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RESEARCH ARTICLE

Genetic variability, association and diversity study among the sunflower genotypes at seedling stage based on different morpho-physiological parameters under polyethylene glycol induced stress

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Abstract

Drought stress directly affects growth along with productivity of plants by altering plant water status. Sunflower (*Helianthus annuus* L.) an oilseed crop, is adversely affected by abiotic stresses. The present study was carried out to study the genetic variability and diversity among the sunflower genotypes at seedling stage based on different morpho-physiological parameters under Polyethylene Glycol(PEG) induced stress. A total of twenty seven genotypes including two hybrids, eight advanced lines and seventeen accessions of sunflower (*Helianthus annuus* L.) were tested at germination and seedling stages in Polyethylene Glycol. Correlation and principle component analysis confirmed that germination percentage, root length, proline content, shoot length, chlorophyll content, stomatal frequency and survival percentage are positively correlated with each other hence, these traits were responsible for most of variation among genotypes. The cluster analysis results showed that genotypes Ausun, line-2, line-8, 17559, 17578, Hysun-33, 17555, and 17587 as more diverse among all the genotypes. These most divergent genotypes could be utilized in the development of inbreeds which could be subsequently used in the heterosis breeding.

Key words: Sunflower, drought, stress, polyethylene glycol

Introduction

Sunflower (*Helianthus annuus* L.) has emerged as an economically important crop of Pakistan. But limited rainfall or shortage of water for irrigation throughout the growing season restrict its seed yield. Water shortage is becoming a key problem for sustainable agriculture in Pakistan. The reduced precipitation, along with high evapo-transpiration is expected to subject natural agricultural vegetation to a great possibility of severe and delayed water stress with every passing year (Shamim *et. al.*, 2013).

Crop responses toward drought stresses involve processes modulated by water shortage at morphological, anatomical, cellular and molecular levels. The changes which occur in the whole plant organs in response to water stress reduce plant photosynthesis resulting in grain yield decline. It would be very useful to develop effectual strategies to reduce drought stress damage crop plants. A strategy involves producing a high yielding genotype with traits leading toward drought tolerance since the genetic mechanism of drought tolerance within crop plants is extremely complex and seed yield

is strongly prejudiced by genotype and environmental conditions (Tardieu and Tuberosa, 2010).

Several morphological and physiological characters affected by drought stress include reduced leaf area, plant height, root length, head diameter, yield per plant, and plant biomass as well as photosynthetic rate. Severe drought stress may possibly result in arresting of photosynthesis, metabolic disturbance and also plant death. (Kumar *et.al.*,2011). High molecular weight Polyethylene Glycol (PEG) has been used to stimulate drought stress in plants. Polyethylene glycol (PEG) of high molecular weight is a non-penetrating osmotic agent lowering the water potential in a way that is similar to soil drying. The ability of Polyethylene glycol of becoming negative water potential can be used as a mean to assume plant tissue response by drought stress. Polyethylene glycol (PEG 4000) is an osmotic agent that does not cause plasmolysis and non-toxic for plants. (Yosephine *et. al.*, 2013). Proline accumulates within plants during the adaption to different types of environmental stress such as drought, salinity, high temperature, nutrients deficiency and exposure to any metals and high acidity. The principle role of proline probably is not to reduce the osmotic potential but to defend enzymes against dehydration and salt accumulation (Faizan *et. al.*, 2012). The present study was carried out to study the genetic diversity and association among different traits in sunflower genotypes based on seedlings parameters and to find out most divergent genotypes under stress.

