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# ANTIOXIDANTS ENHANCED DROUGHT TOLERANCE AND PRODUCTIVITY OF MAIZE UNDER SEMIARID ENVIRONMENTS

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Drought stress during the crop growing season is one of the major threats to higher productivity of maize. The present study was conducted to evaluate the role of beneficial fungus and antioxidants for improving drought tolerance of maize under field conditions. Treatments comprised of three different factors including (1) two levels of available moisture contents (70% and 30% available moisture contents; AMC), (2) seed treatment (untreated seeds and 20 µl $g^{-1}$ fungus treated seeds) and (3) foliar application of antioxidants (glutathione, ascorbic acid) and fungal suspension. Drought stress decreased the morphological, growth and yield related attributes of maize compared with well-watered treatments. Averaged across the years, the reduction in root and shoot length was 30.5% and 20% respectively and the yield was reduced up to 48% under stress. Rate of lipid peroxidation as well as the production of antioxidants was generally increased under drought, however, seed treatment and foliar spray was effective in enhancing the maize growth and yield under stress condition by suppressing the production of reactive oxygen species. Averaged across the years, maize plants emerged from fungus treated seeds recorded significantly lower activities of SOD (79%), POD (41%) and CAT (84%) and enhanced the yield of maize by 48%. All the foliar spray treatments (fungal suspension, glutathione and ascorbic acid) triggered or at least helped in maintaining the antioxidant defense system of maize plants. Averaged across the years; increase in yield of maize due to foliar spray of fungal suspension, glutathione and ascorbic acid was 69%, 53% and 31%, respectively under drought. In crux, seed treatment with fungus, and foliar spray of glutathione were effective in enhancing the maize drought tolerance and such effects were attributed to alleviate the oxidative damage and maintenance of photosynthetic pigments.

**Keywords:** Drought, seed treatment, foliar spray, antioxidants, trichoderma, oxidative damage, maize yield.

## INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop which fulfills man's dietary needs providing predominant caloric requirement. In Pakistan after wheat and rice, it is the third most important cereal crop (Frova *et al.*, 1999). In many countries, maize is usually grown in areas receiving 300 to 500 mm of rainfall, which is near or below the critical level for achieving optimum yield (Monfreda *et al.*, 2008). Erratic rainfall patterns under changing climatic scenario have led to more frequent events of moisture stress in recent past. Spring maize exhibit late maturity due to prolonged cold period at the early crop growing season between the seedling and knee-high stage. Resultantly, a prolonged growing period requires a more water input. Hence, reduction in grain production is associated with the deficiency of water used for irrigation. (Pandit *et al.*, 2017). Drought stress causes 30 to 90 % reduction in yield depending upon the stage of crop (Pandit *et al.*, 2018). Water deficit causes the reduction in rate of crop growth and accumulation of biomass. Water scarcity adversely influences the division and expansion of cells, leaf size, stem growth, root proliferation, stomatal conductance, and nutrient and water relations of plant which ultimately decreases the water use efficiency and reduces the crop yield (Farooq *et al.*, 2009). In maize, the most critical stage for water requirement is pollination, which determines the final productivity of this crop. On the other hand, pre-tasseling and physiological stages are most sensitive stages to drought. Rate of growth is reduced predominantly if drought occurs during 1$^{st}$ to 5$^{th}$ leaf stage of vegetative growth (Pannar, 2012). At most susceptible stages namely vegetative, silking and ear stages, the loss in yield is as high as 25%, 50% and 21%, respectively (Denmead and Shaw, 1960).

Drought stress leads to the higher production of reactive oxygen species (ROS) in plants which can harm the cellular components and cell membranes (Mittler, 2002). Malondialdehyde (MDA) is a product of decomposition of polyunsaturated fatty acids accumulated in biological membranes during drought (Guo *et al*., 2012). Nevertheless, the ROS are scavenged by different enzymatic (e.g. glutathione reductase (GR), peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX)) and non-enzymatic (e.g. ascorbic acid (AsA), reduce glutathione) antioxidants (Hasegawa *et al*., 2000; Gong *et al*., 2005). Enzymes such as SOD, POD, and CAT are the main components of antioxidative defense system in plants during drought (Gogorcena *et al*., 1995). Enhanced levels of

antioxidants in the plants may assure better plant defense against drought induced oxidative stress. The AsA, an antioxidant, is a small, water soluble molecule that plays a significant role in detoxification and neutralization of singlet oxygen and superoxide radicals (Noctor *et al*., 1998). Ascorbate also have a significant role in many developmental processes, division and expansion of cells (Pignocchi *et al*., 2003), modulation of enzymes, gene regulation and redox regulation of antioxidant compounds (Horemans *et al*., 2000). Exogenously applied AsA can enhance plant's stress tolerance and alleviate the oxidative stress (Shalata and Neumann, 2001). Glutathione (GSH) is also a significant enzymatic antioxidant which has an important role in stress tolerance as it maintains the reduced glutathione pool during stress (Pastori *et al.*, 2000). Reduced GSH as an antioxidant in plants defense system plays direct role in reducing most of the ROS (Jiang *et al*., 2012).

The *Trichoderma* is a genus of plant symbiotic, opportunistic, avirulent fungi that has the ability to make colonies in the roots and release compounds that can stimulate the growth and improve the defense system of plants under stress (Harman *et al.*, 2004; Chinnusamy *et al.*, 2004). These species are beneficial endophytic plant symbionts that play a significant role in increasing plant growth and yield, improving uptake of nutrients and water, and enhancing the tolerance against different biotic and abiotic stresses including drought (Bowler *et al.*, 2000; Hermosa *et al.*, 2012). These species can be used as foliar spray or seed treatment. Once the association is developed between fungus and host plant, systemic effects are induced in whole plant. Although *Trichoderma* colonize the plant roots only, their effects occur in above ground parts of plants as well. As a consequence of *Trichoderma*-plant colonization, changes at proteome and transcriptome levels occur in plants due to the fungal released metabolites (Marra *et al*., 2006). Mastouri and Harman (2009) reported that *Trichoderma*-plant interaction mainly occurs in rhizosphere, therefore this mechanism probably resulted in the increased uptake of water due to enhanced root capacity for the absorption of water. However, *Trichoderma* fungal species has been proved to increase drought tolerance in plants as early as germination phase (Mastouri *et al*., 2010).

Though the role of antioxidants and beneficial fungi in abiotic stress (drought) tolerance in crops is well known, the potential of glutathione, ascorbic acid and *Trichoderma harizianum* L. in combination has rarely been investigated for improving drought resistance in maize. Therefore, studies were conducted with the objectives to evaluate the function of beneficial fungus (*T. harizianum*) and antioxidants as a potential tool and an alternative approach to costly and lengthy techniques of breeding and genetics to improve the drought tolerance of maize. Study was also done to explore the possible synergistic role of *Trichoderma* and antioxidants for drought tolerance. Moreover, the possible mechanisms involved in *Trichoderma*-antioxidant mediated drought tolerance in maize were also explored.

