Full Length Research Paper

Collection, evaluation and classification of Iranian confectionary sunflower (*Helianthus annuus* L.) populations using multivaraite statistical techniques

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Characterization of confectionary sunflower germplasm resources is critical to their efficient collection and management as well as for breeding programs. Thirty six (36) confectionary sunflower populations originating from different regions of Northwest Iran were characterized using 15 agro-morphological traits. Among the studied traits, high coefficients of variation were observed for harvest index (48.36), seed yield (42.07) and petiole length (41.63). Cluster analysis using Ward's method classified the 36 populations into four groups. A large number of genotypes was placed in cluster III (16 genotypes) followed by cluster I (13), cluster IV (4) and cluster II (3). Clusters I, III and VI include genotypes from different sources indicating no association between clustering pattern and eco-geographical distribution of genotypes. The maximum inter-cluster distance of 9.03 was observed between clusters II and VI indicating the possibility of high heterosis if individuals from these two clusters are cross-bred. Principal component analysis resulted in the first four components with Eigen value greater than one accounting for 78% of the total variation. The results of PCA were closely in line with those of cluster analysis. These results can now be used by breeders to develop high yielding sunflower hybrids.

Key words: Cluster analysis, confectionary sunflower, genetic variability, principal component analysis.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the 67 species in the genus *Helianthus*. It is a dicotyledonous and diploid plant with 2n =2x = 34 chromosomes (Fick, 1989). There are two types of sunflower, oilseed and confectionary types (Salunkhe et al., 1999). Oilseed sunflower is one of the most important oilseed crops in the world and is the preferred source of oil for domestic consumption and cooking worldwide (Hu et al., 2010). Confectionary sunflower produces large seeds with low oil content and used in baking and snack applications (Lu and Hoeft, 2009). Confectionary kernels are roasted and salted or roasted and no salt added and marketed as edible chips. Confectionary type is one of the most popular and important crop in Iran. It is cultivated in all parts of the country especially in Northwest regions. Exhaustive literature relating to genetic diversity, character association, heterosis and combining ability has been generated for oilseed sunflower type (Fick et al., 1974; Škorić, 1974; Seetharam et al., 1977; Kovačik and Škaloud, 1978; Giriraj et al., 1979; Giriraj et al., 1986; Muppidathi et al., 1995; Alvarez et al., 1996; Chungui et al., 1996; Joshi et al., 1997; Rama et al., 2003; Mohan and Seetharam, 2005; Presotto et al., 2009; Canatamutto et al., 2010, Nooryazdan et al., 2010), while confectionary sunflower has received limited research.

Estimating genetic diversity and determining the relationships among germplasm collections enhances efficient germplasm collection, management (Nisar et al., 2008) and genetic improvement (Geleta et al., 2005). Plant breeders can also use genetic similarity information

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to complement phenotypic information in the development of breeding populations (Nienhuis et al., 1993; Greene et al., 2004; Yüzbaşioğlu et al., 2006). Knowledge of diversity patterns further allows the plant breeders to better understand the evolutionary relationships among accessions (Liu et al., 2003). Future of breeding programs depend on the availability of genetic variability to increase productivity. Morphological characterization is the first step in the description and classifycation of germplasm collections (Smith et al., 1991).

Multivariate statistical techniques, which simultaneously analyze multiple measurements on each individual under investigation, are widely used in analysis of genetic diversity irrespective of whether it is morphological, biochemical or molecular marker-based and subsequently, classification of germplasm collections. Among the multivariate techniques, cluster analysis, principal component analysis (PCA), principal coordinate analysis (PCoA) and multidimensional scaling (MDS) are at present, most commonly employed and appear particularly useful (Mohammadi and Prasanna, 2003). Multivariate analysis has been used frequently for genetic diversity analysis in many crops such as barley (*Hordeum vulgare* L.) (Cross, 1992), Sorghum (*Sorgum bicolor* L. Moench) (Ayana and Bekele, 1999), wheat (*Triticum* spp.) (Hailu et al., 2006), peanut (*Arachis hypogaea* L.) (Upadhyaya et al., 2009) and vineyard peach (*Prunus persica* L. Batsch) (Nikolic et al., 2010). This study presents the first report on the investigation of genetic diversity in confectionary sunflower populations collected from different regions of Iran. Information presented herein, will help the breeder to develop high yielding sunflower hybrids.

