Reprint ISSN 1923-7766 (Web Version)

International Journal of Experimental Agriculture

(Int. J. Expt. Agric.)

Volume: 5 Issue: 3 September 2015

Int. J. Expt. Agric. 5(3): 8-14 (September 2015)

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ISSN-1923-7766 (Online)
Int. J. Expt. Agric. 5(3):8-14(September 2015)

MITIGATING WATER STRESS IN WHEAT BY FOLIAR APPLICATION OF PROLINE

FARHAD[1], M.G. KIBRIA[1], M.H. MIAN[1], Y. MURATA[2] AND M.A. HOQUE[1*]

[1]Department of Soil Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh;
[2]Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan.

*Corresponding author & address: Md. Anamul Hoque, E-mail: anamul71@yahoo.com
Accepted for publication on 15 August 2015

ABSTRACT

Farhad, Kibria MG, Mian MH, Murata Y, Hoque MA (2015) Mitigating water stress in wheat by foliar application of proline. *Int. J. Expt. Agric.* 5(3), 8-14.

A field experiment was conducted to investigate the improvement of water stress tolerance in wheat by exogenous application of proline. Wheat *cv.* BARI Gom-24 was used as a test crop. The experiment was laid out in a split plot design with three replications. Treatment combinations were the different levels of irrigation and proline. There were four levels of irrigation, *viz.* I_0- control (normal irrigations), I_1- water stress at vegetative stage (irrigation missing at vegetative stage), I_2- water stress at flowering stage (irrigation missing at flowering stage), and I_3- 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) which were denoted as P_0, P_{25} and P_{50}. Irrigation and proline were considered as main plot and sub plot treatments, respectively. Water stress caused significant reductions in growth and yield of BARI Gom-24. This reduction was associated with decreased yield components. Water stress also decreased N, P, K and S uptake by wheat. On the other hand, exogenous application of proline resulted in a significant increase in growth, yield components, and grain and straw yields which were positively associated with increased uptake of N, P, K and S. The study showed that interaction effects of exogenous proline and water stress were significant in aspects of higher growth and yields and increased uptake of N, P, K and S in BARI Gom-24. Foliar application of 50 mM proline was found to be more effective in improving water stress tolerance. This study suggests that exogenous proline confers tolerance to water stress in wheat due to increasing nutrient uptake and probably increasing antioxidant defense systems as well.

Key words: *water stress, proline, nutrient uptake, BARI Gom-24*

INTRODUCTION

Drought is one of the major abiotic stresses in agriculture worldwide, limiting crop productivity (Araus *et al.* 2002). Generally, drought stress reduces growth (Garg *et al.* 2004; Samarah *et al.* 2004) and yield of various crops (Dhillon *et al.* 1995). Drought stress reduces the nutrient uptake in plants (Baligar *et al.* 2001). It is known that low water availability under drought stress generally results in reduced total nutrient uptake, and frequently reduces the levels of mineral nutrients in crop plants (Marschner 1995; Baligar *et al.* 2001). It is well evident that drought-stressed plants exhibit various physiological, biochemical and molecular changes to thrive under water limited conditions (Arora *et al*. 2002). Under various environmental stresses, high accumulation of proline is a characteristic feature of most plants (Rhodes *et al*. 1999; Ozturk and Demir, 2002; Hsu *et al*. 2003; Kavi-Kishore *et al*. 2005). High levels of proline enable a plant to maintain low water potentials.

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. It is the second staple food in Bangladesh. The area, production and yield of wheat in Bangladesh are about 0.35 million hectares, 0.99 million tons and 2.78 t ha^{-1} respectively (BBS 2012). Wheat supplies carbohydrate, protein, minerals and vitamins and is preferable to rice for its higher grain protein content. It contributes significantly towards solving food problem and thereby plays a gainful role in the agro-economy of the country.

Water stress is a significant yield-limiting factor in wheat production as this crop is grown during rabi season. Although wheat is a relatively drought-tolerant species, it produces small fraction of yield potential ranging from 0.8 to 1.5 t ha^{-1} under moisture stress on approximately 60 million hectares of developing countries (Morris *et al*. 1991). At the same time, water deficit is also a limitation to wheat productivity in the developing countries.