Materials and methods

The experiment was carried out at the laboratory of Plant Breeding and Molecular Genetics, Faculty of Agriculture, University of Poonch Rawalakot. The material was comprised of twenty seven genotypes of sunflower (*Helianthus annuus* L.) acquired from Oil Seed Research Program (NARC) Islamabad (Table 1). The experiment was tested against drought stress at germination and seedling stages under laboratory conditions (25±3°C). The pots were filled with soil, sand and manure 1:1:1. Polyethylene glycol with a molecular weight of 6000 (PEG-6000) was used as a drought stimulator and five water stress levels of zero (control), -0.35MPa, -0.6MPa, -1.33 MPa was developed by dissolving 5, 10 and 15 g of PEG per 100 mL in distilled water. Five seeds were surface sterilized with 10% sodium hypochlorite solution for five minutes and then washed three times with distilled water. Five seeds of each genotype were planted in each pot. The experiment was laid out in 2x2 factorial completely randomized design with three replications for each experimental unit. The experiment was completed after 30 days of planting.

Germination percentage: Number of seeds germinates were counted daily and data was recorded for 14 days. A seed was considered germinated when both plumule and radical were emerged to a length of 5 mm.

Germination percentage was calculated according to (Jefferson and Penachchio, 2003) by using following formula:

Germination% = (n/N) x 100 where n: number of seeds germinated, N: total number of seed in each pot.

Shoot length: Shoot length of five plants from every pot was measured in cm and their mean was calculated.

Root length: Root length of five plants from every pot was measured in cm and their mean was calculated.

Root fresh weight: Root and Shoot of each plant was separated and fresh weight was determined separately with the help of a digital electrical balance.

Root fresh weight: Root and Shoot of each plant was separated and fresh weight was determined separately with the help of a digital electrical balance.

Shoot dry weight: Shoot of each plant was dried in an oven at 60°C for 24 hours and their dry weight was measured.

Stomatal frequency: The leaf stripes were taken for studying leaf venation and stomata size will was used for counting the stomata low power microscopic field (10x) was used to investigate stomatal frequency.

Root dry weight: Root of each plant was dried in an oven at 60°C for 24 hours and their dry weight was measured.

Chlorophyll contents: The chlorophyll concentrations were determined by the method of Arnon (1949). Chlorophyll contents were estimated from fresh leaves, collected from base, middle and apex of every selected plant from each population under study. Three leaf samples from each plant were subjected to experiment. 1 cm 2 leaf cuttings were soaked in 5 ml acetone in test tube for each sample and left for overnight. Next day greenish liquid from each test tube was collected in cuvette and optical density of that mixture will be taken at two different wavelengths i.e. 663 nm for chlorophyll A and 645 nm for chlorophyll B at spectrophotometer. Observations of optical densities for chlorophyll A and chlorophyll B from all the samples were taken and their mean values were obtained. These values were subjected to the following formula for the final evaluation of total concentrations of chlorophyll for receptive replication of selected populations.

Total chlorophyll = 8.0 × O. D at 663 nm + 20.2 × O. D at 645 nm

The data was analyzed to calculate phenotypic correlation coefficients between the traits (Snedecor, 1956). Simple statistics and numerical taxonomic techniques were utilized for cluster and principle component analysis (Sneath and Sokal, 1973) with the help of computer software Statistica, Past (Hammer *et. al.,* 2001) and SPSS 20. Cluster analysis was conducted on the basis of average distance of k means.

Results and discussions

Drought is the main cause not only of differences between mean yield and potential yield but also of yield variations from year to year and therefore of yield instability (Shamim *et. al.*, 2010).

The mean values for germination percentage showed in (Table 2). It ranged from 67-90% in various sunflower genotypes. Maximum mean value was reported in AUSUN (90%) and minimum in 17562 (67%). The root length had positive significant correlation with germination percentage. (Table 3) In various sunflower genotypes mean values for shoot length varied from 8.7cm-13.0 cm. Negative and non-significant correlation of shoot length was recorded for root length, Stomatal frequency and chlorophyll content. Root length varied from 6.53-10.90 cm, root length showed non significant and negative correlation with Stomatal frequency and chlorophyll content. The increased fresh root weight has been one of the principal factors contributing to genetic yield improvements in sunflower cultivar. Whereas, high fresh root weight demonstrates high yielding ability when cultivars are compared, it can also point out challenges to yield improvement when comparisons are made across differing growing conditions. Mean values of fresh root weight for sunflower genotypes varied in the range of 0.12-0.32 g, fresh root weight had positive non significant correlation with dry shoot weight, dry root weight, Stomatal frequency and chlorophyll content. Mean values for dry root were extremely variable ranging from 0.09 to 0.31 g. Positive correlation of dry root weight was determined for shoot length, root length and Stomatal frequency. Mean values for chlorophyll content ranged from 0.26- 0.49 %

