

Phenotypic and molecular marker distance as a tool for prediction of heterosis and F₁ performance in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions

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Abstract

The aim of this study was to determine phenotypic and genotypic distances in parental lines of diallel population of sunflower, and to identify correlation between both distances with F₁ performance, specific combining ability (SCA) and absolutely mid-parent heterosis. The morphological distance (Euclidean distance; EDs) and genetic distance (Jaccard's distance; JD) among five sunflower parental lines were calculated based on 12 morphological traits and 160 amplified fragment length polymorphism (AFLP) markers in either well-watered and water-stressed conditions. The un-weighted pair-group method using arithmetic average (UPGMA) clustering algorithm, based on Euclidean and Jaccard distances, classified the studied parental lines into 3 groups. In both water treatment conditions, the high values of JD were correlated with high values of ED. Correlation results revealed that both of ED and JD had significant relation with F₁ performance, SCA and heterosis for some traits in both water treatment conditions. Therefore, depending upon target trait, ED and JD could effectively be utilized in order to identify heterosis. Among studied genotypes, LR25 will be regard as a permanent parent in all possible pairwise crosses with other genotypes, to achieve optimum yield in both well-watered and water-stressed conditions.

Keywords: Sunflower, Euclidean distance, Jaccard's distance, amplified fragment length polymorphism, absolute mid-parent heterosis.

Abbreviations: HD: head diameter, HW: head weight, LN: leaf number, APDW: aerial part dry weight, NA: number of achene per head, PH: plant height, SY: seed yield per plant, CC: chlorophyll content, SD: stem diameter, DSF: days to flowering, DFM: days from flowering to physiological maturity, RWC: relative water content.

Introduction

Sunflower (*Helianthus annuus* L.) as a source of vegetable oil and proteins is grown in many parts of the world. It is the fourth important vegetable oil crop (Vollmann and Rajcan, 2009), producing 9 million tonnes per year. Sunflower oil is considered to have a premium quality due to its light colour, mild flavor and ability to withstand at high cooking temperatures. Furthermore, sunflower oil contains fat-soluble vitamins (A, B, E and K) which are related to heart proteins (Evertt et al., 1987; Gossal et al., 1988). Sunflower oil has become the most popular vegetable oil in Iran. Most of our country edible oil requirements are met by means of imports (Economic Survey of Iran) while there is great ability to meet this demand through growing oilseed crops such as sunflower. In addition to principal cultivation, sunflower growth period is such that it can be planted as a second crop after wheat and barley harvest. Sunflower cultivation is hindered by some constraints such as biotic and abiotic stresses. Among abiotic stresses, drought stress is considered as a multidimensional stress which causes world wide reduction in sunflower yield (Dragovic and Maksimovic, 1995). Hybridization and selection in segregating generations may result in obtaining cultivars with good performance in both drought and normal conditions. As sunflower hybrid cultivars are more uniform and higher-yielding than open

pollinated varieties (Beg et al., 1984), heterosis for this cultivated species must be used. Information on genetic distance among lines and populations is prerequisite in hybrid development programs. Evaluation of genetic variation in sunflower germplasm and identification of heterotic groups based on both morphological traits (Kholghi et al., 2011) and molecular markers such as SSR (Darvishzadeh et al., 2010a), ISSR (Garayalde et al., 2011) and AFLP (Hongtrakul et al., 1997) has been carried out. Due to hybrids developing is a costly long term process, heterosis prediction would be desirable. Melchinger et al. (1990) reported that the efficiency of hybrid breeding programs could be increased if the inbred lines could be screened and the superior crosses were predicted before field evaluation. Studies on genetic distance of parental lines as the base of hybrids performance prediction have been undertaken in several crops such as Ethiopian mustard (Teklewold and Becker, 2006), alfalfa (Riday et al., 2003), chickpea (Sant et al., 1999), cotton (Meredith and Brown, 1998), and maize (Ajmone-Marsan et al., 1998; Makumbi et al., 2011). In chickpea, cotton and maize a low correlation was shown between the genetic distance of parental genotypes and the hybrid performance (Sant et al., 1999; Meredith and Brown, 1998; Ajmone-Marsan et al., 1998). However, in some cases significant

correlation has been reported between parental lines diversity with hybrid performance in maize (Rief et al., 2003). Review of literature showed very limited works on the predication of sunflower heterosis and hybrid performance by marker based genetic distance of the parental lines (Tersacet et al., 1994; Cheres et al., 2000). This study was carried out to assess the genetic diversity among some sunflower parental lines using agro-morphological traits and AFLP markers and then, evaluate the association between parental lines genetic diversity with F_1 performance and heterosis under well-watered and water-stressed conditions.

