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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF RICE TO POLYETHYLENE GLYCOL

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Abstract

Effects of water deficit induced by polyethylene glycol (PEG-6000) on rice grown in half strength Hoagland solution was studied in the present investigation. Impacts of different water stress (10, 20 and 30% PEG) on some physiological and biochemical characteristics were determined in this investigation. Results showed that water stress significantly affected most physiological and biochemical characteristics. The increasing water stress declined the root and shoot length and fresh and dry weights of root and shoot. An increase in water stress also resulted in reduction of relative water content, leaf pigments and protein content. Upon dehydration, an incline in reducing and total sugar and proline content were evident in the root and shoot of rice. Water-deficit stress resulted in higher superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities but as the severity of the water stress increased, the SOD activity decreased. These crucial characteristics would be expected to be utilized as screening techniques for water stress tolerance to the future development of new variety.

Introduction

Water availability is becoming an increasing issue for agriculture due to global warming, non-availability of surface water resources, shortage of rainfall and the rising human population. Water stress is one of the major environmental problems in the agricultural field worldwide. Plants experience water stress either when the water supply to their roots becomes limiting or when the transpiration rate becomes intense. In water stress, water may exist in soil solution but plants cannot uptake it (Lisar *et al*. 2012). Nearly 90% of global rural land area is affected by abiotic stress factors at some point throughout the growing period (Cramer *et al*. 2011). Under the on-going climate change scenario in the world, increase of water deficit and frequency has been predicted to further increase in the near future (Farooq *et al*. 2014).

Rice (*Oryza sativa* L.) is one of the chief grains which constitute the staple food of two-third of the world population. In Bangladesh, rice production is not sufficient and rice is imported occasionally to meet the country’s demand. Water stress for agriculture threatens the productivity of rice ecosystem, therefore, new strategy must be sought to save water and sustain rice production. The PEG acts as a non-penetrating osmotic agent resulting into increased solute potential and blockage of absorption of water by root system (Chutia and Borah 2012). Rice was chosen as experimental plant material for the present project because the effects of water stress at physiological and biochemical levels in rice was not considerably understood and such understanding was crucial.

Water stress caused by exogenous application of PEG, significantly decreased root length, shoot length, dry weight and relative water content in cabbage (Sunaina *et al*. 2016). Polyethylene glycol generated osmotic stress decreased the chlorophyll content in *Thymus vulgaris* (Razavizadeh *et al*. 2019). Water deficiency increased sugar accumulation in *Brassica napus* (Ali *et al*. 2014). Due to water stress a significant increase in proline content was observed in sweet basil (Al-Huqail *et al*. 2020). In radish seedlings under water stress, there was remarkable decline in soluble proteins (Balaraju *et al*. 2015).

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Plants have evolved some adaptation mechanisms which may enhance their capability to survive and grow during short- and long-term water stress. The antioxidants enzymes - SOD, POD and CAT play an important role in cleansing those activated oxygen species (Yin *et al.* 2005). Activities of SOD, POD and CAT increased in wheat in response to water stress (Weng *et al.* 2015).

Therefore, this study was aimed to understand the response of rice plants to water stress by examining the physiological (reducing sugar, total sugar, proline, protein, chlorophyll content and activities of different antioxidant enzymes - SOD, POD and CAT) and biochemical (length of root and shoot, length of seedlings, fresh and dry wt. of root and shoot and relative water content), changes on plants after PEG application and also the mechanism of adaptation of plants in water stressed soil. In addition the resistance mechanism of rice and increase rice production in the future to meet national demand, effects of water stress were also examined.

