Exogenously Applied Proline Modulates Drought Tolerance in Maize (Zea mays L)

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT
Improving drought stress tolerance in maize is essential to increase its production and yield worldwide. Thus, the present study was conducted to investigate the improvement of drought tolerance in maize (Zea mays L.) by exogenous application of proline (25 and 50mM) on two maize varieties. Maize plants were subjected to drought stress at various phases of plant growth under pot culture conditions and proline was applied as foliar spray. Water deficit stress caused a significant decrease (by approximately 25%) in growth and yield of both maize varieties by decreasing plant height, cob length, dry root weight, grains per cob and 100-grain weight. Water deficit stress also decreased chlorophyll and intercellular proline contents, and antioxidant enzyme activities viz. catalase (CAT), guaiacol peroxidase (POX) and ascorbate peroxidase (APX). Exogenous application of proline (50 mM) was found to be more effective in increasing growth and yield of both varieties. These increases were positively associated with increased levels (by at least 15%) of chlorophyll and intracellular proline, and enhanced activities of CAT, POX and APX enzymes in both varieties. Interaction effects of exogenous proline and water deficit stress were significant in aspects of higher growth and yields and enhanced levels of chlorophyll, intracellular proline.
proline and antioxidant enzyme activities. Therefore, it is concluded that foliar application of proline improves drought tolerance by modulating chlorophyll and intracellular proline contents, and antioxidant enzyme activities.

Keywords: Drought stress; proline; chlorophyll; antioxidant enzymes.

1. INTRODUCTION

Plants are constantly exposed to various biotic and abiotic stresses affecting growth, development and productivity of higher plants. Of these abiotic stresses, drought is the most devastating one. Drought refers to the absence of adequate moisture for a long period of time which is essential for plant growth and development. Plants show a differential response when they are exposed to water deficit stress condition [1,2]. Water deficit stress conditions affect crop growth and yield by reducing canopy absorption, radiation efficiency, photosynthetically active radiations, chlorophyll biosynthesis, vital membrane degradation, proteins structure destabilization and over-accumulation of reactive oxygen species, etc. [3-6].

Water deficit stress occurring during different development stages of corn may reduce final grain yield to different degrees, and the extent of yield reduction depends not only on the severity of the stress, but also on the stage of the plant development [7]. For the survival of plants under water deficit conditions, various defense and metabolic systems are activated. Plants accumulate osmolytes/compatible solutes, such as proline, glycine betaine, sugars, polyols, and trehalose etc. takes place, which is essential for metabolic and osmotic adjustments in plants [8, 6, 9, and 10]. Plants also protect oxidative damage from water deficit stress by both enzymatic and non-enzymatic systems. The increased levels of ROS can inactivate enzymes, damage important cellular components, which induced plant growth arrest, and even death finally [11]. The CAT, POX and APX play significant roles by ameliorating the water deficit stress [12,13]. Of various compatible solutes, proline acts as a protective molecule that can unite stress generated free radicals and oxygen [14]. Proline, without interfering with the normal metabolic processes, allows the plants to grow or thrive under drought stress [15,16]. Proline accumulation helps maintain cell water status, sub-cellular structures and protect membranes [17-19] and proteins from denaturing effect of the osmotic stress [20]. It is found to be actively associated in plants as defensive response to adverse conditions of salinity, drought, chilling or heat stress [21]. Furthermore, Ali and Ashraf [22] reported that more accumulation of proline was associated with increased biomass production and antioxidant activities under water deficit stress conditions. Therefore, the objective of the present study is to investigate the impact of exogenous application of proline on growth and yield of maize under drought stress condition.

2. MATERIALS AND METHODS

2.1 Pot Experimentation

The pot experiment was carried out at the net-house, Department of Soil Science, BAU, Mymensingh. Two maize cultivars were used as plant materials in this experiment. The total numbers of pots used in this study were 72 (12×3×2). Each plastic pot was 15 cm deep with 17 cm diameter at the top. Top surface area of each pot was 0.23 m². Each pot contained one plant. Considerable spacing was maintained among the pots for convenience of management operations.