Cultural practices are useful strategies to minimize the water deficit and stabilize crop yield. In addition to modifying cultural practices, exogenous application of proline could improve drought tolerance of crops. There are increasing evidences that exogenous application of proline is an alternative approach to minimize the adverse effects of water stress on crop plants (Kavi-Kishore *et al*. 1995). Due to the increasing food demand for increasing population it is necessary to improve drought tolerance in crop with prime importance. Better understanding about the protective roles of proline is imperative for plant tolerance mechanisms to drought stress. Although exogenous proline improves abiotic stress tolerance in plants, there are some reports that high concentration of proline may be detrimental to plants for its inhibitory effects on growth or deleterious effects on cellular metabolisms (Nanjo *et al*. 2003). The main aim of this study was to alleviate the adverse effects of water stress by exogenous application of proline. Therefore, in the present study, we investigated the effect of exogenous proline on the growth, yield and nutrient uptake by wheat under water stress conditions.

MATERIALS AND METHODS

The study was conducted at the Soil Science Field Laboratory of Bangladesh Agricultural University, Mymensingh to investigate whether foliar application of proline minimizes the adverse effects of water stress on the growth and yield of wheat. BARI Gom-24 was used as a test crop and the experiment was laid out in a split

plot design with three replications. Total number of plots was 36. The unit plot size was 1 m × 1 m having 0.3 m spacing between plot to plot and 1 m between block to block. The seeds of BARI Gom-24 were sown in the experimental plots on November 19, 2014 by maintaining a spacing of 30 cm × 20 cm. Treatment combinations were the different levels of irrigation and proline. There were four levels of irrigation *viz.* I_0- control (normal irrigations), I_1- water stress at vegetative stage (irrigation missing at vegetative stage), I_2- water stress at flowering stage (irrigation missing at flowering stage), and I_3- 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 they were denoted as P_0, P_{25} and P_{50}. Irrigation was used as main treatment and proline was used as sub-plot treatment. Thus, there were twelve treatment combinations *viz.* $T_1= I_0P_0$, $T_2= I_0P_{25}$, $T_3= I_0P_{50}$, $T_4= I_1P_0$, $T_5= I_1P_{25}$, $T_6= I_1P_{50}$, $T_7= I_2P_0$, $T_8= I_2P_{25}$, $T_9= I_2P_{50}$, $T_{10}= I_3P_0$, $T_{11}= I_3P_{25}$, $T_{12}= I_3P_{50}$. Proline was applied at vegetative and reproductive stages as foliar spray at a volume of 25 mL per plant as per treatment. For making 25 mM and 50 mM solutions, 2.88 g and 5.76 g proline powder were dissolved in 1000 ml water and 1 ml Tween-20 was properly mixed in it which helps the droplets of proline solution to maintain a close contact with plant leaves.

Full doses of chemical fertilizers *viz.* triple super phosphate (540 g), muriate of potash (430 g), gypsum (300 g), ZnO (4.6 g) and H_3BO_3 (22 g) were added to soils during land preparation (BARC 2012). Urea was applied in three split doses; the first dose (314 g) was applied at 8 DAS, the second dose (314 g) was applied at 35 DAS and the third dose (314 g) was applied at 62 DAS. Intercultural operations such as weeding, pest control, etc. were performed as and when necessary. The crop was harvested at full maturity stage and grain and straw yields were recorded plot wise. The grain and straw samples were weighed carefully, sun-dried to a moisture content of 14% and finally yields were recorded as kg ha^{-1}. Plant height, spike length (cm), number of spikelets $spike^{-1}$, grains $spike^{-1}$ and 1000-grain weight were recorded from each plot. The representative grain and straw samples were dried in an oven at 65^0C for about 24 hours before they were ground by a grinding machine. The prepared samples were stored in paper bags and finally kept into desiccators until analysis. The N, P, K and S contents were determined by following standard methods. Data were analyzed statistically using analysis of variance to examine the treatment effects. The mean differences were adjudged by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) and ranking was indicated by letters.

RESULTS AND DISCUSSION

Growth and yield components of wheat influenced by exogenous proline under water stress

Water stress caused a significant (at 1% level of probability) decrease in plant height, number of spikelet $spike^{-1}$, spike length, number of grains $spike^{-1}$ and thousand (1000)-grain weight of BARI Gom-24 and all these growth and yield components were significantly increased due to application of 0, 25 and 50 mM proline (Table 1). Furthermore, the interaction of exogenous proline and water stress significantly (at 5% level of probability) increased number of spikelet $spike^{-1}$, spike length and number of grains $spike^{-1}$ in wheat variety. The interaction effects of exogenous proline and water stress were not significant in aspect of plant height and thousand (1000)-grain weight of BARI Gom-24. The tallest plant (93.67 cm) was observed with 25 mM proline application under normal irrigation. Again, the highest number of spikelet per spike (18.43), the tallest spike (11.90 cm), the highest number of grains per spike (44.40) and the highest weight of 1000-grain (41.97 g) were found with 50 mM proline application under normal irrigation.