Results

Variation of the traits

Analysis of variance showed significant variability among parental lines and F_1 hybrids for some studied agro-morphological traits in both water treatment conditions (Data not shown). In well-watered condition, mean square values of general combining ability (GCA) were non significant for all studied traits except for number of leave (LN). In this condition, most studied traits including: aerial part dry weight (APDW), plant height (PH), relative water content (RWC), and number of leaves (LN) showed significant specific combining ability effect. In water-stressed condition, variation due to GCA was significant only for aerial part dry weight, head weight and chlorophyll content, while variation due to SCA were significant for all studied traits except for chlorophyll content (CC), plant height (PH), days to flowering (DSF), and seed yield per plant (SY). Means of parental lines, absolute mid-parent heterosis and F_1 performance in both water treatment conditions are displayed in Table 1. Number of achene per head (NA) showed maximum values of heterosis in both well-watered (66.9%) and water-stressed (41.35%) conditions (Table 1). Low and negative heterosis was observed for days to flowering (DSF) in this experiment (Table 1). Among studied traits, relative water content (RWC) had positive heterosis in well-watered condition but negative heterosis in water-stressed condition (Table 1).

Genetic distances among the parents

To evaluate any association between parental lines genetic diversity with F_1 performance and heterosis, both genotypic (Jaccard distance; JD) and phenotypic (Euclidean distance; ED) distances were computed. The phenotypic distance of parental lines (Euclidean distance; ED) were calculated using 12 phenotypic traits in each water treatment conditions, separately. In well-watered condition, Euclidean distance (ED) varied from 2.5 in 'C104 and LR4' to 6.3 in 'LR25 and LR55', respectively. 'C100 and LR55' had the second highest Euclidean distance value (6.0) in this condition. In water-stressed condition, Euclidean distance (ED) varied from 3.8 in both 'LR4 with LR25' and 'C104 with LR25' to 6.1 and 6.0 in 'C100 with LR25' and 'C100 with C104', respectively. Mean of ED among the five parental lines was 4.8 in both water treatment conditions. Genetic distance of parental lines (Jaccard distance; JD) was calculated using 160 polymorphic AFLP markers. Number of bands per primer combination across all parental lines was on average 10 and ranged from 6 (E38M60) to 16 (E32M49). Jaccard's distance values ranged from 0.5 (C100 and C104) to 0.8 (C104 and LR25) with a mean of 0.7. Despite of known pedigree relationship among parental lines, results manifested that there is fairly large

