### Assessment of growth and biochemical indicators for drought tolerance in maize genotypes

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**Abstract**

Ten maize genotypes were assessed in order to evaluate their response for drought tolerance under 100%, 60%, and 50% field capacity. Genetic variation is the unique coping mechanism of maize genotypes that are either tolerant or susceptible to varying degrees of drought. The drought stress significantly reduced the growth properties of all maize genotypes. A significant decrease in growth and the amount of chlorophyll contents was observed in maize genotypes grown under 50% field capacity. Conversely, total soluble sugars (TSS), hydrogen peroxide (H2O2), malondialdehyde (MDA) contents, total flavonoids, anthocyanin, and total phenolic contents all showed a significant increase under drought stress. The results of this study demonstrated that the Gohar-19 genotype of maize was tolerant to drought stress particularly at 60% field capacity with minimum reduction in growth, while the Pak Afghoi genotype of maize showed sensitivity to various levels of drought stress with a significant decrease in all growth attributes. Maize genotypes MMRI-Yellow, Malika, Pak Afghoi, Agaiti-2002, and FH-1046 were found to be sensitive to varying degrees of drought stress, other genotypes of maize, including Neelam, Sahiwal Gold, YH-5427, and AF-5101, were found to be medium tolerant to drought stress. The Gohar-19 was the most tolerant genotype and maintained its chlorophyll contents and development under drought stress.

**Keywords**:- maize genotypes, drought stress, growth indicators, secondary metabolites

**Introduction**

Maize or *Zea mays* L. is the main cereal crop that is grown most commonly worldwide. Maize production increases yearly but at a slow rate to meet the demands of food industry (Ashraf *et al*., 2016). It is used as a feed and fodder crop both commercially and domestically due to its importance and main function in industrial crops. It also acts as a raw material for the production of ethanol. The primary component of a plant body is water, which also serves as the primary cooling agent in plants and is essential to both plant evolution and expansion (Banziger *et al*., 2000). The productivity and yield of maize is reduced by a variety of biotic and abiotic stress factors, including salt, drought, nutritional deficits, pest and insect problems, diseases, and extremes in temperature (Leach *et al*., 2011). The most common of them is drought stress, which lowers the production of maize via a variety of mechanisms by influencing the plant's whole life cycle. Therefore, the productivity and production of maize is more severely affected by drought (Quarrie, 1996). One of the most obvious symptoms of drought stress is the decrease in the relative water contents. a fall in relative water contents causes a reduction in leaf water potential, which in turn causes stomata to become closer together. (Farooq *et al*., 2009). Elevated stomatal resistance leads to a decrease in transpiration rate, which in turn raises leaf temperature. Transpiration is the primary mechanism regulating leaf temperature, hence this is the primary cause of the increase in temperature (Arbona *et al*., 2003). Plants have evolved a variety of water-retention methods to lessen the negative effects of drought stress such as reduction in transpiration rates and enhancing their capacity to extract water from the soil (Vegh, 2013). Additionally, plants use a variety of mechanisms or adaptation processes, including chemical synthesis, accumulation, root system modification, stomatal modulation, and osmotic adjustment (Chaves *et al*., 2003). Development of maize genotypes that are tolerant to drought stress is the greatest strategy to reduce the risk of harvest loss in maize crop under drought stress. A 15% increase in maize yield has been observed since drought-tolerant genotypes have been developed. Furthermore, compared to genotypes that are drought-sensitive, there is a 30% reduction in the risk of harvest failure (Simtowe, 2019). The development of drought-tolerant maize genotypes permit the development of kernels under drought stress by efficient use of water (Blum *et al*., 2009). There are notable hinderances facing the genetics and breeding study, related to this subject matter (Araus *et al*., 2012). In ecosystems facing drought stress, the inheritance of yield and other agronomic qualities is lowered; as a result, the features might not exhibited in subsequent generations. (Lopes *et al*., 2011). One recommended strategy is the ongoing selection of genotypes based on several traits associated with drought resistance (Banziger *et al*., 2006). Maize crop needs heat and water in the right amount and in a balanced manner to produce a higher yield (Chai et al., 2022). Water greatly affects maize yield, or rather, we may argue that water is the fundamental factor influencing maize production. Keeping in view the above facts the present study is designed to explore the drought tolerance potential of maize genotypes based on growth and biochemical indicators (Liu *et al*., 2022).