2.1.1 Plant materials, treatments and design

Two maize varieties (BARI Hybrid Maize-5 and BARI Hybrid Maize-9) were used to validate the effect of exogenous application of proline. These two hybrid varieties were selected as they are mostly grown by the farmers in the field. The factorial experiment was carried out in randomized complete block design (RCBD) with three replications. Treatment combinations were applied at the different levels of irrigation and proline. There were four levels of irrigation, viz. I₀-control (normal irrigations), I₁-water deficit stress at vegetative stage, I₂-water deficit stress at flowering stage and I₃-water deficit stress at vegetative and flowering stages. The 0, 25 and 50mM exogenous proline was applied, denoted as P₀, P₂₅ and P₅₀. However, there were twelve treatment combinations, viz. \( T₁ = I₀P₀; T₂ = I₀P₂₅; T₃ = I₀P₅₀; T₄ = I₁P₀; T₅ = I₁P₂₅; T₆ = I₁P₅₀; T₇ = I₂P₀; T₈ = I₂P₂₅; T₉ = I₂P₅₀; T₁₀ = I₃P₀; T₁₁ = I₃P₂₅; T₁₂ = I₃P₅₀. \)
2.2 Proline Application

For preparation of 25 mM and 50 mM solutions, 2.88 g and 5.76 g of proline powder were dissolved in 1000 ml of water and 1 ml of Tween-20. Tween-20 was used as a sticky substance which helps proline solutions to prevent droplet formation maintaining a close contact with plant leaves. Proline was applied at vegetative and reproductive stages as foliar spray at a volume of 25 mL per plant per treatment.

2.2.1 Analysis of chlorophyll and intracellular proline contents

Chlorophyll and intracellular proline contents were measured from 2nd and 3rd flag green leaf of maize after 15 days’ exogenous proline application.

2.2.2 Chlorophyll content

Chlorophyll content was measured according to method by Porr et al. (1989). An aliquot amount (0.5 g) of fresh green leaf of maize was suspended in 10 ml of 80% acetone, mixed well and kept at room temperature in the dark for 7 days. The supernatant was collected after centrifugation at 5000 rpm for 15 min and the absorbance was read at 645 nm and 663 nm in a spectrophotometer.

2.2.3 Intracellular proline content

Proline content was measured according to the method of Bates et al. (23). An aliquot amount (0.5 g) of fresh green leaf of maize was homogenized in 10 ml of 3% sulfosalicylic acid and the homogenate was centrifuged at 5000 rpm for 15 min. Two milliliters of the supernatant were reacted with 2 ml of 2% ninhydrin and 2 ml of glacial acetic acid for 1 hr at 100°C. The colored reaction mixture was extracted with 4 ml of toluene and the absorbance was recorded at 520 nm. Proline content was calculated from a standard curve.

2.2.4 Extraction and assay of activity of antioxidant enzymes

Performance of antioxidant enzyme activities were measured from green leaves of maize at 15 days after proline application. In this regard, 2 g of the plant materials were homogenized with 10 mL of 0.1M phosphate buffer (pH 6.8) and centrifuged at 2°C for 20 min at 20,000x g in a refrigerated centrifuge. The clear supernatant was used as enzyme extract.

2.2.5 Catalase

Catalase (CAT) activity was determined by the method of Aebi (24). The reaction mixture contained 50 mM Tris–HCl (pH 8.0), 0.25 mM EDTA, 20 mM H2O2 and 25 ml of sample solution. The reaction was started by the addition of H2O2. The activity was calculated from the decrease in absorbance at 240 nm for 2 min when the extinction coefficient was 40 M⁻¹ cm⁻¹.

2.2.6 Guaiacol Peroxidase

Guaiacol peroxidase (POX) activity was determined according to Nakano and Asada (25). The reaction mixture contained 50 mM KH2PO4 buffer (pH 7.0), 0.1 mM EDTA, and 0.1 mM H2O2, and 10 mM guaiacol. The reaction was started by the addition of the sample solution to the reaction mixture. The activity was calculated from the change in absorbance at 470 nm for 30 sec where an extinction coefficient was 26.6 M⁻¹ cm⁻¹.

2.2.7 Ascorbate peroxidase

Ascorbate peroxidase (APX) activity was estimated following the method of Nakano and Asada (25). The reaction mixture contained 50 mM KH2PO4 buffer (pH 7.0), 0.1 mM EDTA, and 0.1 mM H2O2, and 0.5 mM ascorbate. The reaction was started by adding the sample solution to the reaction mixture. The activity was calculated from the change in absorbance at 290 nm for 1 min when the extinction coefficient was 2.8 M⁻¹ cm⁻¹.

2.3 Statistical Analysis

Data were analyzed statistically using two-way analysis of variance (ANOVA) to examine the treatment effects. The mean differences were adjudged by Duncan’s Multiple Range Test (DMRT) (26) and ranking was indicated by letters.