## MATERIALS AND METHODS

***Site description*:** Experiment was conducted at Agronomic Research Area, University of Agriculture, Faisalabad to explore the potential of antioxidants and beneficial fungus for ameliorating the effects of drought in spring maize. The Faisalabad features a semi-arid climate with very hot and humid summers and dry cool winters. The average maximum and minimum temperature in June are 45 °C and 26.9 °C and in January are 19.4 °C and 4.1 °C, respectively. The soil was sandy clay loam with EC ranges between 1.07 to 1.32 dS $m^{-1}$, pH 7.7 to 7.8 and saturation percentage 33 to 36 %.

***Experimental material and soil*:** Seed of maize hybrid (YH-1898) was obtained from Maize and Millet Research Institute, Yousaf Wala, Sahiwal (Punjab-Pakistan). Physical, chemical and hydraulic analyses of the experimental field soil were conducted before the sowing of crop (Table 1).

**Table 1. Physico-chemical properties of soil sampled from the student research area, University of Agriculture, Faisalabad.**

| Sampling | Year 2015 | | Year 2016 | |
|---|---|---|---|---|
| | Depth (cm) | | | |
| | 0-15 | 15-30 | 0-15 | 15-30 |
| Soil textural class | Sandy clay loam | | | |
| EC (dS $m^{-1}$) | 1.32 | 1.15 | 1.12 | 1.07 |
| Ph | 7.70 | 7.80 | 7.80 | 7.70 |
| Saturation (%) | 36.00 | 33.00 | 36.00 | 33.00 |
| Nitrogen (%) | 0.03 | 0.03 | 0.03 | 0.02 |
| Phosphorous (mg $kg^{-1}$) | 22.00 | 19.00 | 24.00 | 21.00 |
| Potassium (mg $kg^{-1}$) | 213.00 | 184.00 | 235.00 | 158.00 |
| Boron (mg $kg^{1}$) | 0.76 | 0.43 | 0.62 | 0.42 |
| Zinc (mg $kg^{1}$) | 1.58 | 1.43 | 1.32 | 1.11 |
| Ferrous (mg $kg^{1}$) | 4.17 | 3.71 | 4.64 | 3.52 |
| Organic matter (%) | 0.52 | 0.47 | 0.56 | 0.45 |
| Bulk density (mg $m^{-3}$) | 1.44 | 1.42 | 1.41 | 1.46 |

Ten representative samples were collected from the soil from 0-15 cm and 15-30 cm depths using soil auger. Composite sample was obtained by mixing of all these initial samples and this composite sample was used for further soil analyses. To determine the percentage of silt and clay, Bouyoucos Hydrometer method was used. Sodium hexametaphosphate was used as dispersing agent. International texture triangle was used to determine the texture class of soil (Moodie and Smith, 1959). Phosphorous availability in the soil was determined by spectrometer according to the Olsen method (Homer and Pratt, 1961). Available potassium in the soil was measured by flame photometer (Mehlich, 1953) and total nitrogen contents were determined by Kjeldhal method (Bremner, 1960). The electrical conductivity (EC) of the saturated soil extract was measured by Field Scout EC 110 Meter following the

protocol of Mehlich (1953). Saturation percentage was found by taking the soil sample from field (Johnson, 1962). Field capacity of the soil was calculated by following the protocol described by Karkanis (1983).

***Weather data*:** Weather data for the both growing seasons (spring 2015 and 2016) were obtained from Weather Station installed at Water Management and Research Center of University of Agriculture, Faisalabad. In 2016, there was less rainfall (25 mm) as compared to 2015. Maximum and minimum temperature was higher by about 1.74°C and 0.52°C, respectively and the year 2016 was slightly warmer than 2015. However, the mean duration of sun shine hours was similar during the both growing seasons. Inter annual variability in distribution and amount of rain was also noticed. During 2015 and 2016 the total rainfall of 117.1 and 91.8 mm, respectively was recorded (Fig. 1).

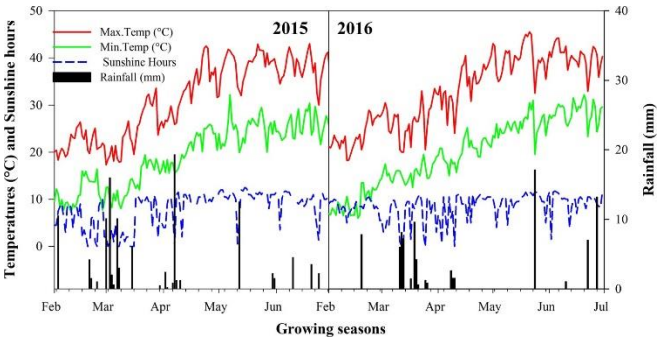


**Figure 1. Daily weather data at experimental site during the growing season in 2015 and 2016**

***Experimental treatments and design*:** Experiments were conducted during two growing seasons i.e., Spring 2015 and 2016. The treatments comprised of three different factors. The main plot factor was drought stress, which consisted of two levels (control (70% available moisture contents (AMC)) and drought stress (30% AMC)). The sub-plot factor was seed treatment which also had two levels, viz. control (untreated) and fungus treated seeds (20 µl $g^{-1}$ of seeds). The third factor was foliar application of antioxidants (50 µM) (glutathione, ascorbic acid and suspension of beneficial fungus *Trichoderma harizianum* L. that was assigned to sub-sub-plots. Foliar application of fungal suspension and antioxidants was done at vegetative stage (30 to 35 days after sowing). The research experiments in both the years were designed in randomized complete block design with split-split plot arrangements having three replicates.

***Crop husbandry*:** A vacated field from previously grown autumn maize was irrigated about 10 days before planting of maize. A fine tilth seedbed was prepared by the cultivation of soil thrice with tractor mounted cultivator. Cultivation was followed by planking. Total 108 plots of 12 $m^2$ (4 m × 3 m; L × W) were prepared. Plots with different moisture levels as per treatment were separated from each other through bunds and non-experimental area (1.5 m) to avoid water flow. Seeds of maize hybrid YH-1898 were sown in mid-February in well prepared field using manual dibbler. In each treatment, four rows were sown in 75 cm spaced crop rows.

