCATATTCCACAA	72-234	3	2	1	66.66
		R: TATACGCACCGGAAAGAAAGTC					
2	ORS-844	F: ACGATGCAAAGAATATACTGCAC	43-118	2	2	0	100.00
		R: CATGTTTAATAGGTTTTAATTCTAGGG					
3	ORS-899	F: GCCACGTATAACTGACTATGACCA	215-898	5	5	0	100.00
		R: CGAATACAGACTCGATAAACGACA					
4	ORS-613	F: GTAAACCCTAGGTCAATTTGCAG	234-559	8	7	1	87.50
		R: ATCTCCGGAAAACATTCTCG					
5	ORS-718	F: CACTTTACGCACACCAAACC	34-285	8	8	0	100.00
		R: ATGCAACACCCGAATCAAAG					
Total				26	24	2	

Number of polymorphic (PB), total number of amplified products (TB), monomorphic bands (MB), percentage of polymorphism (%P)

Table 3: List of ISSR primers, the total number of amplified products (TB), the number of polymorphic (PB) and monomorphic bands (MB) and percentage of polymorphism (%P) obtained by analyzing 13 cultivars of sunflower

S/N	Primer	Primer sequence (5'- 3')	MW (bp)	TB	PB	MB	P (%)
1	ISSR-2	(AG)8 T	103-1009	12	11	1	91.66
2	ISSR-5	(GT)8A	118-1128	9	9	0	100.00
3	UBC-808	(AG)8 C	108-1331	21	20	1	95.23
4	UBC-857	(AC)8YG	194-1078	6	6	0	100.00
5	811	(GA)8C	196-1078	9	9	0	100.00
Total				57	55	2	

Table 4: Comparison of DNA marker systems in *H. annus*

Marker system	Number of primer	Polymorphism (%)	Average number of bands/primer	Average number of polymorphic bands/primer
SSR	5	90.83	5.2	4.8
ISSR	5	97.37	11.4	11.0
SSR+ISSR	10	94.10	8.3	7.9