3. RESULTS

3.1 Effects of Water Deficit Stress and Exogenous Proline on Plant Growth Parameters of Maize Varieties

Imposition of drought stress revealed a significant decrease in plant height, cob length, cob diameter, grains per cob, 1000 grain weight,
root dry weight, shoot dry weight and ultimate yield in BARI Hybrid Maize-5 and BARI Hybrid Maize-9 (Table 1 and 4). But any sort of drought stress after vegetative stage resulted in no cob formation thus only plant height, root dry weight and straw yield was possible to measure at treatment I3 and their reduction was highest under this treatment for both the varieties. On the other hand, exogenous application of 25 mM and 50 mM proline resulted increase in all the parameters in both the varieties. This increment was highest in case of 50 mM proline application in both the varieties except for cob diameter, grains per cob and root dry weight in BARI Hybrid Maize-5 which was highest in case of 25 mM of exogenous proline application (Table 2 and 5).

Interaction effect revealed highest plant height by application of 25 mM proline along with no drought stress i.e. treatment combination I0P25 which was statistically similar to control condition (I0P0) and I0P50 in BARI Hybrid Maize-5 and in case of BARI Hybrid Maize-9, I0P50 gave the highest followed by control condition (I0P0) and I0P25. Cob length and grains per cob was highest in treatment combination I0P50 for both the varieties. Cob diameter was highest in I0P25 followed by I0P0 and I0P50 in case of BARI Hybrid Maize-5 whereas in BARI Hybrid Maize-9 it was highest in I1P50 followed by I0P0, I0P25, and I0P50. Thousand grain weight was highest in control condition in case of BARI Hybrid Maize-9 but BARI Hybrid Maize-5 showed the highest in case of I1P50. Highest root dry weight and straw yield was found by application of 25 mM proline along with no drought stress (Table 3,6).

Imposition of drought stress resulted in significant decrease in the chlorophyll ‘a’, ‘b’ and total chlorophyll contents in the of both maize cultivars (Fig. 1). However, exogenous application of 25 mM and 50 mM proline improved ‘a’, ‘b’ and total chlorophyll contents in water deficit stressed condition but increment of chlorophyll ‘b’ was not significant in BARI hybrid maize-9 (Fig. 2). Similar increment also found in interaction with irrigation (Table 7).

Fig. 1. Effect of water deficit stress on chlorophyll contents

Fig. 2. Effect of exogenous proline on chlorophyll contents
Table 1. Effect of water deficit stress on the growth and yield components of maize

| Water deficit stress | Plant height (cm) | Cob length (cm) | Cob diameter (cm) | Grains per cob | 100 grain weight (g) | Plant height (cm) | Cob length (cm) | Cob diameter (cm) | Grains per cob | 100 grain weight (g) |
|----------------------|------------------|----------------|------------------|----------------|---------------------|------------------|----------------|------------------|----------------|---------------------|
| I₀                  | 169.9a           | 11.2 a         | 4.033 a          | 294.1a         | 15.90a              | 173.0 a          | 12.32 a         | 3.510 a          | 275.4 a        | 16.34 a             |
| I₁                  | 130.3c           | 9.56 b         | 3.503 b          | 217.9b         | 15.25b              | 137.1 c          | 10.19 b         | 3.368 b          | 169.0 b         | 15.25 b             |
| I₂                  | 142.1b           | -              |                |                |                     | 154.2 b          | -              | -                | -              | -                   |
| I₃                  | 105.7d           | -              |                |                |                     | 98.89 d          | -              | -                | -              | -                   |
| SE(±)               | 1.49             | 0.066          | 0.023           | 1.43           | 0.203               | 1.90             | 0.078          | 0.029            | 1.31            | 0.138               |
| CV                  | 3.28             | 3.81           | 3.73            | 3.87           | 7.83                | 4.06             | 4.18          | 5.05             | 3.54            | 5.27                |

I₀-control (normal irrigations), I₁-water deficit stress at vegetative stage, I₂-water deficit stress at flowering stage and I₃-water deficit stress at vegetative and flowering stages SE= standard error of means and CV= coefficient of variation, "-"= indicates no cob yield

Table 2. Effect of exogenous proline application on the growth and yield components of maize

| Proline   | Plant height (cm) | Cob length (cm) | Cob diameter (cm) | Grains per cob | 100 grain weight (g) | Plant height (cm) | Cob length (cm) | Cob diameter (cm) | Grains per cob | 100 grain weight (g) |
|-----------|------------------|----------------|------------------|----------------|---------------------|------------------|----------------|------------------|----------------|---------------------|
| P₀        | 134.1b           | 5.140b         | 1.842 b          | 126.3b         | 7.463 b             | 135.7 b          | 5.368 b         | 1.650 b          | 104.3 b        | 2.120 c             |
| P₂₅       | 137.3ab          | 5.150b         | 1.967 a          | 133.7a         | 7.780ab            | 149.1 a          | 5.692 a         | 1.783 a          | 112.6 a        | 7.220 b             |
| P₅₀       | 139.7 a          | 5.350a         | 1.843 b          | 124.1b         | 8.120 a             | 165.1 c          | 6.723c          | 1.816 c          | 113.8 c        | 8.210 a             |
| SE(±)     | 3.29             | 0.057          | 0.020            | 1.43           | 0.176               | 1.65             | 0.067           | 0.025            | 1.14           | 0.120               |
| CV        | 3.28             | 3.81           | 3.73            | 3.87           | 7.83                | 4.06             | 4.18           | 5.05             | 3.54           | 5.27                |