The total amount of water for irrigation was calculated using cut throat flume. Water was calculated at each stage according to the depth of crop roots in soil. The moisture content was determined before irrigation. Control plots were maintained at 70% available moisture contents (AMC). These plots were irrigated every time after the depletion of 30% available moisture content from the root zone of crop and the drought plots were maintained at 30% AMC. In case of drought, the plots were irrigated after the depletion of 70% AMC from the root zone of crop. The 96 × 36 inches cutthroat flume was installed in the center of the water channel. Readings for the flow of water were measured from the stilling wells at the inlet and outlet sections of the flume. Calculation for the discharge of water flow was done by using the calibration table. Following formula was used to calculate the total time of irrigation (Skogerboe *et al.*, 1967).

$$QT = AD$$

where "t" is the total time in seconds required for irrigation of a given area, "A" is the area for irrigation in $m^2$ and "d" is the depth of applied water in mm. "Q" is discharge from the cut throat flume in $m^3$ $s^{-1}$. The time required for full irrigation was calculated, and flood irrigation method was used to irrigate between the rows. The time of irrigation water for 70%, and 30% was calculated from the time of full irrigation water and reduced accordingly.

Fertilizers as 250:150:75 of N: P: K kg $ha^{-1}$ were added using diammonium phosphate (DAP), urea and sulfate of potash. During seed bed preparation, one-third of nitrogen and whole potassium and phosphorous were applied. Rest of the nitrogen was applied in two equal splits at first (34 days after sowing (DAS)) and second irrigation (DAS), respectively. Standard methods of plant protection were adopted to safeguard the crop against biotic factors like insect pests and diseases. All other agronomic practices except the treatments under study were kept same as per recommendations. Cobs were harvested manually at physiological maturity. Dried cobs were packed in sampling bags. Threshing of kernels was accomplished by using small scale threshing unit.

***Recorded observations*:** Three plants were randomly selected after 45 days of sowing from each experimental unit and root shoot lengths were measured using measuring scale. Root and shoot fresh biomass was measured using an electric balance (TX323L, Shimadzu, Japan). After weighing, plants were dried in oven at 70 °C and dry biomass was also recorded. Biochemical attributes were determined to establish biochemical basis for drought stress tolerance. Fresh leaves of maize plant were grounded in liquid nitrogen using pestle and mortar in 5 milliliter of 50

mM phosphate buffer having pH of 7.8 for the extraction of enzymes. The extracted material was centrifuged for 15 minutes at 4 °C and the supernatant was utilized for further enzymatic assays. Spectrophotometer (UV-4000, ORI, Germany) was used to assay the activity of SOD, POD and CAT (Venisse *et al.*, 2001). To assay the activity of superoxide dismutase (SOD) its inhibition was monitored by practicing the method of Giannopolitis and Ries (1977). Reaction solution was prepared by the addition of 50 µL of extracted enzyme to the reaction mixture containing 1 mL of 50 µM NBT, 1 mL of 1.3 µM riboflavin, 500 µL of 13 mM methionine, 500 µL of 75 mM EDTA, 950 µL of 50 mM phosphate buffer with pH of 7.8. The blue formazone was produced due to the reduction of NBT in light and the increase in absorbance was measured at 560 nm using blue formazone. Reaction solution for peroxidase (POD) was prepared by the addition of 2 mL of 50 mM phosphate buffer with pH 7.0, 500 µLs of 40 mM $H_2O_2$, 400 µLs of 20 mM guaicol (Sigma-G5502) and 100 µLs of extracted enzyme. Change in the absorbance pattern of reaction material at 470 nm was recorded after each 30 seconds for five minutes. Reaction solution for catalase (CAT) contained 2 mL of 50 mM phosphate buffer with pH 7.0, 100 µL of extracted enzymes and 900 µL of 5.9 mM hydrogen peroxide. The catalase activity was expressed in the units (µmol of $H_2O_2$ decomposed per min.) per gram of protein (Dhindsa *et al.*, 1981). To quantify chlorophyll contents, the absorption of the leaves extracted in 80% acetone was recorded at 665 nm (Chl. a) and 649 nm (Chl. b) using the spectrophotometer. Following equation was used to quantify the chlorophyll: Chlorophyll concentration (mg/mL) = 6.63 A665 + 18.08 A649, where A denotes the absorbance at a specific wavelength (Lichtenthaler, 1987). The index used for lipid per oxidation is malondialdehyde (MDA). The MDA contents in maize leaf tissues were determined as per Heath and Parker (1968). Fresh leaf tissues of almost 0.5 g was homogenized in 5 mL of 5 percent w/v TCA and centrifuged at 12000 × for 15 min. The supernatant was mixed with an equal amount of thiobarbutaric acid (TBA). Mixture was boiled for 25 minutes at 100 °C. Reaction mixture was cooled and centrifuged for 5 minutes at 7500 × to purify the solution. Absorbance was taken at 532 and 600 nm. Yield and yield related traits were also recorded following standard procedures. Cob length (CL) was measured by using measuring scale. Number of grains per row was calculated manually. Electric balance was used to calculate thousand grain weight. Biological yield (BY) was calculated by weighing the vegetative as well as reproductive parts of plant. Grain yield (GY) was also calculated using weighing balance.

***Statistical analysis*:** The collected data were analyzed using the Fisher's analysis of variance technique (Steel *et al.*, 1997), and the treatment means were compared by Tukey's honest significant difference (HSD) test at 5% probability level.

## RESULTS

***Morphological parameters*:** Pronounced variation in the morphological parameters of maize was observed under the influence of drought stress, seed treatment and foliar application of beneficial fungus and antioxidants (Table 2).

