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SCREENING OF MORPHOLOGICAL TRAITS IN SUNFLOWER (*Helianthus annus*. L) UNDER NORMAL AND DROUGHT STRESS

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Abstract: Sunflower (*Helianthus annus* L.) is the main oilseed crop throughout the world. Pakistan facing the problem of edible oil shortage. Drought is the major abiotic stress that limits the crop production at drastic level. Screening of tolerant accessions from available germplasm is the basic step in plant breeding. So, there is need to develop drought tolerant plants. Genetically diverse sunflower accessions will be grown at research area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Response of ten sunflower accessions were tested against PEG (Poly Ethylene Glycol) mediated drought stress at germination and seedling stage. The experiment was laid out by completely randomized design with factorial structured treatments. Three treatments i.e., T1= zero (control), T2= -1.33MPa, T3 = -1.62MPa were developed by using 5 and 10% of PEG-6000. Principal component analysis showed that Accessions A21, A22, A23 and A-24 performed well and showed genetic variability among these accessions and can be used for further breeding programs.

Keywords: Drought stress, poly ethylene glycol (PEG-6000), principal component analysis, seedling traits, sunflower accessions

1. INTRODUCTION

About 95% of country's water resources are consumed by agriculture, Climatic changes affect the availability of water at critical times during crop growth, which adversely affect the growth (Bhatti *et al,* 2009). Stress is any effect that has drastic affect to plant. There are two types; biotic and abiotic stress. Biotic stresses include insects, pests and disease. Abiotic stresses include light, temperature, drought and humidity. The major and destructive stress is drought stress, which is due to below average rainfall, low precipitation, high precipitation and low soil moisture.

Drought is a significant environmental pressure that severely diminishes crop production. Regrettably, sunflower, a globally important oilseed source, is highly vulnerable to drought (Razzaq *et al,* 2017). Drought stress leads to a reduction in water potential specifically in sunflower, as indicated by (Ghobadi *et al*, 2013). Water potential serves as a reliable indicator of a plant's response to water stress, and different genotypes exhibit varying levels of water potential due to differences in their ability to absorb water from the soil and reduce water loss through stomata. These stomata help maintain turgor pressure (Siddique *et al,* 2000). Drought has a significant impact on the uptake of minerals and disrupts the balance of nutrients (Gunes *et al,* 2008). However, different species and genotypes within species show varying responses to water scarcity and drought stress (Garg, 2003).

However, not all growth phases are affected in the same way by drought stress, The most essential time to be exposed to drought is during a specific stage or phase, such as anthesis, germination, achene filling, etc., which can reduce sunflower yield by up to 50%. Early-season drought stress inhibits germination, stem growth, and leaf area (Fulda *et al*, 2011), whereas drought stress at anthesis causes the development of empty achenes because of pollen sterility More water is available during the vegetative development phase, which is excellent, but less water is available during the flowering and grain filling stages, which drastically affects the yield because of high transpiration demands (Aboudrare *et al,* 2006).

The initial step in developing drought-tolerant varieties or hybrids involves the careful selection of drought-tolerant accessions from the existing germplasm. Plant breeders have employed various methods to identify accessions and traits that contribute to drought tolerance. However, screening experiments become more challenging due to uncontrolled conditions, soil heterogeneity, a large quantity of plant material, as well as the time and labour required for field experiments. As a result, laboratory experiments are considered more dependable and easier to carry out compared to field trials. In laboratory settings, Polyethylene glycol (PEG-

6000) is commonly utilized as a drought simulator since it is deemed non-penetrable, harmless, and an effective means of inducing osmotic stress resembling drought conditions. (Hu and Jones, 2004, Kaya *et al*, 2006 and Cavallaro *et al,* 2016).

2. MATERIALS AND METHODS

Germplasm consisting of 10 sunflower accessions (Table 1) was collected from Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad.