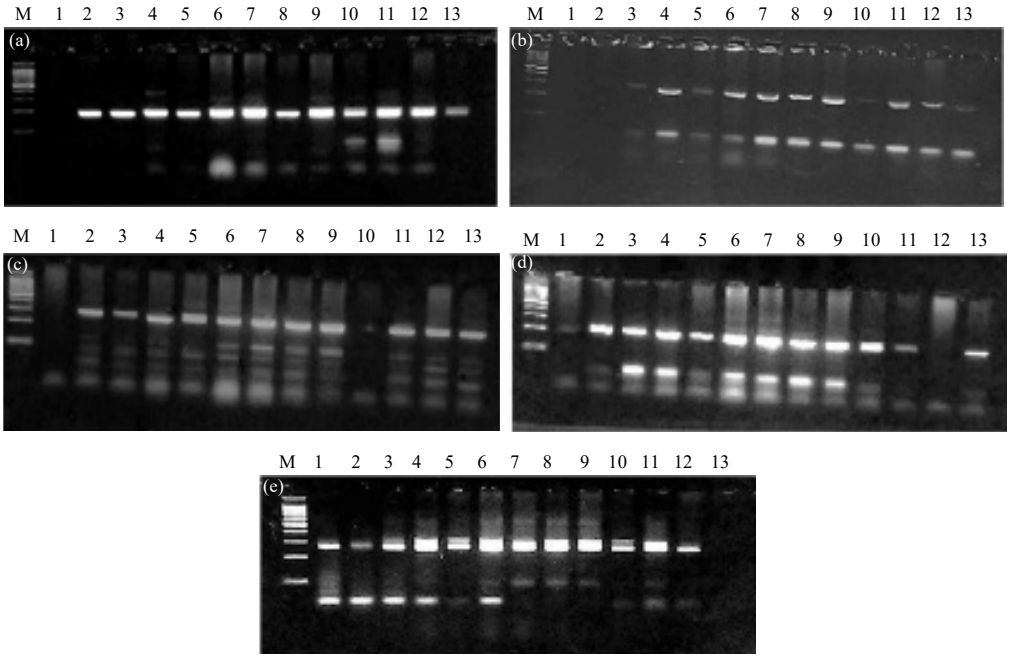

Fig. 1(a-e): SSR-PCR amplification products of 13 accessions of sunflower produced by (a) Primer ORS-818, (b) Primer ORS-844, (c) Primer ORS-899, (d) Primer ORS-613 and (e) Primer ORS-718

Lane M is 1 kb ladder and lanes 1-13 present different sunflower accessions as listed in Table 1

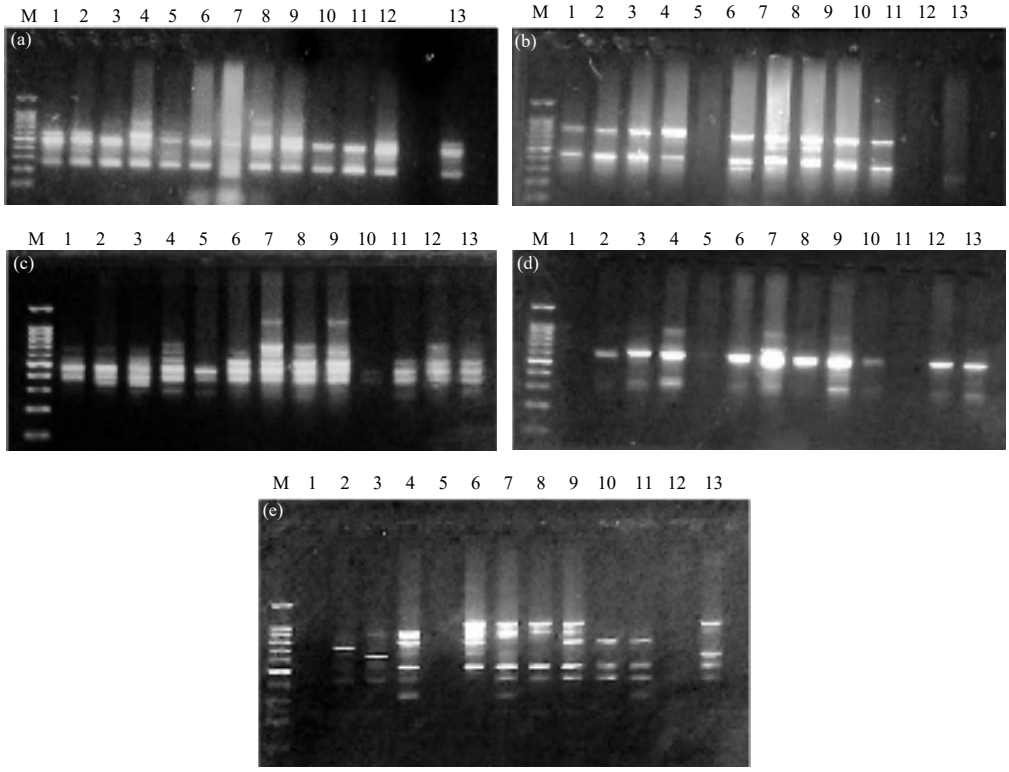

Fig. 2(a-e): ISSR-PCR amplification products of 13 accessions of sunflower produced by (a) Primers ISSR-2, (b) Primer ISSR-5, (c) Primer UBC-808, (d) Primer UBC-857 and (e) Primer 811
Lane M is 1 kb ladder and lanes 1-13 present different sunflower accessions as listed in Table 1