**Table 2. Influence of tested treatments on the morphological attributes of maize**

| Drought | Seed Treatment | Foliar Spray | 2015 | | | | | 2016 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Root length (cm) | Shoot length (cm) | Root fresh weight (g) | Shoot fresh weight (g) | Dry weight of plant (g) | Root length (cm) | Shoot length (cm) | Root fresh weight (g) | Shoot fresh weight (g) | Dry weight of plant (g) |
| 70% AMC | Untreated | Control | 32.50 a-d | 76.67 b-e | 6.50 def | 16.33 c-f | 9.00 b-f | 27.50 b-e | 71.50 b-f | 4.50 d-g | 12.97 a-d | 5.50 c-g |
| | | Fungal Suspension | 39.10 abc | 96.00 a-d | 10.37 a-e | 21.97 bcd | 11.70 abc | 35.00 abc | 86.17 a-e | 7.37 bcd | 14.97 abc | 8.37 bcd |
| | | Glutathione | 41.93 ab | 105.23 ab | 13.13 abc | 26.77 a | 13.50 ab | 39.83 ab | 97.30 ab | 10.13 ab | 17.10 ab | 9.17 abc |
| | | Ascorbic acid | 35.83 a-d | 91.50 a-d | 9.70 b-e | 19.10 b-e | 10.33 b-e | 30.83 a-d | 81.50 a-e | 6.00 g | 14.10 a-d | 7.00 b-f |
| | Fungus Treated | Control | 35.80 a-d | 75.00 b-e | 8.17 c-f | 22.43 a-d | 10.17 b-e | 30.13 a-e | 77.17 a-e | 6.17 c-f | 13.43 a-d | 7.17 b-f |
| | | Fungal suspension | 41.77 ab | 99.00 abc | 12.33 abc | 26.77 ab | 12.33 ab | 38.13 ab | 89.10 a-d | 9.33 abc | 15.77 abc | 9.33 ab |
| | | Glutathione | 44.33 a | 113.83 a | 15.33 a | 31.07 a | 15.50 a | 42.67 a | 108.97 a | 12.00 a | 19.07 a | 12.33 a |
| | | Ascorbic acid | 39.00 abc | 93.33 a-d | 11.17 a-d | 24.77 abc | 11.17 a-d | 36.00 abc | 83.33 a-e | 7.17 bcd | 14.77 abc | 8.17 bcd |
| 30% AMC | Untreated | Control | 15.33 e | 47.67 e | 3.90 f | 9.87 f | 4.10 f | 11.33 f | 41.50 f | 1.73 g | 6.87 d | 2.43 g |
| | | Fungal suspension | 23.17 cde | 68.90 cde | 5.73 ef | 12.00 ef | 5.33 ef | 18.90 def | 59.90 c-f | 2.67 d-g fg | 9.00 cd | 3.67 fg |
| | | Glutathione | 27.67 b-e | 77.00 b-e | 7.93 c-f | 14.83 def | 6.50 def | 21.67 c-f | 78.00 a-e | 3.83 d-g | 10.17 bcd | 4.83 d-g |
| | | Ascorbic acid | 21.33 de | 62.00 de | 4.20 f | 10.53 ef | 4.53 f | 15.33 ef | 53.17 ef | 2.13 g | 8.53 cd | 3.13 g |
| | Fungus Treated | Control | 23.77 cde | 66.67 cde | 8.17 c-f | 13.90 def | 7.17 c-f | 18.77 def | 56.73 def | 3.37 efg | 11.57 a-d | 4.17 efg |
| | | Fungal suspension | 35.50 a-d | 90.53 a-d | 12.30 abc | 18.03 b-f | 9.13 b-f | 29.17 a-e | 80.53 a-e | 4.97 d-g | 14.03 a-d | 6.13 b-g |
| | | Glutathione | 38.67 abc | 99.33 abc | 13.90 ab | 22.47 a-d | 12.73 ab | 33.00 a-d | 91.33 a | 6.57 b-e | 17.23 ab | 7.73 b-e |
| | | Ascorbic acid | 31.50 a-d | 84.67 a-d | 10.73 a-e | 16.37 c-f | 8.57 b-f | 25.17 b-f | 74.67 a-f | 4.40 d-g | 13.37 a-d | 5.57 c-g |

*AMC = available moisture contents, Alphabetical letters (a, b, c, d …) above means show the differences ($P$ _ 0.05) among treatments

The root and shoot length of maize was significantly reduced with decrease in available moisture content (AMC). Compared with control, root and shoot length of maize was decreased by 28% and 21% in 2015 and 33% and 19% in 2016 at 30% AMC, respectively (Table 2). Seed treatment with fungus significantly enhanced the root and shoot length of maize by 72% and 18%, respectively compared with untreated control (Table 2). The positive effects of fungal seed treatment were more prominent under drought stress. Results showed that root shoot length was significantly increased with foliar application of antioxidants and fungus. Averaged across the years, the maximum increase in root and shoot length was observed with foliar spray of glutathione (52% and 49%) followed by fungus (37% and 30%), respectively under drought stress. The interactive effects of drought and fungus treatments on maize root shoot length were also significant during both study years. Interactions of all three factors (drought and foliar spray, fungus and foliar spray, fungus, foliar spray and drought) were non-significant for root shoot length of maize during both study years (Table 2).

Root and shoot fresh weight, and dry weight of maize plant were also significantly reduced with decline in AMC. In 2015, the decrease of 22%, 38% and 38% was observed under drought condition; while, in 2016 the decrease was 45%, 23% and 44% in all three factors (root fresh weight, shoot fresh weight and dry weight of plant) as compared with control (Table 2). Averaged across the years, seed treatment with *T. harizianum* enhanced the RFW, SFW & DW of plant by 29%, 14% and 30%, respectively in drought condition compared with untreated control (Table 2). Results showed that foliar application of antioxidants and fungal suspension significantly improved maize growth attributes under drought stress. Averaged across the years, the maximum increments in RFW, SFW and DW of plant were observed under foliar application of glutathione as compared to foliar spray of fungal suspension and ascorbic acid at both levels of moisture contents however, interactive effects of all three factors remain non-significant (Table 2).

***Biochemical attributes*:** Chlorophyll contents and activities of antioxidants in maize showed significant variations under the influence of drought stress, seed treatment, and foliar application of beneficial fungus and antioxidants. Decrease in chlorophyll contents was noticed for maize plants subjected to drought (30% AMC). Drought stress significantly decreased the chlorophyll a content of leaves by 21% and 22% in 2015 and 2016, while chlorophyll b contents were decreased by 24% and 29%, respectively, compared with control (70% AMC; Table 3). Averaged across the years, chlorophyll a content was significantly improved by seed treatment with fungus by 13% compared with untreated seeds (Table 3). The interactive effects of drought and seed fungal treatments on maize Chl. a was significant ($p \leq 0.05$) during both study year. Higher chlorophyll a pigment (0.43 mg $g^{-1}$ FW) was observed in 2015 as compared to 2016 (0.35 mg $g^{-1}$ FW). Averaged across the years, the highest improvement (26%) in chlorophyll b was observed for fungal treated seeds as