MATERIALS AND METHODS

Plant materials and experimental design

Thirty six (36) confectionary sunflower populations were sampled from different regions of Northwest Iran and investigated during 2009 (Table 1). The experiment was conducted in a randomized complete block design with 4 replications (blocks). Experimental units in each block comprised of one line of four meters long. Row to row and plant to plant spacing was 0.60 and 0.40 m, respectively. Data were collected after flowering stage and after being harvested. Different morphological and agronomical traits were measured that included; days to 50% flowering (dayf), plant height (PH), stem diameter (SD), head diameter (HD), number of leaves (NL), leaf length (LL), leaf width (LW), petiole length (PL), number of filled seeds per head (NFSPH), number of unfilled seeds per head (NUFSPH), total number of seeds per head (TNS), 100-seed weight (HSW), percentage of unfilled seeds (PUFS), seed yield (SY) and harvest index (HI).

Data analysis

Descriptive statistics such as mean, standard deviation and co-efficient of variation for each one of 15 studied traits were calculated. Clustering of genotypes into similarity groups was performed using Ward's hierarchical algorithm based on squared Euclidean distances. For the 15 characters, the data were standar-

dized to have a mean of zero and a variance of one prior to squared Euclidean distance calculation. The pseudo F statistic and the pseudo T^2 statistic (Jobson, 1992) were examined to establish the numbers of clusters using SAS version 9.1 software (SAS Institute, Cary, NC). In order to identify the patterns of morphological variation, principal component analysis (PCA) was conducted. Those PCs with Eigen values greater than one were selected, as proposed by Jeffers (1967). Correlations between the original traits and the respective PCs were calculated. Data were processed using statistic program Minitab 14 (Minitab version 14, Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Descriptive statistics

The highest coefficient of variation correspond to harvest index, seed yield, petiole length, number of unfilled seeds per head and number of filled seeds per head (Table 2). Nooryazdan et al. (2010) reported the highest coefficient of variation for seed weight, leaf and petiole length, plant height, head diameter and sowing-flowering in oily sunflower types. Descriptive statistics analysis such as mean, standard deviation and coefficient of variation, has also been used for the study of genetic diversity in some crops such as garlic (*Allium sativum* L.) (Panthee et al., 2006), groundnut (*Vigna subterranea* (L.) Verdc) (Ntundu et al., 2006) and melon (*Cucumis melo* L.) (Lotti et al., 2008).

Cluster analysis

Cluster analysis classified the 36 confectionary sunflower populations into four groups (Figure 1). Classification was confirmed by pseudo T^2, pseudo F and multivariate analysis of variance (Table 3). The large number of genotypes were included in cluster III (16 genotypes) followed by clusters I (13), IV (4) and II (3). Cluster I, III and IV comprised genotypes from different regions indicating no association between clustering pattern and eco-geographical distribution of genotypes. Similar results were reported in sunflower by Muppidathi et al. (1995), Sankarapandian et al. (1996) and Teklewold et al. (2000). Murthy and Arunachalam (1966) showed that genetic drift and selection in different environments has more influence on genetic diversity than geographic distance. Therefore, selection of parental material for hybridization simply based on geographic diversity may not be beneficial.

Genetic similarity among germplasm from different regions may be attributed to several factors among the introduction of parental material from the same region. There could also be a tendency, particularly among resource-poor farmers in marginal areas, of selecting for the same traits of interest like yield stability, resistance to diseases, resistance to insects and low dependence on the external inputs (Boef et al., 1996).

The maximum inter-cluster distance was observed between clusters II and IV (9.03), while the minimum was