Table.1. Accessions names

Sr. No	Accession names	Sr. NO	Accession names
1	A-21	6	A-26
2	A-22	7	A-27
3	A-23	8	A-28
4	A-24	9	A-29
5	A-25	10	A-30

Screening at seedling stage using PEG mediated drought stress

This research was carried out at the Sunflower Research Lab, located in the Department of Plant Breeding and Genetics at the University of Agriculture, Faisalabad. The study involved testing ten different sunflower accessions for their response to drought stress at the germination and seedling stages using PEG as a mediation agent. The experiment followed a triplicate completely randomized design with factorial structured treatments. Three treatments were applied: T1 represented the control (no stress), T2 represented -1.33MPa stress level, and T3 represented -1.62MPa stress level. The stress levels were achieved by using 5% and 10% concentrations of PEG-6000, as described by Ahmad *et al*. (2009) and Tsago *et al*. (2014), respectively.

The weight of an empty petri dish was determined using a weighing balance. The tare button was pressed to eliminate the weight of the petri dish. PEG was gradually added till the final reading reached 10 grams. The same approach was used to measure 20 grams of PEG. These treatments were made by adding respective concentrations of PEG-6000 in 100 ml of water. The distilled water was added continuously while dissolving PEG-6000 until the final volume became 100ml. 5ml of the solution was applied per treatment per replication on daily basis. Normal distilled water was also applied to the controlled treatments. This was done for 14 days, then the data was recorded for various traits.

3. RESULTS

The analysis of variance and the mean comparisons of tested sunflower significantly differences among genotypes, treatments and their interaction but the average performance of accessions decreased under drought stress, maximum mean value observed under control T1, minimum value under drought stress T2. Mean comparison indicates that genetic variability is present among the genotypes for quantitative trait under study.

Table.1. Mean square values from analysis of variance for stress indexes in sunflower accessions under PEG-6000 simulated drought stress conditions.

Source	DF	GP	SL	RL	RH	DW	FW
Trt	2	1263.9**	36.1158**	49.669**	52.6861**	0.002*5*	0.14365**
Geno	9	28479.2**	4.0275**	8.1673**	11.9957**	0.0023**	0.02111**
trt*geno	18	28875**	1.5629**	3.8831**	3.5596**	0.002**	0.01018**
Error	60	11666.7	0.5947	0.6444	1.1764	0.0009	0.00251
Total	89	70284.7					

*=Significant at 0.05 probability level **= Significant at 0.01 probability level

GP=Seedling germination percentage, SL=Seedling shoot length, RL=Seedling root length, RH=Seedling root hairs, DW=Seedling dry weight, FW=Seedling fresh weight

i. Germination percentage

It ranged from (41.6-86.1%). Accession A-21 had the maximum germination percentage 86.1% while A-28 had a minimum germination percentage (41%). The treatment means ranged from (58-67%). Treatment 1 and 2 has the maximum value (67%) of the mean of treatment while treatment 3 showed a minimum value (58%).

ii. Seedling shoot length(cm)

The means of genotypes ranged from (2.26-4.2). Accession A-23 had the maximum mean value (4.2) while A-24 had the minimum mean value (2.26m) for seedling shoot length. A-23 significantly differs from A-24, A-26, and A-30. The treatments mean ranged from (2.1-4.3). Treatment 3 showed lowest mean value (2.1) while treatment 1 showed maximum mean value (4.3) which showed significant impact of treatments.

iii. Seedling Root length(cm)

The means of genotypes ranged from (2.9-6.4cm). Accession A-28 had the maximum mean value (6.4cm) while A-30 had the minimum mean value (2.9cm) for seedling root length. A-28 significantly differs from A-22, A-26, and A-30. The treatments mean ranged from (3.3-5.9). Treatment 3 showed lowest mean value (3.3) while treatment 1 showed maximum mean value (5.9) which showed significant impact of treatments.

iv. Seedling Root hairs(cm)