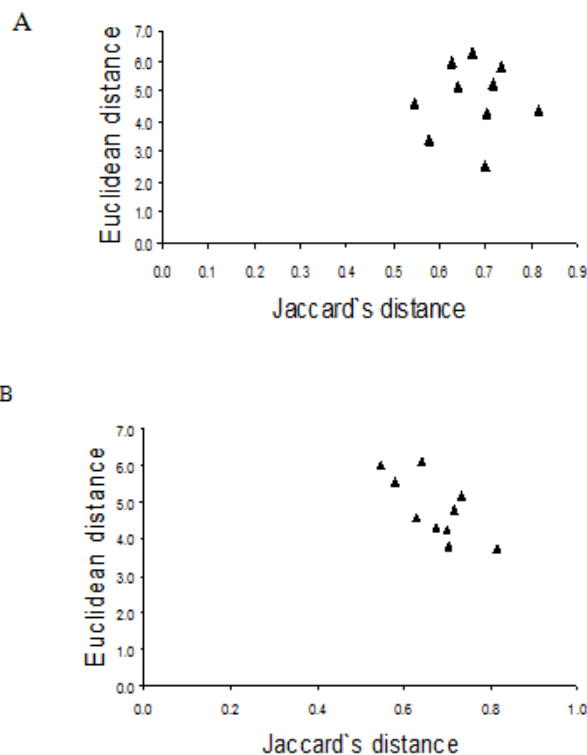


Fig 1. Relationship between the 10 phenotypic (Euclidean distance: EDs) and molecular (Jaccard's distance: JD) distances in sunflower lines under well-watered (A) and water-stressed (B) conditions. The morphological and genetic distance were calculated from all pair-wise combination of the 5 sunflower parental lines based on 12 morphological traits and 160 amplified fragment length polymorphism markers, respectively under well-watered (A) and water-stressed (B) conditions.

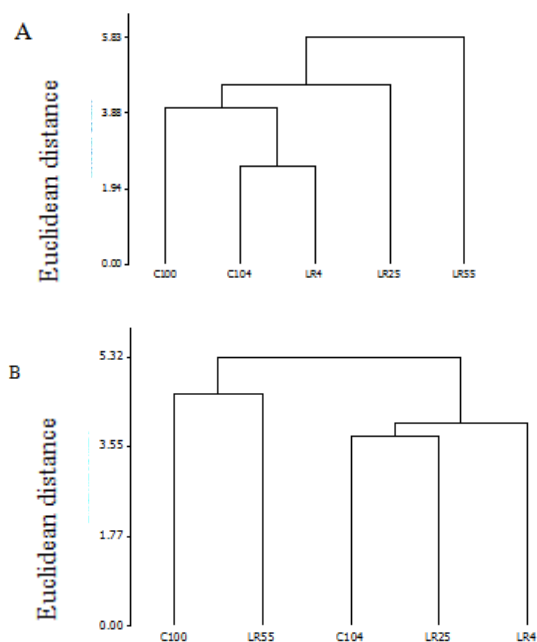


Fig 2. Dendrogram for the 5 sunflower parental lines produced by UPGMA clusters analysis; based on 12 phenotypic traits under well-watered (A) and water-stressed conditions (B) (scale: Euclidean distance).

Table 1. Parental means, F₁ performance and absolute mid-parent heterosis (AMPH) value for 12 studied traits in sunflower grown at well-watered and water stressed conditions.

Traits	Well-watered condition			Water-stressed condition		
	Parental mean	F ₁ performance	AMPH	Parental mean	F ₁ performance	AMPH
NA	91.88	158.83	66.90	66.06	105.34	41.34
SY	4.02	7.86	3.80	1.72	2.83	1.14
LN	17.75	20.58	2.88	20.06	21.73	1.65
HW	9.45	11.17	1.62	5.40	5.76	0.32
HD	6.16	7.19	0.99	4.50	4.75	0.30
APDW	12.60	16.33	3.65	7.06	8.20	1.06
CC	19.93	24.25	4.53	16.47	16.51	0.07
SD	0.61	0.63	0.02	0.49	0.58	0.08
PH	132.73	150.91	18.43	108.06	114.65	6.18
DSF	96.46	95.11	-1.28	96.93	96.18	-0.70
DFM	134.95	136.51	1.28	133.13	134.12	0.92
RWC	77.80	82.00	4.27	65.26	54.90	-10.13

Absolute mid-parent heterosis (AMPH) was calculated as: $AMPH = F_1 - MP$ where $F_1 = F_1$ performance and $MP = (P_1 + P_2)/2$. HD: head diameter, HW: head weight, LN: leaf number, APDW: aerial part dry weight, NA: number of achene per head, PH: plant height, SY: seed yield per plant, CC: chlorophyll content, SD: stem diameter, DSF: days to flowering, DFM: days from flowering to physiological maturity, RWC: relative water content.

Table 2. Phenotypic correlation coefficients of Euclidean and Jaccard's distance with F₁ performance, specific combining ability (SCA) and absolute mid-parent heterosis (AMPH) in sunflower grown at well-watered and water-stressed conditions.

