

IMPACTS OF DROUGHT STRESS ON GROWTH, PROTEIN, PROLINE, PIGMENT CONTENT AND ANTIOXIDANT ENZYME ACTIVITIES IN RICE (*ORYZA SATIVA* L. VAR. *BRR1 DHAN-24*)

SHAMIMA NASRIN, SHUKANTA SAHA*, HASNA HENA BEGUM AND RIFAT SAMAD¹

Department of Botany, Jagannath University, Dhaka-1100, Bangladesh

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Abstract

One of the major problems associated with rice cultivation and production is the deficiency of water resources. In here the effect of drought stress on growth, protein-, proline-, pigment contents and antioxidant enzymes activities in rice var. *BRR1 Dhan-24* was determined and analyzed under drought and well-watered condition. Drought stress caused the decrease of growth and pigment contents - chlorophyll-a, b, a/b, total chlorophyll and carotenoids content of leaves of rice plants. On the other hand, the accumulation of protein, proline contents and antioxidant enzymes activities was increased under drought stress. It may be suggested that antioxidant enzymes activities and proline accumulation were associated with the growth of the plant and consequently with the mechanisms of drought tolerance in rice.

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop in the world and it is the primary source of food and calories for about half of the mankind⁽¹⁾. It is a nutritious cereal crop, provides 20% calories and 15% protein requirements of world population. Rice grows in the tropics, subtropics, semi-arid tropics and temperate regions of the world. More than 90% of the world's rice production is in Asia⁽²⁾. Bangladesh is the 4th largest rice producer in the world. It covers about 77% of agricultural land and engages about 70% of population in rice production activities (Hand Book of Agricultural Technology 2013).

Drought is one of the major abiotic stresses that severely affect and reduce the yield and productivity of food crops worldwide up to 70%⁽³⁾. Droughts, being the most important environmental stress, severely impair plant growth and development, limits plant production and the performance of crop plants, more than any other environmental factors⁽⁴⁾. The response of plants to drought stress is complex and involves changes in their morphology, physiology and metabolism. Reduction of plant

*Author for correspondence: <sahashukanta@gmail.com, saha.s@bot.jnu.ac.bd> ¹Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh.

growth is the most typical symptom of drought stress ⁽⁵⁾. Drought stress also involved with many biochemical, molecular, and physiological changes that influence various cellular and whole plant processes and reduce quality and quantity of yield. Plant tolerance to unfavorable conditions, particularly water deficit, has been associated with proline accumulation and increased antioxidative enzymes ⁽⁶⁾.

The aim of this work was to analyze the effects of drought stress on growth, protein contents, proline accumulation, pigment contents and antioxidant enzyme activities (CAT and SOD) in rice.

Materials and Methods

A pot experiment was carried out at the research garden of the Department of Botany, Jagannath University, Dhaka. Seeds of BRR1 dhan-24 had been collected from Bangladesh Rice Research Institute (BRR1), Gazipur. Half loamy soil and half compost soil were used by mixing. The pot experiment was set up in net house.

The seeds of BRR1 dhan-24 were surface sterilized by agitation in 95% ethanol for 1 min then followed by five washings with sterile water. Seeds were rinsed in distilled water about 30 minutes. These seeds were allowed to germinate on a filter paper in Petri dishes, moistened with 4 ml of distilled water. The Petri dishes were arranged randomly and stored at room temperature ($24 \pm 2^\circ\text{C}$) under dark conditions. The Petri dishes were covered to prevent the loss of moisture by evaporation under laboratory condition. The seeds were germinated within three days and the germinated seeds were sown in the pots. Drought stress was imposed by withholding irrigation at 21 days after sowing in five pots. Water level in well-watered treatment (control) was maintained at 5 cm above the surface of the soil in the rest five pots. Ten pots were used in this experiment. Plants were collected at 12, 15, 18 and 21 days after stress. After taking fresh weight the root and shoot was dried in an oven for 72 hours at 80°C to a constant weight. The experiment was arranged with completely randomized design (CRD) with five replications.