Genetic similarities developed by SSR and ISSR markers: Dice similarity evidence value for the 13 accessions of *H. annuus* examined genetic similarity considering SSR and ISSR processes individually as well as in combination. Genetic distances exhibited by SSR markers varied from 0.1-0.79. 0.1 was the lowest value recorded between *Nagachya* and *Autumn Beauty* genotypes whereas *Sakha 53* and *Red Sun* genotypes showed the highest distance number of 0.79 (Table 5). Based on ISSR markers, a different pattern was revealed by Dice similarity index as the greatest amount (0.77) was recorded between *Sidi Nsir* and *Wild* genotypes while the lowest value (0.0) was between (*Velvet Queen* and *Red Sun*, (Table 6). In a combined analysis of both markers, a higher index value was registered (0.85) between *Arcade de-LT* and *Zahret Median* genotypes, whereas *Velvet Queen* showed the lowest coefficient of 0.15 with *Red Sun* genotype (Table 7).

Phylogenetic analysis: The phylogenetic relations between 13 accessions *of H. annuus* were examined through UPGMA method. All the genotypes may be recognized by SSR and ISSR markers as indicated by the clustering pattern. However, SSR analysis showed a phylogenetic relationship among the genotypes different from that generated by the ISSR analysis.

Based on SSR, the resulting dendrogram gathered the 13 accessions into one main cluster and a solitary genotype of *Red Sun* established a break OUT in cluster I, exhibiting lower genetic identity (0.547) relative with the other genotypes (Fig. 3a). The master cluster II then diverged into two subclusters. The first subcluster IIa included seven genotypes (*Velvet Queen*, *Wild*, *Nagachya*, *Autumn beauty*, *Rumbosol*, *Lemon Queen* and *Sakha-53*), which in turn split into a group of five genotypes with *Velvet Queen* separated as OUT with distance coefficient of 0.241 with regard to the remaining genotypes within this group. Among the other four genotypes, *Wild* and *Nagachya* were the most closer ones as they came in a dichotomous branch with a similarity index of 0.058 whereas *Autumn beauty* and *Rumbosol* revealed a higher mean of dissimilarity value of 0.129 and 0.155 respectively. Within the same subcluster IIa, the two genotypes (*Lemon Queen* and *Sakha-53*) were gathered in one dichotomous branch with dice similarity of 0.151. The second subcluster IIb comprised five genotypes (*Fall Teddy*,

Table 5: Dice similarity coefficient of 13 genotypes based on SSR data analysis

Genotypes	Red sun	Velvet queen	Wild	Autumn beauty	Fall teddy	Lemon queen	Sakha 53	Sidi Nsir	Zriba	Zahret median	Arcade de -LT	Nagachya	Runbosol
Red sun	0												
Velvet queen	0.3262	0											
Wild	0.3262	0.1335	0										
Autumn beauty	0.4854	0.2928	0.1592	0									
Fall teddy	0.5493	0.4700	0.2877	0.2928	0								
Lemon queen	0.6405	0.4478	0.3143	0.3557	0.4478	0							
Sakha 53	0.7921	0.5304	0.3763	0.4020	0.3763	0.1516	0						
Sidi Nsir	0.7085	0.4469	0.2928	0.3185	0.4469	0.2504	0.2842	0					
Zriba	0.6082	0.5289	0.3466	0.2181	0.1924	0.3732	0.3016	0.2181	0				
Zahret median	0.4825	0.6264	0.4032	0.3801	0.2209	0.5352	0.4637	0.3801	0.1257	0			
Arcade de-LT	0.6609	0.5816	0.3993	0.2708	0.3993	0.3081	0.3543	0.1530	0.1705	0.1783	0		
Nagachya	0.3851	0.1924	0.0589	0.1003	0.3466	0.2554	0.3016	0.2181	0.2513	0.4621	0.3040	0	
Rumbosol	0.6082	0.3466	0.1924	0.2181	0.5289	0.2554	0.4352	0.2181	0.4055	0.6853	0.3040	0.1178	0

