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ORIGINAL ARTICLE

Mitigating water stress in wheat (BARI Gom-26) by exogenous application of proline

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ABSTRACT

Wheat, the important cereal crop of Bangladesh, when exposed to drought stress, a significant yield reduction may occur. Literatures showed that proline protects plants against different abiotic stresses like drought. Therefore the present experiment was aimed to investigate the improvement of drought tolerance in wheat by exogenous application of proline. Wheat variety cv. BARI Gom-26 was used as test crop in this study. Treatment combinations consisted of four levels of irrigation (normal irrigation, irrigation missing at vegetative stage, irrigation missing at flowering stage, and irrigation missing at both vegetative and flowering stages) and three concentrations of proline (0, 25 and 50 mM). The results revealed that water stress caused significant reductions in growth, and grain and straw yields of wheat. Water stress also reduced chlorophyll and proline contents, and activities of antioxidant enzymes catalase (CAT), guaiacol peroxidase (POX) and ascorbate peroxidase (APX). On the other hand, exogenous application of proline showed a significant increase in growth and yield of wheat under water stress. These increases were positively correlated with increased levels of chlorophyll and intracellular proline, and enhanced activities of CAT, POX and APX in wheat. The interaction effects of exogenous proline and water stress were significant in aspects of increased plant growth and yield, and enhanced levels of chlorophyll and intracellular proline as well as activities of antioxidant enzymes. Application of 50 mM proline was found to be more effective in improving water stress tolerance. The present study, therefore, suggests that exogenous proline confers tolerance to drought stress in wheat by increasing proline accumulation and antioxidant defense system.

INTRODUCTION

Drought is one of the major abiotic stresses worldwide, limiting crop productivity (Araus et al. 2002). Wheat (Triticum aestivum) is the second most important cereal crop in Bangladesh. Wheat is increasingly cultivated in many areas of the country. Wheat yield potential has been increasing at an annual rate of approximately 0.90% over the last 30 years. Drought is a significant yield-limiting factor in wheat production as this crop is grown during rabi season (Farhad et al. 2015). Water deficit conditions reduce photosynthetic capacity of plants by stomatal closure or metabolic impairments of proteins associated with photosystem and chlorophyll (Athar and Ashraf 2005; Chaves et al. 2009; Pirzad et al. 2011), resulting in lower CO_2 intake (Lawlor and Tezara 2009).

Plants have evolved a variety of adaptive mechanisms to respond to drought stress. One of the main adaptive mechanisms to drought in plants is the accumulation of proline. Increased levels of proline accumulated in plants were correlated with improved drought tolerance (Mani et al. 2002; Yamada et al. 2005). Proline protects plants against various stresses by stabilizing membranes, proteins and enzymes, and preventing photo inhibition (Ashraf and Foolad 2007; Hoque et al. 2008). There are evidences that exogenously applied proline improves abiotic stress tolerance in plants. There is also a report that high concentration of proline may be detrimental to plants, including inhibitory effects on growth or deleterious effects on cellular metabolisms (Nanjo et al. 2003). Exogenous application of proline in low concentration decreased the K^+ efflux from the barley root under salt stress (Cuin and Shabala 2005).

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The protective mechanisms of proline have been increasingly reported in the literature in plants. Ali et al. (2012) has demonstrated that exogenously applied proline improves seed composition, oil quality and antioxidant activity of maize under drought stress. Water stress negatively affected growth and yield attributes of garden cress plants while exogenous proline enhanced the tolerance of crop against water stress (Khalil and El-Noemani 2012). Drought tolerance could be related to increased antioxidant enzyme activities and proline content (Tatari et al. 2012). Kamran et al. (2009) observed that water stress reduced the growth and yield of wheat cultivars whereas exogenous application of proline as pre-sowing seed treatment improved growth and yield of wheat under stress conditions. Water stress-induced reduction in photosynthesis has been ameliorated in maize cultivars by exogenous application of proline (Ali et al. 2007). We have shown that exogenous proline improved salinity and cadmium tolerances and suppressed cell death in cultured tobacco cells by decreasing reactive oxygen species (ROS) accumulation and increasing antioxidant defense systems (Banu et al. 2010; Hoque et al. 2007ab; Islam et al. 2009ab).