According to Chen *et al.*⁽⁷⁾ chlorophyll level in the normal leaves of rice decreases with increasing age. So, chlorophyll-a, b, total chlorophyll content, chlorophyll a/b and carotenoid contents of leaves were determined at early stage for each treatment at 3, 6, 9 and 12 days after stress. The amount of chlorophyll-a and b was determined by using specific absorption co-efficient of Mackinney ⁽⁸⁾ and the formulae of Maclachlan and Zalik⁽⁹⁾. Total chlorophyll content was estimated by using the equation of Hiscox and Israelstam⁽¹⁰⁾. The amount of carotenoid was determined by the equation of Von Wettstein⁽¹¹⁾. Protein content of leaves for each treatment was determined at 12, 15, 18 and 21 days after stress. The method of Lowry *et al.*⁽¹²⁾ was employed to determine the protein content of leaf of rice. The method of Bates *et al.*⁽¹³⁾ was employed to determine the proline content of leaf of rice at 12, 15, 18 and 21 days after stress. The method of Zhang *et al.*⁽¹⁴⁾ was employed to determine the catalase (CAT) activity and superoxide dismutase (SOD) activity of leaf of rice at 15 days after stress.

Results and Discussion

Drought stress decreased the length of root by 49, 71, 64.15 and 68% at 12, 15, 18 and 21 days of treatment, respectively. It decreased the length of shoot by 28.4 - 47.7% from 12 - 21 days of treatment. It also decreased the length of leaves by 31 - 36.38% and breadth of leaves by 22.5 - 56.25% from 12 - 21 days of treatment (Table 1). The fresh weight of the root was decreased by 95.5 - 98.3% from 12 - 21 days of treatment. It decreased fresh weight of shoot by 84.9 - 93.1% from 12 - 21 days of treatment. It also decreased dry matter of root by 90.2, 83.6, 94.3 and 94.7% at 12, 15, 18 and 21 days of treatment respectively and dry weight of shoot by 47.2 - 82.2% from 12 - 21 days after treatment (Table 2).

Table 1. The effect of drought stress on the length of root, shoot and the length, breadth of leaves of rice var. BRRI Dhan-24.

Days after stress	Root length (cm)		Shoot length (cm)		Length of leaves (cm)		Breadth of leaves (cm)	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
12	19.16 ± 1.26	9.76 ± 2.64	51.58 ± 1.02	36.94 ± 1.29	35.86 ± 0.76	24.72 ± 1.02	0.80 ± 0.03	0.62 ± 0.04
15	15.38 ± 0.81	4.86 ± 0.81	49.54 ± 1.67	32.52 ± 1.56	32.58 ± 0.65	21.70 ± 1.05	0.84 ± 0.08	0.60 ± 0.08
18	18.72 ± 1.36	5.84 ± 0.43	49.02 ± 3.47	30.22 ± 1.46	36.22 ± 1.38	24.44 ± 2.06	0.78 ± 0.09	0.42 ± 0.04
21	15.9 ± 1.69	5.70 ± 0.49	56.54 ± 1.88	29.58 ± 0.76	32.16 ± 2.37	20.46 ± 1.13	0.96 ± 0.05	0.42 ± 0.02

Each value is the mean of five replicates; ± standard error.