**Table 3. Influence of tested treatments on the biochemical attributes of maize**

| Drought | Seed treat. | Foliar Spray | 2015 | | | | | | 2016 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Chl.a | Chl.b | SOD | POD | CAT | MDA | Chl.a | Chl.b | SOD | POD | CAT | MDA |
| 70% AMC | Untreated | Control | 0.29bcd | 0.31b-f | 1.24b-e | 0.25ghi | 7.00c-g | 99.3bcd | 0.21b-e | 0.26a-e | 0.88ef | 0.23fg | 3.50def | 94.3bcd |
| | | Fungal Suspension | 0.33a-d | 0.38a-d | 1.52a-d | 0.49bc | 9.37a-e | 77.17ab | 0.27a-e | 0.35abc | 1.22bcd | 0.47bc | 6.37a-d | 72.17ab |
| | | Glutathione | 0.38ab | 0.43ab | 1.96ab | 0.58ab | 11.83ab | 99.30a | 0.31ab | 0.38ab | 1.33abc | 0.56ab | 8.17ab | 67.70a |
| | | Ascorbic acid | 0.29bcd | 0.37a-d | 1.33b-e | 0.37def | 8.33b-f | 77.1abc | 0.25a-e | 0.33a-d | 1.09cde | 0.35de | 5.00b-e | 72.17bc |
| | Fungus Treated | Control | 0.28bcd | 0.33b-f | 1.44a-e | 0.29efg | 8.17b-f | 103bcd | 0.24b-e | 0.28a-e | 1.04de | 0.27def | 5.17 | 94.97cd |
| | | Fungal suspension | 0.35abc | 0.40abc | 2.02ab | 0.50abc | 10.6abc | 77.17ab | 0.30abc | 0.36abc | 1.42ab | 0.48ab | 7.33abc | 72.50ab |
| | | Glutathione | 0.43a | 0.46a | 2.19a | 0.60a | 13.33a | 77.17a | 0.35a | 0.41a | 1.53a | 0.58a | 9.83a | 72.17ab |
| | | Ascorbic acid | 0.28a-d | 0.39a-d | 1.69abc | 0.43cd | 9.17a-e | 77.1abc | 0.28a-e | 0.35abc | 1.29abc | 0.28cd | 6.17a-d | 72.17abc |
| 30% AMC | Untreated | Control | 0.20d | 0.21f | 0.17f | 0.10j | 2.43h | 82.00e | 0.18e | 0.16e | 0.13j | 0.08h | 1.17f | 72.67e |
| | | Fungal suspension | 0.24cd | 0.27c-f | 0.59ef | 0.32d-g | 4.00fgh | 68.5de | 0.21b-e | 0.22b-e | 0.47hi | 0.30def | 1.57ef | 63.50de |
| | | Glutathione | 0.30bcd | 0.32b-f | 0.67def | 0.37def | 5.83d-h | 68.5b-e | 0.25a-e | 0.27a-e | 0.57ghi | 0.35de | 2.83def | 57.50b-d |
| | | Ascorbic acid | 0.22d | 0.24ef | 0.37f | 0.16hij | 3.20gh | 67.50de | 0.19de | 0.19de | 0.34ij | 0.14gh | 1.93ef | 60.83de |
| | Fungus Treated | Control | 0.22d | 0.27def | 0.66ef | 0.16ij | 5.17f-h | 73.33de | 0.20cde | 0.22cde | 0.43hi | 0.14gh | 2.83b-e | 79.67de |
| | | Fungal suspension | 0.29bcd | 0.35a-e | 1.24b-e | 0.39cde | 7.13c-g | 67.7b-e | 0.25a-e | 0.30a-e | 0.77fg | 0.37cd | 4.13c-f | 63.73bcd |
| | | Glutathione | 0.33a-d | 0.37a-d | 1.31b-e | 0.49bc | 9.73a-d | 67.7a-d | 0.29a-d | 0.32a-d | 0.87ef | 0.47bc | 5.73bcd | 59.40a-d |
| | | Ascorbic acid | 0.26bcd | 0.29c-f | 0.90c-f | 0.27fgh | 6.57c-h | 67.7cde | 0.22b-e | 0.24b-e | 0.64fgh | 0.25ef | 3.57def | 62.73cde |

* AMC = available moisture contents, Chl. = chlorophyll, SOD = super oxide dismutase, POD = peroxidase, CAT = catalase, MDA = melondialdehyde, Alphabetical letters (a, b, c, d...) above means show the differences ($P$ _ 0.05) among treatments

compared to untreated seeds grown in drought condition. Foliar application of antioxidants and fungal suspension also improved the tolerance against drought stress. The maximum increase of 44% and 42% was observed for chlorophyll a, while 41% and 50% for chlorophyll b in 2015 and 2016 with foliar spray of glutathione as compared to control (Table 3).

Drought stress increased the SOD, POD and CAT activities by 56, 36 and 43% during 2015, and 57, 35, and 54% during 2016, respectively compared with control (Table 3). Seed treatment with beneficial fungus significantly decreased the activities of antioxidants. Averaged across the years, maize plants emerged from fungus treated seeds recorded significantly lower activities of SOD (79%), POD (41%) and CAT (84%). The interactive effects of drought and fungus treatments were also significant during both study years and the effect of fungal seed treatment was more pronounced under progressive drought levels. Results showed that maximum decrease in the production of ROS was observed with foliar application of glutathione followed by foliar spray of fungus which contributes towards increased drought tolerance (Table 3).

Decrease in available soil moisture content increased the levels of MDA in maize leaves. In 2015, an increase of 18% was observed at 30% AMC over control (70% AMC), while in 2016, the MDA contents were increased by 15% compared with control (Table 3). Fungal seed treatment balanced the level of MDA of maize during both the years. Foliar application of glutathione, fungal suspension and ascorbic acid significantly reduced the MDA contents in maize leaves and the maximum decrease (85% in 2015 and 77% in 2016) was observed with foliar spray of glutathione (Table 3).

***Yield parameters*:** Yield parameters of maize varied under the influence of drought stress, seed treatment and foliar application of beneficial fungus and antioxidants. Cob length, grain rows, number of grain rows per cob, thousand grain weight, biological yield and grain yield of maize showed a significant declining trend with a decrease in available moisture contents. Averaged across the years, drought stress caused the reduction of 24%, 25% and 17% in cob length, grain rows and number of grain rows per cob, respectively compared with control. Similarly, the reductions of 17%, 50% and 48%, respectively in thousand grain weight, biological yield and grain yield of maize were observed under drought stress when compared with control (70% AMC) (Table 4).

Seed treatment with fungus significantly enhanced the yield of maize as compared with untreated seeds (Table 4). Positive effect of fungal seed treatment was more prominent under drought stress. Increase in maize cob length (18%) was observed for fungal treated seeds grown under drought condition as compared with control. Averaged across the years, the increase of 44% was observed in grain yield of crop when seeds were treated with fungus. Foliar spray of glutathione and fungal suspension improved the drought tolerance and increased the yield of maize. Averaged across the years the, maximum increase (45 grains per row) in number of grains was observed with foliar application of glutathione followed by fungus (42 grains per row).