From the above mentioned discussions, it can be easily understood that water stress condition caused a significant yield reduction of wheat and proline plays a vital role in minimizing the damage caused by water stress condition. So, the main aim of this study was to elucidate the role of proline in drought tolerance mechanism at physiological and biochemical levels. As wheat is an important cereal crop in Bangladesh and its production is affected by drought stress, the experiments were conducted with a view to observe the effect of exogenous proline on minimizing the damage caused by drought stress.

METHODOLOGY

Soil Collection and Pot Preparation

Pot experiments were carried out at the net house of the Department of Soil Science, Bangladesh Agricultural University, Mymensingh. Soils were collected from 0-15 cm depth from Bangladesh Agricultural University farm. Each plastic pot was prepared with 8 kg soils. The soil was silt loam having pH 6.15, electrical conductivity 0.17 dSm-1, exchangeable Na 0.35 meq 100 g-1 soil, total N 0.11% and organic matter 1.90%.

Plant Materials and Treatments

Wheat cultivar viz. BARI Gom-26 was used as plant materials in this experiment. Treatment combinations were the different levels of irrigation and proline. There were four levels of irrigation viz. I0-control (normal irrigations), I1-water stress at vegetative stage (irrigation missing at vegetative stage), I2-water stress at flowering stage (irrigation missing at flowering stage) and I3- water stress at vegetative and flowering stages (irrigation missing at both vegetative and flowering stages). There were three levels of proline (0, 25 and 50 mM) and denoted as P_0, P_{25} and P_{50}. Different doses of proline containing 0.1% Tween-20 were sprayed on the leaves at a volume of 25 mL per plant as per treatments at vegetative and reproductive stages. The experiment was laid out in a completely randomized design with four replications.

Management Practices, Crop Harvesting and Data Recording

Tape water was used as irrigation. Fertilizations and other management practices were performed as and when required based on BARC fertilizer Recommendation Guide 2012. At 15 days after proline application, healthy green leaves were collected from wheat plants to determine chlorophyll and proline contents, and activity of antioxidant enzymes. The crop was harvested at full maturity. The maturity of crop was determined when about 90% grains became golden yellow. Grain and straw yields and plant parameters were recorded.

Assay of Chlorophyll Contents

Chlorophyll content was measured according to Porra et al. (1989). An aliquot amount of fresh green leaf 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. The absorbance was recorded at 645 nm and 663 nm using a spectrophotometer (model 336001, Spectronic Instruments, USA).

Assay of Intracellular Proline Contents

Proline content was determined following the method of Bates et al. (1973). An aliquot amount of fresh green leaf was homogenized in 10 mL of 3% sulfosalicylic acid and then the homogenate was centrifuged at 5000 rpm for 15 min. Other details of the assay were followed as described by Islam (Islam et al. 2009a).

Preparation of Enzyme Extract and Assay of Antioxidant Enzymes

An aliquot amount of fresh green leaf was homogenized with 5 mL of 50 mM Tris–HCl buffer (pH 8.0) for CAT, and 50 mM KH_2PO4 buffer (pH 7.0) for POX and APX. The homogenate was centrifuged at 5000 rpm for 20 min and the supernatant was then used as enzyme extract. CAT (EC: 1.11.1.6) activity was assayed as described by Islam et al. (2009a). POX (EC: 1.11.1.7) and APX (EC: 1.11.1.11) activities were assayed as described by Hoque et al. (2007b).

Statistical Analysis

Data were analyzed statistically using analysis of variance with the help of MSTAT-C software package. The significant differences between mean values were compared by Duncan's Multiple Range Test as described by Gomez and Gomez (1984). Differences at $p \leq 0.05$ were considered significant.