Well-watered condition (A)						
Traits	Euclidean distance			Jaccard's distance		
	F ₁ performance	SCA	AMPH	F ₁ performance	SCA	AMPH
NA	-0.64*	-0.12	-0.72**	0.14	-0.73**	0.09
SY	-0.15	0.45	-0.27	0.57*	-0.37	0.60*
LN	-0.33	0.41	-0.17	-0.46	-0.28	-0.37
HW	0.02	-0.21	0.67*	0.54	0.38	0.42
HD	-0.30	0.20	-0.02	0.53	-0.86**	0.52
APDW	0.02	0.03	0.37	0.50	-0.58*	0.52
CC	-0.13	-0.04	-0.06	0.61**	-0.69*	0.58*
SD	0.03	-0.03	0.18	0.27	-0.44	0.51
PH	-0.54	0.39	0.08	0.41	-0.74**	0.53
DSF	-0.55*	-0.56*	-0.38	0.05	-0.61*	0.24
DFM	-0.37	0.11	0.27	0.38	-0.50	-0.09
RWC	-0.38	-0.28	-0.58*	0.11	-0.75**	-0.32
Water-stressed condition (B)						
Traits	Euclidean distance			Jaccard's distance		
	F ₁ performance	SCA	AMPH	F ₁ performance	SCA	AMPH
NA	-0.60*	0.45	-0.19	0.52	-0.44	0.11
SY	-0.53	-0.03	-0.18	0.78**	-0.26	0.50
LN	0.72**	0.14	0.71**	-0.65*	-0.49	-0.64*
HW	0.12	0.08	0.56	-0.10	0.19	-0.63*
HD	-0.52	0.08	0.30	0.45	0.28	-0.35
APDW	0.64*	-0.26	0.81**	-0.41	0.14	-0.68*
CC	-0.21	0.04	-0.16	0.23	-0.15	0.19
SD	0.17	-0.47	0.39	-0.09	0.65*	-0.52
PH	0.46	0.28	0.54	-0.60*	-0.21	-0.72**
DSF	0.27	0.72**	0.29	-0.09	-0.84**	-0.09
DFM	0.11	0.31	0.78**	0.25	-0.20	-0.45
RWC	-0.10	0.68*	-0.56*	-0.34	-0.85**	0.21

* and ** significant at $P \leq 0.05$ and $P \leq 0.01$ respectively. HD: head diameter, HW: head weight, LN: leaf number, APDW: aerial part dry weight, NA: number of achene per head, PH: plant height, SY: seed yield per plant, CC: chlorophyll content, SD: stem diameter, DSF: days to flowering, DFM: days from flowering to physiological maturity, RWC: relative water content.

estimates of ED and JD between studied sunflower genotypes. There were not strong linear relationships among ED and JD, in both water treatment conditions (Figure 1 A, B).

Cluster analysis

The un-weighted pair-group method using arithmetic average (UPGMA) dendrograms representing relationships between

parental lines based on Euclidean and Jaccard distances are shown in Figures 2 and 3, respectively. Clustering based on JD and EDs in well-watered and water-stressed conditions established genotypes in three distinct groups. Classification of parental genotypes based on Euclidean distance in well-watered condition was the same to the grouping achieved by

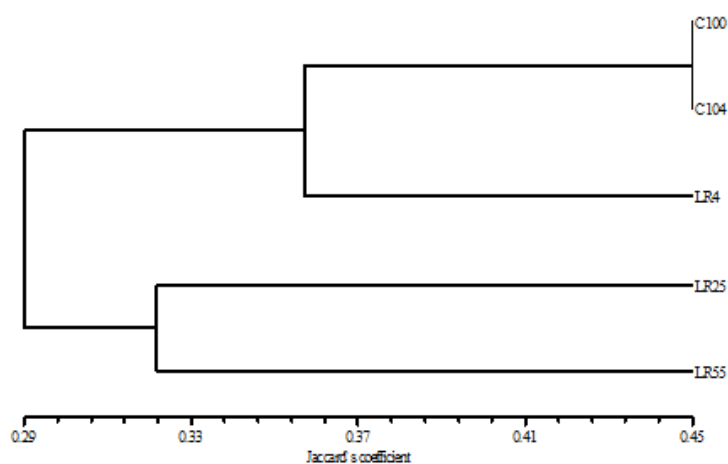


Fig 3. Dendrogram for the 5 sunflower parental lines produced by UPGMA clusters analysis based on 160 polymorphic AFLP markers (scale: Jaccard's coefficient of similarity)

using Jaccard distance, where C100, C104 and LR4 were located in the same group and LR25 and LR55 located in other two separate groups. For water-stressed condition, classification pattern of parental genotypes based on ED distance was different as compared to JD classification, resulting in grouping of C104, LR4 and LR25 in the same cluster; and C100 and LR55 located in other two separate groups.