Benmoussa and Achouch (2005) also showed that water deficit had significant effects on grain yield, plant height, number of days per heading and lodging. Shao *et al*. (2004) showed that the percentage of ripened grains, 1000 grain weight and grain yield of rice under water stress were lower than those under well-watered conditions. Similar results were reported by Gupta *et al*. (1995); Islam *et al*. (1994); Akram (2011) and Bakul *et al*. (2009).

Table 1. Effect of exogenous proline on growth and yield components of wheat under water stress

	Treatments	Plant height(cm)	Number of spikelets per spike	Spike length(cm)	Number of grains per spike	1000- grain weight(g)
Irrigation	I_0	93.50a	18.36a	11.84a	44.36a	41.84a
	I_1	89.33c	16.78c	10.90b	41.71c	40.81a
	I_2	91.84b	17.28b	10.84bc	42.82b	40.97a
	I_3	86.38d	16.37c	10.43c	38.82d	38.73b
	SE (±)	1.499	0.147	0.128	0.333	0.884
	Level of significance	**	**	**	**	**
Proline	P_0	89.42b	16.52b	10.33b	39.80b	39.67b
	P_{25}	90.27ab	17.43a	11.29a	42.58a	40.90a
	P_{50}	91.10a	17.63a	11.39a	43.40a	41.19a
	SE (±)	2.147	0.218	0.162	1.835	0.680
	Level of significance	*	**	**	**	**
Interaction of Irrigation and proline	I_0P_0	93.23	18.30ab	11.77abc	44.27a	41.80
	I_0P_{25}	93.67	18.33ab	11.86ab	44.40a	41.77
	I_0P_{50}	93.60	18.43a	11.90a	44.40a	41.97
	I_1P_0	88.20	15.93d	9.967ef	38.20de	39.53
	I_1P_{25}	89.00	17.07c	11.23abcd	42.93ab	41.38
	I_1P_{50}	90.80	17.33c	11.50abcd	44.00a	41.52
	I_2P_0	90.93	16.73cd	10.27e	40.33cd	40.15
	I_2P_{25}	92.20	17.53bc	11.10bcd	43.87a	41.35
	I_2P_{50}	92.40	17.57abc	11.17abcd	44.27a	41.40
	I_3P_0	85.33	15.10e	9.333f	36.40e	37.22
	I_3P_{25}	86.20	16.80c	10.97d	39.13cd	39.12
	I_3P_{50}	87.60	17.20c	11.00cd	40.93bc	39.87
	SE (±)	2.147	0.218	0.162	1.835	0.680
	Level of significance	NS	*	*	*	NS

** = Significant at 1% level of probability
* = Significant at 5% level of probability
NS = Not significant
SE = Standard error of means and CV = Coefficient of variation

Effects of exogenous proline on grain and straw yields of wheat under water stress

Grain yield

Plants exposed with water stress significantly decreased grain yield of BARI Gom-24 (Figure 1). Foliar application of proline over plant leaves resulted in a significant increase in grain yield (Figure 2). The interaction effects of proline and water stress were significant, resulting in increased grain yield of wheat (Figure 3). The highest grain yield (3593 kg ha^{-1}) was found with foliar application of 50 mM proline under normal irrigation. The lowest grain yield (2817 kg ha^{-1}) was observed when irrigation was missing both at vegetative and flowering stages with no proline application (Figure 3). Guttieri *et al*. (2001) observed that grain yield of wheat was significantly reduced under water stress condition due to decrease in the grain weight of each spike during grain filling period.

Straw yield

Water stress caused a drastic decrease in straw yield of BARI Gom-24 (Figure 1). The lowest straw yield (4523 kg ha^{-1}) was observed when irrigation was missing at vegetative and flowering stages (Figure 1). Application of proline significantly increased straw yield of wheat (Figure 2). The highest straw yield (5157 kg ha^{-1}) was found with foliar application of 25 mM proline under normal irrigated conditions. Straw yield was also increased significantly due to the interaction effects of exogenous proline and water stress (Figure 3). It was noted that 50 mM proline showed higher straw yield than 25 mM proline under water stress condition. These findings of the present study were similar to the findings of some earlier studies in which foliar application of proline alleviated the adverse effects of water stress on the yield (grain and straw) of rice plants (Kavi-Kishore *et al*. 1995) and *Allenrolfea occidentalis* (Chrominski *et al*. 1989).