P₀= No exogenous proline application, P₂₅= 25 mM exogenous proline application and P₅₀=50 mM exogenous proline application, SE= standard error of means and CV= coefficient of variation
Table 3. Interaction effects water deficit stress and exogenous proline application on the growth and yield components of maize

| Treatment combination | BARI hybrid Maize-5 | BARI hybrid Maize-9 |
|-----------------------|---------------------|---------------------|
|                       | Plant height (cm)   | Cob length (cm)     | Cob diameter (cm) | Grains per cob | 100 grain weight (g) | Plant height (cm) | Cob length (cm) | Cob diameter (cm) | Grains per cob | 100 grain weight (g) |
| P0                    | 170.0a              | 11.33b              | 4.000 b           | 283.7 b         | 15.91ab         | 167.0 b            | 12.80 a          | 3.533ab          | 265.3 b         | 17.28a             |
| P25                   | 170.7a              | 10.53c              | 4.200 a           | 285.0 b         | 15.95b          | 163.3 b            | 11.67 b          | 3.467ab          | 259.0 b         | 16.07bc            |
| P50                   | 169.0a              | 12.0a               | 3.900 b           | 313.7 a         | 15.83ab         | 188.7 a            | 12.50 a          | 3.530ab          | 302.0 a         | 15.68c             |
| P0                    | 116.7d              | 9.23e               | 3.370 d           | 182.7 e         | 141.7d          | 152.0 c            | -                | -                | 148.3 e         | 13.20d             |
| P25                   | 129.3c              | 10.07d              | 3.670 c           | 249.7 c         | 15.17d          | 134.3 d            | 9.80 d           | 3.133c           | 158.3 d         | 16.77ab            |
| P50                   | 145.0b              | 9.40e               | 3.470 d           | 16.65a          | 14.7d           | 15.65a             | 10.27 b          | 3.600a           | 148.3 e         | 13.20d             |
|                      | 141.3b              | -                   | -                 | -               | -               | -                  | -                | -                | -                | -                  |
|                      | 142.7b              | -                   | -                 | -               | -               | -                  | -                | -                | -                | -                  |
|                      | 142.3b              | -                   | -                 | -               | -               | -                  | -                | -                | -                | -                  |
|                      | 108.3e              | -                   | -                 | -               | -               | -                  | -                | -                | -                | -                  |
|                      | 106.3e              | -                   | -                 | -               | -               | -                  | -                | -                | -                | -                  |
|                      | 102.3e              | -                   | -                 | -               | -               | -                  | -                | -                | -                | -                  |
|                      | 2.59                | 0.114               | 0.040             | 2.86            | 0.351           | 3.30               | 0.135            | 0.050            | 2.27            | 0.240              |
| CV                    | 3.28                | 3.81                | 3.73              | 3.87            | 7.83            | 4.06               | 4.18             | 5.05             | 3.54            | 5.27               |

SE= standard error of means and CV= coefficient of variation, "-"= indicates no cob yield

Table 4. Effect of different level of irrigation on growth and yield of maize cultivars

| Water deficit stress | BARI Hybrid Maize-5 | BARI Hybrid Maize-9 |
|----------------------|----------------------|----------------------|
|                      | Root dry weight (g/pot) | Straw Yield (g/pot) | Grain Yield (g/pot) | Root dry weight (g/pot) | Straw Yield (g/pot) | Grain Yield (g/pot) |
| I0                   | 3.923a               | 45.77a               | 44.72a              | 2.720a               | 47.72a             | 42.17a             |
| I1                   | 1.603b               | 33.22b               | 28.78b              | 1.777b               | 34.23b             | 31.60b             |
| I2                   | 1.166c               | 29.64c               | ND                  | 1.130c               | 31.37c             | ND                 |
| I3                   | 0.87d                | 12.70d               | ND                  | 0.993d               | 12.47d             | ND                 |
| SE(±)                | 0.011                | 0.48                 | 0.213               | 0.025                | 0.20               | 0.258              |
| CV (%)               | 1.88                 | 4.75                 | 3.48                | 4.60                 | 1.91               | 4.21               |

