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**ANALYSIS OF THE RESPONSE OF THE SUNFLOWER  
(HELIANTHUS ANNUUS L.) GENOTYPES UNDER  
POLYETHYLENE GLYCOL MEDIATED DROUGHT  
STRESS IN LABORATORY CONDITIONS  
OF RAWALAKOT AZAD JAMMU & KASHMIR  
PAKISTAN**

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**Abstract:** Drought stress directly affects growth along with productivity of plants by altering plant water status. The early effect of drought on plant growth is inhibition of shoot as well as root growth. Sunflower (*Helianthus annuus* L.) an oilseed crop, is adversely affected by abiotic stresses. The present study was carried out to characterize the genetic variability for seedling and morpho-physiological parameters in different sunflower genotypes under well watered and water stressed conditions. In this study a total of twenty seven genotypes including two hybrids, eight advanced lines and seventeen accessions of sunflower (*Helianthus annuus* L.) were tested against drought stress at germination and seedling stages. The material was sown within pots at the laboratory of Plant Breeding and Molecular Genetics, University of Poonch, Faculty of Agriculture Rawalakot. Five seeds of each genotype were planted in each pot. The experiment was laid out in 2x2 factorial completely randomized design with three replications. Significant means were calculated among traits using analysis of variance (ANOVA) whereas, correlation and Principle component analysis also confirmed that germination percentage, root length, proline content, shoot length, chlorophyll content, stomatal frequency and survival percentage are positively linked with each other hence these traits were responsible for most of variation among genotypes. The cluster analysis verified Ausun, line-2, line-8, 17559, 17578, Hysun-33, 17555, and 17587 as more diverse among all the genotypes.

**Key Words:** Sunflower, Genotypes, Polyethylene-Glycol

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### **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 analysis was carried out to characterize the genetic variability for seedling and physio-chemical parameters in different sunflower genotypes under well watered and water stressed condition. To find favorable tolerant genotypes under various levels of water 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. 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.

#### **A). Seedling Traits**

##### **1) Germination Percentage:**

Number of seeds germinates were counted daily and data was recorded for 14 days. A seed was consider 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:

$$\text{Germination \%} = (n/N) \times 100$$

where n: number of seeds germinated, N: total number of seed in each pot.

##### **2) Shoot length**

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

##### **3) Root length**

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

##### **4) 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.

##### **5) 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.

##### **6) Shoot dry weight**

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

##### **7) Stomatal frequency**

The leaf stripes were taken for studying leaf venation and stomata size will be used for counting the stomata

Low power microscopic field (10x) was used to investigate stomatal frequency.

##### **8) Root dry weight**

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

##### **9) 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<sup>2</sup> 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 =  $8.0 \times O.D$  at 663 nm +  $20.2 \times O.D$  at 645 nm

#### **Statistical Analysis**

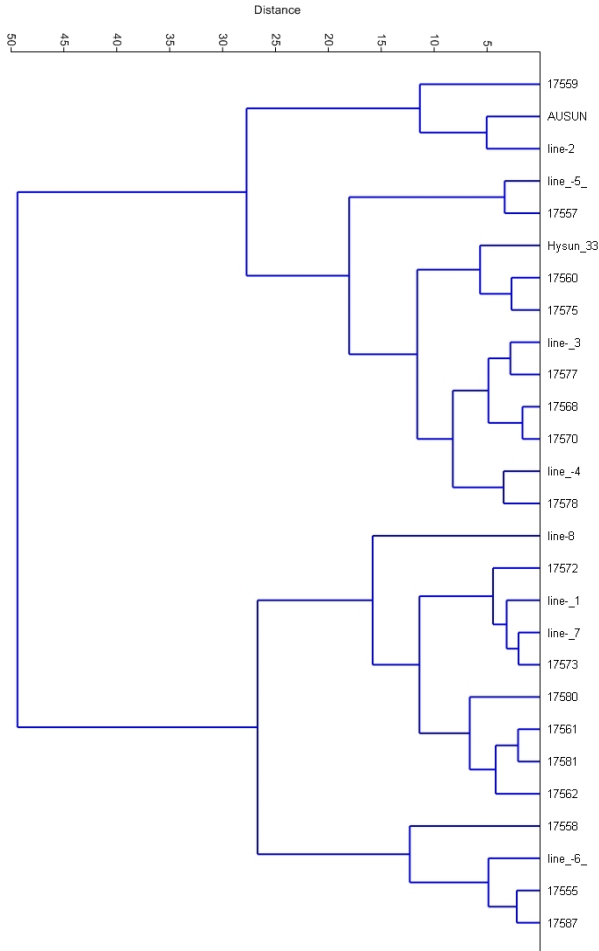
The data was analyzed to calculate phenotypic correlation coefficients between the traits (Snedecor, 1956). Simple statistics and numerical taxonomic techniques were utilized for cluster and principle component analysis (Sneath and Sokal, 1973) with the help of computer software Statistica, Past (Hammer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

#### **Results and Discussions**

The tree diagram based on 27 sunflower genotypes was displayed in figure 1. The figure indicated two main clusters at linkage distance 40.

The clusters were named as cluster I, cluster II. Cluster I was further classified into two sub-clusters Ia and Ib. Sub-cluster Ia consisted of three genotypes including 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, 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. , 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. and line-6, 17555

17587 showed the same linkage distance and show no diversity. Among all sunflower twenty seven genotypes i.e. 17559, Hysun-33, line-8, and 17558 showed more diversity as compared to others.



**Figure. 1 Dendrogram based on Euclidean distance in sunflower Genotypes**

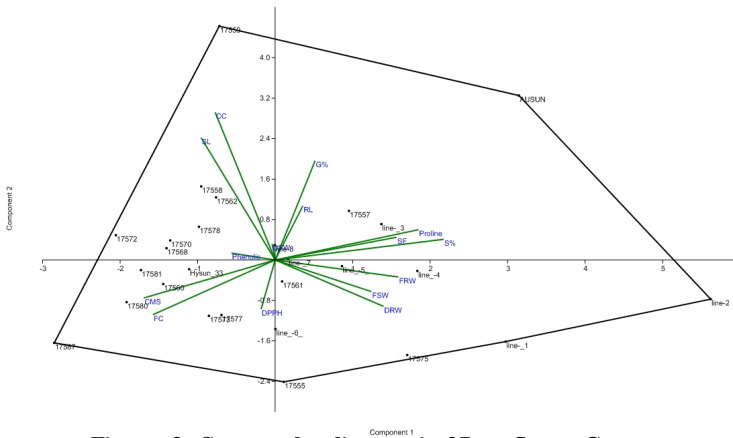


Figure. 2. Scatter plot diagram in 27 sunflower Genotypes

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