Table1: List of sunflower genotypes used in the present study

S.No	Genotype	Source	S.No	Genotype	Source
1	Ausun (Hybrid)	NARC	15	17561	NARC
2	Line 1	NARC	16	17562	NARC
3	Line 2	NARC	17	17568	NARC
4	Line 3	NARC	18	17570	NARC
5	Line 4	NARC	19	17572	NARC
6	Line 5	NARC	20	17573	NARC
7	Line 6	NARC	21	17575	NARC
8	Line 7	NARC	22	17577	NARC
9	Line 8	NARC	23	17578	NARC
10	17555	NARC	24	17580	NARC
11	17557	NARC	25	17581	NARC
12	17558	NARC	26	17587	NARC
13	17559	NARC	27	Hysun-33(Hybrid)	NARC
14	17560	NARC			

Table 2: Mean values of sunflower genotypes under PEG mediated drought stress at seedling stage

Genotypes	G	SL	RL	FRW	DRW	FSW	DSW	S F	CC
1. AUSUN	90	11.0	7.63	0.22	0.11	0.73	0.042	8.3	1.9
2. line -1	77	9.0	7.97	0.18	0.10	0.68	0.025	8.3	0.2
3. line-2	70	12.7	7.47	0.22	0.31	0.68	0.025	7.3	0.3
4. line- 3	73	10.7	9.10	0.19	0.30	0.61	0.021	9.7	1.33
5. line- 4	73	12.3	9.03	0.31	0.31	0.58	0.027	9.3	0.3
6. line -5	77	12.0	8.93	0.20	0.29	0.54	0.027	10.0	0.2
7. line- 6	80	11.7	9.60	0.21	0.12	0.47	0.019	7.3	0.4
8. line- 7	77	12.3	8.73	0.13	0.14	0.46	0.030	7.7	0.5
9. line-8	77	10.0	9.63	0.15	0.11	0.46	0.037	9.3	0.4
10. 17555	77	11.0	8.40	0.13	0.28	0.44	0.017	7.3	0.4
11.17557	80	10.3	6.97	0.13	0.10	0.43	0.016	9.7	0.3
12. 17558	80	12.0	6.53	0.14	0.13	0.41	0.022	9.0	0.2
13. 17559	73	13.3	7.20	0.13	0.14	0.38	0.021	9.3	0.3
14. 17560	80	12.0	9.63	0.13	0.12	0.37	0.021	6.7	0.4
15. 17561	77	11.3	10.90	0.12	0.10	0.36	0.021	7.3	0.3
16. 17562	67	13.0	10.83	0.15	0.09	0.35	0.021	8.7	0.3
17. 17568	70	10.3	10.43	0.13	0.15	0.33	0.022	8.3	0.4
18. 17570	67	11.7	8.03	0.13	0.10	0.32	0.029	8.7	0.4
19. 17572	70	13.0	7.13	0.12	0.10	0.32	0.025	8.7	0.4
20.17573	83	11.3	9.03	0.13	0.30	0.31	0.017	9.7	0.4
21. 17575	80	9.0	10.10	0.17	0.32	0.31	0.037	7.3	0.4
22. 17577	80	9.3	7.57	0.19	0.30	0.28	0.028	8.3	0.4
23. 17578	77	8.7	7.30	0.13	0.13	0.27	0.038	9.7	0.4
24. 17580	83	11.3	8.93	0.13	0.09	0.25	0.032	9.3	0.5
25. 17581	73	11.7	9.33	0.14	0.13	0.20	0.014	9.0	0.5
26. 17587	73	11.3	9.43	0.32	0.14	0.19	0.023	8.7	0.3
27. Hysun 33	73	14.3	9.37	0.20	0.10	0.12	0.024	9.3	0.6