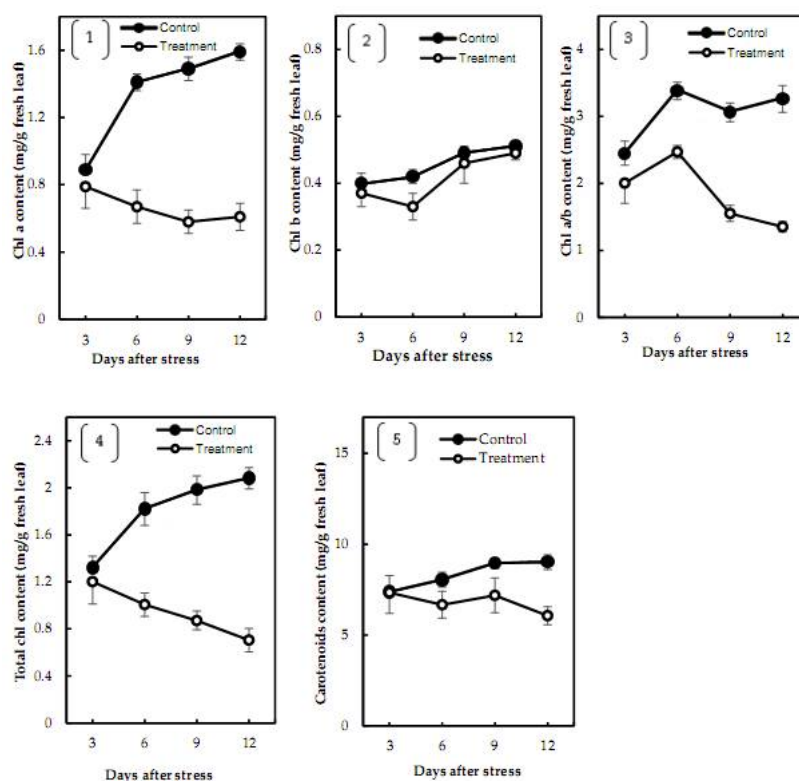
Drought stress decreased chlorophyll-a content by 11.2 to 61.6% from 3 to 12 days of treatment (Fig. 1). It decreased chlorophyll-b content by 21.4% at 6 days and the inhibitory effect was maintained up to 12 days of treatment (Fig. 2). It decreased chlorophyll a/b ratio by 18.2 to 58.5% from 3 to 12 days of treatment (Fig. 3). It also decreased total chlorophyll content by 9.01 to 66.2% from 3 to 12 days of drought stressed condition (Fig. 4) and carotenoids content by 0.41 to 32.7% from 3 to 12 days of treatment (Fig. 5). This result was supported by Mannivannan *et al.* who showed that a decrease in chlorophyll content upon drought stress on *Vigna unguiculata* L.⁽¹⁵⁾

Drought stress increased protein content in the leaf of rice by 17.5 to 54.2% from 12 to 21 days of treatment (Fig. 6). The changes in the total protein content during drought stress are an indicator of their drought tolerance ability defined by their genetic composition⁽¹⁶⁾. On the contrary, the capacity for protein synthesis decreases in response to water stress studied by Hsiao TC *et al.* in *Zea mays*⁽¹⁷⁾.

Table 2. The effect of drought stress on fresh and dry weight of root and shoot of rice var. BRR1 dhan-24.

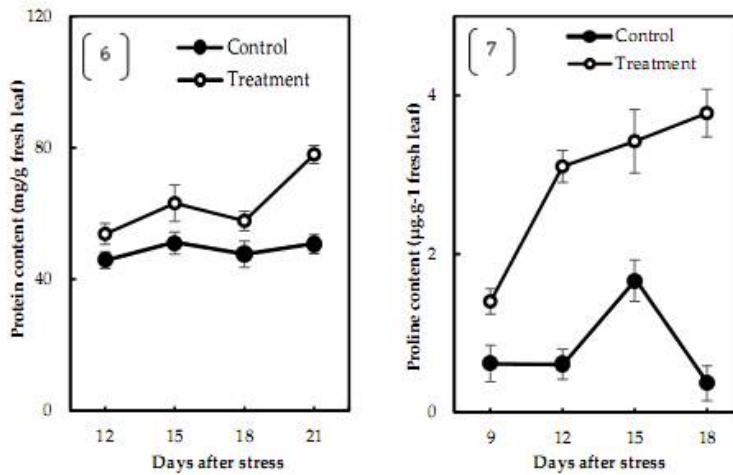
Days after stress	Fresh weight (gm)				Dry weight (gm)			
	Root		Shoot		Root		Shoot	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
12	0.880 ± 0.065	0.040 ± 0.006	1.286 ± 0.073	0.521 ± 0.058	0.194 ± 0.026	0.019 ± 0.003	0.246 ± 0.016	0.130 ± 0.015
15	0.479 ± 0.083	0.019 ± 0.003	1.348 ± 0.221	0.325 ± 0.044	0.073 ± 0.010	0.012 ± 0.002	0.278 ± 0.046	0.092 ± 0.010
18	0.702 ± 0.114	0.017 ± 0.003	1.427 ± 0.297	0.192 ± 0.031	0.130 ± 0.022	0.007 ± 0.002	0.266 ± 0.054	0.065 ± 0.010
21	0.831 ± 0.233	0.014 ± 0.001	2.307 ± 0.319	0.159 ± 0.007	0.159 ± 0.054	0.009 ± 0.001	0.458 ± 0.068	0.082 ± 0.003

Each value is the mean of five replicates; ± standard error.

