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AGRO-MORPHOLOGICAL VARIATION AND GENETIC DIVERSITY ASSESSMENT OF TUNISIAN SUNFLOWER (*HELIANTHUS ANNUUS* L.) ACCESSIONS USING MICROSATELLITE MARKERS

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is considered as one of the most important oilseed crops in the world. In Tunisia, sunflower is cultivated in the Northern region of the country using local populations which are genetically not well studied. This study was conducted to evaluate the genetic variation of 33 accessions (26 local and 7 introduced) using 23 agro-morphological traits and 15 simple sequence repeats (SSR) markers. The variance analysis of the agro-morphological traits showed a significant variation among sunflower accessions. The relationship among the sunflower accessions was also performed by using the principal component analysis; the two first axes explained 54.4% of the total variability and showed that the accessions spread into five groups. The groups (G3 and G4) could be used to improve sunflower varieties with high performance especially for the diameter of the head and the weight of 1000 seeds characters. Ten among the 15 SSR primers used revealed clear polymorphic bands and were able to amplify 29 alleles with an average of 2.9 alleles per locus. The percentage of total polymorphism, the polymorphism information content (PIC) and the dissimilarity coefficient values varied from 50 to 100% with an average of 91%, from 0.35 to 0.75 with an average of 0.50, and from 0.00 to 0.81 with an average of 0.41, respectively. Cluster analysis of the SSR markers grouped accessions into 3 distinct groups. A significant correlation was observed among SSR markers and morphological traits. These results found in this study may be helpful for improvement of sunflower breeding programs.

Keywords: *Helianthus annuus*; genetic diversity; microsatellite; morphological characterisation; cluster analysis.

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INTRODUCTION

The cultivated sunflower (*Helianthus annuus* L.) is a diploid species (2n = 34), allogamous, a member of the *Asteraceae* family. It occupies an important place in several countries of the world with around 51.95 million tones global production in 2018 (FAOSTAT, 2018). The part of sunflower edible oil represents 12% in 2017 of the world consumption, it ranks fourth behind palm, soybean and canola oil (Rauf *et al*., 2017).

In Tunisia, sunflower is being cultivated in the Northern favourable area of the country where farmers, in absence of hybrid varieties, are using their own and local produced seeds. Exploring the genetic diversity/variation of these local populations could provide information on agro-morphological performances and potential traits associated with the specific adaptation to local environments.

The genetic variation of cultivated sunflower in Tunisia remains unknown. Unlike their biology and physiology, few studies interested on the evaluation of their genetic diversity. The characterization of genetic diversity and the determination of genetic relationships of the germplasm are important to investigate. Several criteria for plant genetic diversity estimation can be used (morphological, cytological, biochemical and molecular markers) (Nadeem *et al*., 2018). Morphological traits have been commonly used to estimate relationships between genotype (Andersson *et al*., 2006). The breeder could evaluate genetic diversity of their plant material based on data of biometric measurements. Morphological markers have been applied in sunflower breeding program to indentify genetic variability (Muller *et al*., 2006; Presotto *et al*., 2009; Ghaffari *et al*., 2020).

In the other hand, molecular marker technology has been considered as a very effective tool for evaluating genetic diversity for many plant species. The simple sequence repeats (SSR) markers have been widely used for assessment of genetic diversity because they are co-dominant, easy to score and highly polymorphic markers (Lichtenzveig *et al*., 2005). These markers have been successfully used in *H. annuus* to assess molecular

variability (Muller *et al*., 2010; Filippi *et al*., 2015; Bulatova *et al*., 2020).

In the present study, we used the morphological traits and SSR markers to investigate genetic variability among 33 sunflower accessions in order to develop a breeding program and to improve selection efficiency.

MATERIALS AND METHODS

Plant Material: Plant material included 26 accessions of sunflower collected from different locations in the main growing areas and 7 introduced sunflower accessions used as references (Table 1). The 26 sunflower accessions were collected by the National Agricultural Research Institute of Tunisia (INRAT) in collaboration with the National Gene Bank (BNG) while the introduced accessions were kindly provided by the National Plant Germplasm System (NPGS) and the Plant Gene Resources of Canada (PGRC).

Agro-Morphological Characterization: The field experiment was carried out in Beja (Latitude: 36°42'N and Longitude: 9°05'E, in the North West of Tunisia). The experimental site was characterized by sub-humid climate with cold winter and hot summer and an average annual precipitation of 560 mm.

The trial was established according to a randomized complete block design (RCBD) including 3 blocks. The tested accessions were 33 entries sowed in single row in each block. The seeds of each sunflower accession were sown directly in the field with 80 cm row spacing and 60 cm separation from plant to plant in each row.

No fertilizer or other chemical treatments were applied. The trial was planted in mid March 2016 and harvested by hand in July 2016. Observations covered 23 morphological descriptors including 16 quantitative and 7 qualitative traits (Table 2). Five plants from each row were used to carry out all the measurements. Observations were performed at the maturation stage.

Molecular Characterization: Genomic DNA was isolated from 2-weeks-old seedlings leaves according to Saghai-Maroof *et al*. (1984). DNA quality was examined and estimated using 0.8% agarose gel electrophoresis and stored at -20ºC. A set of fifteen simple sequence repeat (SSR) primer pairs (Table 3) were used for molecular analyses. Nine primer pairs were acquired from Tang *et al*. (2002): ORS 1265, ORS 928, ORS 844, ORS 878, ORS 598, ORS 920, ORS 423, ORS 160 and ha3555, and 6 primers pairs were acquired from Poormohamed Kiani *et al*. (2007): ha2682, ha4142, ha1604, ha3638, ha4136 and ORS 718.

The PCRs were carried out in a final volume of 25 µl containing 5 µl of PCR buffer (1x), 1.25 µl of $MgCl_2$ (2.5 mM), 0.25 µl of dNTPs (100 µM), 0.625 µl of each primer (0.25 µM), 0.2 µl of Taq DNA polymerase (1U) and 5 µl of template DNA (5 ng/µl). The PCR amplifications were carried out in a thermocycler (Boi_Rad C100^{TM}). The touchdown PCR were used for the amplification of all investigated SSRs as: 95ºC for 3 minutes, 1 cycle of 94 ºC for 30 seconds, 64ºC for 30 seconds, 72ºC for 45 seconds and was followed by 10 cycles with a decrease of annealing temperature at 1ºC per cycle. This was followed by 33 cycles of 94ºC for 30 seconds, 54ºC for 30 seconds and 72ºC for 45 seconds. The final extension was 20 minutes at 72ºC (Tang *et al*., 2002). Amplified PCR products were separated by electrophoresis using 2% agarose gel (1xTBE buffer), stained by ethidium bromide (0.5µg/ml) and visualized under ultraviolet light. All PCR reactions and electrophoreses were performed twice and independently scored. The 100 bp marker was used as a molecular size marker.

Statistical Analysis: For quantitative morphological traits data, an analysis of variance (ANOVA) was conducted using PROC anova procedure of SAS software version 9.3. Means were compared with Duncan's test at alpha = 0.05. Data means were then used to conduct a principal component analysis (PCA) using PROC PRINCOMP procedure of SAS software version 9.3.considering the following variables: Leaf width, Leaf length, Stem diameter, Head diameter, Sterile spot diameter, Number of lines per head, Number of grains per head, Weight of grains per head, Hulls weight, Kernels weight, Leaves number, Plant height, Grains length, Grains width, Bract tip length of the head and 1000 grains weight. For qualitative morphological traits data, some descriptive statistics were used to compare Tunisian and introduced accessions.

Molecular data were analysed using the NTSYS-pc software version 2.1 as described by Rohlf (2000). A 0/0.5/1 (absence/allele in heterozygosity/allele in homozogosity) matrix was constructed. From these data, a dissimilarity matrix was calculated with the SIMGEND module using the Nei coefficient (Nei, 1972). Cluster analyses were carried out using UPGMA (Unweighted Pair-Group Method with Arithmetic mean) method.

The percentage of polymorphism (PP) was calculated as follows: PP= number of polymorphic bands/total number of bands x100. Polymorphism information content (PIC) was calculated for each marker as described by Anderson *et al*. (1992) using the following formula:

$$PICj = 1 - \sum_{i=1}^{n} Pi^2$$

Where Pi: the frequency of the i^{th} allele revealed by the j^{th} primer, i: i^{th} allele for the j^{th} primer and n: the total number of alleles for the j^{th} primer.