Table 6: Dice similarity coefficient of 13 genotypes based on ISSR data analysis

Genotypes	Red sun	Velvet queen	Wild	Autumn beauty	Fall teddy	Lemon queen	Sakha 53	Sidi Nsir	Zriba	Zahret median	Arcade de -LT	Nagachya	Runbosol
Red sun	0												
Velvet queen	0	0											
Wild	0.4236	0.4236	0										
Autumn beauty	0.2554	0.2554	0.6791	0									
Fall teddy	0.3466	0.3466	0.4825	0.6020	0								
Lemon queen	0.4904	0.4904	0.4032	0.7458	0.5493	0							
Sakha 53	0.2554	0.2554	0.6791	0.5108	0.6020	0.7458	0						
Sidi Nsir	0.3466	0.3466	0.7702	0.6020	0.6931	0.5493	0.6020	0					
Zriba	0.3466	0.3466	0.4825	0.6020	0.4055	0.5493	0.6020	0.6931	0				
Zahret median	0.2554	0.2554	0.1682	0.5108	0.6020	0.4581	0.5108	0.6020	0.6020	0			
Arcade de-LT	0.3466	0.3466	0.2594	0.6020	0.4055	0.3262	0.6020	0.4055	0.4055	0.6020	0		
Nagachya	0.2554	0.2554	0.6791	0.5108	0.6020	0.7458	0.5108	0.6020	0.3143	0.5108	0.6020	0	
Rumbosol	0.2554	0.2554	0.6791	0.5108	0.6020	0.4581	0.5108	0.3143	0.6020	0.5108	0.3143	0.5108	0

Table 7: Dice similarity coefficient of 13 genotypes based on ISSR+SSR data analysis

Genotypes	Red sun	Velvet queen	Wild	Autumn beauty	Fall teddy	Lemon queen	Sakha 53	Sidi Nsir	Zriba	Zahret median	Arcade de -LT	Nagachya	Runbosol
Red sun	0												
Velvet queen	0.1542	0											
Wild	0.4236	0.2695	0										
Autumn beauty	0.3325	0.3325	0.6020	0									
Fall teddy	0.5625	0.3801	0.4955	0.7408	0								
Lemon queen	0.5007	0.3466	0.3648	0.6791	0.4390	0							
Sakha 53	0.4236	0.2695	0.5390	0.6020	0.4955	0.3648	0						
Sidi Nsir	0.3801	0.2260	0.4955	0.5585	0.4520	0.3213	0.3620	0					
Zriba	0.3801	0.3801	0.4955	0.5585	0.6061	0.5726	0.4955	0.6061	0				
Zahret median	0.3365	0.3365	0.2695	0.5148	0.7856<	0.5007	0.6060	0.5625	0.5625	0			
Arcade de-LT	0.5596	0.5596	0.4236	0.7380	0.5625	0.5007	0.8291	0.5625	0.5625	0.8473	0		
Nagachya	0.5148	0.3325	0.6020	0.6931	0.5585	0.6791	0.6020	0.5585	0.5585	0.7380	0.7380	0	
Rumbosol	0.4825	0.4825	0.7520	0.6609	0.7085	0.6060	0.7520	0.4854	0.7085	0.7702	0.2594	0.6609	0