**Table 4: Influence of tested treatments on the agronomic and yield attributes of maize**

| Drought | Seed Treat. | Foliar Spray | 2015 | | | | | | 2016 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cob Length (cm) | No. of Grain Rows | Grains per Row | Thousand Grain Weight (g) | Biological yield (t ha$^{-1}$) | Grain Yield (t ha$^{-1}$) | Cob Length (cm) | No. of Grain Rows | Grains per Row | Thousand Grain Weight (g) | Biological yield (t ha$^{-1}$) | Grain Yield (t ha$^{-1}$) |
| 70% AMC | Untreated | Control | 18.6bcd | 12.6a-d | 36.3abc | 266b-e | 8.01b-e | 3.67a-f | 17.6b-e | 12.6b-g | 30.6bcd | 239.0ef | 7.01b-e | 3.34bcd |
| | | Fungal Suspension | 24.1abc | 15.3ab | 42.67ab | 365abc | 11.2abc | 4.8abc | 22.6a-d | 14.6a-d | 39.6abc | 331abc | 10.1abc | 4.70ab |
| | | Glutathione | 26.50ab | 17.33a | 47.00ab | 381.3ab | 12.29a | 5.34ab | 25.50ab | 16.0ab | 43.00ab | 358.0a | 11.29ab | 5.47a |
| | | Ascorbic acid | 23.0a-d | 14a-d | 42.67ab | 322a-d | 10.5abc | 4.1a-e | 22.0a-e | 14a-e | 36.3abc | 309a-d | 9.26a-d | 4.0abc |
| | Fungus Treated | Control | 20.6a-d | 13.3a-d | 38.6abc | 275a-e | 9.34a-d | 3.3a-g | 19.8a-e | 13.3a-f | 36.6abc | 255def | 7.68b-e | 3.01bcd |
| | | Fungal suspension | 26.08ab | 16ab | 45.00ab | 376.6ab | 12.24a | 4.91ab | 24.1abc | 15.3abc | 43.33ab | 357.0a | 10.9abc | 4.6ab |
| | | Glutathione | 28.67a | 18.0a | 50.00a | 395.6a | 13.53a | 5.76a | 28.00a | 17.33a | 48.00a | 370.6a | 12.62a | 5.37a |
| | | Ascorbic acid | 24.5abc | 14.a-d | 43.67ab | 335a-d | 11.59ab | 4.5a-d | 23.5a-d | 14.6a-d | 40.6abc | 315a-d | 10.3abc | 4.03abc |
| 30% AMC | Untreated | Control | 14.17d | 8.67d | 24.67c | 194.0e | 3.68f | 1.20g | 13.17e | 8.67g | 19.6d | 207.f | 2.59f | 0.98e |
| | | Fungal suspension | 17.17cd | 11.3bcd | 36.6abc | 292a-e | 5.24def | 2.1efg | 16.1cde | 10.6d-g | 32.6a-d | 292b-e | 4.49ef | 1.83de |
| | | Glutathione | 19.6bcd | 14a-d | 40.0abc | 315a-e | 6.29def | 2.6c-g | 18.6b-e | 12.6b-g | 35.0a-d | 327abc | 5.53def | 2.9b-e |
| | | Ascorbic acid | 16.50cd | 8.67d | 32.3bc | 216.3de | 4.59ef | 1.85fg | 15.5cde | 10efg | 28.3bcd | 274cde | 4.00ef | 1.72de |
| | Fungus Treated | Control | 16.50cd | 9.33cd | 31.67bc | 245cde | 4.68ef | 1.87fg | 15.00de | 9.33fg | 24.6cd | 283cde | 3.34ef | 1.67de |
| | | Fungus suspension | 20.6a-d | 12.6a-d | 41.67ab | 344abc | 7.08c-f | 3.2b-g | 19.1a-e | 12b-g | 38.6abc | 316a-d | 6.57c-f | 2.8b-e |
| | | Glutathione | 23.5abc | 14.6abc | 44.67ab | 363abc | 7.62b-f | 3.83a-f | 22.0a-e | 14a-e | 44ab | 348.3ab | 7.19b-e | 3.6a-d |
| | | Ascorbic acid | 20.0a-d | 10.6bcd | 39.6abc | 300a-e | 5.59def | 2.4d-g | 18.5b-e | 11.3c-g | 34.6a-d | 322abc | 4.86ef | 2.2cde |

* AMC = available moisture contents, Alphabetical letters (a, b, c, d...) above means show the differences ($P$ _ 0.05) among treatments

Thousand grain weight was also increased and the maximum increase was 46% due to foliar application of glutathione as compared to the control. Similarly, the results showed that the maximum biological yield (7.62 t $ha^{-1}$) and grain yield (3.83 t $ha^{-1}$) were observed at foliar spray of glutathione followed by fungus (7.08 t $ha^{-1}$ and 3.17 t $ha^{-1}$) in 2015 (Table 4). Interactive influence of soil moisture contents and foliar sprays, seed treatment and foliar sprays, and seed treatment, foliar spray and available soil moisture were non-significant during both study years.

## DISCUSSION

Results regarding various morphological, biochemical and agronomic parameters of maize grown under drought stress suggested the effectiveness of beneficial fungus and antioxidants in ameliorating the adverse effect of drought on crop growth. These improvements were observed in seed treated and foliar sprayed plots because of the application of beneficial fungus *T. harizianum* and antioxidants as stress mitigating tools. Contrarily, plant without seed treatment and foliar spray showed a reduction in studied parameters under drought presumably because of water stress induced by limited available moisture. The reduction in root (31%) and shoot (20%) length as well as their biomass (41%) were apparent under limited availability of moisture content during two years (2015 and 2016) of study. Drought stress reduces the cell division and size which resultantly decreases the plant growth (Nonami, 1998). Drought stress is well known to decrease the root density, volume (Nejad *et al.*, 2010) and root biomass accumulation (Tahir and Mehid, 2001). Farooq *et al*. (2009) stated that the decrease in growth and biomass accumulation of plants are the common adverse effects of drought stress.

In the present study, root and shoot growth was improved by the application of fungal seed treatment and foliar spray of fungal suspension and antioxidants. When fungi make a chemical communication inside the plant, fungus gets more access to nutrients and start proliferating. On the contrary, plant benefits from this association through the increase in root and shoot growth, enhanced uptake of nutrients and resistance to diseases (Harman *et al*., 2004).

Plants cells respond to oxidative stress by maintaining antioxidant defense molecules at levels that reflect ambient environmental conditions and scavenge the extra ROS. In the present study, limited availability of moisture reduced the activity of antioxidant enzymes including superoxide dismutase, peroxidase and catalase during both of study years (2015 and 2016), which indicated the poor antioxidative defense system of maize under stress conditions. Application of beneficial fungus and antioxidants increased the activities of these antioxidants (SOD, POD and CAT), which was concomitant with better tolerance and growth of maize in these treatments. *Trichoderma* species play a significant role in antioxidative defense mechanisms, through enhanced expression of specific genes encoding the component enzymes (Mastouri *et al*., 2010). Proteomics of plant roots which were inoculated with *Trichoderma* strains showed the enhanced production of ROS scavenging enzymes as glutathione-reductase (GR), glutathione-S-transferase (GST), POD and other detoxifying enzymes (Shoresh *et al*., 2008). Our results also showed that lipid peroxidation, as indicated by MDA contents, was higher in drought stressed leaves of maize, however, application of fungal suspension and antioxidants was effective in reducing the MDA contents in maize leaves (Table 3). Better antioxidative defense system in plants treated with fungal suspension and antioxidants effectively modulated the ROS production and reduced the lipid peroxidation rate. Chugh *et al*. (2013) reported that under drought, the MDA contents of tolerant genotypes were lower which represents that higher antioxidant capability have scavenged the ROS and prevented the damage to cellular membranes.