A Mantel test was conducted using XLSTAT software for correlation between morphological and SSRs markers.

RESULTS

Morphological data: Analysis on variance of quantitative parameters showed a significant ($P < 0.05$) effect of the block factor on Leaf width, Leaf length, Stem diameter, Head diameter, Sterile spot diameter, Number of lines per head, Number of grains per head, Weight of grains per head, Hulls weight and Kernels weight. However, block factor have a non-significant effect on Leaves number, Plant height, Grains length, Grains width, Bract tip length of the head and 1000 grains weight. Variance analysis (ANOVA) revealed also highly significant effect of sunflower accession on all the quantitative characters (Table 4). Comparison of the mean values quantitative morphological traits revealed that the highest coefficient of variation (32.8%) was observed for the number of leaves, whereas the smallest ones were observed for seed length and seed width (9.7% and 9.5%, respectively). The difference between the minimum and the maximum was higher in all traits studied. The mean values of the quantitative morphological traits measured for all sunflower accessions are presented in Table 5 and showed a wide polymorphism for all characters.

The performed (PCA) analysis showed that the two first axes explained 54.43% of the total variability (Fig. 1). The first axis of PCA represents the direction of maximum variation through the data. Next, another axis is added orthogonal to the first and positioned to represent the next highest variation through the data.

The first axe expressed the largest percentage of variability (38.57%). This axe was positively correlated with all quantitative parameters except the leaves number (LN). The second axe explained 15.86% of variability. It was positively defined by the following parameters: grain width (GW), hulls weight (HW), kernels weight (KW), 1000 grains weight (W1000G), grains length (GL) as well as head diameter (HD). It was negatively correlated with plant height (PH), number of grains per head (NGH), number of lines per head (NLH), stem diameter (SD), leaves width (LW), leaves length (LL), bract tip length of the head (BTL), weight of grain per head (WGH) and sterile spot diameter of the head (SSD). The projection of patterns of accessions in the layout generated by the axis 1 and 2 showed a distribution of the 33 accessions in five main groups (Figure 1). The first group (G1), was composed by three accessions (TL15, TL17 and TL18) introduced from abroad. They were characterized by reduced diameter of the stem (2.24, 2.4 and 2.23 respectively). Moreover, TL17 and TL18 were distinguished from the other accessions by high number of leaves (40 and 39, respectively), reduced leaf length (15.44 and 14.89, respectively) and reduced leaf width (13.56 and 13, respectively). The second group (G2) included TL12, TL13, TL16 and TL23. All these accessions except TL23 were introduced from abroad. These accessions were characterized by important sterile spot diameter of the head (3, 3.72, 3.17 and 3.83, respectively) and low 1000 grains weight (69.56, 59.12, 52.56 and 75.14, respectively). The accessions TL1, TL3, TL8 and TL33 belonged to the third group (G3). These accessions were defined by large leaf length (24.78, 25.33, 23.78 and 28.56, respectively), high leaf width (23.56, 25.67, 23.44 and 26.56 respectively), high seed weight of head (106.34, 88.88, 76.7 and 73.33, respectively), large stem diameter (3.09, 3.17, 3.71 and 3.24, respectively) and large head diameter (22.56, 24.67, 19.94 and 19, respectively). The fourth group (G4) was represented by TL21 and TL30 accessions. These accessions were characterized by the largest seed kernel weight (0.7 and 0.71, respectively), the highest seed shell weight (0.71 and 0.43, respectively) and the highest 1000 grains weight (117 and 103.41, respectively). The group (G5) contained the remaining landraces accessions and one introduced accession (TL14).

The qualitative traits, showed high variability among the accessions (Table 4). In fact, the grain shape is a trait that could discriminate sunflower accessions derived from distinct origin (Fig. 2). In this respect, the grains were mainly broad ovoid (80.7%) for Tunisian accessions and mainly elongated (77.15%) for introduced accessions. The study of the frequency of the heads shape showed that landraces accessions were characterized by 87% strongly convex heads while the introduced accessions had 75% of the weakly convex heads. According to the character “bract shape of head”, the Tunisian accessions represented 79.3% of the accessions having a clearly rounded bract. The introduced accessions (85.7% of the accessions) showed a neither clearly elongated nor clearly rounded bract shape. For "heads attitude" trait, the majority of the landraces (89%) had a turned down heads while the introduced accessions (82.8%) showed an inclined attitude. The local accessions were grouped into two groups, according to the colour of the seeds. The first group represented the majority of accessions (73%) had a black colour whereas the second group had a grey colour. The introduced accessions had a black colour.The trait of the presence of stripes on grain margins allowed us to distinguish various groups of accessions. The landraces accessions were distributed into three distinct groups. The first group represented 69% of the accessions with none or very weakly expressed stripes. The second group represented 23% of the accessions with seeds presenting stripes on margins poorly expressed. Whereas, the third group represented only 8% of the accessions with seeds having expressed stripes. While all of the introduced accessions were characterized by seeds having stripes very weakly expressed or absent on the edges.

The frequencies of stripes among margins grain showed three distinct classes on local landraces. The main one (60%) characterized by seeds having expressed

weakly stripes or absent on the edges. Only 3.85% of the evaluated accessions had seeds with strongly expressed stripes between margins. However, 36.15% of the landraces had seeds with weakly expressed stripes between margins. The introduced accessions had seeds weakly expressed stripes or absent.

Molecular data: Fifteen SSR primers pairs were used in this study and 10 of them produced clear polymorphic bands, whereas 5 primer pairs produced monomorphic bands (Table 3). Among the total of 34 bands, only 25 were polymorphic. The total number of alleles amplified by the 10 polymorphic primers was 29 with an average of 2.9 alleles per locus. The SSR primer ha1604 showed the most polymorphic microsatellite with high number of amplified alleles (5 alleles/locus). The less polymorphic were ORS 1265, ha2682, ha3638, ha4142 SSR primers with 2alleles/locus (Table3). The results depicted that the percentage of polymorphism ranged from 50 to 100% with an average of 91%. Among the 10 primers used, 8 showed 100% polymorphism. The PIC values ranged from 0.35 for the ha2682 primer to 0.75 for the ha4136 primer with an average of 0.50 (Table 3).

The analyses of SSR profiles obtained allowed to produce distance matrix for the genetic dissimilarity among sunflower accessions. The matrix obtained (Table 6) showed that the genetic variance distance ranged between 0.00 and 0.81, with an average of 0.41. The highest genetic distances were observed among the following genotypic components: (TL12-TL17: 0.81), (TL9-TL12: 0.77), (TL14-TL17: 0.75), (TL12-TL18: 0.72), (TL11-TL12: 0.66), (TL12-TL31: 0.62), (TL12-TL27: 0.62), (TL12-TL23: 0.62) and (TL6-TL17: 0.62). These ones showed that these coefficients reflected a great divergence between the accessions. However, the least coefficients of dissimilarity were observed for the following genotypic components: (TL3-TL21: 0.00), (TL3-TL25: 0.00), (TL19-TL20:0.00), (TL21-TL25: 0.00), (TL22-TL26: 0.00), (TL23-TL27: 0.00), (TL4-TL8: 0.03), (TL7-TL26: 0.03), (TL7-TL22: 0.03), (TL7-TL8: 0.03), (TL8-TL33: 0.03), (TL8-TL31: 0.03), (TL22-TL33: 0.03), (TL22-TL27: 0.03), (TL22-TL23: 0.03), (TL23-TL26: 0.03), (TL26-TL33: 0.03) and (TL26-TL27: 0.03). These ones proved that these individuals had the highest similarity in their genetic bases.

The UPGMA dendrogram analysis revealed three major groups (A, B and C) considering a fixed point of dissimilarity at 0.23 (Fig. 3). The first group (A) contained 28 accessions. This group included the introduced accessions (TL13, TL16 and TL18) as well as the rest of the local accessions. The second group (B) contained two accessions: One TL6 from Tunisia and TL15 from Romania. The third group (C) contained the accessionsTL12 and TL14 respectively from Spain and the former Soviet Union. Whereas, the accession TL17 originated from United States, was clustered out of the three mentioned groups.