Where, G: Germination (%), SL: Shoot length (cm), RL: Root length (cm), FRL: Fresh root weight (g), DRL: Dry root weight (g), FSW: Fresh shoot weight (g), DSW: Dry shoot weight (g), SF: Stomatal frequency (%), CC: Chlorophyll content (%)

stomatal frequency had highly significant as well as positive correlation with chlorophyll content. Similar kinds of results were reported in the studies of Canavar *et. al.*, (2014), Ahmed *et al*. (2014), Reza *et. al.*, (2013) and Iraj *et. al.*, (2014).

Cluster analysis

Cluster analysis was carried to group the sunflower genotypes presented in tabl4. The tree diagram based on 27 sunflower genotypes was displayed in figure 1. The figure indicated two main clusters at linkage distance 40. Three major clusters viz., cluster I, cluster II. Cluster I was further classified into two sub-clusters Ia and Ib. Sub-cluster Ia grouped three genotypes 17559, Ausun and line-2. Among these three genotypes 17559 was an outlier and showed diversity while Ausun and line-2 were at the same linkage distance and are closely related with each other. Sub-cluster Ib was further classified into two groups. Group I consisted of two genotypes i.e. line-5 and 17557. In this group both i.e line-5 and 17557 were at the same linkage distance and show no variation. Group II contained nine genotypes i.e.Hysun-33, 17560, 17575, line-3, 17577, 17568, 17570, line-4 and 17578. In this group 17560 and 17575 were at the same linkage distance while Hysun-33 was an outlier and showed diversity. Line-3 and 17577 also showed the same linkage distance in this group. Among all the outliers in this cluster 17559 showed maximum divergence. Cluster II was further divided into two sub-clusters IIa and IIb. Sub-cluster IIa comprised of nine genotypes i.e. line-8, 17572, line-1, line-7, 17573, 17560, 17561, 17581, 17562. Line-8 was an outlier and showed variation. The line-1, line-7, 17573 were at the same linkage distance, while 17560, 17561, 17581, 17562 also show no significant variation show the same linkage distance. Sub-cluster IIb consisted of four genotypes 17558, line-6, 17555, and 17587. Among these genotypes 17558 was an outlier and showed diversity. Among all sunflower twenty seven genotypes i.e. 17559, Hysun-33, line-8, and 17558 showed more diversity as compared to others. Nadeem *et. al.*, (2011), Abdi *et. al.*, (2012) and Khamsee *et. al.*, (2012) support our present findings.

Correlation and principle component analysis showed that germination percentage, root length, Proline content, shoot length, chlorophyll content, stomatal frequency and survival percentage have significant relationship with each other; hence these traits were responsible for most of variation among genotypes fig 2. Biplot verified Ausun, line 2, line 1, 17559, 17555, 17572, 15557, 17575 and 17550 as more diverse among all the genotypes. The quantification of genetic diversity by principle component analysis made it possible to select genetically diverse parents for hybrid production. Genetic diversity is important to select appropriate inbred for hybridization (Xia *et al*., 2005; Reddy *et. al.*, 2012).

It was concluded that genotypes which grouped under different clusters are diverse and can be used in future sunflower breeding. The present investigation is in accordance with Reddy *et. al.*, (2012), Khamsee *et. al.*, (2012), Mudassar et al. (2013), Mehdi et al. (2013), Andrea *et. al.*, (2013) and Reza *et. al.*, (2013) who had reported similar results which in agreement with present investigation.