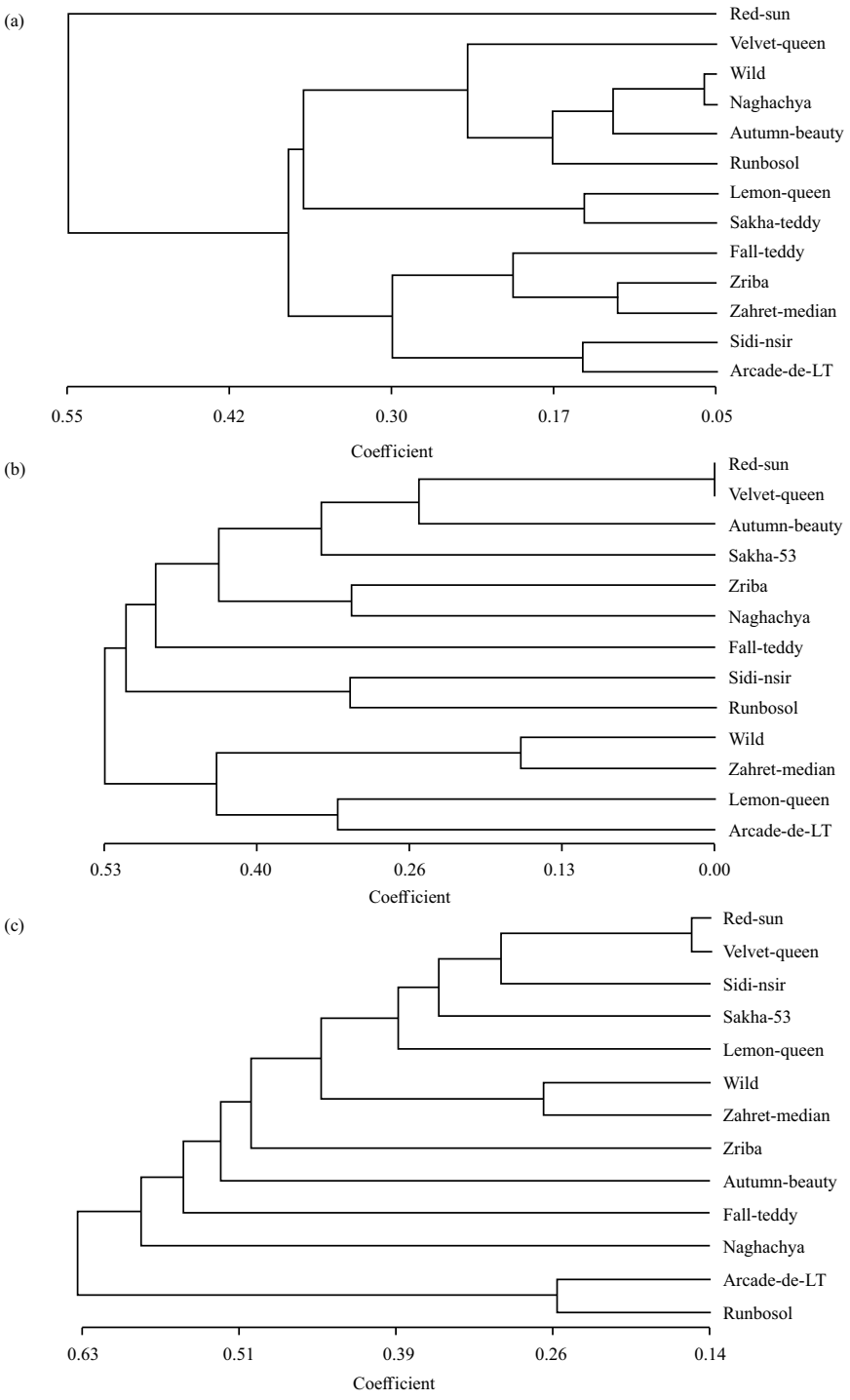

Fig. 3(a-c): Dendrograms gaining from 13 accessions of sunflower with UPGMA based on similarity coefficient. (a) SSR data-based dendrogram; (b)ISSR data-based dendrogram; (c) combined (SSR and ISSR) data based dendrogram

Zrbia and *Zahret Median*, *Sidi Nsir* and *Arcade de LT*). The two genotypes *Sidi Nsir* and *Arcade de LT were* separated in a dichotomous branch with a genetic identity of 0.153, whereas the three remaining taxa were grouped in a dichotomous branch included *Zrbia* and *Zahret Median* genotypes and *Fall Teddy* separated as OUT genotype.