Correlation between SSR and morphological traits: Mantel test was applied to see for possible correlations among distance matrices based on molecular and morphological data (Fig. 4). The Mantel test was significant at the 5% threshold (r = 0.164 and p = 0.013).

Table 1: List of 33 *Helianthus annuus* L. accessions used for diversity analysis.

Accessions no.	Codes of NPGS and PGRC	Origins	Latitudes (N)	Longitudes (E)	Altitudes (m)
TL1	-	Tunisia	36° 53′ 46.405″	9° 26′ 43.522″	258
TL2	-	Tunisia	36° 53′ 03.856″	9° 26′ 00.820″	263
TL3	-	Tunisia	36° 48′ 52.197″	9° 21′ 37.392″	335
TL4	-	Tunisia	36° 48′ 52.197″	9° 21′ 37.392″	335
TL5	-	Tunisia	36° 51′ 37.225″	9° 18′ 15.015″	335
TL6	-	Tunisia	36° 47′ 06.360″	9° 19′ 24.550″	307
TL7	-	Tunisia	36° 44′ 50.068″	9° 04′ 30.182″	341
TL8	-	Tunisia	36° 45′ 59.276″	9° 03′ 44.647″	351
TL9	-	Tunisia	36° 44′ 50.068″	9° 04′ 30.182″	341
TL10	-	Tunisia	36° 50′ 33.992″	9° 12′ 36.437″	370
TL11	-	Tunisia	36° 50′ 33.992″	9° 12′ 36.437″	370
TL12	PI 633614	Spain	-	-	-
TL13	PI 257641	former Soviet Union	-	-	-
TL14	PI 257643	former Soviet Union	-	-	-
TL15	PI 431521	Romania	-	-	-
TL16	PI 617027	United States	-	-	-
TL17	PI 607925	United States	-	-	-
TL18	CN 37370	Canada	-	-	-
TL19	-	Tunisia	36° 53′ 46.405″	9° 26′ 43.522″	258

TL20	-	Tunisia	36° 45′ 47.657″	9° 11′ 43.281″	225
TL21	-	Tunisia	36° 45′ 47.657″	9° 11′ 43.281″	225
TL22	-	Tunisia	36° 51′ 46.655″	9° 22′ 58.578″	370
TL23	-	Tunisia	36° 47′ 06.360″	9° 19′ 24.550″	307
TL24	-	Tunisia	36° 45′ 01.597″	9° 10′ 15.707″	314
TL25	-	Tunisia	36° 46′ 39.454″	9° 08′ 09.588″	266
TL26	-	Tunisia	36° 44′ 50.068″	9° 04′ 30.182″	341
TL27	-	Tunisia	36° 43′ 15.577″	9° 05′ 12.526″	315
TL28	-	Tunisia	36° 43′ 31.962″	9° 08′ 40.498″	445
TL29	-	Tunisia	36° 48′ 37.964″	9° 11′ 07.264″	270
TL30	-	Tunisia	36° 48′ 37.964″	9° 11′ 07.264″	270
TL31	-	Tunisia	36° 50′ 33.997″	9° 12′ 36.499″	374
TL32	-	Tunisia	36° 50′ 33.992″	9° 12′ 36.437″	370
TL33	-	Tunisia	36° 47′ 27.886″	9° 16′ 47.552″	360

Table 2: Morphological descriptors measured on 33 *Helianthus annuus* L. accessions.

Type of descriptors	Descriptors	Abbreviations	Scale / unit
Quantitative	Leaves number	LN	-----
	Leaf length	LL	(cm)
	Leaf width	LW	(cm)
	Plant height	PH	(m)
	Stem diameter	SD	(cm)
	Head diameter	HD	(cm)
	Sterile spot diameter	SSD	(cm)
	Bract tip length of the head	BTL	(cm)
	Number of grains per head	NGH	-----
	Weight of grains per head	WGH	(g)
	Number of lines per head	NLH	-----
	1000 grains weight	W1000G	(g)
	Hulls weight	HW	(g)
	Kernels weight	KW	(g)
	Grains length	GL	(mm)
	Grains width	GW	(mm)
Qualitative	Head attitude	-	1= inclined; 2= vertical; 3= half-turned down; 4= turned down; 5= over turned.
	Head shape	-	1= weakly convex; 2= strongly convex.
	Bract shape	-	1= neither clearly elongated nor clearly rounded; 2= clearly rounded.
	Grain shape	-	1= elongated; 2= narrow ovoid; 3= broad ovoid; 4= rounded.
	Grain main color	-	1= grey; 2= black.
	Grain stripes on margins	-	1= none or very weakly expressed; 2= weakly expressed; 3= strongly expressed.
	Grain stripes between margins	-	1= none or very weakly expressed; 2= weakly expressed; 3= strongly expressed.

Table 3: Information on SSR primers pairs detected among the 33 *Halianthus annuus* L. accessions.

Primers	Forward (5'-3')	Reverse (5'-3')	NBA	NPB	PP (%)	PIC
ORS 1265	GGGTTTAGCAAATAATAGGCACA	ACCCTTGGAGTTTAGGGATCA	2	2	100	0.50
ORS 928	CATGGTTATTTTGGTTTGGGTTT	GCTATTATCATGTCCTTGTCCTTTT	3	3	100	0.53
ORS 844	ACGATGCAAAGAATATACTGCAC	CATGTTTAATAGGTTTTAATTCTAGGG	3	3	100	0.52
ORS 878	TGCAAGGTATCCATATTCCACAA	TATACGCACCGGAAAGAAAGTC	3	3	100	0.48
ORS 598	CCAAATGTGAGGTGGGAGAA	ATAGTCCCTGACGTGGATGG	3	3	100	0.38
ha2682	CACAATCGTTTCTTTCCAAAA	ACCCATATGCCCACTCATAA	2	2	100	0.35
ha4142	GAGTCGACATTTTCGGAAATCG	CTTCATCTTCTGACACCCAAC	2	2	100	0.44
ha1604	GCAAATGCACTAAAGGCCCC	CCCTACTCAAACCTTACCTC	5	3	60	0.56
ha3638	GACATAATCACTAGTTGTTGGTGC	CTCCTCCCACCTCAACAATTTC	2	2	100	0.51
ha4136	CCTATTCCTGATAATTCACTAAGC	GGTAGCATGCTTACATTAAGATG	4	2	50	0.75
ORS 920	CGTTGGACGAAGAACTTGATTT	ACTTCCGTTTGTTCCGAGCTT	1	0	0	0.00
ORS 718	CACTTTACGCACACCAAACC	ATGCAACACCCGAATCAAAG	1	0	0	0.00
ORS 423	TCATATGGAGGGATCTGTTGG	AAGCAACCATAATGCATCAGAA	1	0	0	0.00
ORS 160	TCCCTTCCTTTCATCGTCTGCT	TGGCAATTTGCCAAGGACC	1	0	0	0.00
ha3555	GATATCTCTCATAAGTGCCG	GGTCTTGTGATGACGAA	1	0	0	0.00
Total/ Average	-----	------	34	25	91	0.50

NBA (number of bands amplified); NPB (number of polymorphic bands); PP (percentage of polymorphism); PIC (polymorphism information content).The primers ORS 920, ORS 718, ORS 423, ORS 160 and ha3555 were excluded from calculation of the average values since they did not produce polymorphic bands.

Table 4: Range of variation for various morphological traits measured in 33 *Helianthus annuus* L. accessions.