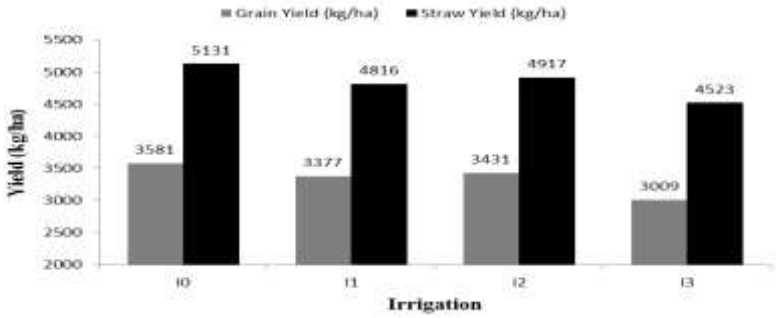

Fig. 1. Effects of water stress on grain and straw yields of wheat (BARI Gom-24)

Here,
I_0- control (normal irrigations)
I_1- water stress at vegetative stage (irrigation missing at vegetative stage)
I_2- water stress at flowering stage (irrigation missing at flowering stage)
I_3- water stress at vegetative and flowering stages (irrigation missing at both vegetative and flowering stages)

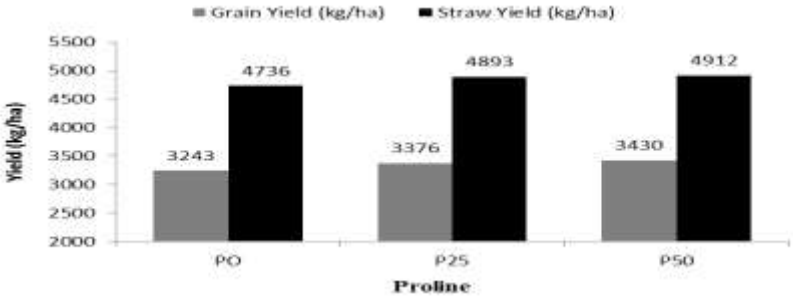

Fig. 2. Effects of exogenous proline on grain and straw yields of wheat

Here,
P_0- no proline
P_{25}- 25 mM proline
P_{50}- 50 mM proline

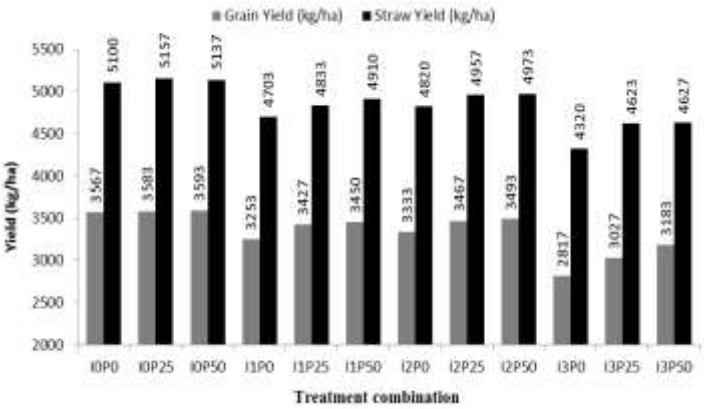

Fig. 3. Interaction effects of exogenous proline and water stress on grain and straw yields of wheat (BARI Gom-24)

Effects of exogenous proline on nutrient contents of wheat under water stress conditions

Neither water stress nor proline application showed any significant effect on nutrient contents (N, P, K, and S contents) in grain and straw of BARI Gom-24 (data not shown). The interaction effects of proline and water stress were also insignificant. The results were in agreement with Abd El-Samad *et al.* (2011).

Effects of exogenous proline on nutrient uptake by wheat under water stress conditions

Nitrogen uptake

Nitrogen uptake by grain and straw was significantly decreased due to water stress in BARI Gom-24 and proline application significantly increased N uptake by grain as well as straw (data not shown). Total N uptake was also significantly decreased due to water stress and proline application significantly increased total N uptake in wheat (Table 2). The highest total N uptake (106.2 kg ha^{-1}) was found at 50 mM proline application under normal irrigation. The interaction effects of exogenous proline and water stress were significant in aspect of total N uptake by BARI Gom-24 variety.

