International Journal of Plant & Soil Science

33(19): 198-212, 2021; Article no.IJPSS.73222
ISSN: 2320-7035

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

Shamim Akram[1], Mohammad Golam Kibria[2], Yoshiyuki Murata[3] and Md. Anamul Hoque[2]*

[1]Soil Science Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202, Bangladesh.
[2]Department of Soil Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[3]Graduate School of Environmental and Life Science, Okayama University, Okayama 700-8530, Japan.

Authors' contributions

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

Article Information

DOI: 10.9734/IJPSS/2021/v33i1930619
Editor(s):
(1) Prof. Surendra Singh Bargali, Kumaun University, India.
Reviewers:
(1) Habibu Bashir Labaran, Rabiu Musa Kwankwaso College Of Advanced And Remedial Studies, Nigeria.
(2) Nour Ali Sajedi, Islamic Azad University, Iran.
Complete Peer review History: https://www.sdiarticle4.com/review-history/73222

Original Research Article

Received 06 July 2021
Accepted 16 September 2021
Published 18 September 2021

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

---

Corresponding author: E-mail: anamul71@bau.edu.bd;

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 accumulates 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, and10]. 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 m^2. 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. I_0-control (normal irrigations), I_1-water deficit stress at vegetative stage, I_2-water deficit stress at flowering stage and I_3-water deficit stress at vegetative and flowering stages. The 0, 25 and 50mM exogenous proline was applied, denoted as P_0, P_{25} and P_{50}. However, there were twelve treatment combinations, viz. T_1= I_0P_0; T_2= I_0P_{25}; T_3= I_0P_{50}; T_4= I_1P_0; T_5= I_1P_{25}; T_6= I_1P_{50}; T_7= I_2P_0; T_8= I_2P_{25}; T_9= I_2P_{50}; T_{10}= I_3P_0; T_{11}= I_3P_{25} and T_{12}= I_3P_{50}.

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 Porra *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 acid 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 2^0C 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 contained50 mM Tris–HCl (pH 8.0), 0.25 mM EDTA, 20 mM H_2O_2 and 25 ml of sample solution. The reaction was started by the addition of H_2O_2. 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 KH_2PO_4 buffer (pH 7.0), 0.1 mM EDTA, and 0.1 mM H_2O_2, 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 mM^{-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 KH_2PO_4 buffer (pH 7.0), 0.1 mM EDTA, and 0.1 mM H_2O_2, 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 mM^{-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 I_3 and their reduction was highest under this treatment for both the varieties. On the other hand, exogenous application of 25mM and 50mM 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 25mM proline along with no drought stress i.e. treatment combination I_0P_{25} which was statistically similar to control condition (I_0P_0) and I_0P_{50} in BARI Hybrid Maiz-5 and in case of BARI Hybrid Maiz-9, I_0P_{50} gave the highest followed by control condition (I_0P_0) and I_0P_{25}. Cob length and grains per cob was highest in treatment combination I_0P_{50} for both the varieties. Cob diameter was highest in I_0P_{25} followed by I_0P_0 and I_0P_{50} in case of BARI Hybrid Maiz-5 whereas in BARI Hybrid Maiz-9 it was highest in I_1P_{50} followed by I_0P_0, I_0P_{25}, and I_0P_{50}. Thousand grain weight was highest in control condition in case of BARI Hybrid Maiz-9 but BARI Hybrid Maiz-5 showed the highest in case of I_1P_{50}. Highest root dry weight and straw yield was found by application of 25mM 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).