Table 1. Geographical origin of studied populations of confectionary sunflower

Code	Location	Altitude (m)	Latitude (E)	Longitude (N)
01	Salmas 2	1382	44°46′	38°12′
02	Khoy 11	1147	44°56′50.46″	38°33′09.60″
03	Khoy 5	1125	44°57′18.20″	38°35′38.34″
04	Nushin Shahr	1311	45°3′15.39″	37°43′50.53′
05	Sadaghiyan	1381	44°50′	38°13′
06	Miyane	1091	47°42′34.95″	37°25′07.53″
07	Khoy 7	1148	44°55′50.46″	38°32′09.60″
08	Maranghalou 1	1320	45°12′	36°38′
09	Boukan	1353	46°12′31″	36°31′13″
10	Yamchi 1	1348	45°46′10.69″	38°25′30.42″
11	Yamchi 3	1348	45°46′10.69″	38°25′30.42″
12	AS613	-	-	-
13	Khoy 8	1147	44°56′50.46″	38°33′09.60″
14	Khoy 3	1125	44°57′36.68″	
15	Khoy 6	1125	44°57′18.20″	38°35′38.34″
16	Maranghalou 2	1320	45°12′	36°38′
17	Saghghez	1466	46°16′14.42″	36°14′12.73″
18	Ghezel Je 3	1384	45°06′	38°17‴
19	Khoy 10	1147	44°56′50.46″	38°33′09.60″
20	Khoy 4	1211	44°53′21.92‴″	38°31′01.15″
21	Salmas 1	1382	44°46′	38°12′
22	Urmia 2	1321	45°06′	37°33′
23	Maranghalou 3	1320	45°12′	36°38′
24	Khoy 9	1125	44°57′18.20″	38°35′38.34″
25	Urmia 1	1321	45°06′	37°33′
26	Khoy 1	1148	44°55′50.46″	38°32′09.60″
27	Barough	1321	45°06′	37°33′
28	Khoy 2	1148	44°55′50.46″	38°32′09.60″
29	Yamchi 5	1348	45°46′10.69″	38°25′30.42″
30	Yamchi 4	1348	45°46′10.69″	38°25′30.42″
31	Ordakli	1349	45°45′10.69″	38°24′30.42″
32	Ghezel Je 1	1384	45°06′	38°17‴
33	Ghezel Je 2	1384	45°06′	38°17‴
34	Yamchi 2	1348	45°46′10.69″	38°25′30.42″
35	Dizaj	1321	45°06′	37°33′
36	Koshk Saray	1346	45°34′	38°27′

between clusters I and III (3.21). Crossing between individuals from clusters with maximum inter-cluster distance may result in high heterosis. Even though the genetic mechanisms that explain heterosis are not fully understood, it is well documented that crosses between unrelated and consequently, genetically distant parents, show greater hybrid vigor than crosses between closely related parents (Stuber, 1994). The minimum inter-cluster distance value found between cluster I and III (3.20585) suggested a close relationship between individuals placed in clusters.

Comparison of cluster means for characters under study marked considerable genetic differences between them (Table 4). Cluster IV had higher values for the plant height, stem diameter, head diameter, number of leaves, leaf length, leaf width, petiole length, days to 50% flowering, number of filled seeds per head, seed yield and harvest index (Table 4). In other hand, clusters I and II had the lowest values for these traits (Table 4). Thus, the genotypes with contrast mean performance from

Table 2. Agro-morphological characteristics of the 36 confectionary sunflower populations.

Variable	Maximum	Minimum	Range	Mean	SE	St. dev.	CV
SH	227.11	149.16	77.95	190.25	2.76	16.55	8.70
SD	3.62	2.23	1.39	2.73	0.05	0.32	11.85
HD	20.13	12.49	7.64	16.61	0.27	1.63	9.83
NL	36.00	24.15	11.85	32.77	0.35	2.08	6.36
LL	30.83	20.54	10.29	25.54	0.42	2.51	9.83
LW	23.31	13.34	9.97	17.87	0.33	1.98	11.09
PL	43.89	8.38	35.51	13.21	0.92	5.50	41.63
Dayf	96.50	82.50	14.00	87.98	0.61	3.67	4.17
Hs	13.01	5.37	7.64	9.07	0.28	1.66	18.35
PSN	519.10	83.10	436.00	292.70	18.40	110.20	37.65
ESN	667.80	88.60	579.20	284.60	18.80	112.70	39.59
TSN	984.80	352.80	631.90	570.50	22.90	137.20	24.05
PofE	82.59	26.70	55.89	48.32	2.31	13.87	28.70
SY	2364.90	205.90	2159.00	1101.00	77.20	463.20	42.07
HI	22.51	2.65	19.86	10.54	0.85	5.10	48.36