On the other hand, the phylogenetic relationships among the various accessions revealed by ISSR analysis have been

fairly distinctive from that generated by SSR markers. A dendrogram of ISSR gathered the 13 accessions into two main clusters (Fig. 3b). Genotypes within these two master clusters I, II was then split into two subclusters (Ia, Ib and IIa, IIb). Subcluster Ia included three genotypes (*Red Sun*, *Velvet Queen* and *Autumn beauty*) and a single genotype (*Sakha-53*) formed a breakaway in cluster Ia revealing less similarity coefficient (0.340) with the three genotypes. Meanwhile, *Red Sun* and *Velvet Queen* were close to each other with a similarity index equal to 0 (Table 6). subcluster Ia also grouped *Nagachya* with *Zrbia* together in a dichotomous branch as well as *Fall Teddy* which created a separate OUT as exhibited by minimum identity coefficient (0.484) with the remaining 6 genotypes in the same subcluster. Subcluster Ib included only a dichotomous branch with *Sidi Nsir* and *Rumbosol* with Dice index of 0.314. On the other hand, cluster II was subdivided into two subclusters of the remaining four taxa in the form of two dichotomous branches. Subcluster IIa gathered *wild* with *Zahret Median* whereas *Lemon Queen* and *Arcade de LT* were included in subcluster IIb.

Another UPGMA cluster analysis combined the ISSR and SSR data, (Fig. 3c). The type of clustering through the genotypes in the collective test was identical to the ISSR dendrogram in grouping four genotypes in dichotomous branches (*Red Sun* / *Velvet Queen* and *Wild*/ *Zahret Median*), while the SSR dendrogram exhibited a different type of clustering in sunflower genotypes. In general, collective data in a cluster analysis of the two markers formed a dendrogram that broke the genotypes into two clusters. The first cluster (I) involved 11 taxa of sunflower whereas the remaining two genotypes (*Acrade-de LT* and *Rumbosol*) were comprised in the second cluster II in a dichotomous branch with a similarity index of 0.259. Within cluster I, *Wild* and *Zahret Median* genotypes recorded similarity coefficient of 0.269 as they were adjacent to each other and *Velvet-Queen* and *Red Sun* were also grouped in the same branch with a similarity coefficient of 0.154. The other seven genotypes were distant from those four genotypes as they separated as OUT (Fig. 3c).

DISCUSSION

In many studies, genetic and population diversity is commonly estimated using SSR and ISSR markers derived information[39]. Both marker systems were proved as successful tools to assess genetic variation and to resolve the relationships through phylogeny in *H. annuus* L.[40,41]. Polymorphism is a result of divergence in DNA sequences and indicates genetic diversity among individuals. In this study, the results showed high polymorphism among the genotypes of *H. annuus*, which reflects the presence of wide genetic variability. This elevated degree of polymorphism might be referred to as the different locations of the genotypes or/and their origin knowledge[42]. Previous studies reported polymorphism percentage of 91.30%[40] and 54.05%[33] in *H. annuus*. In this study, both SSR and ISSR markers were confirmed to have efficiency for estimation of genetic variation in sunflower as they exceeded up to 97% of polymorphism. SSR marker has the advantage as being a co-dominant marker that detects heterozygote from the homozygote genotypes[25,40]. On the other hand, the higher percent of polymorphism generated by ISSR may be due to the change in the attaching positions of the primer as a result of alterations in the microsatellite sequence[43]. This finding is in accordance with polymorphism percentage revealed by ISSR marker among 24 different *P. lentiscus* wild genotypes, in comparison to other molecular markers[44,45].