Limited availability of moisture reduced the chlorophyll contents in maize plants during both the study years. However, such negative effects were mitigated to a significant extent by the application of fungal seed treatment and foliar spray of fungal suspension and antioxidants (Tables 3). Ghannoum (2009) reported that drought induced degradation of cellular protein or decline in the synthesis of nitrates causes the reduction in chlorophyll contents. Water requirement of plants is decreased by reducing the leaf area and probability of plant survival is increased under stress (Belaygue *et al*., 1996). Nevertheless, decrease in chlorophyll contents, and photosynthetic activity reduced the final grain yield (Flagella *et al*., 2002). *Trichoderma* gives protection to plant structures and functions against ROS by promoting the ROS scavenging mechanism under drought stress, thus enhances the chlorophyll synthesis and photosynthetic capability of plants (Mastouri *et al*., 2010).

In the present study, reductions in grain yield of maize were apparent under limited availability of moisture during both study years (2015 and 2016). However, maize yield was improved by the application of fungal seed treatment and foliar spray of fungal suspension and antioxidants. Drought stress reduces the activities of various enzymes involved in starch synthesis and assimilates partitioning, which limit the grain filling and final productivity of maize. Yadav *et al*. (2004) reported that reduction in re-mobilization of photosynthetic assimilates under water deficit conditions reduced the grain size of pearl millet. Under drought, the rate of grain filling declines due to a reduction in the activity of sucrose and starch synthesizing enzymes (Anjum *et al*., 2011). Yield is reduced due to reduction in flower production and grain filling period with smaller and fewer grains under drought stress. Reduction of the activities of various enzymes involved in the synthesis of starch, and in assimilate partitioning limit the grain filling in maize which

ultimately lowers crop yields (Ahmadi and Baker, 2001). Study revealed that the drought induced reduction in maize yield was due to the higher production of ROS, which affected the growth and yield of crop via their effects on cellular components and mechanisms. The ROS are scavenged by different enzymatic and non-enzymatic antioxidants (Hasegawa *et al*., 2000). Enhanced activities of the various antioxidant enzymes under drought have been reported (Nayyar and Gupta, 2006). However, the application of *T. harizianum* and antioxidants played a significant role in improving the defense system of plants by higher antioxidant activities and scavenging of reactive oxygen species which contribute towards drought resistance in maize.

***Conclusions:*** Drought stress severely hampered the growth and yield of maize; however, seed treatment with beneficial fungus (*Trichoderma harizianum* L.) and foliar spray of fungal suspension and antioxidants particularly glutathione was effective in enhancing the maize growth and yield under drought. The drought stress caused the maximum lipid peroxidation as well as the production of ROS was increased under drought condition. Maize plants derived from fungus treated seeds recorded lower production of MDA compared with untreated seeds. Foliar sprays of fungal suspension and antioxidants in maize crop were also effective in alleviating the detrimental effects of drought by lowering the production of MDA and ROS. Synergistic role of *Trichoderma* and antioxidants for drought tolerance was also seen as the maximum increase in yield was observed in case of glutathione sprayed plants grown from fungus treated seeds. The possible mechanisms involved in *Trichoderma*-antioxidant mediated drought tolerance in maize was that the treated seeds and foliar sprayed plants were found to trigger or at least maintain the antioxidant defense system of maize crop. So, these findings suggested that the maize tolerance against drought stress was mainly due to better alleviation of oxidative damage and maintenance of photosynthetic pigments.

## REFERENCES


Ahmadi, A. and D.A. Baker. 2001. The effect of water stress on the sucrose to starch pathway. Plant Growth Regul 35:81-91.

Anjum, S.A., L.C. Wang, M. Farooq, I. Khan and L.L. Xue. 2011. Methyl jasmonate-induced alteration in lipid peroxidation, antioxidative defense system and yield in soyabean under drought. J. Agron. Crop Sci., doi:10.1111/j. 1439-037X.2010.00468.x.

Belaygue, C., J. Wery, A.A. Cowan and F. Tardieu. 1996. Contribution of leaf expansion, rate of leaf appearance, and stolon branching to growth of plant leaf area under water deficit in white clover. Crop Sci 36:1240-1246.

Bowler, C. and R. Fluhr. 2000. The role of calcium and activated oxygens as signals for controlling cross-tolerance. Trends Plant Sci. 5:241-246.

Bremner, J.M. 1960. Determination of nitrogen in soil by the Kjeldahl method. J Agric Sci. 55:11-33.

Chinnusamy, V., K. Schumaker and J.K. Zhu. 2004. Molecular genetic perspectives on cross-talk and specificity in abiotic stress signaling in plants. J Exp Bot. 55:225-236.

Chugh, V., N. Kaur, M.S. Grewal and A.K. Kupta. 2013. Differential antioxidative response of tolerant and sensitive maize (*Zea mays* L.) genotypes of drought stress at reproductive stage. J Biochem Biophys. 50:150-158.

Denmead, O.T. and R.H. Shaw. 1960. The effects of soil moisture stress at different stages of growth on the development and yield of corn. Agron J. 52:272-274.

Dhindsa, R.S., P. Dhindsa and T.A. Thorpe.1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot. 32:93-101.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra. 2009. Plant drought stress: effects, mechanisms and management. Agron Sustain Dev.29:185-212.

Flagella, Z., T. Rotunno, E. Tarantino, R. Di Caterina and A.D. Caro. 2002. Changes in seed yield and oil fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to the sowing date and the water regime. Eur J Agron.17:221-230.

Frova, C., P. Krajewski, N.D. Fonzo, M. Villa and M. Sari-Gorla. 1999. Genetic analysis of drought tolerance in maize by molecular markers. I. yield components. Theor Appl Gene. 99:280-288.

Ghannoum, O. 2009. C4 photosynthesis and water stress. Ann Bot.103:635-644.

Giannopolitis, C. N. and S. K. Ries. 1977. Superoxide Dismutases: II. Purification and Quantitative Relationship with Water-soluble Protein in Seedlings. Plant Physiol. 59: 315-318.