Phosphorus uptake

P uptake by grain and straw was significantly decreased due to water stress in BARI Gom-24 and proline application significantly increased P uptake by grain and straw under water stress conditions (data not shown). Total P uptake in BARI Gom-24 was significantly decreased due to water stress (Table 2). Proline application significantly increased total P uptake in wheat where the highest total P uptake (13.68 kg ha^{-1}) was found at 50 mM proline application with normal irrigation. Total P uptake was also significantly increased due to interaction effects of exogenous proline and water stress in BARI Gom-24.

Potassium uptake

Potassium uptake by grain and straw was significantly decreased due to water stress and application of proline significantly increased K uptake by grain as well as straw of wheat (data not shown). Total K uptake was significantly decreased by water stress (Table 2). Proline application resulted in a significant increase in total K uptake by wheat where the highest total K uptake (104.3 kg ha^{-1}) was found at 50 mM proline application with water stress at vegetative stage (irrigation missing at vegetative stage). The interaction effects of exogenous proline and water stress also significantly increased total K uptake by BARI Gom-24.

Sulphur uptake

Sulphur uptake by grain and straw of wheat was significantly decreased by water stress and proline application significantly increased S uptake by grain and straw (data not shown). Total S uptake was also significantly decreased due to water stress in BARI Gom-24 and proline application showed a significant increase in total S uptake (Table 2). The highest total S uptake (11.33 kg ha^{-1}) was found at 25 mM proline application with control irrigation treatment. The interaction effects of exogenous proline and water stress were significant in aspect of total S uptake by wheat (Table 2).

There are some reports suggesting that proline minimizes the adverse effects of various stresses on plants by affecting the uptake and accumulation of inorganic nutrients (Ali *et al.* 2008). Similar to our results, Abd El-Samad *et al.* (2011) reported that application of proline increased N, P and K nutrient uptake in rice plants. Soad and Shetea (2007) also showed that proline application increased N and P uptake in soybean (*Glycine max* L.).

Table 2. Effect of exogenous proline on total N, P, K and S uptake by wheat under water stress condition

	Treatments	Total N uptake (kg ha^{-1})	Total P uptake (kg ha^{-1})	Total K uptake (kg ha^{-1})	Total S uptake (kg ha^{-1})
Irrigation	I_0	101.1a	13.55a	101.4a	11.16a
	I_1	94.87b	9.827c	99.03a	9.252c
	I_2	93.89b	10.75b	85.96b	9.870b
	I_3	87.74c	9.965c	80.41c	8.908d
	SE (±)	1.368	0.386	12.075	0.031
	Level of significance	**	**	**	**
Proline	P_0	86.55c	9.772c	86.37c	9.073b
	P_{25}	96.37b	11.41b	91.74b	10.05a
	P_{50}	100.3a	11.89a	96.95a	10.27a
	SE (±)	0.777	0.149	9.278	0.062
	Level of significance	**	**	**	**
Interaction of Irrigation and Proline	I_0P_0	94.10e	13.41a	101.8ab	10.86b
	I_0P_{25}	102.9b	13.56a	100.9ab	11.33a
	I_0P_{50}	106.2a	13.68a	101.5ab	11.30a
	I_1P_0	87.13g	7.490g	94.55c	8.400g
	I_1P_{25}	97.48d	10.72cd	98.26bc	9.500ef
	I_1P_{50}	100.0c	11.27bc	104.3a	9.857de
	I_2P_0	85.26h	9.702e	78.58e	9.090f
	I_2P_{25}	95.50e	11.06bc	84.14d	10.12cd
	I_2P_{50}	100.9c	11.49b	95.16c	10.40c
	I_3P_0	79.72i	8.485f	70.59f	7.940h
	I_3P_{25}	89.58f	10.31de	83.72de	9.270f
	I_3P_{50}	93.92e	11.10bc	86.91d	9.515ef
	SE (±)	0.777	0.149	9.278	0.062
	Level of significance	*	**	**	*

** = Significant at 1% level of probability
* = Significant at 5% level of probability
NS = Not significant
SE = Standard error of means and CV = Coefficient of variation

CONCLUSION

Exogenous application of proline is a vital approach to alleviate the adverse effects of water stress on crop plants. Exogenous proline showed significant increases in growth, and grain and straw yields of wheat which were positively associated with increased nutrient uptake under water stress. It can be concluded that exogenous proline can minimize the adverse effects of water stress on plants.

ACKNOWLEDGEMENT

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

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