Quantitative traits	CV%	Mean	Max	Min	MS (P Values)	
					Accession	Block
Leaves number	32.84	25.12	40.44	17. 67	384.10 (<.0001)	82.06 (0.32)
Leaf width (cm)	13.77	21.25	26.56	13	88.51 (<.0001)	51.25 (0.003)
Leaf length (cm)	13.48	22.42	28.56	14.44	89.13 (<.0001)	44.87 (0.008)
Plant height (m)	12.14	1.33	1.57	0.96	0.20 (<.0001)	0.04 (0.23)
Grains length (mm)	9.70	11.20	12.69	8.27	6.56 (<.0001)	0.6 (0.60)

Grains width (mm)	9.48	6.90	8.5	2.77	10.86 (<.0001)	1.17 (0.07)
Stem diameter (cm)	15.88	2.79	3.71	2.23	1.05 (<.0001)	0.76 (0.02)
Head diameter (cm)	15.35	17.80	24.67	9	73.73 (<.0001)	24.68 (0.04)
Sterile spot diameter (cm)	17.87	2.81	3.83	1.51	2.12 (<.0001)	1.27 (0.007)
Number of lines per head	15.39	19.05	26.33	8	116.27 (<.0001)	27.48 (0.04)
Bract tip length of the head (cm)	18.81	2.44	3.08	1	1.77 (<.0001)	0.31 (0.24)
Number of grains per head	14.79	788	1261	165	396983.03 (<.0001)	46093.4 (0.03)
Weight of grains per head (g)	17.27	63.99	106.34	15	2880.15 (<.0001)	727.91 (0.003)
1000 grains weight (g)	16.34	82.96	117	42.56	2122.27 (<.0001)	487.95 (0.07)
Hulls weight (g)	25.84	0.30	0.70	0.15	0.09 (<.0001)	0.02 (0.04)
Kernels weight (g)	17.68	0.54	0.71	0.30	0.09 (<.0001)	0.054 (0.003)

Qualitative traits	**Tunisian accessions**	**Introduced accessions**
Head attitude	turned down= 89%; half-turned down= 5.4%; over turned= 3%; vertical= 2.6%.	inclined= 82.8%; turned down= 8.6%; vertical= 4.3%; half-turned down= 4.3%.
Head shape	Strongly convex= 87%; weakly convex= 13%.	weakly convex= 75%; Strongly convex= 25%.
Bract shape	Clearly rounded= 79.3%; neither clearly elongated nor clearly rounded= 20.7%.	neither clearly elongated nor clearly rounded= 85.7%; clearly rounded= 14.3%.
Grain shape	broad ovoid= 80.7%; narrow ovoid= 10.8%; rounded= 7.7%; elongated= 0.8%.	elongated= 77.15%; narrow ovoid= 20.6%; broad ovoid= 2.25%.
Grain main color	black= 73%; grey= 27%.	black= 100%.
Grain stripes on margins	none or very weakly expressed= 69%; weakly expressed= 23%;strongly expressed= 8%.	none or very weakly expressed= 100%.
Grain stripes between margins	none or very weakly expressed= 60%; weakly expressed= 36.15%; strongly expressed= 3.85%.	none or very weakly expressed= 100%.

CV (coefficient of variance); Max (maximum); Min (minimum); MS (mean squares); P (probability).

Table 5: Means of the quantitative morphological traits measured on the 33 sunflower accessions according to Duncan's test at P < 0.05