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Table3: Simple correlation coefficients for morphological traits in sunflower genotypes

	G	S	SFW	RFW	DSW	DRW	SL	RL	SF	CC
G	1									
S	.310	1								
SFW	-.017	-.306	1							
RFW	-.142	.303	-.059	1						
DSW	-.198	.203	.106	.194	1					
DRW	-.038	.105	-.038	.062	.321	1				
SL	.074	.059	.132	-.205	-.221	.089	1			
RL	.500**	.245	-.140	-.202	-.200	.082	-.230	1		
SF	.170	.288	.046	.270	.359*	.126	-.078	.125	1	
CC	.226	.112	-.089	.038	.232	-.096	-.279	-.270	.480**	1

Where, G: Germination (%), SL: Shoot length (cm), RL: Root length (cm), FRL: Fresh root weight (g), DRL: Dry root weight (g), FSW: Fresh shoot weight (g), DSW: Dry shoot weight (g), SF: Stomatal frequency (%), CC: Chlorophyll content (%)

Figure. 1 Dendogram based on Euclidean distances displaying diversity among geotypes

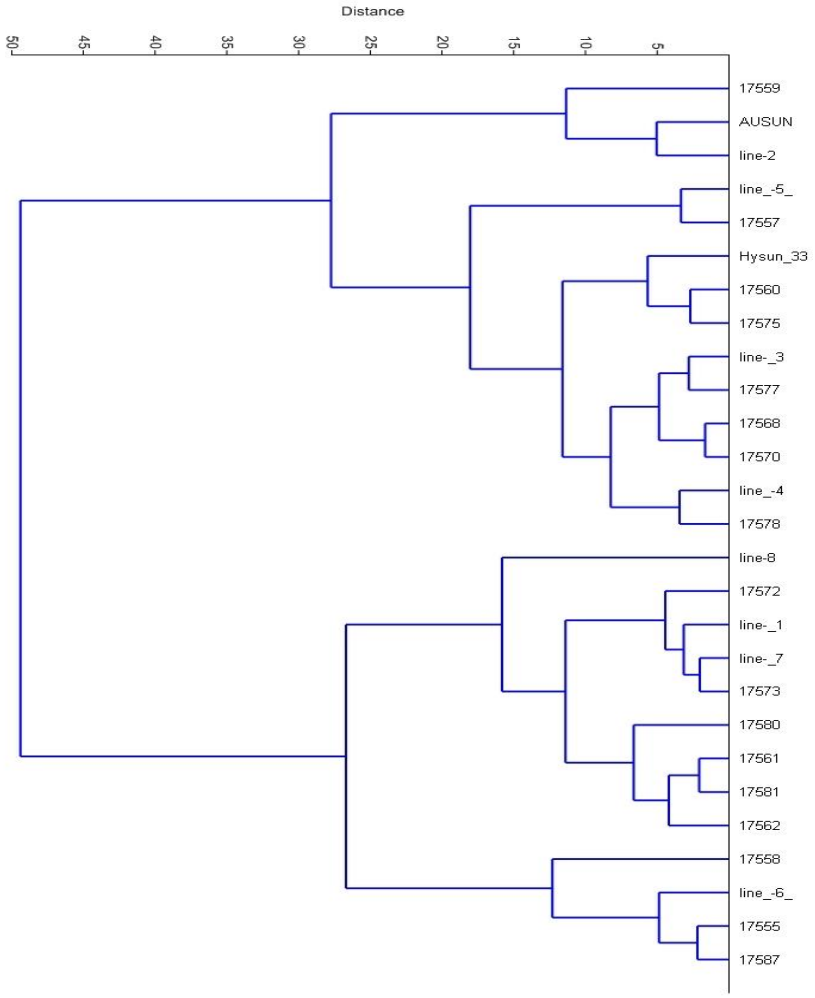
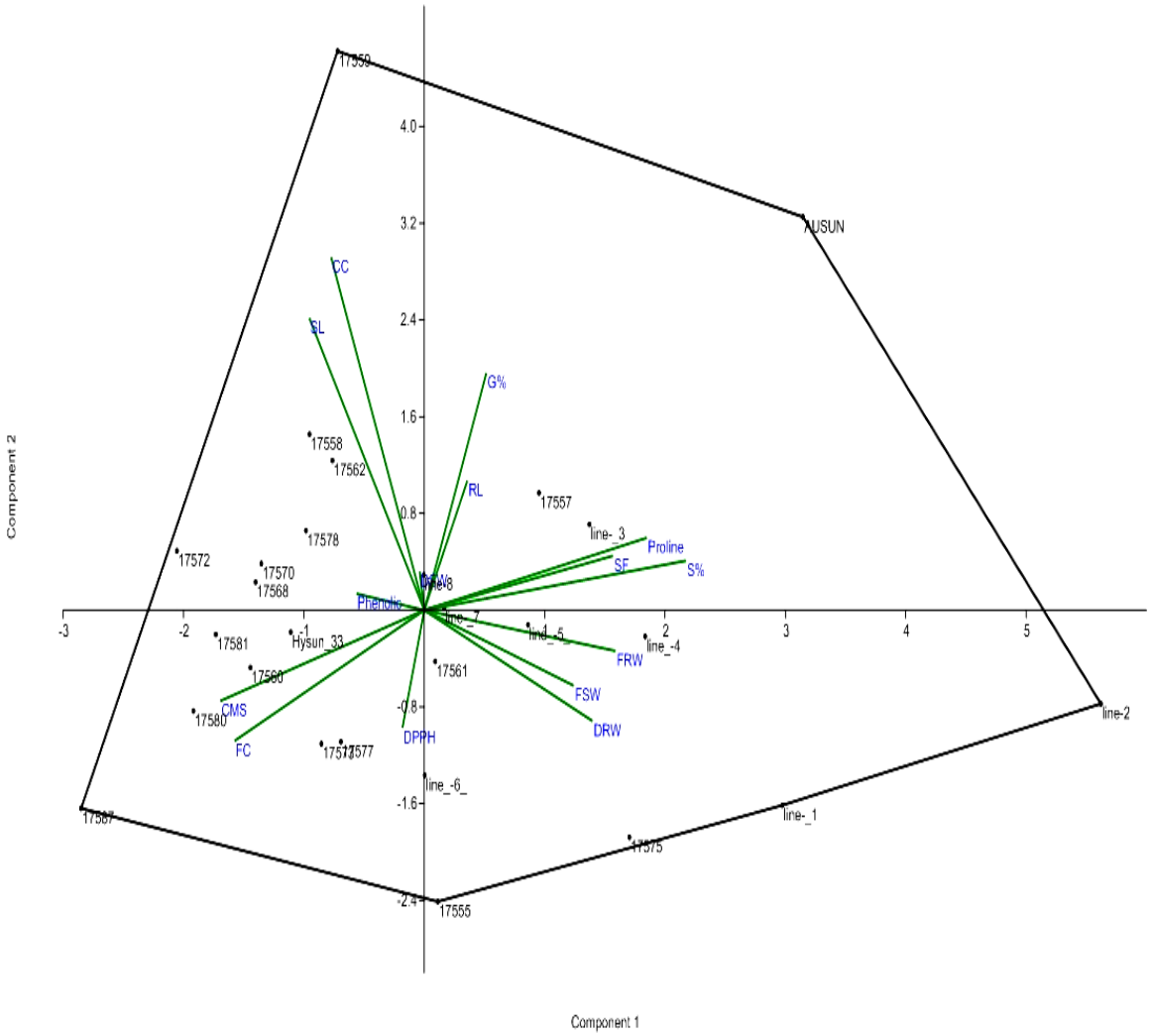


Table 4: Grouping of the sunflower genotypes in different clusters

Cluster No.	Grouping of the sunflower genotypes in respective cluster
I	17568, LINE-8, 17570, Ausun, 17577, line-3, 17587 17578, 17575, 17560, Hysun-33, line-4, 17555 and line-2
II	17559 and 17558
III	17580, 17581, 17582, 17561, 17572, line-7, line-6, 17573, line-5, line-1 and 17557

Fig: 2 Scatter plot diagram in sunflower genotypes



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