**Materials and Methods**

The experiment was conducted in the experimental area of Government College University Faisalabad. Seeds of the maize genotypes (Gohar-19, Sahiwal Gold, Malka-2016, Neelam, MMRI-Yellow, Pak Afghoi, YH-5427, Agaiti-2002, FH-1046, and AF-5101) were acquired from the Maize & Millet Research Institute (MMRI), Yusafwala, Sahiwal. Pots filled with 8 kg soil were used to sow the seeds, the design used was completely randomized design (CRD) with three replications. Seven-day-old plants were then subjected to three different intensities of drought stress: 100% control, 60%, and 50% field capacity.

**Growth Attributes**:- After twenty days of germination, one plant was taken out of each pot to measure the growth parameters, including shoot and root length, fresh biomass of the shoots, and roots. After allowing the plants to air dry, the dry weights of each shoot and root was measured in grams and plants were then placed in an oven by adjusting at 65ºC for 72 hours.

**Biochemical Attribute**s:- **Chlorophyll contents** ("a", "b", and carotenoids) were measured using Arnon's method (1949). Leaves were taken off in order to measure the amount of chlorophyll. Fresh leaves from each treatment were weighed (approximately 0.1 g), removed, and their chlorophyll content was measured by crushing the leaves in 80% acetone at 0.4 °C. The solution was then left overnight and centrifuged at 10,000 rpm for five mints in order to extract the supernatant. By a spectrophotometer (Hitachi-U2001, Tokyo, Japan), supernatant absorbance was measured at three distinct wavelengths: 645, 663, and 480 nm. The formulas were employed to analyze the amounts of chlorophyll "a" and "b" as follows:

Chl. a= [12.7 x OD 663 – 2.69 x OD 645] × V (ml)/1000× W (g)

Chl. b= [22.9 x OD 645 – 4.68 x OD 663] × V (ml)/1000× W (g)

Kirk & Allen (1965) method was used for the determination of carotenoids.

**Carotenoids** (mg ml-1) = A.car/Em100% (Emission =2500) \* 100

**Total Phenolic Contents**:- The total amount of phenolic contents in the leaf was calculated using the Julkenen-Titto (1985) method. The fresh leaf material (0.5 g) was grounded and homogenized in an 80% concentration of acetone solution for each replicate. After that, the material was centrifuged at about 10,000 x g for 10 minutes to extract the supernatant. About, 2 ml of plant extract and 1 ml of folin reagent were added to the mixture in the test tube. Added 20% of 5 ml sodium carbonate, to raise the total volume to roughly 10 ml. The solution was carefully homogenized. Finally, using a spectrophotometer, absorbance at 570 nm was measured.

**Total Soluble Sugars**:- An 80% methanol solution was used for grinding a plant sample in order to estimate the total amount of soluble sugars. Added 3 ml of Anthrone reagent to 0.1 ml of plant extract material. Anthrone reagent was prepared by adding about 0.1g of anthrone to 70% H2SO4. The solution was then submerged in water for ten minutes. Samples were taken out of the water bath. Mixture was left to stand for half an hour at room temperature. Readings were recorded at 625 nm.