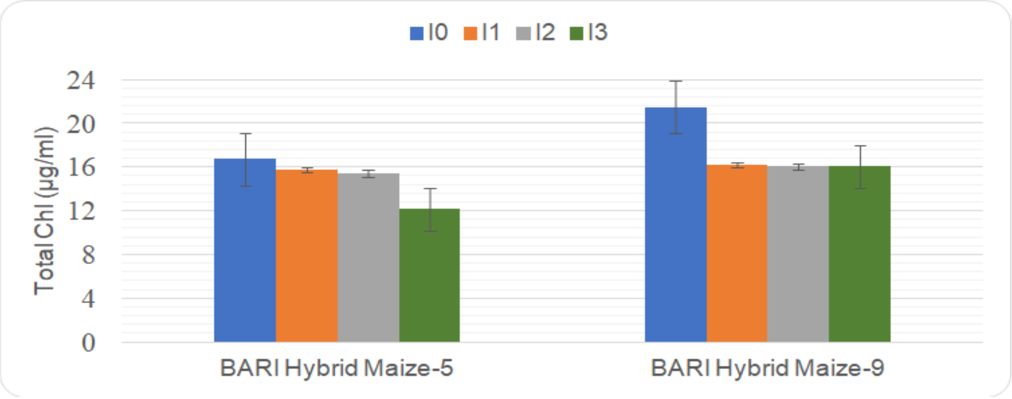

Fig. 1. Effect of water deficit stress on chlorophyll contents

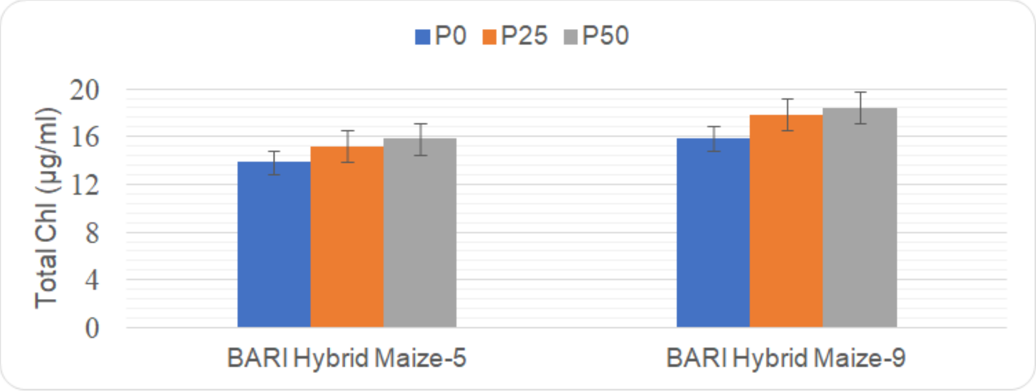

Fig. 2. Effect of exogenous proline on chlorophyll contents

Table 1. Effect of water deficit stress on the growth and yield components of maize

Water deficit stress	**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)**
I_0	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
I_1	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
I_2	142.1b	-	-	-	-	154.2 b	-	-	-	-
I_3	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

I_0-control (normal irrigations), I_1-water deficit stress at vegetative stage, I_2-water deficit stress at flowering stage and I_3-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	**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)**
$\mathbf{P_0}$	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
$\mathbf{P_{25}}$	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
$\mathbf{P_{50}}$	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(±)	1.29	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

P_0= No exogenous proline application, P_{25}= 25 mM exogenous proline application and P_{50}=50mM 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)
I_0P_0	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
I_0P_{25}	170.7a	10.53c	4.200 a	285.0 b	15.95ab	163.3 b	11.67 b	3.467ab	259.0 b	16.07bc
I_0P_{50}	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
I_1P_0	116.7d	9.23e	3.370 d	221.3 d	13.94c	135.3 d	10.50 c	3.370b	200.3 c	15.77c
I_1P_{25}	129.3c	10.07d	3.670 c	249.7 c	15.17b	134.3 d	9.80 d	3.133c	158.3 d	16.77ab
I_1P_{50}	145.0b	9.40e	3.470 d	182.7 e	16.65a	141.7 d	10.27 c	3.600a	148.3 e	13.20d
I_2P_0	141.3b	-	-	-	-	152.0 c	-	-	-	-
I_2P_{25}	142.7b	-	-	-	-	144.0cd	-	-	-	-
I_2P_{50}	142.3b	-	-	-	-	166.7 b	-	-	-	-
I_3P_0	108.3e	-	-	-	-	96.33 e	-	-	-	-
I_3P_{25}	106.3e	-	-	-	-	101.0 e	-	-	-	-
I_3P_{50}	102.3e	-	-	-	-	99.33 e	-	-	-	-
SE(±)	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)
I_0	3.923a	45.77a	44.72a	2.720a	47.72a	42.17a
I_1	1.603b	33.22b	28.78b	1.777b	34.23b	31.60b
I_2	1.166c	29.64c	ND	1.130c	31.37c	ND
I_3	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

I_0-control (normal irrigations), I_1-water deficit stress at vegetative stage, I_2-water deficit stress at flowering stage and I_3-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	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)
P_0	1.819b	28.36c	16.96c	1.580b	29.22c	17.22c
P_{25}	1.928a	30.58b	18.55b	1.673a	31.55b	18.52b
P_{50}	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	4.60	1.91	4.21