The means of genotypes ranged from (3.1-6.7cm). Accession A-21 had the maximum mean value (6.7cm) while A-26 had the minimum mean value (3.1cm) for seedling root hairs. A-21 significantly differs from A-22, A-26, and A-30. The treatments mean ranged from (3.2-5.8). Treatment 3 showed lowest mean value (3.3) while treatment 1 showed maximum mean value (5.9) which showed significant impact of treatments.

v. Seedling Dry Weight(g)

The means of genotypes ranged from (0.009-0.062g). Accession A-26 had the maximum mean value (0.062g) while A-30 had the minimum mean value (0.009g) for seedling dry weight. A-26 significantly differs from A-22, A-26, and A-30. The treatments mean ranged from (0.03-0.05). Treatment 1 showed maximum value while treatment 2 showed minimum value.

vi. Seedling Fresh weight(g)

The means of genotypes ranged from (0.13-0.27g). Accession A-22 had the maximum mean value (0.27g) while A-25 had the minimum mean value (0.13g) for seedling dry weight. A-22 significantly differs from A-24, A-26, and A-30. The treatments mean ranged from (0.13-0.26). Treatment 1 showed maximum value while treatment 3 showed minimum value.

Principal Component Analysis

In PCA 1 all the accessions had more than one eigen value in biplot cumulative variance percentage in dimension 1 had (80.6%) and in dimension 2 had (13%). Biplot for the principal components (PCA 1 (To),PCA 2 (T2) and PCA 3 (T3) are presented in Figure 1,Figure 2 and Figure 3 respectively.Accessions,A-26,A27,A28 and A-30 were drought tolerant accessions as these are falling in Quadrate 1 where all the indexes had showed positive response towards drought tolerance. Accessions A-21, A22 and A-24 falls in quadrate 2 and these are drought tolerant. Accessions A-25 and A-29 were considered the most drought sensitive in all treatment from Quadrate IV. In PCA 2 all the accessions had more than one eigen value, in biplot for cumulative variance percentage in dimension 1 had (47.4%) and in dimension 2 had (20.2%). Accessions A-23 were drought tolerant accessions as it is falling in Quadrate 1. Accessions A-26,A-29 are drought tolerant as falls in quadrate 2.Accessions A-24,A-25,A-27 and A-30 were considered the most drought sensitive as these were falls in quadrate In PCA 3 all the accessions had more than one eigen value in biplot cumulative variance percentage in dimension 1 had (30%) and in dimension 2 had (25.7%). Accessions A-23, A-26, A29 and A-30 were drought tolerant accessions falls in quadrate 1, Accessions A-21 and A-22 falls in quadrate 2, Accessions A-24 and A-25 falls in quadrate 3. Accessions A-27 and A-28 falls in quadrate IV. Accessions A-23, A26, A-29 and A-30 had positive respond for yield per plant and germination percentage. Accessions A21 and A-22 had positive response for leaf area, number of achene and filled achenes. Accessions A-24 and A-25 had positive response for plant height, number of leaves and head diameter. The accessions A-22, A-24, A-25, A-27 and A-30 were considered the most drought sensitive in all treatment from Quadrate IV.