**Flavonoid Contents**:- Karadeniz et al. (2005) devised the technique to determine flavonoid contents. About 1g of fresh leaves were weighed and grinded in 20 ml of 80% methanol. To obtain clear supernatant, the grounded material was then filtered. Added 0.5 ml of the filtered material, 3 ml of distilled water, and 0.3 ml of 5% NaNO2. The solution was left at 25 ºC for about five minutes. Added 0.6 ml of AlCl3 to the solution and 2 ml of 1M NaOH. The solution was diluted by adding distilled water, with a final volume of 10 ml. Measurements were recorded at 510 nm. Using the standard calibration curve derived from rutin, the amount of flavonoids was calculated.

**Malondialdehyde contents**:- Malondialdehyde content were measured using the Cakmak & Horst (1991) method. Initially, 3ml of 0.1% (w/v) TCA solution were used to grind the 1.0-gram leaf samples. Next, for 15 minutes at 20,000 rpm, the homogenized plant sample was centrifuged to extract the supernatant. A test tube was filled with approximately 0.5 ml of the supernatant and 3 ml of 0.5% TBA, yielding 20% trichloroacetic acid. The mixture in the test tubes was then brought to a boil in a water bath for 50 minutes. The water bath was kept at a temperature of 95°C. Later that, the test tubes were taken out of the water bath and allowed to cool for a bit. Finally, the MDA concentration was measured at 532 and 600 nm wavelength.

**H2O2 contents:-** The Velikova et al. (2000) method was applied to determine H2O2 contents. About 6% TCA was used to grind the leaf material. Added 0.5 milliliters of the plant extract, mix it with 0.5 milliliters of potassium phosphate buffer (pH of 7.0), and 1 milliliter (1M) of potassium iodide. The measurements were recorded at 390 nm.

**Total soluble protein**:- Bradford's (1976) technique was applied to calculate the total soluble protein. It was measured at 595 nm wavelength.

**Anthocyanin contents** were measured by Mirecki & Teramura (1984). About 250 µl of acidic methanol was added, along with leaf sample. Using a mortar and pestle, the plant sample was grinded in ice and incubated at 4˚C. The extract was centrifuged at 14,000 rpm for five minutes in order to get a clear supernatant. Finally, the measurement was recorded at 530 nm and 657 nm wavelength.

**Total Free Amino Acids:-** The amount of total free amino acid was calculated by Hamilton & Van Slyke (1943) method. For this estimation, approximately 1 milliliter of plant extract was taken, and 1 milliliter of pyridine (1%) and 1 milliliter of nin-hydrin (2%) were added to the solution. The test tubes were then submerged in a water bath and allowed to boil for 30 minutes at 95°C. Finally, using a spectrophotometer, measurements were recorded at 570 nm.