P_0= No exogenous proline application, P_{25}= 25 mM exogenous proline application and P_{50}=50mM 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	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)
I_0P_0	3.89b	45.76a	43.95b	2.72 a	47.67a	41.72a
I_0P_{25}	3.95a	45.80a	44.69ab	2.71 a	47.74a	41.89a
I_0P_{50}	3.93ab	45.74a	45.51a	2.73 a	47.75a	42.91a
I_1P_0	1.45d	28.65ef	23.88e	1.65 bc	31.39d	27.17d
I_1P_{25}	1.69c	33.64c	29.52d	1.84 b	34.77c	32.19c
I_1P_{50}	1.67c	37.37b	32.95c	1.84 b	36.52b	35.45b
I_2P_0	1.07f	26.47f	ND	1.04 e	27.96f	ND
I_2P_{25}	1.20e	30.35de	ND	1.12 de	30.14e	ND
I_2P_{50}	1.23e	32.09cd	ND	1.23 d	36.01b	ND
I_3P_0	0.870g	12.56g	ND	0.910 f	9.84h	ND
I_3P_{25}	0.870g	12.53g	ND	1.02 e	13.56g	ND
I_3P_{50}	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)
I_0P_0	6.41cdef	10.37a	16.65a	6.41f	7.05ab	14.10b	21.15b	6.15g
I_0P_{25}	6.71bc	10.19a	16.90a	6.50f	6.62bcde	14.65ab	21.27b	6.29g
I_0P_{50}	6.84b	9.910ab	16.75a	6.65f	6.95abc	15.04a	21.99a	6.92f
I_1P_0	6.19f	8.470d	14.66c	7.53d	5.90f	8.76e	14.66f	7.06f
I_1P_{25}	6.68bc	9.140c	15.82b	7.99c	6.74bcd	9.49de	16.21e	7.41de
I_1P_{50}	6.59bcd	10.30a	16.89a	8.44b	7.39a	10.10cd	17.49cd	7.96c
I_2P_0	6.56bcde	8.12d	14.68c	6.69f	6.34de	7.31f	13.65g	7.23ef
I_2P_{25}	6.25ef	9.42bc	15.67b	7.15e	6.76bcd	10.26cd	17.02d	7.86c
I_2P_{50}	6.30def	9.50bc	15.80b	7.81c	7.00abc	10.60c	17.60c	7.88c
I_3P_0	5.32g	4.53g	9.85e	6.98e	6.20ef	7.94f	14.14fg	7.64cd
I_3P_{25}	6.62bcd	5.70f	12.32d	8.63b	6.74bcd	10.28cd	17.02d	8.44b
I_3P_{50}	7.45a	6.64e	14.09c	9.30a	6.55cde	10.45c	17.00d	8.78a
SE(±)	0.103	0.175	0.203	0.094	0.139	0.270	0.181	0.103
CV(%)	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.