Materials and Methods

Rice (*O. sativa* var. BRRI Dhan-53) was taken as experimental plant material. The present seeds of rice were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur. The experiments were conducted at Department of Botany, University of Dhaka, Dhaka, Bangladesh. The seeds were surface sterilized with 5.25% sodium hypochlorite solution and then the seeds were spread over cotton gauge placed in a plastic lid having holes and was placed upon the beaker filled with half strength Hoagland solution (Hoagland and Arnon 1950). After 48 hrs of sowing, the seeds were germinated which were then transferred to light bank. Rice seedlings were grown at a day/night temperature of $30 \pm 1^{o}C/25^{o}C \pm 1^{o}C$ and day/night length of 14 hrs/10 hrs. Fourteen days old seedlings were transferred in 10, 20 and 30% polyethylene glycol (PEG-6000) solution which were used as treatment. Untreated seedlings in nutrient solution were taken as control. Solution was replaced after every 48 hrs and were continuously aerated through bubbler with the help of air compressor. Roots and shoots were collected in triplicate after 24, 48 and 72 hrs of water stress treatment.

Length of root and shoot of the seedlings were measured in cm with a scale. Fresh and dry weights of samples were recorded with an electronic balance. The relative water content (RWC) was calculated as per Bars and Weatherly (1962).

Chlorophyll content was measured using specific absorption co-efficient method of Mckinney (1940) and calculated using the formula of Maclachlan and Zalik (1963). The amount of carotenoid was determined by the equation of Von Wettstein (1957). Reducing and total sugar were extracted by boiling fresh tissue in 80% ethanol and were determined by Somogyi-Nelson (Nelson 1944, Somogyi 1952) method and Dubois *et al.* (1956) method, respectively. Determination of protein and proline were done according to the method of Lowry *et al.* (1951) and Bates *et al.* (1973), respectively. Activities of different antioxidant enzymes (SOD, POD and CAT) were estimated following the protocol of Zhang *et al.* (1995), Barber (1980) and Zhang *et al.* (2005), respectively.

Results and Discussion

The increase in water deficit in rice by PEG noticeably reduced the length of root as compared to control. Polyethylene glycol (PEG 30%) decreased root length of rice by 27.8 to 37.0% from 24 to 72 hrs of treatment. Similarly, 30% PEG caused 25.4 to 40.0% decrease of shoot length of rice from 24 to 72 hrs of application. Exposure to 20 and 30% PEG resulted in 20.0 to 30% and 28.85 to 40.22% decrease of seedling length of rice, respectively, from 24 to 72 hrs of treatment (Fig. 1a and b). According to Faisal *et al.* (2019), root and shoot lengths were restricted in wheat in PEG-mediated osmotic deficit.

Water stress decreased the fresh weight of root and shoot in rice. Maximum decrease of the fresh weight of root of rice was observed at 30 % PEG which ranged from 59.0 to 72.0% from 24 to 72 hrs of application. Similarly, in shoot 20% PEG inhibited the fresh weight by 64.8% at 72 hrs of exposure (Fig. 2a and b). A similar trend about reduction in the fresh and dry weight under PEG-mediated stress conditions was reported by Hellal *et al.* (2018) in Egyptian barley.