RESULTS

Effect of Proline on Growth and Yield of Wheat Varieties under Water Stress

Plant height

Water stress caused a significant decrease in plant height of BARI Gom-26 (Table 1). Application of proline (25 and 50 mM) resulted in significant increases of plant height of wheat (Table 2). Application of 50 mM proline produced the tallest plants. The interaction effects of exogenous proline and water stress on plant height were also significant.

Spike length

A reduction of spike length in wheat (Table 1) was observed in response to water stress. The reduction was insignificant in BARI Gom-26. An increased spike length was observed due to application of proline in wheat (Table 2) but this increase was insignificant in BARI Gom-26.

Number of spikelet spike^{-1}

Water stress also caused a significant decrease in number of spikelet per spike of wheat (Table 1). Foliar application of proline resulted in increases in number of spikelet of wheat (Table 2).

Grains spike^{-1}

Plants exposed with varying levels of water stresses significantly decreased grains in BARI Gom-26 (Table 1). Application of proline significantly increased grains in wheat (Table 2). It is also noted that 50 mM proline application produced higher numbers of grains.

100-grain weight

The 100-grain weight was significantly decreased due to water stress in wheat (Table 1). Proline application significantly increased 100-grain weight of BARI Gom-26 (Table 2). Plants treated with 50 mM proline increased 100-grain weight in wheat under water stress.

Root biomass

Water stress caused a significant decrease in dry root weight of BARI Gom-26 (Table 1). Application of proline significantly increased dry root weight of wheat under water stress condition (Table 2). The highest dry root weight was observed in 50 mM proline-treated plants in wheat.

Straw yield

A drastic decrease in straw yield was observed due to water stress in BARI Gom-26 (Table 1). Application of proline significantly increased straw yield wheat (Table 2). The highest straw yield was found in BARI Gom-26 due to the foliar application of 50 mM proline.

Grain yield

A significant decrease in grain yield of BARI Gom-26 (Table 1) varieties was observed in response to water stress. Application of proline significantly increased grain yield of wheat (Table 2). The highest grain yield was observed in wheat due to the foliar application of 50 mM proline under water stress.

Table 1. Effect of water stress on growth and yield of BARI Gom-26

Treatments	Plant height	Spike length	Spikelet spike^{-1} (no.)	Grains Spike^{-1} (no.)	100-grain weight (g)	Dry root weight (g)	Straw yield (g pot^{-1})	Grain yield (g pot^{-1})
I_0	65.67a	5.39	10.00a	31.33a	5.24ab	1.27a	15.00a	6.52a
I_1	58.04b	5.12	9.67b	27.33b	5.34a	1.09b	13.33b	5.78b
I_2	56.83c	5.00	9.00c	27.00bc	5.08b	0.88bc	10.00c	3.48c
I_3	51.89d	5.08	8.67d	22.00c	3.65c	0.72c	9.00d	3.04d
SE±	1.23	0.12	0.12	0.77	0.15	0.04	0.54	0.26
CV (%)	6.40	2.89	4.21	4.96	3.94	4.92	4.26	7.86

In a column, figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT) at 5% level of probability. SE= Standard Error and CV= Coefficient of variation

Table 2. Effect of exogenous proline on growth and yield of BARI Gom-26

Treatments	Plant height	Spike length	Spikelet spike^{-1} (no.)	Grains Spike^{-1} (no.)	100-grain weight (g)	Dry root weight (g)	Straw yield (g pot^{-1})	Grain yield (g pot^{-1})
P_0	52.52c	5.04	9.00b	24.00c	4.68b	0.93b	10.75b	4.28c
P_{25}	60.02b	5.12	9.50a	27.83b	4.91a	1.02a	12.25ab	4.81b
P_{50}	61.78a	5.29	9.50a	28.92a	4.89a	1.03a	12.50a	5.02a
SE±	0.20	0.32	0.03	0.96	0.31	1.13	0.16	0.89
CV (%)	6.40	2.89	4.21	4.96	3.94	4.92	4.26	7.86

In a column, figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT) at 5% level of probability. SE= Standard Error and CV= Coefficient of variation.