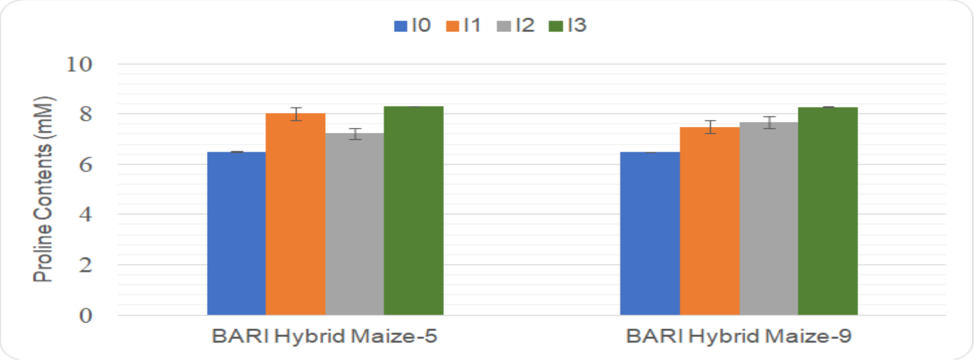

Fig. 3. Effect of water deficit stress on proline contents

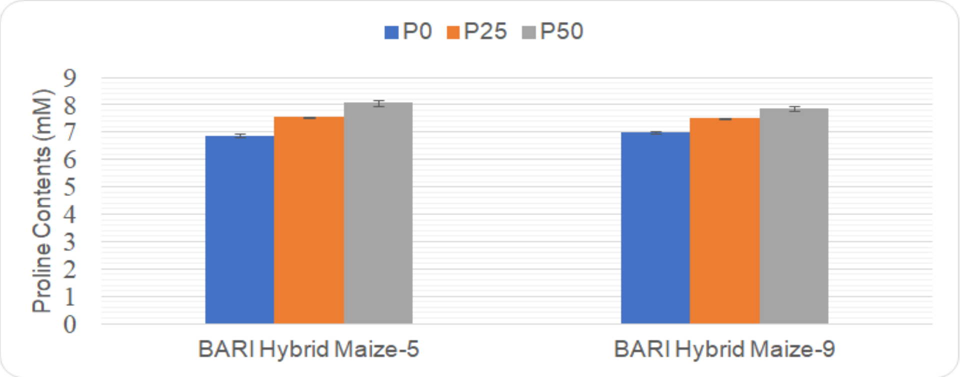

Fig. 4. Effect of exogenous proline on proline contents

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)
I_0	6.167a	100.7c	4.734c	4.756a	68.67c	3.340d
I_1	5.633b	117.3a	5.577a	4.433b	75.67a	4.267a
I_2	5.067c	112.3b	5.183b	4.400b	72.00b	4.113b
I_3	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	3.58	1.72	3.72

I_0-control (normal irrigations), I_1-water deficit stress at vegetative stage, I_2-water deficit stress at flowering stage and I_3-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)
P_0	5.475c	99.25c	4.722b	3.642b	62.00b	3.530b
P_{25}	6.150b	105.5b	5.210a	4.500a	72.50a	4.017a
P_{50}	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	3.58	1.72	3.72

P_0= No exogenous proline application, P_{25}= 25 mM exogenous proline application and P_{50}=50mM 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)**
I_0P_0	5.90cd	98.00f	4.71cd	4.47b	64.00f	3.28f
I_0P_{25}	6.20bcd	101.00e	4.71cd	4.90a	71.00d	3.34ef
I_0P_{50}	6.40ab	103.00e	4.78c	4.90a	71.00d	3.40ef
I_1P_0	5.30f	112.00c	5.04c	3.80c	67.00e	3.85d
I_1P_{25}	5.80de	118.00b	5.84a	4.80a	79.00ab	4.38ab
I_1P_{50}	5.80de	122.00a	5.85a	4.70ab	81.00a	4.57a
I_2P_0	5.40ef	106.00d	4.42d	3.60cd	64.00f	3.57e
I_2P_{25}	6.30abc	114.00c	5.42b	4.80a	75.00c	4.35ab
I_2P_{50}	6.50ab	117.00b	5.71ab	4.80a	77.00bc	4.42ab
I_3P_0	5.30f	81.00i	4.71cd	2.70e	53.00g	3.42ef
I_3P_{25}	6.30abc	89.00h	4.87c	3.50d	65.00ef	4.00cd
I_3P_{50}	6.70a	92.00g	4.94c	3.60cd	63.00f	4.17bc
SE(±)	0.134	0.894	0.108	0.087	0.685	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]; Nounjan 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]. Sofy 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. Nounjan 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_2O_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_2O_2, a dominant and detrimental oxidizing factor to H_2O 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_2O_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_2O_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.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Bangladesh Agricultural University Research System, Bangladesh.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lea PJ, Parry MAJ, Medrano H. Improving resistance to drought and salinity in plants. Annal. App. Biol. 2004;144:249-250.
2. Asadi M, Reza HE (2021). Response of sorghum genotypes to water deficit stress under different CO2 and nitrogen levels,Plant Physiology and Biochemistry,158, Pages 255-264.
3. Earl HJ, Davis RF. Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize. Agron J. 2003; 95(3):688–696.
4. Ashraf M, Harris PJC. Photosynthesis under stressful environments: An overview. Photosynthetica. 2013; 51:163–190.
5. Kosar F, Akram NA, Ashraf M. Exogenously-applied 5-aminolevulinic acid modulates some key physiological characteristics and antioxidative defense system in spring wheat (*Triticum aestivum* L.) seedlings under water stress. S Afr J Bot .2015;96:71–77.
6. Akram NA, Shafiq S, Ashraf M, Aisha R, Sajid MA. Drought induced anatomical changes in radish (*Raphanus sativus* L.) leaves supplied with trehalose through different modes. Arid Land Res Manag. 2016;30(4):412–420.
7. Claasen, M.M., Shaw, R.H. Water deficit effects on grain. II. Grain components. Agron. J. 1970;62, 652–655.
8. Shafiq S, Akram NA, Ashraf M. Does exogenously-applied trehalose alter oxidative defense system in the edible part of radish (*Raphanus sativus* L.) under water-deficit conditions? Sci Hort.2015; 185:68–75.
9. Jiang XM, Shiran B, Wan JL, Lewis DC, Jenkins CLD, Condon AG, Richards RA, Dolferus R. Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. Plant, Cell and Environment.2010;33:926-942.
10. Liang X, Zhang L, Natarajan SK, Becker DF. Proline mechanisms of stress survival. Antioxidants & Redox Signaling. 2013; 19:998-1011.
11. Arora A, Sairam RK, Srivastava GC. Oxidative stress and antioxidant systems in plants. Current Science.2002;82:1227-1238.
12. Shao B, Yuxiu Y, Chen X, Wanq Z, Yanq J, Zhu Q, Zhanq H. Effect of water stress during grain filling on the grain yield & quality of two-line hybrid rice. Journal of Yanq Zhau University, Aaricultural and Life Sciences.2004;25(1):46-50.
13. Yadav RS, Gayadin AK, Jaiswal AK. Morphophysiological changes variable yield of wheat genotypes under moisture stress conditions. Indian Journal of Plant Physiology.2001;5(4): 390-394.
14. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot.2012; 1:26.
15. Verbruggen N, Hermans C. Proline accumulation in plants: a review. Amino Acids.2008;35(4):753–759.
16. Chun SC, Paramasivan M and Chandrasekaran M. Proline Accumulation Influenced by Osmotic Stress in Arbuscular Mycorrhizal Symbiotic Plants. Front. Microbiol. 2018;9:2525.
DOI: 10.3389/fmicb.2018.02525
17. Nayyar H, Walia DP. Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and absisic acid. Biologia Plantarum.2003;46:275-279.