Correlation of parental distances with F_1 performance, specific combining ability (SCA) and absolute mid-parent heterosis (AMPH)

Association of phenotypic (ED) and genetic (JD) distances with F_1 performance, SCA and absolute mid-parent heterosis (AMPH) are displayed in Table 2. In well-watered condition (Table 2A), F_1 performance had generally negative and positive relations with ED and JD, respectively. There were negative and significant correlation between ED and F_1 performance only for number of achene per head and days to flowering, while significant and positive correlation was found between JD and F_1 performance for seed yield per plant (SY) and chlorophyll content (CC). The correlation of JD with SCA was mostly negative and significant for all studied traits. Oppositely, ED had negative and significant relation with SCA just in days to flowering (DSF) trait. There was negative and significant correlation between ED and AMPH in traits including number of achene per head (NA) and relative water content (RWC) and positive and significant correlation in head weight (HW) trait. JD was positively and significantly correlated with AMPH in seed yield per plant and chlorophyll content (CC) traits. In water-stressed condition (Table 2B), F_1 performance showed positive and significant correlation with ED in number of leaves (LN) and aerial part dry weight (APDW) traits but negative and significant correlation in number of achene per head (NA) trait. JD correlated positively and significantly with F_1 performance only in number of achene per head (NA) trait. ED, positively and significantly and JD, negatively and significantly correlated with SCA values in traits including days to flowering (DSF) and relative water content (RWC). By the way, JD had positive and significant correlation with SCA in stem diameter (SD). AMPH had positive and

significant correlation with ED in number of leaves (LN), aerial part dry weight (APDW) and days from flowering to physiological maturity (DFM) traits. Negative and significant correlation was found between JD with AMPH in number of leaves (LN), head weight (HW), aerial part dry weight (APDW) and plant height (PH) traits.

Discussion

For breeders it is important to have genetic variation for target traits. In agreement to previous studies (Darvishzadeh et al., 2010b; Dong et al., 2007; Kholghi et al., 2011), agromorphological and AFLP marker attributes could properly used for evaluation of sunflower germplasm, reporting high level of genetic variation among parental genotypes. Genetic variation and high parental distance are prerequisite for expression of heterosis. Using parental distances based on both morphological and molecular traits, is useful for identifying parents that would produce crosses exhibiting optimal level of heterosis without making all possible crosses among the potential parents (Riday et al., 2003; Teklewold and Becker, 2006). According to finding of Xu et al., (2004), classification based on molecular marker data (JD) was accommodated with phenotypic data (ED) and parental heterotic groups based on agro-morphological traits in normal condition and AFLP markers were coincident with together. Due to the modifications in physiological response of sunflower genotypes to drought stress (Cellier et al., 1998), heterotic pattern in drought condition was differed. Regarding to maximum genotypic and phenotypic distance of parent LR25 with genotypes belong to other heterotic groups, it could efficiently used in producing sunflower hybrid with good performance in both normal and drought conditions. Recently (Darvishzadeh et al., 2010b) by using various stress tolerance indices depicted that the crosses 'LR4 × LR25' and 'C100 × LR25' are drought tolerant sunflower genotypes and genotype 'LR4 × LR25' has reasonable yield in both water-stressed and non-stressed conditions. Linear relationship among ED and JD was observed only in water-stressed condition. However, the high values of JD were correlated with high values of ED in both well-watered and water-stressed conditions. It was in accordance with Teklewold and Becker, (2006) that reported strong association between ED and JD in Ethiopian mustard. Burstin and Charcosset, (1997) revealed that correlation between molecular marker distance and phenotypic distance is not necessarily significant. The non linear relationship between ED and JD implies the existence of large number of loci contributed to the variation of the traits used to estimate distances in sunflower genotypes. This is also possible to infer that there is no or slight linkage disequilibrium between AFLP markers and QTLs controlling traits. Commonly, due to inbreeding depression in open-pollinated plants such as sunflower, correlation values obtained between hybrid performances with inbred lines was low. Makumbi et al., (2011) and Jagosz, (2011) strongly emphasized on this item in maize and carrot. For some traits in both water treatment conditions, ED (agro-morphological distances) or JD (genetic distance) showed significant correlations with F_1 performance, SCA and AMPH. Generally, there was not observed any positive and significant correlation between ED and JD with SCA for seed weight and number of achene per head in both conditions. Gvozdenovic et al., (2009) found non significant correlation among genetic distance and SCA in sunflower. In contrast, Bocianowski et al., (2008) using diallel analysis based on F_1 and F_2 generations of spring rape, proved