Effects of Proline on Biochemical Attributes of Wheat under Water Stress

Chlorophyll contents

Water stress caused a significant reduction in chlorophyll contents of BARI Gom-26 (Figure 1). Conversely, application of proline significantly increased total chlorophyll contents in wheat under water stress conditions (Figure 2). The highest chlorophyll content in BARI Gom-26 was observed with proline application in normal irrigation condition (Table 3).

Table 3. Chlorophyll, proline and antioxidant enzymes activities of BARI Gom-26

Treatments	Chlorophyll (µg mL^{-1})	Proline (mM)	CAT (mmol min^{-1} g^{-1} FW)	POX (µmol min^{-1} g^{-1} FW)	APX (µmol min^{-1} g^{-1} FW)
I_0P_0	21.16b	3.93bc	2.53cd	95.50d	14.80def
I_0P_{25}	23.27a	4.48ab	2.47d	121.20a	14.60def
I_0P_{50}	24.01a	4.49ab	2.46d	121.00a	13.70ef
I_1P_0	14.67e	2.19e	3.24b	94.50d	12.00f
I_1P_{25}	16.22de	2.76de	3.89a	103.20c	24.40a
I_1P_{50}	18.50c	5.12a	3.88a	109.70b	25.10a
I_2P_0	18.89c	3.77bcd	3.29b	43.30h	18.90bcd
I_2P_{25}	18.40cd	4.34ab	3.49b	49.20g	23.10ab
I_2P_{50}	17.08cd	4.79ab	3.57ab	62.50f	24.30a
I_3P_0	17.96cd	3.08cde	2.74c	37.30i	16.30c-f
I_3P_{25}	18.85c	3.93bc	3.12bc	64.00f	18.60b-e
I_3P_{50}	17.22cd	4.26ab	3.20b	69.30e	20.30abc
SE (±)	0.16	0.03	0.96	0.31	0.89
CV (%)	4.26	4.21	21.86	3.94	7.86

In a column, figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT) at 5% level of probability. SE= Standard Error and CV= Coefficient of variation

Intracellular proline contents

Water stress caused a significant reduction in intracellular proline content in BARI Gom-26 (Figure 3). Application of proline significantly increased intracellular proline contents in wheat (Figure 4). The highest intracellular proline content was found in wheat by application of 50 mM proline (Table 3).

Effects of proline on the activities of antioxidant enzymes

Water stress significantly decreased the activities of CAT, POX and APX in BARI Gom-26 (Figure 5). CAT, POX and APX activities increased due to the application of proline in wheat (Figure 6). Most cases, the highest CAT, POX and APX activities were observed by foliar application of 50 mM proline. POX and APX activities were also increased due to the interaction of exogenous proline and water stress in wheat (Table 3). CAT activity was also increased by proline application under water stress conditions but this increase was not significant and exogenous application of 50 mM proline showed a higher POX and APX activities in wheat under water stress conditions.

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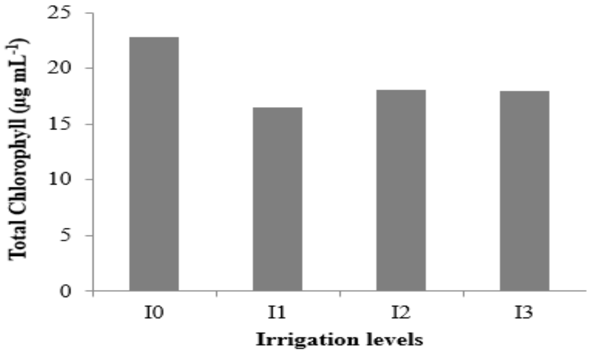