18. Ozturk L, Demir Y. In vivo and In vitro protective role of proline. Plant Growth Regulation.2002;38: 259-264.
19. Peymaninia Y, Valizadeh M, Shahryari R, Ahmadizadeh M. Evaluation of morpho-physiological responses of wheat genotypes against drought stress in presence of a leonardite derived humic fertilizer under greenhouse condition. The Journal of Animal and Plant Sciences.2012;22(4):1142-1149.
20. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany.2007;59;206–216.
21. Farooq, M M. Hussain, H. Rehman, K. Jabran and M.B. Khan. Glycine betaine improves chilling tolerance in hybrid maize. J. Agron. Crop Sci..2008;194:152-160.
22. Ali Q, Ashraf M. Induction of drought tolerance in maize (Zea mays L.) due to exogenous application of trehalose: growth, photosynthesis, water relations and oxidative defense mechanism. J. Agron. Crop Sci.2011; 10:1439-48.
23. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil.1973:205–207.
24. Aebi H. Catalase. Methods in Enzymology.1984;105:121–126.
25. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology.1981;22:867–880.
26. Gomez KA, Gomez AA. Statistical procedures for agricultural research. A Wiley International Science Published by John Wiley and Sons New work, Brisbane Singapore.1984,139-240.
27. Bhusan D, Das DK, Hossain M, Murata Y, Hoque MA. Improvement of salt tolerance in rice (Oryza sativa L.) by increasing antioxidant defense systems using exogenous application of proline. Australian Journal of Crop Science. 2016;10(1): 50-56
28. Hoque MA, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y. Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. Journal of Plant Physiology.2008;165:813–824.
29. Banu MNA, Hoque MA, Sugimoto-Watanabe M, Mastuoka K, Nakamura Y, Shimoishi Y, Murata Y. Proline and glycine betaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress. Journal of Plant Physiology.2009;166:146–156.
30. Hossain MA, Fujita M. Evidence for a role of exogenous glycinebetaine and proline in antioxidant defense and methylglyoxal detoxification systems in mung bean seedlings under salt stress. Physiol Mol Biol Plants. 2010; 16:19–29.
31. Ahmed CB, Magdich S, Rouina BB, Sensoy S, Boukhris M, Abdullah FB. Exogenous proline effects on water relations and ions contents in leaves and roots of young olive. Amino Acids. 2011; 40:565–573.
32. Nounjan N, Nghia PT, Theerakulpisut P. Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. Journal of Plant Physiology.2012;169(6):596-604.
33. Hasanuzzaman M, Alam MM, Rahman A, Hasanuzzaman M, Nahar K, Fujita M. Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt induced oxidative stress in two rice (Oryza sativa L.) varieties. Biomed Res Int.Vol.2014.
34. Devar, M.; Jafar, A.; Marefat, G.; Mohsen, H. and Ali, K. Is salinity tolerance related to Na accumulation in maize cultivars (*Zea mays* L.) *Life Science Journa.2013;*10(1s):72-75.
35. Taie HAA, Abdelhamid MT, Dawood MG, Nassar RMA. Pre-sowing seed treatment with proline improves some physiological, biochemical and anatomical attributes of faba bean plants under sea water stress. Journal of Applied Sciences Research. 2013;9(4):2853–2867.
36. El-Beltagi, HS, Mohamed HI and Sofy, MR. Role of ascorbic acid, glutathione and proline applied as singly or in sequence combination in improving chickpea plant through physiological change and antioxidant defense under different levels of irrigation intervals. Molecules 2020, 25, 1702.
37. Ghafoor R, Akram NA, Rashid M, Ashraf M, Iqbal M, Lixin Z. Exogenously applied proline induced changes in key anatomical features and physiobiochemical attributes