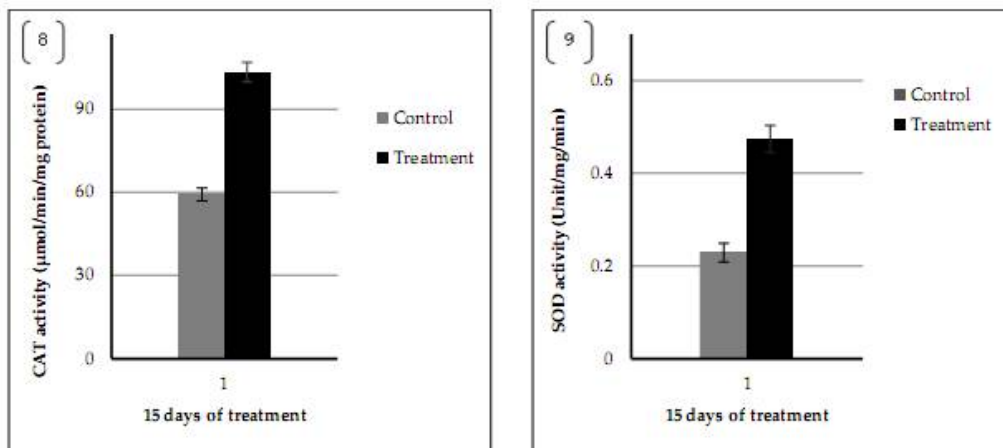


Figs 1-5: 1. The effect of drought stress on chlorophyll-a content of leaf of rice. Each value is the mean of five replicates, the bars represent ± standard error. 2. The effect of drought stress on chlorophyll-b content of leaf of rice. Otherwise as in Fig. 1. 3. The effect of drought stress on chlorophyll a/b ratio of leaf of rice. Otherwise as in Fig. 1. 4. The effect of drought stress on total chlorophyll content of leaf of rice. Otherwise as in Fig. 1. 5. The effect of drought stress on carotenoid contents of leaf of rice. Otherwise as in Fig. 1.

Proline accumulation in the leaf of rice was increased by 1.3 to 10.2- folds from 9 to 18 days of treatment (Fig. 7). Mafakheri *et al.*⁽¹⁸⁾ showed drought stress increased proline content about 10-folds in chickpea. The increase in proline level may help to maintain osmotic potential in cytoplasm of cells which is important for survival of plants under stress⁽¹⁹⁾.



Figs 6-7: 6. The effect of drought stress on the accumulation of protein content in the leaf of rice. Otherwise as in Fig. 1. 7. The effect of drought stress on the accumulation of proline content in the leaf of rice. Otherwise as in Fig. 1.



Figs 8-9: 8. The effect of drought stress on catalase activity in the leaf of rice. Each value is the mean of five replicates; the bars represent ± standard error. 9. The effect of drought stress on superoxide dismutase activity in the leaf of rice. Otherwise as in Fig. 8.

Drought stress increased catalase activity in the leaf of rice by 74.70% at 15 days of treatment (Fig. 8). CAT activity was increased in the drought tolerant varieties shown by Pazirandeh⁽²⁰⁾. It increased superoxide dismutase activity in the leaf of rice by 1.1-folds at

15 days of treatment (Fig. 9). Mahdi Ebrahimi *et al.* ⁽²¹⁾ showed that SOD activity was increased along with the enhancement of drought stress on *Calendula officinalis* L.

It may be suggested that antioxidant enzymes activities (CAT and SOD) and proline accumulation were associated with the growth of the plant and consequently with the mechanisms of drought tolerance in rice.

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