Figure 1. Effect of water stress on total chlorophyll content

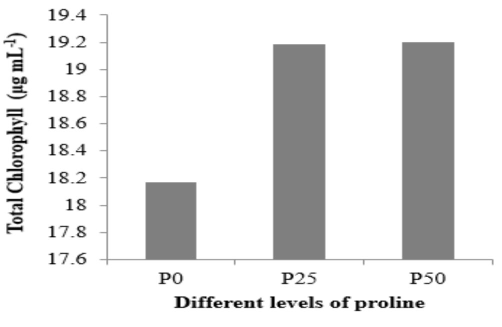

Figure 2. Effect of proline on total Chlorophyll content

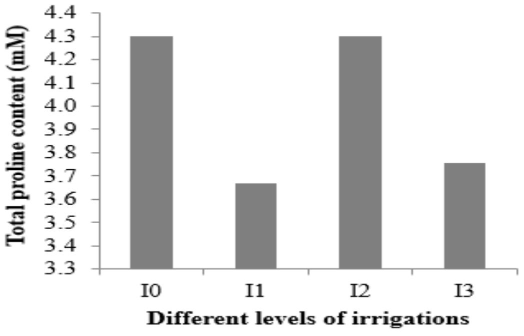

Figure 3. Effect of water stress on proline content

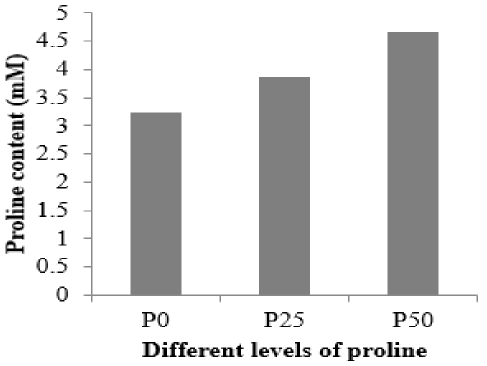

Figure 4. Effect of exogenous proline on intracellular proline content

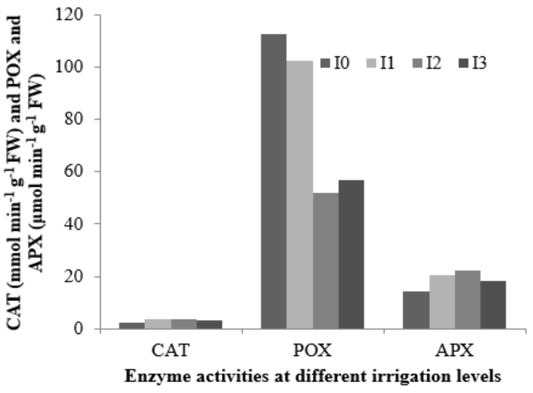

Figure 5. Effects of water stress on the activities of antioxidant enzymes of wheat

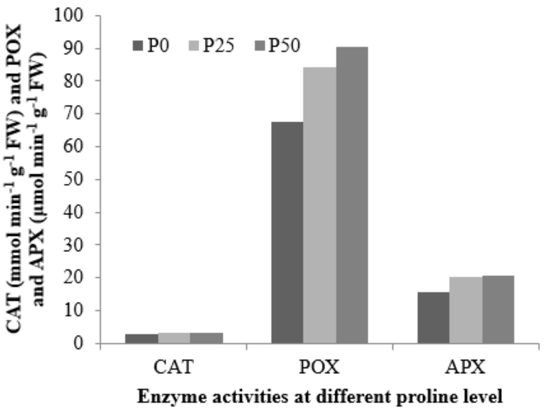

Figure 6. Effects of proline on the activities of antioxidant enzymes of wheat

DISCUSSION

The protective role of proline against water stress in plants was widely reported. We previously reported that exogenous proline suppressed cell death and improved drought tolerance in wheat under field condition (Farhad et al. 2015). In the present study, we clarified the protective effects of exogenous proline on wheat against water stress (Table 1 and 2). A large body of evidences reported that exogenous proline improved growth of wheat plants including rice in response to water stress (Hasanuzzaman et al. 2014). Talat et al. (2013) also reported that exogenous application of proline increased dry root weight of wheat. The present findings are similar to some earlier studies in which foliar applied proline alleviated the adverse effects of water stress on the growth and/or yield of wheat (Kavi Kishore et al. 1995). It was found that water stress in wheat growth can be mitigated by exogenous application of 50 mM proline.