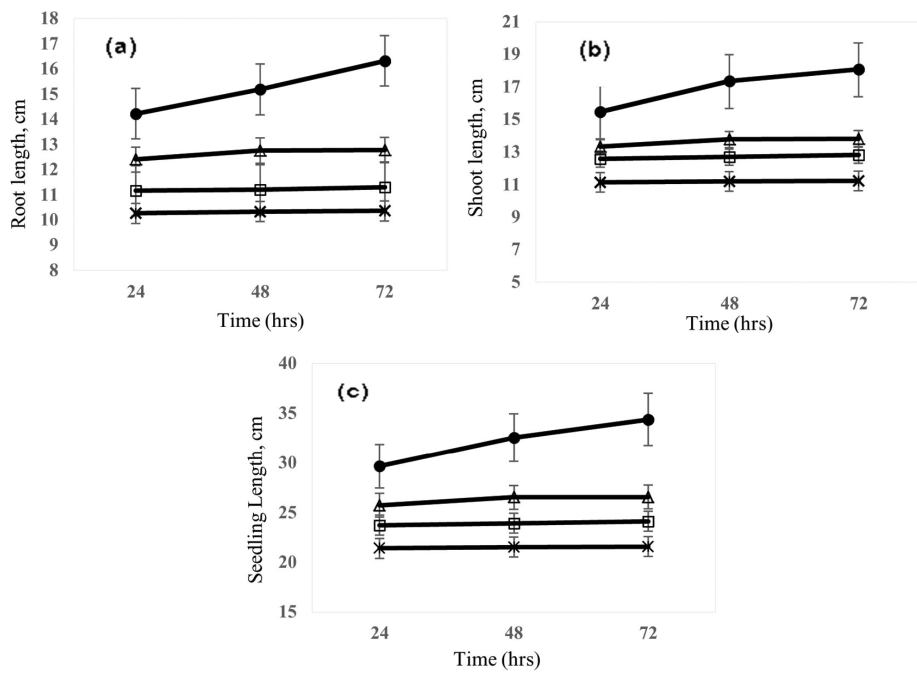

Fig. 1. The effect of different concentrations of water stress on the length of the (a) root, (b) shoot and (c) seedling of rice seedlings grown in solution culture. ● represents control; Δ 10% PEG; □ 20% PEG and × 30% PEG. Each value is the mean of three replicates ± standard error.

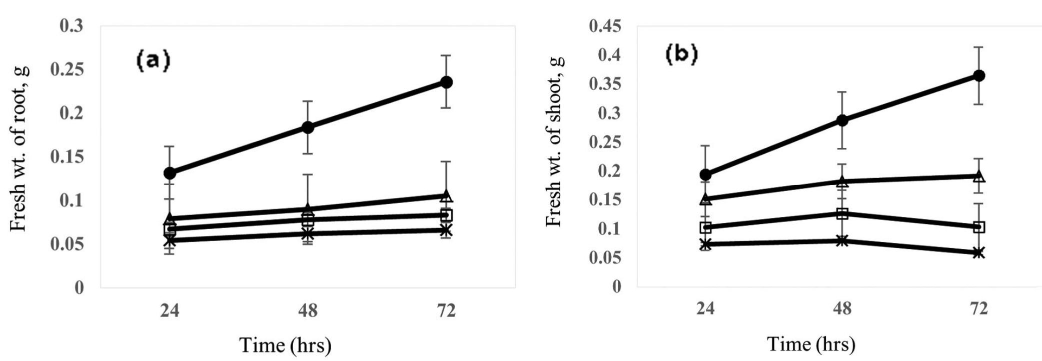

Fig. 2. The effect of different concentrations of water stress on fresh weight of the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

Polyethylene glycol, at concentrations of 10 to 30%, decreased the dry weight of root of rice seedlings by 28.57 to 61.82% from 24 to 72 hrs of treatment. In rice seedlings, a maximum of 48.21 to 59.33% decrease in the shoot dry weight was observed following 30% PEG treatment (Fig. 3a and b). Similarly, when plants were subjected to both mild and severe water deficit regimes, root and shoot dry weight decreased significantly in wheat (Faisal *et al.* 2017).

A sharp decrease in relative water content (RWC) with the increased PEG concentration was noted in rice seedlings. Water deficit decreased RWC of root by 23.26 to 95.0% due to 10 to 30% PEG treatment from 24 to 72 hrs of treatment. Results revealed that decreased in RWC of shoot had similar trend as obtained in decrease in RWC of root and the range varied from 36.79 to 93.24% (Fig. 4a and b). Zhang *et al.* (2018) also observed the decrease of RWC with increased water stress in alfalfa plants.