**Results**

The root and shoot lengths were decreased with the increase in the degree of drought stress (fig.1). A discernible decline was noted in each genotype of maize, with the greatest decline in Malika, MMRI-Yellow Pak Afghoi in all growth parameters. In contrast, genotypes YH-5427, Neelam, and AF-5101 exhibited resistance against drought stress, whereas MMRI-Yellow, Agaiti-2002, and FH-1046 showed a considerable decline in all growth parameters. Analysis revealed that under varying (100% control, 60% and 50% FC) drought stress conditions, all genotypes of maize (Gohar-19, Sahiwal Gold, Malika-2016, Neelam, MMRI-Yellow, Pak Afghoi, YH-5427, Agaiti-2002, FH-1046, and AF-5101) showed a reduction in fresh and dry biomass of both shoots and roots (fig.2). As compared to other maize genotypes, the Pak Afghoi genotype had a notable fall in shoot fresh and dry weight, although the Gohar-19 genotype exhibited the least amount of a decline. The Sahiwal Gold, Neelam, YH-5427, and AF-5101 maize genotypes showed the least amount of decline, whilst the Pak Afghoi genotype showed the most drop. The data shown in Figure 3 indicated that, all maize genotypes cultivated under normal conditions showed a considerable rise in the concentration of Chl.a, Chl.b, and total chlorophyll contents. Significant decrease was seen in the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll was observed with an increase in drought stress; however, the Pak Afghoi maize genotype showed particularly a considerable decrease. Under normal conditions, the concentration of leaf carotenoids was high in Gohar-19, which showed the largest rise in leaf carotenoids. All maize genotypes had a notable decline in leaf carotenoids with the increase in drought stress, however Pak Afghoi showed a particularly notable decline in leaf carotenoids levels when compared to other maize genotypes. All kinds of maize genotypes had low total anthocyanin contents when grown under controlled conditions, however all genotypes showed a notable rise in total anthocyanin contents with the increase in drought stress. Additionally, a notable rise was noted in the Sahiwal Gold genotype of maize, whereas the FH-1046 genotype showed the lowest level under 60 % and 50 % FC. Under drought stress, the concentration of total free amino acid increased noticeably. Total free amino acid content of all maize genotypes were increased; however, the Agaiti-2002 genotype exhibited a greater increase in total free amino acid contents (Fig. 4). Under controlled conditions, quantity of total phenolic contents was remarkably high in all kinds of maize genotypes (Fig. 4). Conversely, as the level of drought stress increased, a gradual rise in phenolic compounds was noted in all kinds of maize. Comparing, the Gohar-19 genotype exhibited a greater amount of total phenolic compounds. Other genotypes that exhibited increased level of total phenolic compound are Neelum and FH-1046. However, with the increase in drought stress, all genotypes showed noticeable increase in total flavonoid contents, with the Malika showing higher levels than the others. All maize genotypes had increased total soluble protein contents under controlled conditions, with the Gohar-19 genotype exhibited the highest total soluble protein content. The concentration of total soluble protein was decreased with the increase in the intensity of drought stress. MMRI-Yellow had the greatest fall in total soluble protein content, whereas Gohar-19 demonstrated the lowest reduction. Under normal circumstances, all maize genotypes had significant amounts of total soluble sugar contents; however, as the degree of drought stress is elevated, the concentration of total soluble sugars in all maize genotypes increased gradually. Compared to other maize genotypes, Agaiti-2002 exhibited the highest increase in total soluble sugar content, while MMRI-Yellow maize genotype showed the lowest increase. Under controlled conditions, the concentration of H2O2 was variable in all genotypes of maize. However, when the degree of drought stress was increased, the Malika genotype of maize exhibited the highest concentration of H2O2, whereas the AF-5101 showed the least value. Under controlled circumstances, the MDA content of the maize genotypes did not vary significantly. Nearly all maize genotypes had elevated MDA levels with the increase in drought stress level, YH-5427 and Pak Afghoi having particularly high MDA contents. However, a minimal increase was noted in the maize genotype AF-5101 (fig. 5).



**Fig.1.** Effect of drought stress onroot length and shoot length of ten genotypes of maize (*Zea mays* L.).



**Fig.2.** Effect of drought stress onshoot fresh weight, shoot dry weight, root fresh weight and root dry weight of ten genotypes of maize (*Zea mays* L.).



**Fig.3.** Effect of drought stress onChl a, Chl b, total Chl. and leaf carotenoid contents of ten genotypes of maize (*Zea mays* L.).



**Fig.4.** Effect of drought stress onanthocyanin contents, total free amino acid, total phenolic contents and total flavonoids contents of ten genotypes of maize (*Zea mays* L.).



**Fig.5.** Effect of drought stress ontotal soluble proteins, total soluble sugar, hydrogen peroxide and malondialdehyde contents of ten genotypes of maize (*Zea mays* L.).