Chlorophyll is one of the most important pigment components, providing photosynthetic ability of a plant. Chlorophyll content varies due to water stress condition, eventually affecting plant growth and development (Table 3). The decreased chlorophyll content under drought condition could be due to impaired biosynthesis or increased pigment degradation. Drought induced reduction in photosynthesis can also be attributed to decrease in chlorophyll content (Baker et al. 2007). In this experiment, water stress caused a significant reduction in chlorophyll contents of BARI Gom-26 (Figure 1) varieties. Conversely, application of proline significantly increased total chlorophyll contents in wheat under water stress conditions (Figure 2). Photosynthetic pigments like chlorophyll a and b decreased in both maize cultivars due to water stress, which is in agreement with some previous studies on different crops such as Vicia faba (Gadallah 1999), wheat (Waseem et al. 2006) and canola (Kauser et al 2006). These results indicated that increased growth of rice plants during water stress in the presence of proline and we found that if we apply 50 mM exogenous proline, it can successfully tolerate water stress conditions.

The increased accumulation of proline in plants was correlated with improved stress tolerance (Hasanuzzaman et al. 2014). Our previous studies in wheat under field condition showed that exogenous proline increased intracellular proline levels and improved drought tolerance (Farhad et al. 2015), indicating that accumulation of proline played pivotal role in adaptation to drought stress. Drought stress caused a significant reduction in intracellular proline content in BARI Gom-26 (Figure 3). Application of proline significantly increased intracellular proline contents in wheat (Figure 4). Nounjan and Theerakulpisut (2012) also showed that exogenous application of proline increased endogenous proline in Thai aromatic rice pants. Proline was suggested to function as an antioxidant in protecting cells against various abiotic stresses since proline scavenged free radicals and suppressed ROS accumulation (Banu et al. 2010; Hoque et al. 2007ab).

Increased antioxidant defense mechanism was positively associated with the decreased oxidative damage and improved stress tolerance in plants (Hasegawa et al. 2000; Mittova et al. 2003). In order to elucidate the role of proline in antioxidant defense mechanism and antioxidant enzyme activities were measured. The metabolism of H_2O_2 is mainly dependent on the activity of antioxidant enzymes CAT and POXs localized in all compartments of plant cells. APX is considered as a major H_2O_2-scavenging enzyme in plants. We found that water stress significantly decreased the activities of CAT, POX and APX in BARI Gom-26 (Figure 5). CAT, POX and APX activities increased due to the application of proline in wheat (Figure 6). Mittler and Zilinskas (1994) observed that CAT activity increased during drought stress. Nounjan and Theerakulpisut (2012) showed a decrease in CAT activity due to exogenous application of proline and further showed that proline application decreased APX activity.

Overall, drought stress adversely affects the plant biomass, yield attributes and grain yield of wheat cultivar BARI Gom-26. In all of the previous studies, it has been reported that exogenous application of proline protects plant from water stress but not specified the rate and time of application. Here, in this study we found that exogenous application of 50 mM proline at both vegetative and flowering stages confers tolerance to drought stress of wheat by increasing proline accumulation and antioxidant defense mechanisms.

CONCLUSION

Water stress decreased the growth and yield of wheat. Chlorophyll and intracellular proline contents also decreased due to water stress. On the other hand, exogenous application of proline showed a significant increase in growth, and grain and straw yields of wheat under water stress. In most of cases, exogenous proline application increased chlorophyll and intracellular proline contents in wheat during water stress. Additionally, exogenous proline enhanced the activities of CAT, POX and APX enzymes. Overall, foliar application of 50 mM proline was found to be more effective in improving water stress tolerance. It can be concluded that exogenous proline confers tolerance to drought in wheat by increasing chlorophyll and endogenous proline contents, and antioxidant defense systems. Better understanding about the physiological and biochemical functions of proline is important to select and breed plants for plant tolerance mechanisms to drought stress.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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