SH, Plant height; SD, stem diameter; HD, head diameter; NL, number of leaves; LL, leaf length; LW, leaf width; PL, petiole length; Dayf, days to 50% flowering; PSN, number of filled seeds per head; ESN, number of unfilled seeds per head; TSN, total number of seeds per head; HS, 100-seed weight; PofE, percentage of unfilled seeds; SY, seed yield; HI, harvest index; CV, coeffcient of variation.
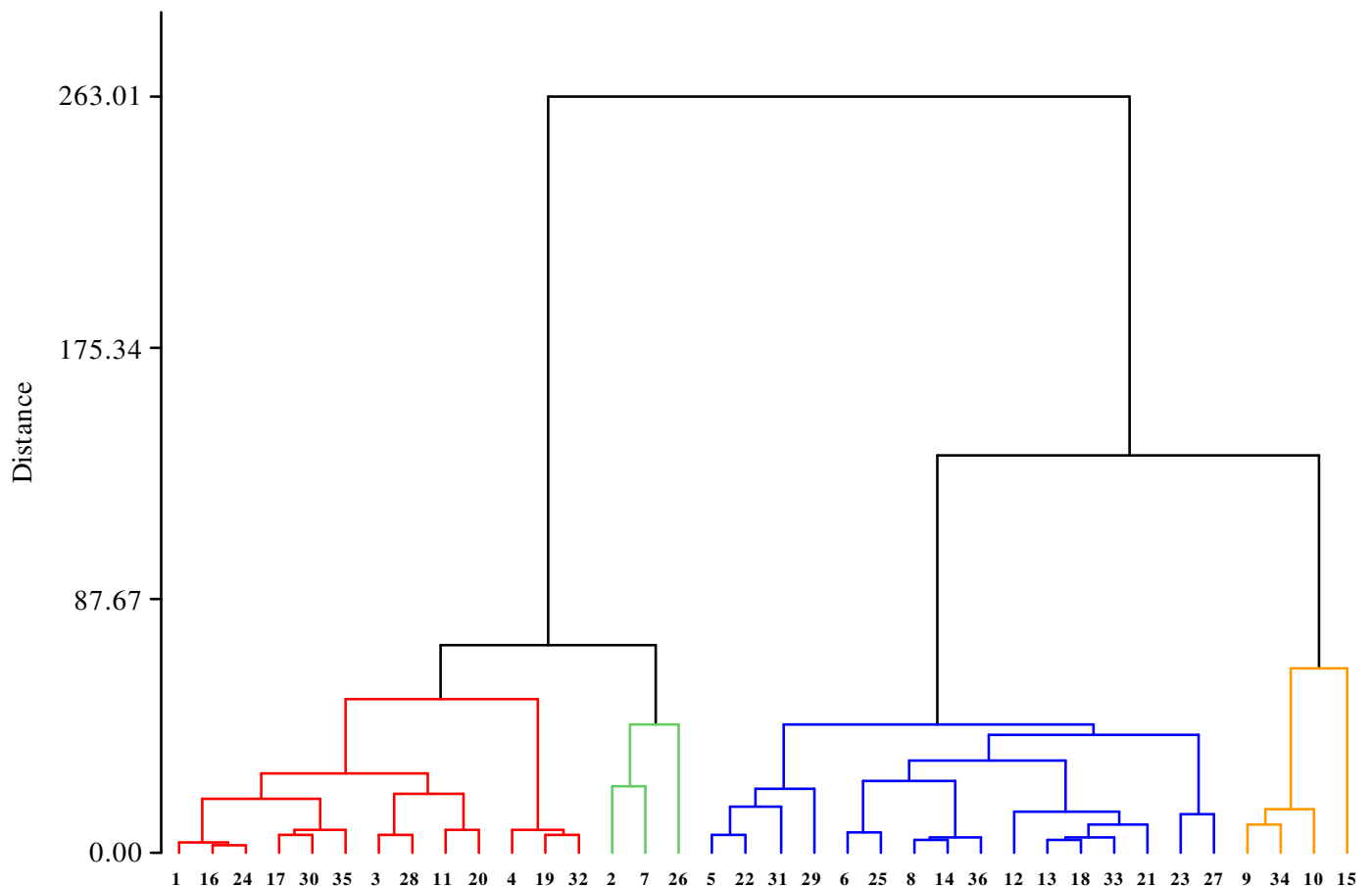
Figure 1. Dendrogram for the 36 confectionary sunflower populations collected from different regions of Iran, produced by Ward's clusters analysis; based on 15 morphological traits (scale: Squared Euclidean distance).