I0-control (normal irrigations), I1-water deficit stress at vegetative stage, I2-water deficit stress at flowering stage and I3-water deficit stress at vegetative and flowering stages SE= standard error of means and CV= coefficient of variation, ND= No data (no cob yield)
Table 5. Effect of exogenous application of proline on growth and yield of maize cultivars

| Proline levels | Root dry weight (g/pot) | Straw Yield (g/pot) | Grain Yield (g/pot) | Root dry weight (g/pot) | Straw Yield (g/pot) | Grain Yield (g/pot) |
|----------------|-------------------------|---------------------|---------------------|-------------------------|---------------------|---------------------|
| P0             | 1.819b                  | 28.36c              | 16.96c              | 1.580b                  | 29.22c              | 17.22c              |
| P25            | 1.928a                  | 30.58b              | 18.55b              | 1.673a                  | 31.55b              | 18.52b              |
| P50            | 1.925a                  | 32.05a              | 19.61a              | 1.712a                  | 33.58a              | 19.59a              |
| SE(±)          | 0.010                   | 0.416               | 0.184               | 0.022                   | 0.173               | 0.224               |
| CV (%)         | 1.88                    | 4.75                | 3.48                | 4.80                    | 1.91                | 4.21                |

P0 = No exogenous proline application, P25 = 25 mM exogenous proline application and P50 = 50 mM exogenous proline application, SE = standard error of means and CV = coefficient of variation, *±* = indicates no cob yield

Table 6. Interaction effects of irrigation and exogenous proline on growth and yield of maize cultivars

| Treatment combinations | Root dry weight (g/pot) | Straw Yield (g/pot) | Grain Yield (g/pot) | Root dry weight (g/pot) | Straw Yield (g/pot) | Grain Yield (g/pot) |
|------------------------|-------------------------|---------------------|---------------------|-------------------------|---------------------|---------------------|
| I0P0                   | 3.89b                   | 45.76a              | 43.95b              | 2.72 a                  | 47.67a              | 41.72a              |
| I0P25                  | 3.95a                   | 45.80a              | 44.69ab             | 2.71 a                  | 47.74a              | 41.89a              |
| I0P50                  | 3.93ab                  | 45.74a              | 45.51a              | 2.73 a                  | 47.75a              | 42.91a              |
| I1P0                   | 1.45d                   | 28.65ef             | 23.88e              | 1.65 bc                 | 31.39d              | 27.17d              |
| I1P25                  | 1.69c                   | 33.64c              | 29.52d              | 1.84 b                  | 34.77c              | 32.19c              |
| I1P50                  | 1.67c                   | 37.37f              | 32.95c              | 1.84 b                  | 36.52b              | 35.45b              |
| I2P0                   | 1.07f                   | 26.47f              | ND                  | 1.04 e                  | 27.96f              | ND                  |
| I2P25                  | 1.20e                   | 30.35de             | ND                  | 1.12 de                 | 30.14e              | ND                  |
| I2P50                  | 1.23e                   | 32.09cd             | ND                  | 1.23 d                  | 36.01b              | ND                  |
| I3P0                   | 0.870g                  | 12.56g              | ND                  | 0.910f                  | 9.84h               | ND                  |
| I3P25                  | 0.870g                  | 12.53g              | ND                  | 1.02 e                  | 13.56g              | ND                  |
| I3P50                  | 0.870g                  | 13.01g              | ND                  | 1.05 e                  | 14.02g              | ND                  |
| SE(±)                  | 0.020                   | 0.832               | 0.369               | 0.043                   | 0.347               | 0.448               |
| CV (%)                 | 1.88                    | 4.75                | 3.48                | 4.60                    | 1.91                | 4.21                |

SE = standard error of means and CV = coefficient of variation, ND = No data (no cob yield)
### Table 7. Combined effects of exogenous proline application and irrigation on chlorophyll and intercellular proline content