Accessions	LN	LW (cm)	LL (cm)	GL (mm)	GW (mm)	NGH	WGH (g)	W1000G (g)	HW (g)	KW (g)	PH (m)	SD (cm)	HD (cm)	SSD (cm)	BTL (cm)	NLH
TL1	20.67^{ef}	23.56^{af}	24.78^{be}	11.44^{bh}	7.77^{be}	1108.56^{b}	106.34^{a}	95.15^{be}	0.31^{ch}	0.66^{ac}	1.33^{dg}	3.09^{be}	22.56^{ab}	2.22^{ij}	2.44^{cg}	22.44^{bd}
TL2	19.67^{ef}	19.33^{hk}	22.33^{ch}	10.43^{gh}	7.49^{dg}	585.11^{jk}	56.28^{ik}	101.94^{bd}	0.23^{hl}	0.54^{dj}	1.10^{ik}	2.28^{h}	15.72^{gj}	3^{cg}	2.28^{eh}	15^{jl}
TL3	24.67^{df}	25.67^{ab}	25.33^{bd}	11.17^{dh}	8.5^{a}	930^{df}	88.88^{b}	95.88^{be}	0.33^{ce}	0.71^{a}	1.48^{ad}	3.17^{bd}	24.67^{a}	2.5^{gj}	2.75^{ae}	24^{ab}
TL4	21.44^{ef}	21.56^{dk}	21.78^{eh}	11.68^{ag}	7.85^{ad}	742.44^{gi}	60.67^{gj}	82.48^{ej}	0.36^{bc}	0.64^{ad}	1.36^{cg}	2.4^{gh}	17.44^{eh}	2.06^{j}	2.13^{fh}	19.22^{di}
TL5	24.78^{df}	22.33^{ci}	22.78^{bh}	12^{ae}	6.59^{hj}	724.33^{gi}	62.58^{fj}	85.86^{eh}	0.24^{fk}	0.54^{dj}	1.37^{bg}	2.82^{bg}	16.89^{fj}	3.5^{ac}	3.08^{a}	17.67^{hj}
TL6	24.22^{df}	23^{bg}	24.33^{be}	10.71^{fh}	7.21^{dh}	1006.22^{bd}	80.06^{bd}	80.71^{ej}	0.26^{dj}	0.45^{il}	1.56^{a}	2.79^{bg}	21.89^{bc}	2.17^{ij}	2.47^{cg}	23.33^{bc}
TL7	22.56^{df}	19.33^{hk}	19.67^{h}	11.14^{dh}	6.29^{j}	645.56^{ik}	53.79^{jk}	84.94^{ei}	0.20^{il}	0.48^{gk}	1.37^{bg}	2.67^{eh}	15.22^{hj}	2.67^{ei}	1.8^{hi}	18.33^{gi}
TL8	29de	23.44^{af}	23.78^{bf}	11.9^{af}	6.98^{fj}	999.22^{bd}	76.69^{ce}	76.67^{fj}	0.33^{ce}	0.62^{ae}	1.54^{ab}	3.71^{a}	19.94^{k}	2.83^{dh}	3^{ab}	26.33^{a}
TL9	22.67^{df}	23.33^{bg}	22^{dh}	10.32^{hi}	7.49^{dg}	1004.56^{bd}	72.73^{cf}	72.25^{gk}	0.31^{ch}	0.55^{di}	1.21^{gi}	2.68^{dh}	18.33^{dg}	2.61^{fi}	2.17^{fh}	20.44^{ch}
TL10	24.44^{df}	22.67^{bh}	23^{bh}	11.75^{af}	7.23^{dh}	596.89^{jk}	63.54^{fj}	106.28^{ab}	0.33^{cd}	0.6^{bf}	1.52^{ac}	2.83^{bg}	17.17^{ei}	2.5^{gj}	2.67^{af}	17.67^{hj}
TL11	26.67^{df}	21.11^{ek}	24^{bf}	10.98^{dh}	6.78^{gj}	752^{gi}	59.85^{hk}	83.83^{ej}	0.27^{dj}	0.57^{ch}	1.31^{dg}	2.81^{bg}	19.39^{ce}	3.28^{bd}	2.72^{ae}	21.22^{bg}
TL12	26.44^{df}	20.33^{fk}	22.89^{bh}	9.55^{i}	2.77^{l}	696.56^{hj}	48.82^{kl}	69.56^{ik}	0.15^{l}	0.3^{n}	1.35^{cg}	2.46^{fh}	9^{k}	3^{cg}	1^{j}	12.33^{l}
TL13	24.78^{df}	20^{gk}	20.56^{fh}	11.15^{dh}	5.5^{j}	1261.33^{a}	73.67^{cf}	59.12^{kl}	0.20^{il}	0.36^{ln}	1.35^{cg}	2.64^{eh}	17.72^{eh}	3.72^{ab}	2.58^{af}	20.44^{ch}
TL14	17.67^{ef}	22.22^{ci}	24^{bf}	12.42^{ac}	6.56^{hj}	530.89^{k}	41.46^{l}	77.21^{fj}	0.32^{cf}	0.52^{ej}	1.04^{jk}	2.26^{h}	13.94^{j}	2.33^{hj}	2.03^{gh}	13.89^{kl}
TL15	24.56^{df}	15^{l}	14.44^{i}	11.01^{dh}	8.27^{ac}	557.33^{k}	23.75^{mn}	42.56^{m}	0.43^{b}	0.56^{ci}	0.96^{k}	2.24^{h}	14.22^{ij}	3.17^{cf}	2.16^{fh}	15^{jl}
TL16	28.78^{de}	19.56^{hk}	20.11^{hg}	10.78^{eh}	6.7^{hj}	552^{k}	28.14^{m}	52.56^{lm}	0.16^{kl}	0.34^{mn}	1.28^{eh}	2.73^{ch}	14^{j}	3.17^{cf}	2.5^{bg}	23.33^{bc}
TL17	40.44^{b}	13.56^{l}	15.44^{i}	11.31^{ch}	5.13^{j}	1059.11^{bc}	74.98^{cf}	70.77^{hk}	0.19^{jl}	0.52^{ej}	1.03^{jk}	2.4^{gh}	19^{cf}	1.51^{k}	1.5^{i}	8^{m}
TL18	39.44^{bc}	13^{l}	14.89^{i}	8.27^{j}	5.02^{k}	165^{l}	15^{n}	90.66^{cf}	0.25^{dj}	0.4^{km}	1.13^{hj}	2.23^{h}	15.17^{hj}	3^{cg}	2.67^{af}	18.56^{fi}
TL19	22.44^{df}	21.22^{dk}	23.44^{bg}	11.73^{af}	6.88^{gj}	842.56^{eg}	69.48^{dh}	82.45^{ej}	0.25^{dj}	0.52^{ej}	1.49^{ad}	2.93^{bf}	18.44^{dg}	2.89^{dh}	3.06^{a}	22^{be}
TL20	20.78^{ef}	21.44^{dk}	22.67^{bh}	11.71^{af}	6.66^{hj}	843.11^{eg}	59.24^{hk}	69.4^{jk}	0.25^{fj}	0.52^{ej}	1.33^{cg}	2.64^{eh}	16.89^{ei}	2.78^{dh}	2.91^{ac}	21.11^{bg}
TL21	31.67^{cd}	23.33^{bg}	25.67^{ac}	11.57^{ah}	8.43^{ab}	597.56^{jk}	66.56^{ei}	117^{a}	0.71^{a}	0.69^{ab}	1.11^{ik}	3.2^{bc}	20^{be}	2.89^{dh}	2.67^{af}	21.67^{bf}
TL22	26.33^{df}	24.44^{ae}	25.22^{be}	11.1^{dh}	7.1^{ei}	778.78^{gh}	63.6^{fj}	83.62^{ej}	0.3^{ch}	0.58^{ch}	1.38^{bg}	3.28^{b}	18.3^{dg}	2.83^{dh}	2.33^{dg}	20^{di}
TL23	24.33^{df}	24.56^{ad}	25.22^{be}	10.42^{gi}	6.3^{j}	809.33^{fh}	59.55^{hk}	75.14^{gj}	0.23^{gk}	0.44^{jl}	1.48^{ad}	3.06^{be}	19.06^{ce}	3.83^{a}	2.83^{ad}	19.72^{di}
TL24	24.11^{df}	18.44^{k}	20.11^{hg}	10.76^{eh}	7.26^{dh}	727.78^{gi}	63.85^{fj}	87.77^{dg}	0.25^{dj}	0.58^{ch}	1.43^{ae}	2.84^{bg}	17.06^{ei}	2.33^{hj}	2.19^{fh}	16.78^{ik}
TL25	23.33^{df}	22.22^{ci}	20.56^{fh}	10.92^{eh}	7.69^{ce}	851^{eg}	69.05^{dh}	80.65^{ej}	0.26^{dj}	0.51^{ej}	1.37^{bg}	3.02^{be}	18.44^{dg}	2.89^{dh}	2.4^{cg}	19.5^{di}
TL26	25.22^{df}	22.56^{bh}	25.11^{be}	12.26^{ad}	7.27^{dg}	946.89^{ce}	76.62^{ce}	82.28^{ej}	0.29^{ci}	0.54^{dj}	1.42^{af}	2.8^{bg}	17.83^{eh}	3^{cg}	2.5^{bg}	18.39^{fi}
TL27	25.67^{df}	18.67^{jk}	20.67^{fh}	12.69^{a}	6.69^{hj}	978.56^{cd}	84.1^{bc}	85.85^{eh}	0.3^{ci}	0.49^{gk}	1.33^{dg}	2.93^{bf}	16^{fj}	2.5^{gi}	2.17^{fh}	19^{ei}
TL28	26.89^{df}	22^{di}	23.67^{bf}	11.21^{ch}	7.63^{cf}	822.22^{eh}	70.61^{dh}	85.66^{eh}	0.28^{ci}	0.59^{cg}	1.39^{ag}	2.83^{bg}	19.38^{ce}	3.28^{bd}	2.5^{bg}	17.67^{hj}
TL29	22.11^{df}	21.78^{dj}	23.44^{bg}	10.96^{dh}	7.02^{fj}	934.44^{df}	69.5^{dh}	73.66^{gj}	0.24^{ej}	0.47^{hk}	1.35^{cg}	2.6^{eh}	17.39^{eh}	2.89^{dg}	2.58^{af}	22.17^{be}
TL30	24.78^{df}	19^{ik}	20.11^{hg}	12.59^{ab}	7.5^{df}	636.67^{ik}	65.66^{ej}	103.41^{ac}	0.43^{b}	0.7^{ab}	1.35^{cg}	2.57^{eh}	17.56^{eh}	2.5^{gj}	2.75^{ad}	17.44^{hj}
TL31	23.33^{df}	20.89^{fk}	23.33^{bg}	11.53^{ah}	7.49^{dg}	695.78^{hj}	62.67^{fj}	90.85^{cf}	0.32^{cg}	0.54^{dj}	1.24^{fi}	2.73^{ch}	18.67^{dg}	2.5^{gj}	2.25^{eh}	16.67^{ik}
TL32	22.78^{df}	25.44^{ac}	26^{ab}	11.07^{dh}	6.47^{ij}	824^{eh}	70.78^{dh}	84.4^{ej}	0.27^{dj}	0.56^{ci}	1.36^{cg}	3.22^{bc}	21.11^{bd}	3.17^{cf}	2.61^{af}	19.89^{di}
TL33	24.33^{df}	26.56^{a}	28.56^{a}	11.19^{dh}	7.14^{di}	698.56^{hj}	73.33^{cf}	107.26^{ab}	0.41^{b}	0.64^{ad}	1.48^{ad}	3.24^{b}	19^{cf}	3.22^{be}	3^{ab}	19.5^{di}

LN (Leaves number); LL (Leaf length); LW (Leaf width); PH (Plant height); SD (Stem diameter); HD (Head diameter); SSD (Sterile spot diameter); BTL (Bract tip length of the head); NGH (Number of grains per head); WGH (Weight of grains per head); NLH (Number of lines per head); W1000G (1000 grains weight); HW (Hulls weight); KW (Kernels weight); GL (Grains length); GW (Grains width).

Table 6: Matrix of correlations generated by the SSR data and representing the coefficient of dissimilarity existing between 33 sunflower accessions.