Table 1 Mean square values exhibiting growth and biochemical attributes of maize genotypes under drought stress.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **SOV** | **df** | **SFW** | **SDW** | **RFW** | **RDW** | **SL** | **RL** |
| Genotypes (G) | 9 | 1.627\*\*\* | 0.004\*\*\* | 0.335\*\*\* | 4.993ns | 396.856\*\*\* | 98.597\*\*\* |
| Stress (S) | 2 | 3.915\*\*\* | 0.022\*\*\* | 0.684\*\*\* | 0.003\* | 1527.816\*\*\* | 580.02\*\*\* |
| G × S | 18 | 0.274\*\*\* | 0.0012\*\*\* | 0.052\*\*\* | 2.312ns | 63.1931\*\*\* | 21.170\*\*\* |
| Error | 60 | 0.0078 | 4.277 | 0.005 | 8.022 | 2.733 | 1.45988 |
| **SOV** | **df** | **Chl. a** | **Chl. b** | **T. Chl** | **Car.** | **Anthocyanin** | **TFA** |
| Genotypes (G) | 9 | 0.255\*\*\* | 0.038\*\*\* | 0.2909\*\*\* | 0.001\*\*\* | 0.318\*\*\* | 1.2409\*\*\* |
| Stress (S) | 2 | 3.985\*\*\* | 0.3805\*\*\* | 6.827\*\*\* | 0.008\*\*\* | 4.632\*\*\* | 14.303\*\*\* |
| G × S | 18 | 0.093\*\*\* | 0.022\*8\* | 0.115\*\*\* | 0.001\*\*\* | 0.192\*\*\* | 0.708\*\*\* |
| Error | 60 | 0.028 | 0.006 | 0.029 | 1.038 | 0.042 | 0.113 |
| **SOV** | **df** | **TPC** | **TFC** | **TSP** | **TSS** | **H2O2** | **MDA** |
| Genotypes (G) | 9 | 64.395\*\*\* | 2.039\*\*\* | 0.308ns | 5.472\*\*\* | 11.810\*\*\* | 4.1103\*\*\* |
| Stress (S) | 2 | 266.359\*\*\* | 8.003\*\*\* | 3.0701\*\*\* | 38.271\*\*\* | 61.038\*\*\* | 14.032\*\*\* |
| G × S | 18 | 32.265\*\*\* | 0.3207\*\*\* | 0.179ns | 2.184\*\*\* | 3.316\*\*\* | 0.663\* |
| Error | 60 | 3.295 | 0.031 | 0.182 | 0.477 | 0.311 | 0.361 |