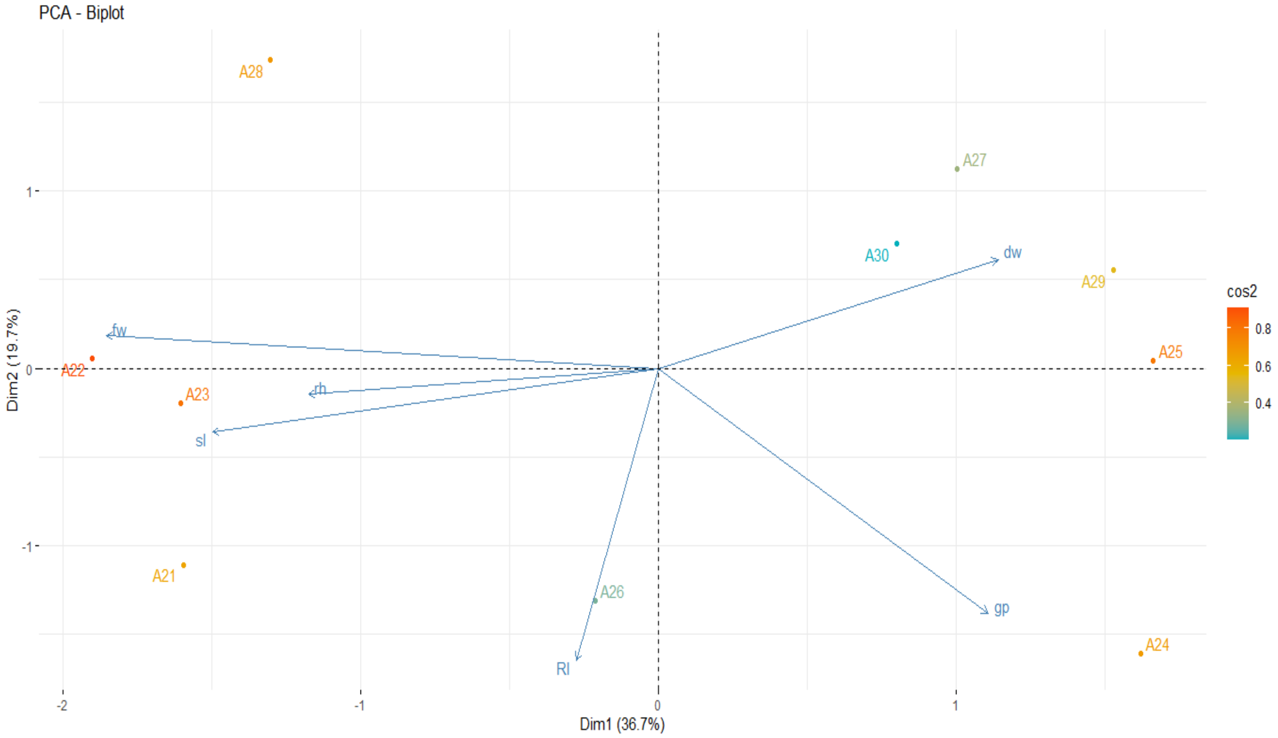

Figure 1. Principal Component Analysis of Stress Indexes of T1 In Sunflower Accessions