	TL1	TL2	TL3	TL4	TL5	TL6	TL7	TL8	TL9	TL10	TL11	TL12	TL13	TL14	TL15	TL16	TL17	TL18	TL19	TL20	TL21	TL22	TL23	TL24	TL25	TL26	TL27	TL28	TL29	TL30	TL31	TL32	TL33
TL1	0.00																																
TL2	0.04	0.00																															
TL3	0.16	0.12	0.00																														
TL4	0.23	0.18	0.22	0.00																													
TL5	0.18	0.22	0.08	0.33	0.00																												
TL6	0.33	0.37	0.2	0.37	0.22	0.00																											
TL7	0.16	0.2	0.15	0.07	0.16	0.2	0.00																										
TL8	0.2	0.15	0.11	0.03	0.2	0.24	0.03	0.00																									
TL9	0.26	0.2	0.15	0.14	0.16	0.4	0.15	0.11	0.00																								
TL10	0.13	0.08	0.04	0.1	0.13	0.26	0.12	0.07	0.12	0.00																							
TL11	0.33	0.26	0.12	0.27	0.13	0.21	0.2	0.15	0.12	0.17	0.00																						
TL12	0.55	0.59	0.56	0.57	0.62	0.3	0.5	0.53	0.77	0.52	0.66	0.00																					
TL13	0.18	0.22	0.16	0.23	0.09	0.33	0.16	0.2	0.16	0.13	0.22	0.48	0.00																				
TL14	0.36	0.3	0.25	0.32	0.36	0.4	0.34	0.29	0.43	0.21	0.5	0.21	0.26	0.00																			
TL15	0.46	0.4	0.25	0.32	0.36	0.13	0.25	0.21	0.34	0.3	0.17	0.48	0.58	0.53	0.00																		
TL16	0.28	0.23	0.18	0.1	0.28	0.32	0.1	0.07	0.18	0.14	0.23	0.45	0.23	0.24	0.24	0.00																	
TL17	0.36	0.4	0.34	0.41	0.36	0.62	0.34	0.37	0.34	0.3	0.4	0.81	0.46	0.75	0.44	0.41	0.00																
TL18	0.36	0.3	0.17	0.41	0.17	0.5	0.34	0.29	0.17	0.21	0.13	0.72	0.17	0.44	0.44	0.24	0.35	0.00															
TL19	0.13	0.17	0.04	0.27	0.04	0.17	0.12	0.15	0.2	0.08	0.17	0.52	0.13	0.3	0.3	0.23	0.3	0.21	0.00														
TL20	0.13	0.17	0.04	0.27	0.04	0.17	0.12	0.15	0.2	0.08	0.17	0.52	0.13	0.3	0.3	0.23	0.3	0.21	0,00	0.00													
TL21	0.16	0.12	0,00	0.22	0.08	0.2	0.15	0.11	0.15	0.04	0.12	0.56	0.16	0.25	0.25	0.18	0.34	0.17	0.04	0.04	0.00												
TL22	0.2	0.24	0.11	0.18	0.11	0.15	0.03	0.07	0.19	0.15	0.15	0.53	0.2	0.37	0.21	0.14	0.37	0.29	0.07	0.07	0.11	0.00											
TL23	0.26	0.3	0.15	0.22	0.08	0.2	0.07	0.11	0.15	0.2	0.12	0.62	0.16	0.43	0.25	0.18	0.43	0.25	0.12	0.12	0.15	0.03	0.00										
TL24	0.2	0.24	0.11	0.26	0.11	0.24	0.11	0.14	0.19	0.15	0.15	0.53	0.2	0.37	0.21	0.14	0.21	0.13	0.07	0.07	0.11	0.07	0.11	0.00									
TL25	0.16	0.12	0,00	0.22	0.08	0.2	0.15	0.11	0.15	0.04	0.12	0.56	0.16	0.25	0.25	0.18	0.34	0.17	0.04	0.04	0,00	0.11	0.15	0.11	0.00								
TL26	0.2	0.24	0.11	0.18	0.11	0.15	0.03	0.07	0.19	0.15	0.15	0.53	0.2	0.37	0.21	0.14	0.37	0.29	0.07	0.07	0.11	0,00	0.03	0.07	0.11	0.00							
TL27	0.26	0.3	0.15	0.22	0.08	0.2	0.07	0.11	0.15	0.2	0.12	0.62	0.16	0.43	0.25	0.18	0.43	0.25	0.12	0.12	0.15	0.03	0,00	0.11	0.15	0.03	0.00						
TL28	0.26	0.21	0.1	0.32	0.17	0.4	0.25	0.21	0.17	0.13	0.13	0.79	0.26	0.53	0.35	0.28	0.27	0.13	0.13	0.13	0.1	0.21	0.25	0.13	0.1	0.21	0.25	0.00					
TL29	0.22	0.26	0.12	0.37	0.04	0.26	0.2	0.24	0.2	0.17	0.17	0.52	0.04	0.3	0.5	0.27	0.5	0.13	0.08	0.08	0.12	0.15	0.12	0.15	0.12	0.15	0.12	0.21	0.00				
TL30	0.13	0.17	0.12	0.1	0.13	0.17	0.04	0.07	0.2	0.08	0.26	0.4	0.13	0.21	0.3	0.14	0.4	0.4	0.08	0.08	0.12	0.07	0.12	0.15	0.12	0.07	0.12	0.3	0.17	0.00			
TL31	0.16	0.12	0.07	0.07	0.16	0.3	0.07	0.03	0.07	0.04	0.12	0.62	0.16	0.34	0.25	0.1	0.25	0.17	0.12	0.12	0.07	0.11	0.15	0.11	0.07	0.11	0.15	0.1	0.2	0.12	0.00		
TL32	0.26	0.3	0.24	0.14	0.26	0.16	0.07	0.11	0.24	0.2	0.2	0.34	0.16	0.34	0.29	0.14	0.54	0.34	0.2	0.2	0.24	0.11	0.15	0.19	0.24	0.11	0.15	0.34	0.2	0.12	0.15	0.00	
TL33	0.23	0.18	0.07	0.13	0.14	0.18	0.07	0.03	0.14	0.1	0.1	0.55	0.23	0.32	0.16	0.1	0.41	0.24	0.1	0.1	0.07	0.03	0.07	0.1	0.07	0.03	0.07	0.16	0.18	0.1	0.07	0.14	0.00

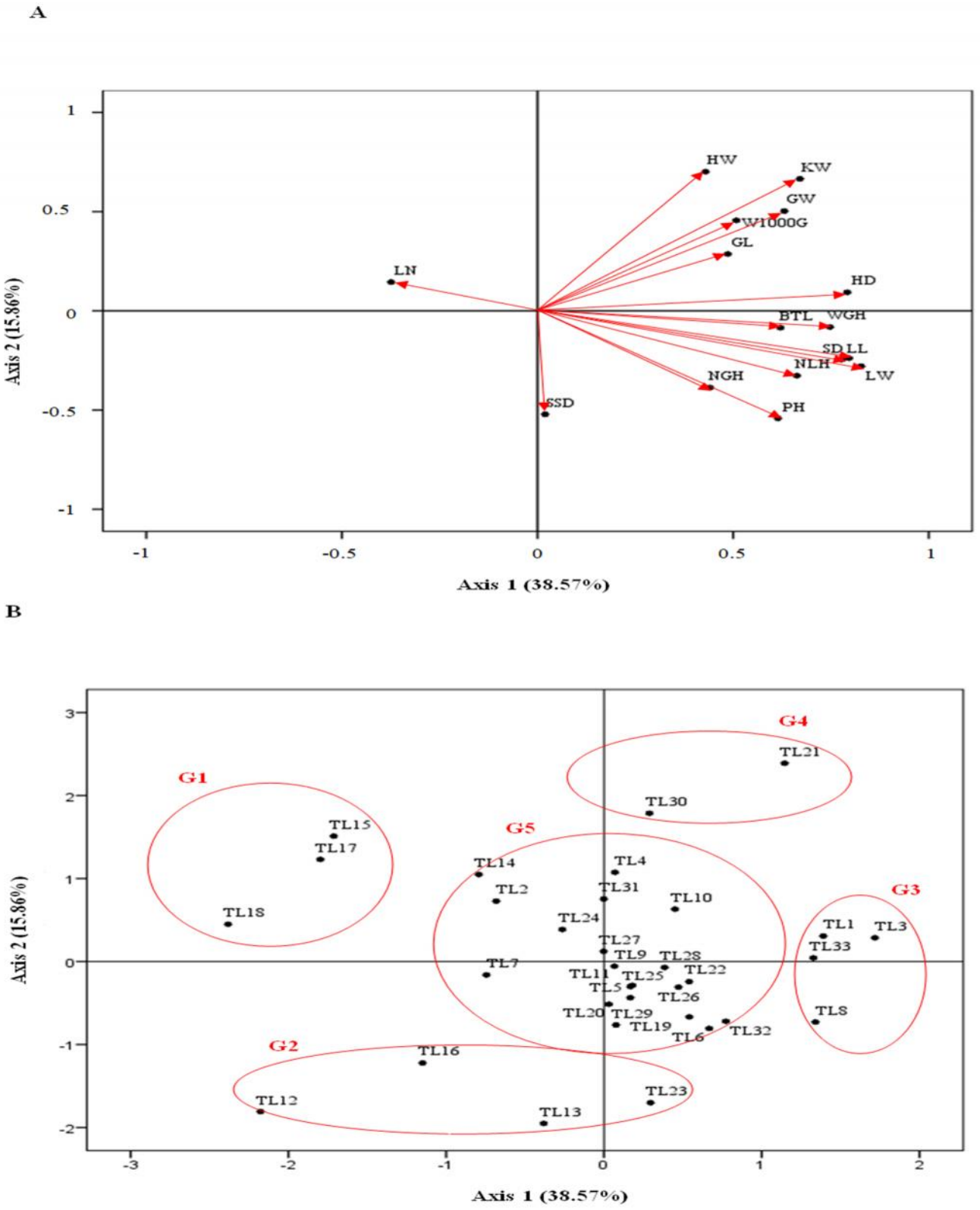

Fig. 1. A two-dimensional principal component analysis (PCA) of 33 sunflower accessions based on quantitative morphological trait values. (A) Projection of the agro-morphological descriptors in the layout generated by the axis 1 and 2. (B) Projection of the accessions studied in the layout generated by the axis 1 and 2.