Table3. AFLP primer combinations and their polymorphic markers used for genotyping parental lines of sunflower.

Primer combination	No. of polymorphic bands
E32M49 (E _{AAC} × M _{CAG})	16
E32M49 (E _{AAC} × M _{CAG})	11
E33M48 (E _{AAG} × M _{CAC})	9
E33M48 (E _{AAG} × M _{CAC})	11
E35M48 (E _{ACA} × M _{CAC})	10
E38M48 (E _{ACT} × M _{CAC})	8
E38M50 (E _{ACT} × M _{CAT})	11
E38M50 (E _{ACT} × M _{CAT})	9
E38M60 (E _{ACT} × M _{CTC})	6
E40M47 (E _{AGC} × M _{CAA})	7
E40M47 (E _{AGC} × M _{CAA})	13
E40M62 (E _{AGC} × M _{CTT})	15
E40M62 (E _{AGC} × M _{CTT})	7
E40M50 (E _{AGC} × M _{CAT})	7
E41M59 (E _{AGG} × M _{CTA})	7
E41M62 (E _{AGG} × M _{CTT})	13
Total	160

Core sequences for the primer sets (with selective nucleotides shown as N) are: *EcoRI* 5'-GACTGCGTACCAATTCNNN-3'; *MseI* 5'-GATGAGTCTGAGTAANNN-3'.

that there is significant correlation between Mahalanobis distance of parental lines and SCA. In above mentioned study, they observed significant correlation between ED with heterosis for number of achene per head and JD with heterosis for seed yield per plant in well-watered condition. Similarly, a significant correlation was found between the genetic distance of the sunflower parents and hybrid heterosis for seed yield by Cheres et al., (2000). Also, Riday et al., (2003) and Bocianowski et al., (2008) found significant correlation for heterosis with phenotypic distance in Medicago and spring rape. Both ED and JD had not any significant correlation with number of achene per head and seed yield per plant of sunflower.

Conclusion

Like many other crops, agro-morphological traits and AFLP markers could efficiently be used to evaluate sunflower germplasm and identify heterotic pattern. Classification of parental lines in drought condition was different as compared to normal condition. In this study, there was not observed strong linear correlation among genetic distance with morphological distance especially in well-watered condition. Unlike previous inferences in sunflower, both of ED and JD had significant relation with F₁ performance, SCA and heterosis in some traits in both water treatment conditions.

Materials and methods

Plant material and experimental design

Five sunflower recombinant inbred lines (RILs) out of 100 were selected on the basis of their contrasting responses to water stress and different agronomical characteristics revealed in previous experiments (Poormohammad Kiani et al., 2007a, b; 2008; 2009). The RILs were F₉ pure lines which were developed through single seed descent from F₂ plants derived from a cross between 'PAC2' and 'RHA266'. RHA266 was obtained from a cross between wild *H. annuus* and Peredovik from USDA, and PAC2 is an INRA-France inbred line from a cross between *H. petiolaris* and 'HA61' (Gentzittel et al., 1995). This public RILs population is widely used for genetic analysis of complex traits in sunflower (Darvishzadeh et al., 2007; Davar et al., 2010;