Gogorcena, Y., I. Iturbe-Ormaetxe, P.R. Escuredo, M. Becana. 1995. Antioxidant defenses against activated oxygen in pea nodules subjected to water stress. Plant Physiol. 108:753-759.

Gong, H., X. Zhu, K. Chen, S. Wang and C. Zhang. 2005. Silicon alleviates oxidative damage of wheat plants in pots under drought. Plant Sci. 169:31-321.

Guo, Y., S. Gui, A. Gao, X. Tang, Y. Hu, W. Huang, Z. Jia, M. Li and H.L. Sun. 2012. Frequent mutations of genes encoding ubiquitin-mediated proteolysis pathway components in clear cell renal cell carcinoma. Nat. Genet. 44:17-19.

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004. Trichoderma species-opportunistic,

avirulent plant symbionts. Nature Reviews Microbiol 2:43-56.

Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert. 2000. Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol. 51:463-49.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 125:189-198.

Hermosa, R., A. Viterbo, I. Chet and E. Monte. 2012. Plant-beneficial effects of Trichoderma and of its genes. Microbiol. 158:17-25.

Homer, C. and F.P. Pratt. 1961. Methods of analysis for soils, plants and waters. Agricultural Research Division 3rd ed. Wiley New Yok.pp. 1-76.

Horemans, N., C.H. Foyer and H. Asard. 2000. Transport and action of ascorbate at the plant plasma membrane. Trends Plant Sci. 5:263-267.

Jiang, K., D.P. Lepak, K. Han, Y. Hong, A. Kim and A. Winkler. 2012. Clarifying the construct of human resource systems: Relating human resource management to employee performance. Hum Resour Manag Rev. 22:73-85.

Johnson, A.I. 1962. Methods of measuring soil moisture in the field, 2nd ed. Government Printing Office USA.pp. 1-24.

Karkanis, P.G. 1983. Determining field capacity and wilting point using soil saturation by capillary rise. Can Agric Eng. 25:19-21.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Meth Enzymo. 148:350-382.

Marra, R., P. Ambrosino, V. Carbone, F. Vinale, S.L. Woo, M. Ruocco and M. Lorito. 2006. Study of the three-way interaction between *Trichoderma atroviride*, plant and fungal pathogens by using a proteomic approach. Curr Genet. 50:307-321.

Mastouri, F. and G.E. Harman. 2009. Beneficial microorganism *Trichoderma harzianum* induces tolerance to multiple environmental and physiological stresses during germination in seeds and seedlings. In IS-MPMI 2009 XIV Congress Quebec Canada.

Mastouri, F., T. Bjorkman and G.E. Harman. 2010. Seed treatment with Trichoderma harizianum alleviates biotic, abiotic and physiological stresses in germinating seeds and seedlings. Phytopathology. 100:1213-1221.

Mehlich, A. 1953. Determination of P, Ca, Mg, K, Na, and NH4. North Carolina Soil Test Division USA.pp 23-89.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410.

Monfreda, C., N. Ramankutty and J.A. Foley. 2008. Farming the planet; 2. Geographic distribution of crop areas, yields, physiological types, and net primary production in the year 2000 Global Biogeochem. Cycles.pp. 22.

Moodie, G., W.R.M. Smith. 1959. Laboratory manual for soil fertility. Department of Agronomy, Washington State University USA.pp.31-39.

Nayyar, H. and D. Gupta. 2006. Differential Sensitivity of C3 and C4 Plants to Water Deficit Stress: association with oxidative stress and antioxidants. Environ Exp Bot 58:106-113.

Nejad, S.K., A. Bakhshande, S.B. Nasab and K. Payande. 2010. Effect of drought stress on corn growth. Rep Opin 2:1-7.

Noctor, G., M. Strohm, L. Jouanin, K.J. Kunert, C.H. Foyer and H. Rennenberg. 1998. Synthesis of glutathione in leaves of transgenic poplar (*Populus tremula x P. alba*) overexpressing glutamylcysteine synthetase. Plant Physiol. 112:1071.

Nonami, H. 1998. Plant water relations and control of cell elongation at low water potentials. J Plant Res. 111:373-382.

Pandit, N.R., J. Mulder, S.E. Hale, H.P. Schmidt and G. Cornelissen. 2017. Biochar form Kon Tiki" flame curtain and other kilns; effects of nutrient enrichment and kiln type on crop yield and soil chemistry. PLoS One 12:e0176378. doi:10.1371/ journal.pone.0176378.

Pandit, 2018. Gene action and combining ability for dual purpose traits in maize (*Zea mays* L.) under water deficit stress prevailing in eastern India. Range Manag Agrofor.39:29-37.

Pannar, 2012. Quality seed, know the maize plant. Pannar Seeds Private Limited.

Pastori, G., C.H. Foyer and P. Mullineaux. 2000. Low temperature-induced changes in the distribution of $H_2O_2$ and antioxidants between the bundle sheath and mesophyll cells of maize leaves. J Exp Bot. 51:10-7113.

Pignocchi, C., J.M. Fletcher, J.E. Wilkinson, J.D. Barnes and C.H. Foyer. 2003. The function of ascorbate oxidase in tobacco. Plant Physiol. 132**:**1631-1641.

Shalata, A. and P.M. Neumann. 2001. Exogenous ascorbic acid (vitamin C) increases resistance to stress and reduces lipid peroxidation. J Exp Bot. 52:2207-2221.

Shoresh, M., G.E. Harman and F. Mastouri. 2008. Induced systemic resistance and plant responses to fungal biocontrol agents. Ann Rev Phytopathol. 48:21-43.

Skogerboe, G.V., M.L. Hyatt, R.K. Anderson and K.O. Eggleston. 1967. Design and calibration of submerged open channel flow measurement structures: part 3-cutthroat flumes, 3rd ed. Utah State University Logan, Utah.pp.1-36.

Steel, R.G., J.H. Torrie, D.A. Dickey. 1997. Principles and procedures of statistics: A biometrical approach. 3rd ed. McGraw Hill Book Co Inc New York.pp.s 400-428.

Tahir, M.H.N. and S.S. Mehid. 2001. Evaluation of open pollinated sunflower (*Helianthus annuus* L.)

populations under water stress and normal conditions. Int J Agric Biol. 3:236-238.

Venisse, J.S., G. Gullner and M.N. Brisset. 2001. Evidence for the involvement of an oxidative stress in the initiation of infection of pear by *Erwinia amylovora*. Plant Physiol. 125:2164-2172.

Yadav, R.S., C.T. Hash, F.R. Bidinger, K.M. Devos and C.J. Howarth. 2004. Genomic regions associated with grain yield and aspects of post flowering drought tolerance in pearl millet across environments and tester background. Euphytica. 136:265-277.

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