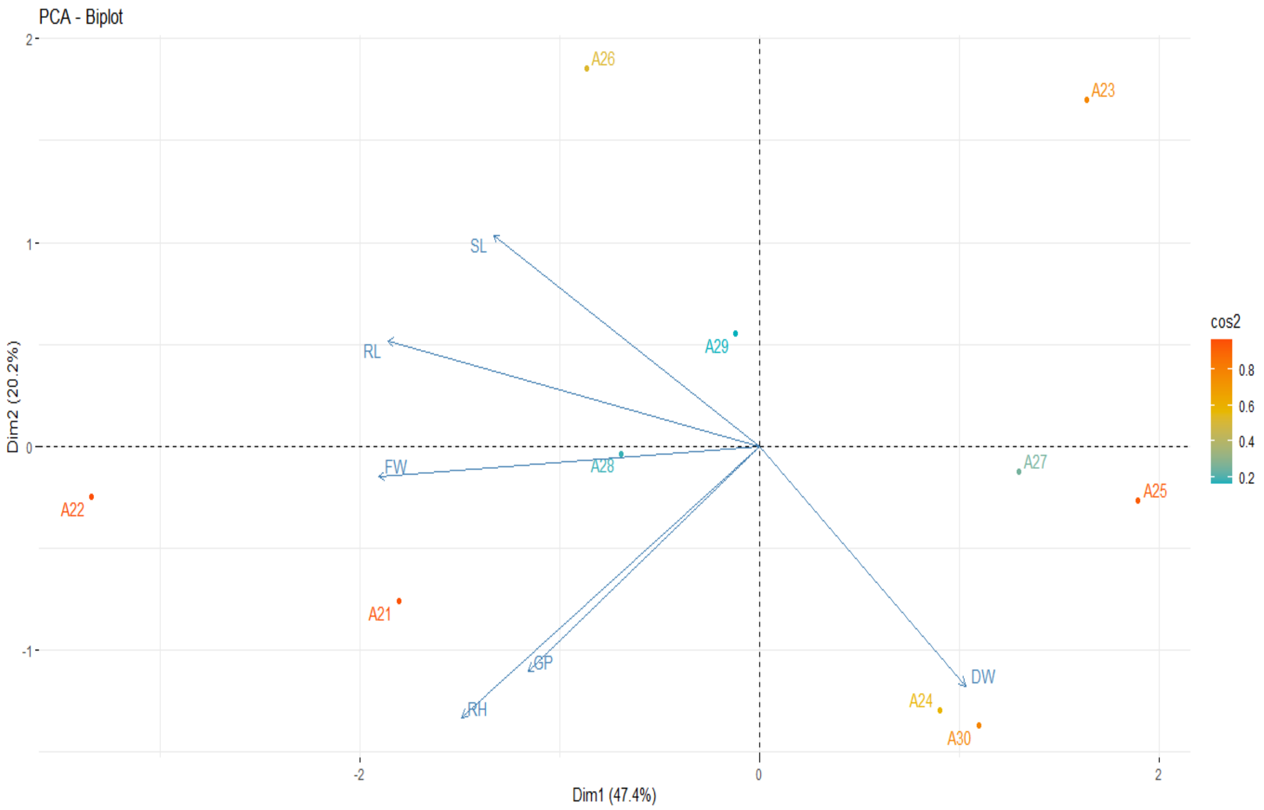

Figure 2. Principal Component Analysis of Stress Indexes of T2 In Sunflower Accessions