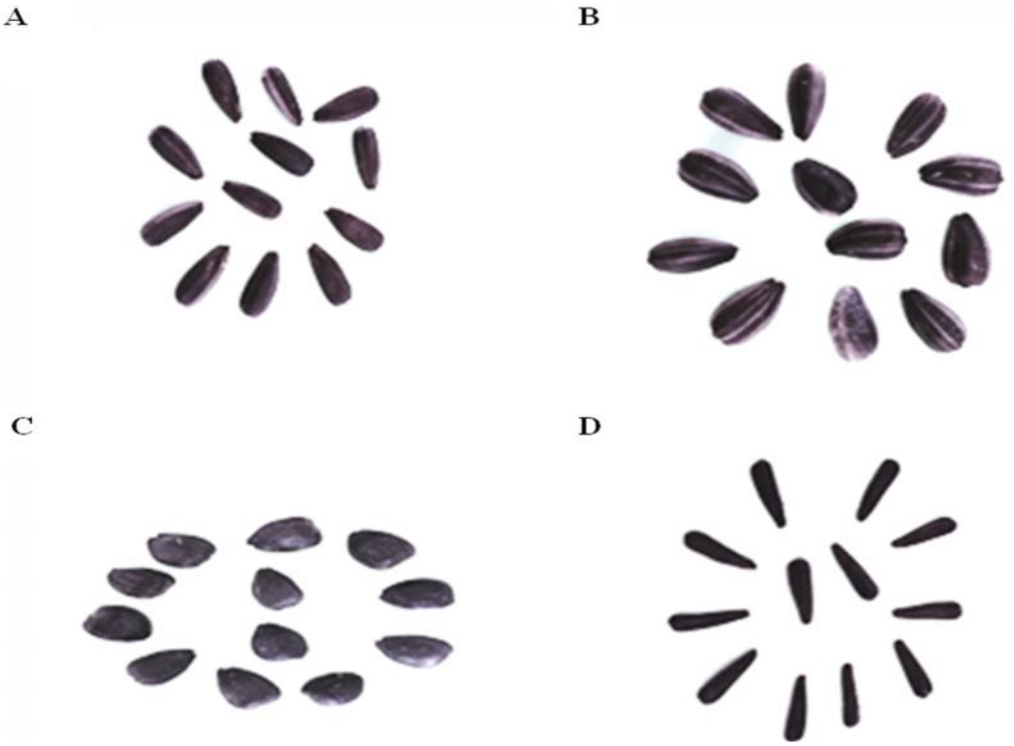

Fig. 2. Various shapes of grains for 33 sunflower accessions. (A) Narrow ovoid. (B) Broad ovoid. (C) Rounded. (D) Elongated.

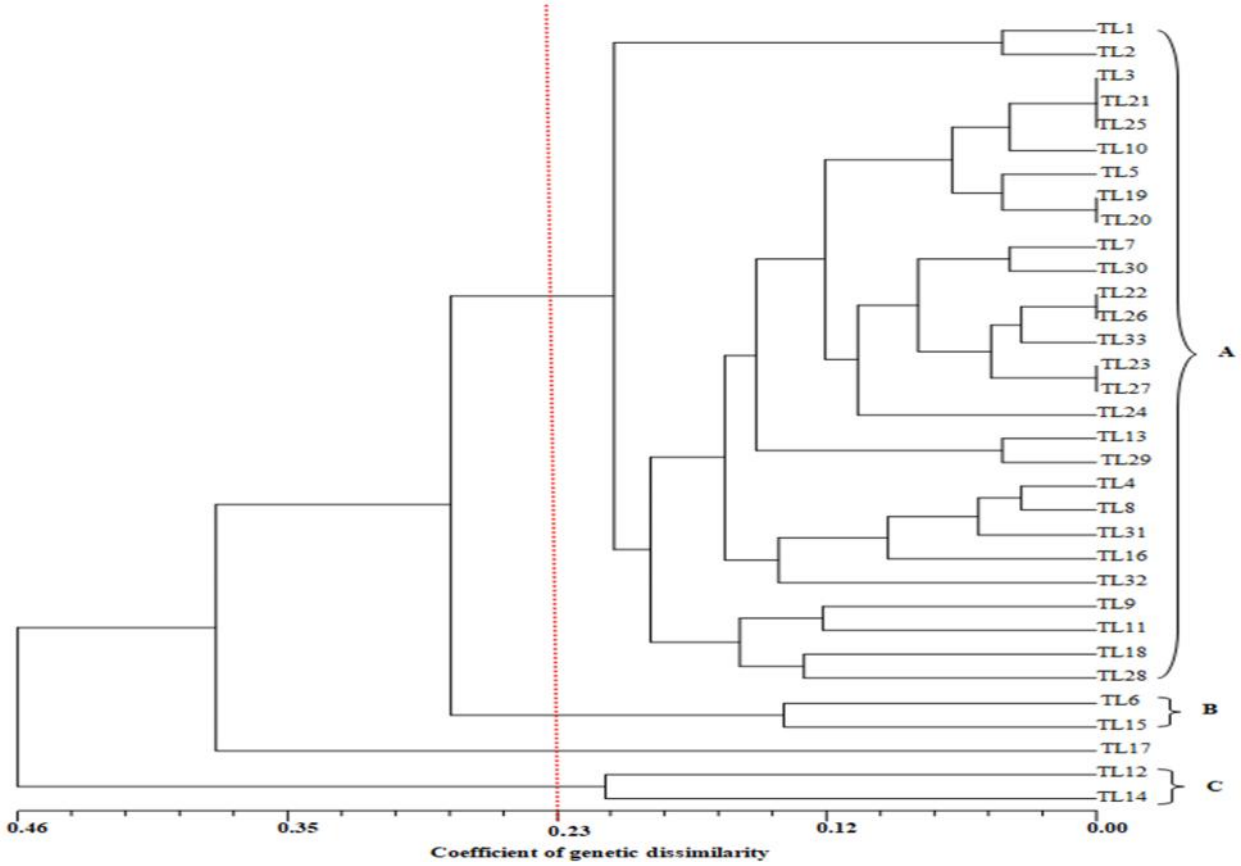

Fig. 3. Dendrogram of dissimilarity obtained by UPGMA method of 33 sunflower accessions based on SSR data.