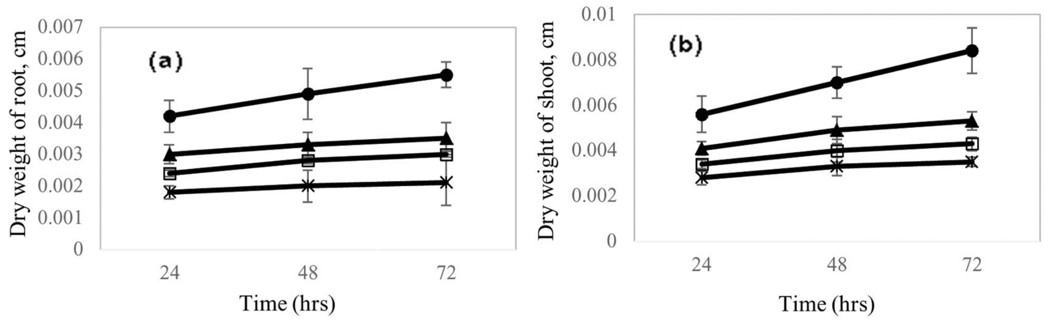

Fig. 3. The effect of different concentrations of water stress on dry weight of the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

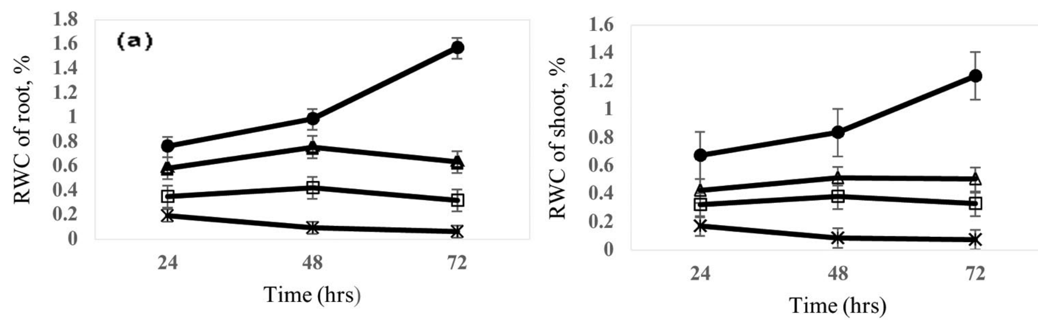

Fig. 4. The effect of different concentrations of water stress on RWC of the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

Water stress imposed by PEG-6000 effects the photosynthetic pigments. A 38.46 to 70.51% inhibition of chlorophyll a content in the leaves of rice was obtained following 20% PEG treatment from 24 to 72 hrs of exposure (Fig. 5a). A maximum of 34.64 to 60.16% inhibition of Chlorophyll b content in the leaves of rice was observed when exposed to 30% PEG from 24 to 72 hrs of application (Fig. 5b). Polyethylene glycol (PEG 10-30%) decreased carotenoid content in the leaves of rice plants from 24 to 72 hrs of treatment. 30% PEG caused 31.0 to 59.27% inhibition of carotenoids in the leaves from 24 to 72 hrs of application (Fig. 5c). Meher *et al.* (2018) also observed that chlorophyll a and b were reduced significantly in peanut to PEG induced water stress condition.

Maximum stimulation of reducing sugar accumulation in the root of rice was 88.0% at 72 hrs following water stress application (Fig. 6a). In the shoot of rice, similar stimulation of the accumulation of reducing sugar was also observed due to water stress treatment (Fig. 6a and b). This result is in agreement with the work of Ali *et al.* (2014) who reported that water deficiency resulted in an increase in the accumulation of reducing sugar in *Brassica napus*.