in water stressed oat (*Avena sativa* L.) plants. Physiol Mol Biol Plants. 2019; 25:1121–35.

38. Dolatabadian A, Modarres SAM, Sharifi M. Effect of salicylic acid and salt on wheat seed germination. Acta Agric Scand Sect B Soil Plant Sci. 2009;59:456–464.
39. Moaveni P (2011) Effect of water deficit stress on some physiological traits of wheat (*Triticum aestivum* L.). Agric Sci Res J 1:64–68
40. Pattanagul W. Exogenous abscisic acid enhances sugar accumulation in rice. Asian J Plant Sci. 2011;10(3):212–219.
41. Shafiq S, Akram NA, Ashraf M, Arshad A (2014) Synergistic effects of drought and ascorbic acid on growth, mineral nutrients and oxidative defense system in canola (Brassica napus L.) plants. Acta Physiol Plant 36:1539–1553.
42. Sofy, Mahmoud R; Seleiman, Mahmoud F; Alhammad, Bushra A; Alharbi, Basmah M; Mohamed, Heba I. Minimizing Adverse E_ects of Pb on Maize Plants by Combined Treatment with Jasmonic, Salicylic Acids and Proline. Agronomy.2020;10(5):699.
43. Flexas J, Bota J, Cifre J. Understanding down regulation of photosynthesis under water stress, future prospects and searching for physiological tools for irrigation management. Annals of Applied Biology.2004;273-283.
44. Athar HR, Ashraf M. Photosynthesis under drought stress. In: Handbook of Photosynthesis, (Ed.): M. Pessarakli, CRC Press, Taylor and Francis Group, NY.2005;793-804.
45. Ali Q, Ashraf M, Athar HUR. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. Pak J Bot.2007;39:1133–1144
46. Deivanai S, Xavier R, Vinod V, Timalata K,Lim OF. Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. Journal of Stress Physiology and Biochemistry. 2011; 7(4):157-174.
47. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel, J, Ahmad, A. Role of proline under changing environments: A review. Plant. Signal. Behav. 2012, 7:1456–1466.
48. De Freitas, P.A.F.; de Souza Miranda, R.; Marques, E.C.; Prisco, J.T.; Gomes Filho, E. Salt tolerance induced by exogenous proline in maize is related to low oxidative damage and favorable ionic homeostasis. J. Plant Growth Regul. 2018; 37, 911–924.
49. Desoky, E.M.; Merwad, A.M.; Rady, M.M. Natural biostimulants improve saline soil characteristics and salt stressed-sorghum performance. Commun. Soil Sci. Plant Anal. 2018, 49:967–983.
50. Radhakrishnan, R.; Lee, I. Spermine promotes acclimation to osmotic stress by modifying antioxidant, abscisic acid, and jasmonic acid signals in soybean. J. Plant Growth Regul. 2013, 32:22–30.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/73222