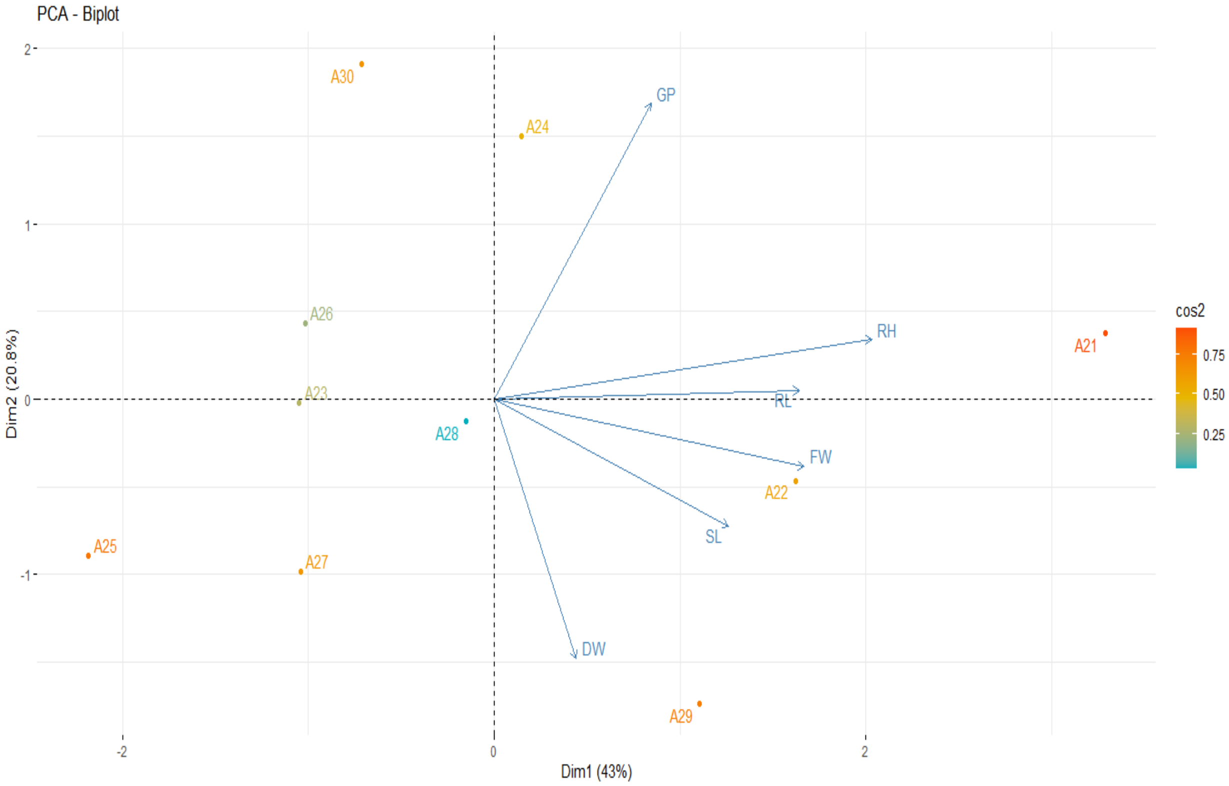

Figure 3. Principal Component Analysis of Stress Indexes of T3 In Sunflower Accessions.

4. DISCUSSIONS

The results indicated that the accessions had significant genetic variability for all the seedling traits. All the seedling traits i.e., germination percentage, shoot length, root length, number of leaves, leaf area, seedling fresh weight and seedling dry weight got decreased when the drought stress levels were increased except germination percentage. Researchers Hsio (1973), Ahmad *et al.* (2009), Rauf and Sadaqat (2008), Rauf (2008), Hossain *et al.* (2010), Mehrpouyan *et al.* (2010), Ghaffari *et al.* (2012), Zlatev and Lidon (2012), Hussain *et al*. (2013), and Cavallaro *et al. (*2016) have also noted a decrease in indexes when the drought stress is increased.

The analysis of variance and the mean comparisons of tested sunflower significantly differences among genotypes, treatments and their interaction like plant height, head diameter, number of leaves, leaf area, number of achenes, filled achene but the average performance of accessions decreased under drought stress. Peckan (2016.) found that mean comparisons of tested sunflower significantly difference among genotypes, treatments and their interaction like plant height, head diameter and number of leaves. However, while there were observed increase at 1st stress, there were some decreases at 2nd and 3rd stress conditions. Similar condition publishes. Sarimirad (2019) The significance of the environmental effect is evident in the combined analysis of variance, indicating that drought stress has had a significant impact on all traits. Consequently, the implementation of stress conditions highlights the disparities among cultivars, and assessing cultivars under stressful environments can aid in selecting appropriate ones. However, the genotype's influence was found to be insignificant for certain traits, suggesting that the performance of all characteristics declines compared to normal conditions. Their findings demonstrate that the maximum decrease in performance is observed in seed yield per plant.

Principal component analysis showed that Accession A-24 and A25 performed under normal showed positive response for plant height, seed yield per plant, leaf area and filled achene percentage, under T1 Accessions A21, A22 and A-23 performed well and show positive response for the head diameter and number of leaves. Under T2 Accessions A21, A22, A23 performed well and showed genetic variability among these accessions and can be used for further breeding programs.

5. CONCLUSION

Except for germination percentage, the presence of genetic variability was observed among all the accessions for all traits. This observation indicates that A-21, A-22, and A-23 exhibited tolerance to drought, while A-24 and A-25 displayed positive responses under normal conditions. These specific accessions can be utilized in future research on drought stress and the development of sunflower varieties with enhanced drought tolerance. Among the studied traits, excluding germination, it was determined that they serve as more dependable indicators of drought stress.

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