Table 3. Values of pseudo F and T2 Hotelling for determining optimum number of clusters and distances between cluster centroid.

Number of cluster	Pseudo F	Pseudo T^2 hotelling	Distance	Value
7	27.80	32.50	Cluster 1: Cluster 2	3.85
6	30.40	9.30	Cluster 1: Cluster 3	3.21
5	30.80	12.80	Cluster 1: Cluster 4	6.13
4	38.70	3.90	Cluster 2:Cluster 3	5.58
3	27.60	40.00	Cluster 2:Cluster 4	9.03
2	47.70	5.60	Cluster 3:Cluster 4	4.65
1	0.00	47.70		

Table 4. Duncan`s multiple range test for comparing mean of clusters in 36 sunflower populations.

Cluster	Number of genotype	SH	SD	HD	NL	LL	LW	PL	Dayf	hs	PSN	ESN	TSN	PofE	SY	HI
I	13	178 [c]	2.45 [c]	15.62 [c]	32.19 [b]	24.12 [b]	16.96 [c]	11.8 [b]	87 [b]	8.6 [a]	284 [b]	223 [b]	493 [bc]	42.79 [bc]	1002 [b]	9.06 [b]
II	3	184 [bc]	2.54 [c]	13.87 [d]	28.6 [c]	21.94 [b]	15.91 [c]	11.7 [b]	92 [a]	6.1 [a]	169 [b]	277 [ab]	443 [c]	57.52 [a]	388.4 [c]	3.48 [c]
III	16	196 [b]	2.87 [b]	17.38 [b]	33.52 [ab]	26.86 [a]	18.6 [ab]	12.7 [b]	88 [ab]	9.7 [a]	279 [b]	357 [a]	634 [ab]	55.45 [ab]	1099 [b]	10.54 [b]
IV	4	213 [a]	3.21 [a]	18.81 [a]	34.82 [a]	27.56 [a]	19.36 [a]	21 [a]	88 [a]	10.5 [ab]	466 [a]	200 [b]	664 [a]	30.84 [c]	1964 [a]	20.65 [a]

SH, Plant height; SD, stem diameter; HD, head diameter; NL, number of leaves; LL, leaf length; LW, leaf width; PL, petiole length; Dayf, days to 50% flowering; PSN, number of filled seeds per head; ESN, number of unfilled seeds per head; TSN, total number of seeds per head; HS, 100-seed weight; PofE, percentage of unfilled seeds; SW, seed yield; HI, harvest index.

these clusters may be identified as potential parents and could be utilized in the development of new varieties.

Principal component analysis

Principal component analysis was used to identify the most significant variables in the data set. Principal component analysis revealed that, the first four principle components (PCs) with Eigen values greater than one accounted for 78% of the total variation (Table 5).

Correlations coefficients between the original data of studied traits and the respective PCs were calculated (Table 5). The first component (PC1), explaining 40.2% of the total variation was negatively associated with head diameter, seed yield, harvest index, stem diameter and number of leaf. This means that, genotypes with high values of PC1 have lower seed yield and vice versa. The second component (PC2) accounted for 18.1% of the total variation and was negatively correlated with the number and percentage of unfilled seeds, whereas positively associated with seed yield and harvest index. This means that, genotypes with high values of PC2 have lower number of unfilled seeds, higher seed yield and harvest index. The third component (PC3) was associated with 100 seed weight and day to 50% flowering, while PC4 was correlated with days to 50% flowering, number of unfilled seed and petiole length.

Distribution of the 36 confectionary sunflower populations along the first and second principal components based on measured morphological characters is presented in Figure 2. Based on principal component analysis and scatter plot, the genotypes 9, 10, 15 and 34 that have low PC1 values and high PC2 values, have great value in confectionary sunflower yield breeding pro-grams (Figure 2). The results of PCA were closely correlated with the clustering pattern. PCA has previously been used in sunflower which could reduce the number of traits to be studied in sunflower germplasm (Dong et al., 2007;

Table 5. Eigen values and proportion of total variability as well as correlation between the original variables and the first four principal components (PC) in 36 confectionary sunflower populations used in this study.