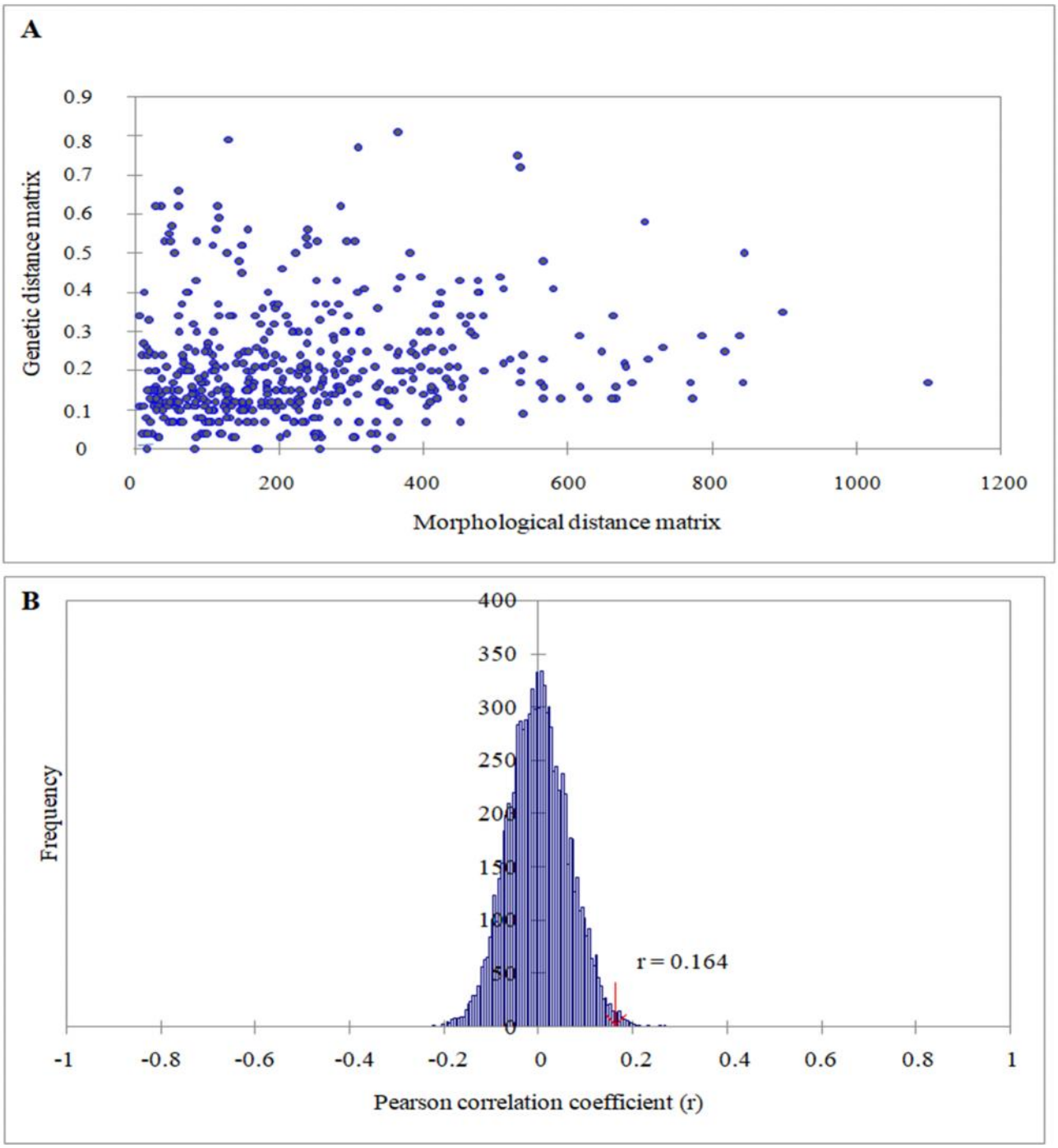

Fig. 4. (A) Matrix and (B) histogram of correlation of the Mantel test assessing the relationship between genetic distance and morphological distance for 33 sunflower accessions.

DISCUSSION

Genetic diversity is the key success for crop improvement strategy (Terzić *et al*., 2020). The sunflower genetic resources of the local landraces were unevaluated. Evaluation of genetic variability either through morphological or molecular characterization could be considered as a basic step for plant genetic resources. A total of 33 accessions of sunflower was characterised by using morphological and morphometric traits as well SSR markers.

All of the quantitative traits analyses revealed high variability of genetic heterogeneities among the accessions. The 33 accessions were showed on the PCA layout representing the two first axes (54.43% of total variation) with high variation. Five groups were discriminated. G3 and G4 groups include accessions having high values for several traits of interest. These two groups could make up a genetic pool for these criteria to be used in breeding program. Several authors made selection of sunflower based on different morphological traits such as head diameter, 1000 grains weight and plant height (Ahmadpour *et al*. 2013; Saremirad and Mostafavi, 2020; Sasikala *et al*., 2020).

Morphological characterization of these accessions showed that the most distinctive qualitative characters were the attitude of the heads, the presence of stripes on the seeds and the shape of the seeds. Similar results of distinctive qualitative characters among sunflower of different origins were reported by Yue *et al*. (2009). Observation on sunflower seeds has revealed a high level of morphological polymorphism. In fact, we observed four different shapes of seeds: ovoid narrow, elongated, rounded and ovoid broad. 80.7% of local accessions are characterized by broad ovoid seeds. In fact, the shape of the seeds could be considered as distinguishing feature of the landraces compared to the introduced ones. Yield and seed shape were used as good criteria for improving the local population by farmers to meet consumption requirement.

Thirty-three sunflower accessions were analysed using 15 microsatellite markers to assess the genetic variability within our collection. Among the fifteen SSR primers used, 10 were polymorphic with good amplification. The average number of alleles per locus is 2.9 which is close to that reported by Mwangi *et al*. (2019) (2.7) using 32 SSR markers for 24 sunflower accessions. Our study showed that the average of polymorphism rate is 91% indicating high variability among the accessions. Similar observations were reported by Tufan and Erdoğan (2017) with 87.8% of polymorphism among 22 faba bean genotypes using 25 SSR markers and Bouabid *et al*. (2018) with 89% of polymorphism among 13 Narbon vetch accessions using 13 SSR markers.

The PIC values were used to estimate the quality of an SSR marker for genetic studies and for accessing genetic variation among accessions. We observed the highest PIC for ha4136 (0.75), ORS 928 (0.53) and ORS 844 (0.52), while the lower one was for ha2682 and ORS 598 (0.35 and 0.38, respectively). Similar results on high PIC values were reported by Darvishzadeh *et al*. (2010) using 38 SSRs markers for characterization 28 genotypes of sunflower. They obtained the highest PIC values by using ORS 844 and ORS 928 primers (0.49 and 0.47, respectively). These PIC values are different to those obtained by Aghdam *et al*. (2020), who used SSR markers in their studies of sunflower genotypes collected from different sites of the world.

Based on molecular data, three groups could be discriminated. Overall, our results indicated that the sunflower accessions were not grouped according to their geographical origin. Most of landraces collected from different sites are in the same group (A). Therefore, the lowest dissimilarity coefficient (0.03) was observed among local accessions. It can be deduced that these accessions are from the same gene pool. The genetic proximity recorded between these accessions can be explained by seeds exchange among farmers from different cropping areas (Rebaa *et al*., 2017). In absence of selected seed industry, farmers will buy their seeds from the local market. As sunflower is allogamous, farmers could not maintain pure seeds. The TL13, TL16 and TL18 accessions originated respectively from former Soviet Union, USA and Canada are classified with the most of all local accessions in the same group (A). This denotes that geographical origin doesn't have a main effect on the observed genetic distances. These remarks were also observed for TL6 (local) and TL15 (Romania). This result concurs with those found by Lazrek *et al*. (2009) and Yahia *et al*. (2014), who used molecular markers with plant species (*Medicago truncatula* and *Vicia faba* L., respectively) and showed the presence of divergence between genetic clusters and geographical origins for the different cultivars studied. The group C included two introduced accessions (TL12 and TL14). The TL17 accession from the United States clustered out of the others groups. TL12 and TL17 accessions showed the highest dissimilarity coefficients compared to others accessions. These results reflect a clear genetic divergence among these introduced accessions and the rest of the introductions.

The statistical analysis of correlation between the matrices of the different used parameters, based on the Mantel test, supports our results showing significant correlation among the morphological and molecular markers. According to Carvalho *et al*. (2017), this correlation indicates that both approaches are important and can be useful for sunflower genetic diversity studies and breeding programs.

Conclusions: Molecular and agro-morphological markers are complementary for better characterizing and studying the genetic diversity of sunflower. The morphological characterization allowed us the identification of an important agro-morphological variability among the accessions for improving the sunflower breeding programs. This study showed for the molecular level, a considerable genetic variability among these accessions. There is no clear correlation between geographical origin and grouping based on molecular data. This denotes that the selection of genotypes for genetic improvement should focus on genetic diversity rather than geographic

distance. Overall, molecular analysis allowed a better understanding the genetic structure of these accessions. This can present a starting point for a new breeding program and an investigative useful alleles involved in the resistance to certain diseases or in the nutritional oil quality of sunflower.

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