| Treatment combination | BARI Hybrid Maize-5 | BARI Hybrid Maize-9 |  
|-----------------------|---------------------|---------------------|  
|                       | Chl-a (μg/ml)       | Chl-b (μg/ml)       | Total chl (μg/ml) | Proline (mM) | Chl-a (μg/ml) | Chl-b (μg/ml) | Total chl (μg/ml) | Proline (mM) |  
| I0P0                  | 6.41cdef            | 10.37a              | 16.65a            | 6.41f        | 7.05ab       | 14.10b        | 21.15b            | 6.15g        |  
| I0P25                 | 6.71bc              | 9.910ab             | 16.75a            | 6.65f        | 6.95abc      | 15.04a        | 21.99a            | 6.92f        |  
| I0P50                 | 6.19f               | 8.470d              | 14.66c            | 7.53d        | 5.90f        | 8.76e         | 14.66f            | 7.06f        |  
| I1P0                  | 6.68bc              | 9.140c              | 15.82bc           | 7.99c        | 6.74bcd      | 9.49de        | 16.21e            | 7.41de       |  
| I1P25                 | 6.59bcd             | 10.30a              | 16.89a            | 8.44b        | 7.39a        | 10.10bcd      | 17.49bcd           | 7.96c        |  
| I1P50                 | 6.56bcde            | 8.12d               | 14.68c            | 6.69f        | 6.34de       | 7.31f         | 13.65g            | 7.23ef       |  
| I2P0                  | 6.25ef              | 9.42bc              | 15.67b            | 7.15e        | 6.76bcd      | 10.26bcd      | 17.02d            | 7.86c        |  
| I2P25                 | 6.30def             | 9.50bc              | 15.80b            | 7.81c        | 7.00abc      | 10.60c        | 17.60c            | 7.88c        |  
| I2P50                 | 5.32g               | 4.53g               | 9.85e             | 6.98e        | 6.20ef       | 7.94f         | 14.14fg           | 7.64cd       |  
| I3P0                  | 6.62bcd             | 5.70f               | 12.32d            | 8.63b        | 6.74bcd      | 10.28bcd      | 17.02d            | 8.44b        |  
| I3P25                 | 7.45a               | 6.64e               | 14.09c            | 9.30a        | 6.55cde      | 10.45c        | 17.00d            | 8.78a        |  
| I3P50                 | 0.103               | 0.175               | 0.203             | 0.094        | 0.139        | 0.270         | 0.181             | 0.103        |  
| SE(%)                 | 2.76                | 3.57                | 2.35              | 2.18         | 3.61         | 4.35          | 1.80              | 2.39         |  

SE= standard error of means and CV= coefficient of variation
Drought stress caused a significant decrease in intracellular proline content in BARI hybrid maize-5 and BARI hybrid maize-9 varieties. Application of proline significantly increased intracellular proline contents in both varieties (Fig. 3 and 4). The highest intracellular proline content was found in both maize cultivars due to the foliar application of 50 mM proline.

The interaction effects of exogenous proline and water deficit stress was significant in aspect of intracellular proline content. The highest intracellular proline was found by application of 50 mM proline under water deficit stress conditions (Table 8).

Water deficit stress caused a significant decrease in CAT, POX and APX activities in both BARI Hybrid maize-5 and BARI Hybrid maize-9 varieties (Table 8). CAT, POX and APX activities increased due to the application of exogenous proline in both varieties (Table 9). In most of cases, the highest CAT, POX and APX activities were found by foliar application of 50 mM proline. POX and APX was also induced due to the interaction of exogenous proline and water deficit stress in both varieties (Table 10). CAT and APX activities were also increased but insignificant in BARI Hybrid maize-9. Exogenous application of 50 mM proline showed a higher CAT and APX activities in both varieties under water deficit stress conditions.

4. DISCUSSION

Drought tolerance likely to be enhanced by applying proline to the foliage. In this present study, applying proline increased maize plant growth and physiological responses in comparison to no proline application. The findings are also in agreement with Bhusan et al. [27] who reported improvement of salt tolerance in rice, and Shao et al. [12] who also reported a significant increase in drought tolerance of maize by exogenous application of proline. The increased plant growth might be associated with increasing physiological and biochemical parameters such as chlorophyll content, intercellular proline and antioxidant enzymatic activities.

![Fig. 3. Effect of water deficit stress on proline contents](image)

![Fig. 4. Effect of exogenous proline on proline contents](image)
Table 8. Effect of water deficit stress on antioxidant enzyme activities

| Water deficit stress | BARI Hybrid Maize-5 |  | BARI Hybrid Maize-9 |  |
|----------------------|----------------------|------------------|----------------------|------------------|
|                      | Catalase (mmol/min/g FW) | Peroxidase (μmol/min/g FW) | Ascorbate peroxidase (μmol/min/g FW) | Catalase (mmol/min/g FW) | Peroxidase (μmol/min/g FW) | Ascorbate peroxidase (μmol/min/g FW) |
| I₀                   | 6.167a               | 100.7c           | 4.734c               | 4.756a           | 68.57c             | 3.340d             |
| I₁                   | 5.633b               | 117.3a           | 5.577a               | 4.433b           | 75.67a             | 4.267a             |
| I₂                   | 5.067c               | 112.3b           | 5.183b               | 4.400b           | 72.00b             | 4.113b             |
| I₃                   | 5.100c               | 87.33d           | 4.841c               | 3.267c           | 60.33d             | 3.863c             |
| SE(±)                | 0.077                | 0.516            | 0.062                | 0.050            | 0.396              | 0.048              |
| CV(%)                | 3.89                 | 1.48             | 3.70                 | 1.72             | 3.72               |                   |