Awareness of genetic distance between genotypes is beneficial in providing an additional functional sampling of genotypes and breeding applications of germplasm for the improvement of populations[42,46]. A connection of molecular diversity with geographical sites have been established in many previous phylogenetic studies[47-50]. In this study, SSR, ISSR whether individually or combined proved a variable pattern of genetic relatedness regarding the geographical distribution. Considering SSR data analysis, it can be deduced that some of sunflower populations belonging to distant geographical locations (e.g., *Sakha 53* and *Red Sun*) revealed higher genetic variation compared to those from close allocations (e.g., *Zahret Madian*, *Zriba*).Even though the two distant accessions (*Nagachya* and *Wild*) exhibited a higher genetic similarity represented by low Dice similarity value. On the other hand, ISSR data analysis exhibited higher genetic similarity between two genotypes (*Red Sun* and *Velvet Queen*) from the same geographical region, whereas distantly distributed genotypes such as *Wild* and *Sidi Nisr* showed genetic dissimilarity as revealed by high Dice similarity value. This could be attributed to limiting gene stream by seed migration through geographical barriers. As suggested by Siragusa *et al.*[51], effective segregation of tightly attached accessions could be verified by combined data from more than one molecular marker. In the same context, combined SSR and ISSR data confirmed the genetic relatedness of both *Red Sun* and *Velvet Queen* genotypes whereas high genetic dissimilarity was greatly represented between *Sakha 53* and *Acrade de LT* as they were from different geographical regions. Nevertheless, these findings are inconsistent with the lower recorded genetic similarity index between the two genotypes (*Zahret Median* and *Acrade de LT*), irrespective of the geographical

origin. Hence, the position of those genotypes in different zones and/or their origin history resulted in genetic diversity among the sunflower genotypes.

Furthermore, UPGMA analysis showed no obvious manner of clustering according to the geographical sites. As indicated by the Dice coefficient, cluster tests exhibited that accessions from a single geographical area collected with each other in some situations while they were positioned in dissimilar clusters in others. Phylogenetic analysis of both markers allocated sunflower cultivars as sister groups in two clusters indicating that they have the same progenitor. However, each analysis showed a variable pattern of monophyletic groups which is fortified by similarity coefficient values. SSR dendrogram exhibited four monophyletic groups whereas ISSR phylogenetic construction generated five different monophyletic groups. Combined ISSR and SSR data reduced the number of monophyletic groups to three, two of them coincided with those generated from ISSR phenogram.

Regardless of its origin genotype, genetic variability within sunflower accessions could be attributed to insertion/deletion or genetic recombination[52], high allogamy quality of the sunflower plant[53], crossed mating system[54], diversity in selection, adaptation, migration, surroundings and human actions[55], incomplete genome covering due to the small number of studied samples and markers[56,57]. The indicated non-considerable relation between genetic similarity and geographical distance is consistent with phylogenetic studies on other plant species, e.g., canola[42], *Melocanna*[58], castor [59]. Furthermore, this study detected the presence of different genetical subdivision of groups within the investigated sunflower cultivars regardless of whether they were naturally occurring (*Wild and Lemon Queen*) or cultivated. In disturbed habitats, likely, wild sunflower is mainly grown as a weed and so have been expanded via anthropogenic activities[60-62]. This diversion is probably owing to the time of development and variations in the breeding history of the cultivated species.

CONCLUSION

Based on SSR and ISSR markers, this study aimed to deduce the genetic variation of sunflower accessions gathered from different geographic areas including wild and cultivated cultivars. In comparison to SSR, ISSR markers were proved as preferable candidates for supplementary investigations on genetic diversity in *H. annus* because of their higher clonal polymorphism. No remarkable association between genetic diversity and geographic distribution was recorded in this study. Therefore, more extensive analysis with a higher number of molecular markers and genotypes will be required to clarify the existence of genetic variance in *H. annus* germplasm.

SIGNIFICANCE STATEMENT

This study discovers the allocation and extension of genetic diversity on 13 sunflowers (*Helianthus annuus*) genotypes from different regions. This study will help the researcher to uncover the critical areas of the wide genetic basis for outlook sunflower that many researchers were not able to explore. Thus, a new theory on germplasm breeding programs may be arrived at.

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