Poormohammad Kiani et al., 2007a, b; 2008; 2009). The five genotypes were grown and crossed in a diallel mating system without reciprocals to produce 10 F₁ hybrid combinations. The parental genotypes and their F₁ hybrids were grown in the greenhouse under controlled conditions. Plants were individually grown in plastic pots containing a mixture of 40% soil, 40% compost and 20% sand as described by Poormohammad Kiani et al., (2007a, b). Temperature was maintained at 25/18 ± 2°C (day/night) and relative humidity at about 65/85 ± 5%. Supplementary light was provided to obtain 16 h light period. Fifteen genotypes including 10 F₁ hybrids plus 5 parental lines were evaluated in well-watered and water-stressed conditions. In each condition, the genotypes were evaluated using a randomized complete block design with three replications. To simulate natural water deficit conditions similar to field, a progressive water stress from mild stress to severe stress was imposed on 45-day-old plants at stage near flower bud formation (R₁) for a period of 12 days (Poormohammad Kiani et al., 2007a, b). Both well-watered and water-stressed plants were weighed and water lost was replaced carefully. Well-watered (control) plants received sufficient water to maintain soil water content close to field capacity. Water-stressed plants were subjected to a progressive water stress and irrigated with a water volume of 60%, 50% and 40% of field capacity (each 4 days) during 12 days and continued up to harvest. Days to flowering (DSF), days from flowering to physiological maturity (DFM), head diameter (HD), head weight (HW), leaf number (LN), aerial part dry weight (APDW), number of achene (NA), plant height (PH), stem diameter (SD), and seed yield per plant (SY) were measured. Morphological traits were measured in full flowering stage and traits related to seed were recorded after seed harvesting. Relative water content (RWC) was determined on most fully expanded leaf as $RWC = (FW - DW) / (TW - DW)$, where: FW is fresh weight and TW is turgid weight after 24h rehydration at 4°C in dark room by placing the petioles in a container, with distilled water. DW is dry weight after oven drying for 24h at 80°C. The greenness of the upper most fully expanded leaves as an indicator of total chlorophyll content (CC) was determined using a portable chlorophyll meter, SPAD-502, Soil-Plant Analysis Development Section, Minolta Camera, Osaka, Japan, in SPAD values according to Castelli et al., (1996).

DNA extraction and PCR amplification

Genomic DNA of the plant material was extracted according to Fulton et al. (1995). Different *MseI/EcoRI* primer combinations were used for AFLP genotyping (Table 3, <http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html>). The AFLP procedure was conducted as described by Rachid Al-Chaarani et al., (2004).

Data analysis

Combining ability analysis was conducted based on Griffing's method 2 model I (Griffing, 1956), by using the SAS program for Griffing's diallel analysis (Zhang et al., 2005). Banding profiles generated by AFLP assays were separately compiled into a data matrix on the basis of presence (1) or absence (0) of bands. The binary matrices were used to estimate DNA polymorphisms and genetic relatedness of sunflower genotypes. Similarity matrices were calculated by using Jaccard's similarity coefficients (Jaccard, 1908). Jaccard's distance (JD) was calculated as $1 - \text{Jaccard's similarity coefficients}$. Euclidean distance (ED) was computed from the 12 phenotypic traits after standardization (subtracting the mean value and dividing it by the standard deviation) (Sneath and Sokal, 1973). The distance matrix from phenotypic traits and the similarity matrix from molecular markers were used to construct dendrograms based on un-weighted pair-group method using arithmetic average (UPGMA) algorithm. Absolute mid-parent heterosis (AMPH) was calculated as: $\text{AMPH} = F_1 - \text{MP}$ where $F_1 = F_1$ performance and $\text{MP} = (P_1 + P_2) / 2$ (Teklewold and Becker, 2006). Data analysis was performed using SPSS 16.0 statistical software.

Acknowledgments

The author is gratefully thanked to the Urmia University, Urmia, Iran for the financial support of this work. The author thanks Dr H. Hatami Maleky (Department of Agronomy and Plant Breeding, University of Maragheh, Maragheh, Iran) for statistical analysis and critical reading the manuscript And Dr. H. Laurentin (Departamento de Ciencias Biológicas, Decanato de Agronomía, Universidad Centroccidental Lisandro Alvarado, Barquisimeto, Venezuela) for English corrections.

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