I₀-control (normal irrigations), I₁-water deficit stress at vegetative stage, I₂-water deficit stress at flowering stage and I₃-water deficit stress at vegetative and flowering stages SE= standard error of means and CV= coefficient of variation

Table 9. Effect of proline on antioxidant enzyme activities

| Proline | BARI Hybrid Maize-5 |  | BARI Hybrid Maize-9 |  |
|---------|----------------------|------------------|----------------------|------------------|
|         | Catalase (mmol/min/g FW) | Peroxidase (μmol/min/g FW) | Ascorbate peroxidase (μmol/min/g FW) | Catalase (mmol/min/g FW) | Peroxidase (μmol/min/g FW) | Ascorbate peroxidase (μmol/min/g FW) |
| P₀      | 5.475c               | 99.25c           | 4.722b               | 3.642b           | 62.00b             | 3.530b             |
| P₂₅     | 6.150b               | 105.5b           | 5.210a               | 4.500a           | 72.50a             | 4.017a             |
| P₅₀     | 6.350a               | 108.5a           | 5.320a               | 4.500a           | 73.00a             | 4.140a             |
| SE(±)   | 0.067                | 0.447            | 0.054                | 0.043            | 0.343              | 0.041              |
| CV(%)   | 3.89                 | 1.48             | 3.70                 | 1.72             | 3.72               |                   |

P₀-No exogenous proline application, P₂₅= 25 mM exogenous proline application and P₅₀=50 mM exogenous proline application, SE= standard error of means and CV= coefficient of variation
### Table 10. Interaction effects of exogenous proline and water on antioxidant enzyme activities

| Treatment combination | BARI Hybrid Maize-5 | | | BARI Hybrid Maize-9 | | |
|-----------------------|---------------------|----------------|---------------------|---------------------|----------------|
|                       | Catalase (mmol/min/g FW) | Peroxidase (μmol/min/g FW) | Ascorbate peroxidase (μmol/min/g FW) | Catalase (mmol/min/g FW) | Peroxidase (μmol/min/g FW) | Ascorbate peroxidase (μmol/min/g FW) |
| I₀P₀                  | 5.90cd              | 98.00f          | 4.71cd              | 4.47b              | 64.00f          | 3.26f          |
| I₀P₂₅                 | 6.20bcd             | 101.00e         | 4.71cd              | 4.90a              | 71.00d          | 3.34ef         |
| I₀P₅₀                 | 6.40ab              | 103.00e         | 4.78c               | 4.90a              | 71.00d          | 3.40ef         |
| I₂P₀                  | 5.30f               | 112.00c         | 5.04c               | 3.80c              | 67.00e          | 3.85d          |
| I₂P₂₅                 | 5.80de              | 118.00b         | 5.84a               | 4.80a              | 79.00ab         | 4.38ab         |
| I₂P₅₀                 | 5.80de              | 122.00a         | 5.85a               | 4.70ab             | 81.00a          | 4.57a          |
| I₃P₀                  | 5.40ef              | 106.00d         | 4.42d               | 3.60cd             | 64.00f          | 3.57e          |
| I₃P₂₅                 | 6.30abc             | 114.00c         | 5.42b               | 4.80a              | 75.00c          | 4.35ab         |
| I₃P₅₀                 | 6.50ab              | 117.00b         | 5.71ab              | 4.80a              | 77.00bc         | 4.42ab         |
| I₅₀P₀                 | 5.30f               | 81.00i          | 4.71cd              | 2.70e              | 53.00g          | 3.42ef         |
| I₅₀P₂₅                | 6.30abc             | 89.00h          | 4.87c               | 3.50d              | 65.00ef         | 4.00cd         |
| I₅₀P₅₀                | 6.70a               | 92.00g          | 4.94c               | 3.60cd             | 63.00f          | 4.17bc         |
| SE(μ)                 | 0.134               | 0.894           | 0.108               | 0.087              | 0.885           | 0.083          |
| CV(%)                 | 3.89                | 1.48            | 3.70                | 3.58               | 1.72            | 3.72           |

**SE=** standard error of means and **CV=** coefficient of variation
Plants have developed a variety of adaptive mechanisms to respond to drought. One of the main adaptive mechanisms to drought in plants is the accumulation of proline. In addition to its role as an osmoprotectant, proline counteracts the adverse effects of various stresses by reducing cellular damage and increasing antioxidant defense systems [28,29]. The protective mechanisms of proline have been increasingly reported in the literature in plants against various stresses. In this study, drought caused decreased in growth and yield of maize which is increased significantly with the increasing doses of exogenous proline application.