**Discussion**

The findings of this study exhibited that water stress negatively impacted growth, productivity, physiological and biochemical attributes in all genotypes of maize. The growth characteristics were considerably reduced under conditions of water shortage. Restrictions in root structure, which limit the passage of water and nutrients for regular metabolic processes, may be the source of the decrease in maize growth brought on by water stress (Husain *et al*., 2019). The three main processes that drive plant growth are differentiation, expansion, and cell division. Under water shortage, poor growth of plants was observed due to decrease in the process of mitosis and cell elongation (Hussain *et al*., 2008). Lack of water restricts the growth of plant cell primarily owing to reduced turgidity (Taiz & Zeiger, 2006). Water restricting situations slow down cell growth mostly since there is not enough water flowing from the xylem to the adjacent cells. Shortage of water results in fewer and smaller leaves. Normally, the turgor pressure and assimilate supply determine how much a leaf expands. Abridged turgidity of cell and a slower photosynthetic rate under stress conditions are the main factors limiting leaf growth. Certain morphological traits, like shoot and root length, shoot and root fresh and dry weight are decreased under drought stress. Similar outcomes were reported by Zhao *et al*. (2006). Under drought stress, all kinds of maize genotypes showed a considerable drop in their absorptions of chlorophyll a and b. Chlorophyll a and particularly chlorophyll b were significantly impacted by drought stress. On the other hand, Sahiwal Gold, Gohar-19, Malika, Neelum, and MMRI-Yellow maize genotypes showed a notable decrease in chlorophyll b. According to reports, plants under drought stress maintained larger concentrations of chlorophyll a than chlorophyll b (Jain *et al*., 2010). Thylakoid membranes and photosynthetic pigments are damaged by drought (Anjum *et al*., 2011). There have also been reports of decreased chlorophyll contents under drought stress (Din *et al*., 2011). The most recent data showed a greater decline in chlorophyll-b, which suggests that some genotypes are more susceptible to drought stress. It has been reported that the photosynthetic pigments are reliable markers to determine the conflicting effects of drought stress. Similar to this, drought stress has a detrimental impact on plants' overall chlorophyll contents and gas exchange characteristics. In our research drought stress decreased the amount of total leaf carotenoids in all maize genotypes. Carotenoids play a variety of roles in drought tolerance, such as protection against oxidative damage is brought on by tolerance to drought stress and harvesting light. Consequently, the plant's metabolic system may suffer total destruction if the carotenoid level is decreased. Our findings are consistent with those of Havaux (1998) and Kiani *et al*. (2008), who found that drought stress decreased total leaf carotenoids and chlorophyll. Our findings correlate with that of Koutoua *et al*. (2016), who found that drought reduced tomato plant height, carotenoids, and specific weight. Under limited water conditions, the anthocyanin contents of all genotypes of maize showed a considerable increase. In response to abiotic stresses such as drought, excessive salinity, light, and cold, plants produce anthocyanin, which are frequently associated with increased stress tolerance. Zhao *et al.* (2022) and Cao *et al*. (2022) found comparable results, indicating that anthocyanin concentration rises in response to drought stress. Under drought stress, maize genotypes exhibited an increase in total free amino acid content as reported by Ma *et al*., (2016). Additionally, our results are comparable with Obata *et al*. (2015) and Chmielewska *et al*. (2016). Plants containing phenolic compounds have a number of secondary metabolites that are involved in preventing oxidative damages (Krol *et al*., 2014) caused by stress and have antioxidant qualities. The total phenolic contents of plants can decrease or increase in response to stress conditions (Al Hassan *et al*., 2015 & Gharibi *et al*., 2015). The total phenolic compound in all genotypes of maize increased significantly in our study, which was in accordance with findings from (Rivas-Ubach *et al*., 2012 & Fraire-Velazquez & Balderas-Hernandez, 2013) and Weidner *et al*. (2009), who reported increased total phenolic compound in grapevine roots under drought stress. According to our research, the total flavonoid content of all maize cultivars showed a considerable increase as the degree of drought stress increased. The outcomes of our research show similarity with Gao *et al*. (2020), who found that drought prompted the accretion of secondary metabolites, for instance flavonoids, in two different Adonis species. Talbi *et al*. (2020) reported that drought stress markedly increased the antioxidant capacity and flavonoid accumulation in the Saharan plant *Oudeneya africana*, improving plant adaptation to abiotic stress. A change in total soluble protein is observed in all genotypes of maize under drought. Riccardi *et al*. (1998) reported that total soluble proteins of two genotypes of *Zea mays* in the leaves and roots increased initially, then decreased under drought stress. All genotypes of maize showed an increase in total soluble sugar content when subjected to drought stress. These findings show resemblance to Sperdouli *et al*. (2012). It is also well known that sugars accumulate in response to drought stress (Zanloo *et al*., 2008, Watanabe, 2000). It is commonly known that soluble sugars play a complicated and vital function in plant metabolism as byproducts of hydrolytic activities, substrates in biosynthetic processes, producers of energy, as well as in systems that sense and interact with sugar. According to recent claims (Kishor *et al*., 2005), even sugar flow may serve as a signal for metabolic regulation when conditions are stressed due to drought. Additionally, soluble sugars can act as an ordinary osmo-protectant, keeping turgor pressure constant and stabilizing cellular membranes. As a byproduct of lipid peroxidation, MDA is frequently employed for assessing oxidative stress in situations of water stress (Farooq *et al*., 2010). Drought is the major, of many abiotic stressors that causes creation of ROS, which damage the membrane in diverse ways. One such ROS is H2O2 a recent study found a correlation between increased H2O2 content and higher MDA concentration in plants under drought stress. All kinds of maize genotypes showed a notable rise in MDA and H2O2 levels in response to drought stress.

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