Item	PC axis			
	1	2	3	4
Eigen value	6.0333	2.7166	1.6646	1.2856
Proportion	0.402	0.181	0.111	0.086
Cumulative	0.402	0.583	0.694	0.78
Variable	Eigen vector			
	PC1	PC2	PC3	PC4
SH	-0.28	-0.12	0.2	0.18
SD	-0.3	-0.16	0.29	0.18
HD	-0.35	-0.07	-0.17	-0.02
NL	-0.3	-0.22	-0.14	0.04
LL	-0.27	-0.24	-0.21	0.31
LW	-0.25	-0.14	0.13	0.26
PL	-0.2	0.08	-0.25	0.35
Dayf	0.1	-0.01	0.43	0.55
Hs	-0.24	-0.08	-0.5	-0.1
PSN	-0.28	0.34	0.3	-0.18
ESN	-0.03	-0.52	0.15	-0.32
TSN	-0.26	-0.18	0.38	-0.4
PofE	0.14	-0.54	-0.05	-0.05
SY	-0.34	0.26	0.07	-0.17
HI	-0.33	0.21	-0.07	-0.12

SH, Plant height; SD, stem diameter; HD, head diameter; NL, number of leaves; LL, leaf length; LW, leaf width; PL, petiole length; Dayf, days to 50% flowering; PSN, number of filled seeds per head; ESN, number of unfilled seeds per head; TSN, total number of seeds per head; HS, 100-seed weight; PofE, percentage of unfilled seeds; SW, seed yield; HI, harvest index.
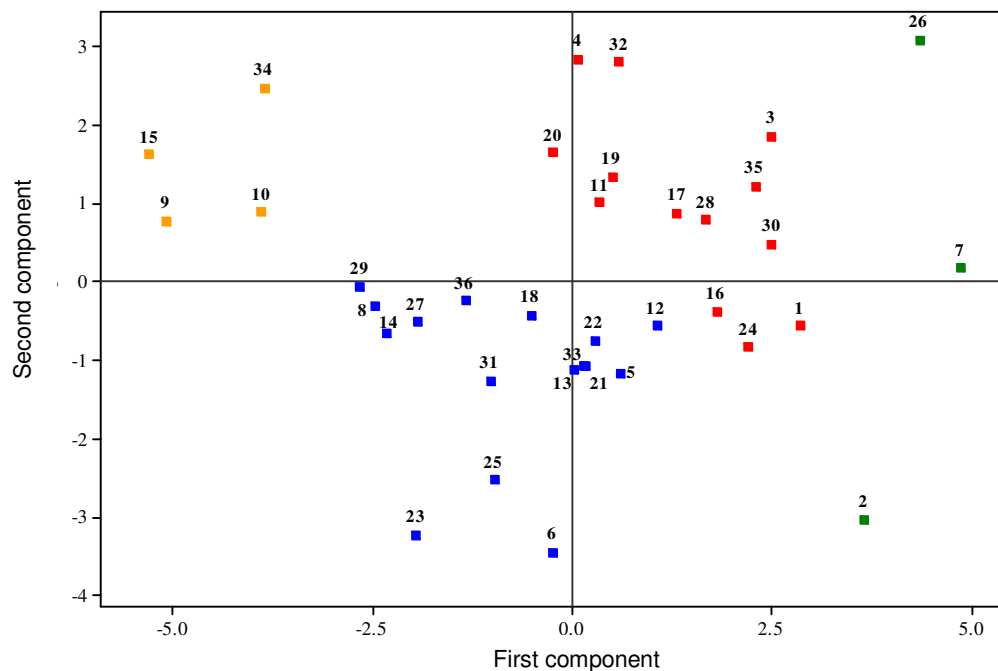
Figure 2. Scatter plot of 36 confectionary sunflower populations using first and second components of principal components analysis; using 15 morphological characteristics.

Nooryazdan et al., 2010).

Conclusions

There is high genetic variability in the studied sunflower populations even though it was not uniformly distributed across the regions. It is suggested that, selection of parents for hybridization need to be based on genetic diversity instead of geographic diversity. The maximum inter-cluster distance was observed between clusters II and IV (9.03) and crossing between individuals from these clusters may result in high heterosis for yield and yield components such as head diameter.

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