Furthermore, exogenous proline application increased plant height of both maize cultivars under drought stress conditions. Hossain and Fujita [30]; Ahmed et al. [31]; Nounjian and Theerakulpisut [32]; Hasanuzzaman et al. [33] reported that proline improved growth and alleviated the adverse effects of salt stress of plants. Dry root weight was also increased due to increasing concentration of proline from 25 mM to 50 mM in both the varieties but the extent was low. Devar et al. [34] also reported that exogenous application of proline increased the dry root weight of maize cultivars. Effectiveness of proline applied as a foliar spray depends on the type of species, plant developmental stage, time of application and concentration [20]. Concerning the effect of proline, Taie et al. [35] found a similar trend of increased growth parameters (plant height, number of leaves, and shoot dry weight). Bhusan et al. [27] reported exogenous proline (25-50 mM) application significantly increased root and shoot growth as well as grain yield in rice. El-Beltagi et al. [36] also reported positive stimulation in yield attributes of chickpea where among different levels of proline used for the experiment and Ghafoor et al. [37] found 40mM proline considerably improve the growth of oat.

The reduction in growth observed in the present investigation subjected to excess drought is often associated with a decrease in rate of photosynthetic capacity. Photosynthetic pigments like chlorophyll ‘a’ and ‘b’ decreased in both maize cultivars due to water deficit stress, which agreed with some previous studies on different crops e.g., maize [38], wheat [39], rice [40], and canola [41]. Soly et al. [42] also reported reduction in photosynthetic pigments under Pb stressed maize plant. Reduction in photosynthetic rate occurs due to stomatal closure under water deficit conditions which may limit CO$_2$ diffusion into the leaves [43,44]. Application of 50mM proline increased both the chlorophyll pigments in both the variety which is parallel to Ali et. al. [45] who reported increased chlorophyll by applying 30 and 60mM proline. Deivanai et al. [46] also reported increased chlorophyll on two Malaysian rice. El-Beltagi et al. [36] reported, proline application causes significant increase in all photosynthetic pigments whereas Ghafoor et. al. [37] found increase in chlorophyll ‘b’ only in oat.

Intercellular proline content decreased due to water deficit stress in both varieties and found increased by exogenous application of proline. Hayat et. al. [47] and Bhusan et al. [27] also reported similar increase of proline under stress condition. Proline accumulates in larger amount than other amino acids and regulates osmotic potential of the cell. It is also hypothesized that proline, besides being an osmolyte is also involved in scavenging free radicals and protects plant cell against adverse effect of drought by maintaining osmotic balance. The interaction effects of exogenous proline and water deficit stress also increased proline in both varieties. Nounjian et al. [32] also showed that exogenous application of proline increased endogenous proline accumulation in Thai aromatic rice pants.

In this study, drought stress caused a significant decrease in major H$_2$O$_2$ scavenging antioxidant enzymes CAT but increase in POX and APX activities in both maize varieties which is similar to finding of Ghafoor et al. [37] in oat and El-Beltagi et al. [36] in chickpea. In most of the cases, application of exogenous proline increases CAT, POX and APX enzyme activities in both maize varieties. Bhusan et. al. [27] reported 25 and 50mM proline application increase antioxidant enzyme activities. De Freitas et al. [48] found that treatment with proline caused significant differences in APX activity in maize plants under salt stress. According to Desoky et al. [49], CAT and APX changed the switching of H$_2$O$_2$, a dominant and detrimental oxidizing factor to H$_2$O and O$_2$. Radhakrishnan et al. [50] reported that Increased CAT, SOD and POX activity could effectively detoxify harmful ROS effects. As the ROS including H$_2$O$_2$ is a highly electron receiving agent. It receives the electron from other molecules of plant cell and damage them. Antioxidant defense mechanism is a system that protects H$_2$O$_2$ to receive the electron from cell molecule and mitigate the cell from drought.
stress. Thus, exogenous proline confers tolerance to drought stress of maize by increasing proline accumulation and antioxidant defense mechanisms.

5. CONCLUSION

Water deficit stress caused a significant reduction in growth and development as well as yield of both (BARI Hybrid Maize-5 and BARI Hybrid Maize-9) varieties. Exogenous application of proline application confers tolerance to drought stress in maize cultivars by increasing chlorophyll, endogenous proline contents and enhancing antioxidant defense systems and resulted increased plant growth and physiological responses.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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