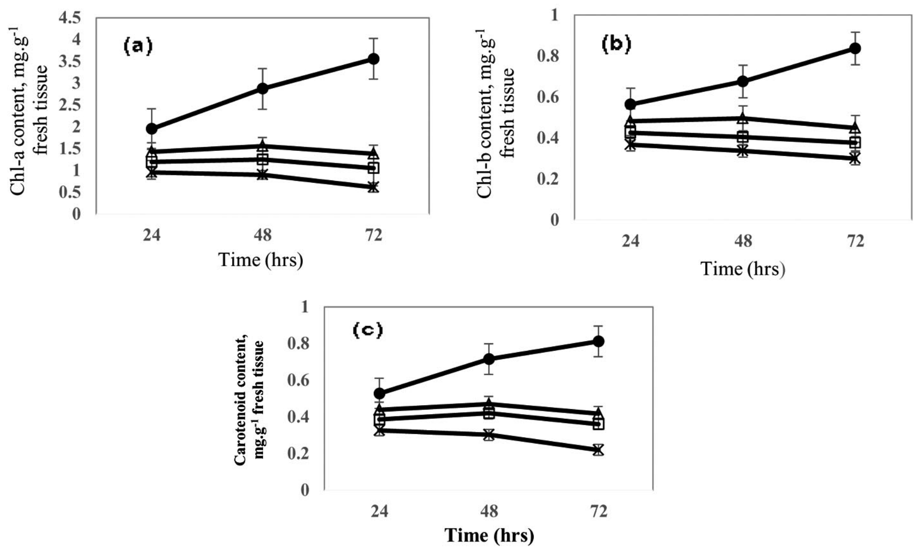

Fig. 5. The effect of different concentrations of water stress on (a) chlorophyll a, (b) chlorophyll b and (c) carotenoid content in the shoot of rice seedlings. Otherwise as Fig. 1.

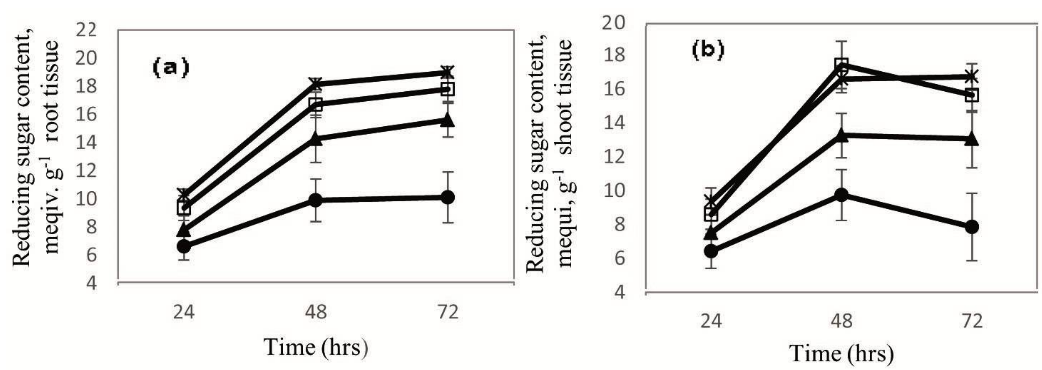

Fig. 6. The effect of different concentrations of water stress on the accumulation of reducing sugar in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

Polythelene glycol of 10 to 30%, increased total sugar content in the root of rice seedlings from 24 to 72 hrs of application and 20% PEG increased total sugar content in the root by 20.0 and 50.13% at 24 and 72 hrs of treatment, respectively. In the shoot of rice seedlings, all the concentrations of PEG (10-30%) increased total sugar content from 24 to 72 hrs of treatment (Fig. 7a and b). Yooyongwech *et al.* (2017) reported that total soluble sugar was significantly affected by water stress in sweet potato which is in agreement with the present findings.

Rice plants subjected to water stress for 72 hrs produced significantly higher amounts of proline in the root compared to that of control plants and 30% PEG increased accumulation of proline in the root by 37.64 to 51.40 from 24 to 72 hrs of application. Due to water deficiency, proline content was also increased in the shoot of rice but the rate of stimulation was lesser in the shoot than that of the root (Fig. 8a and b). The observation is in agreement with the findings of Li *et al.* (2017), who also observed the increased accumulation of proline with the increase in the intensity of drought stress in sweet corn.

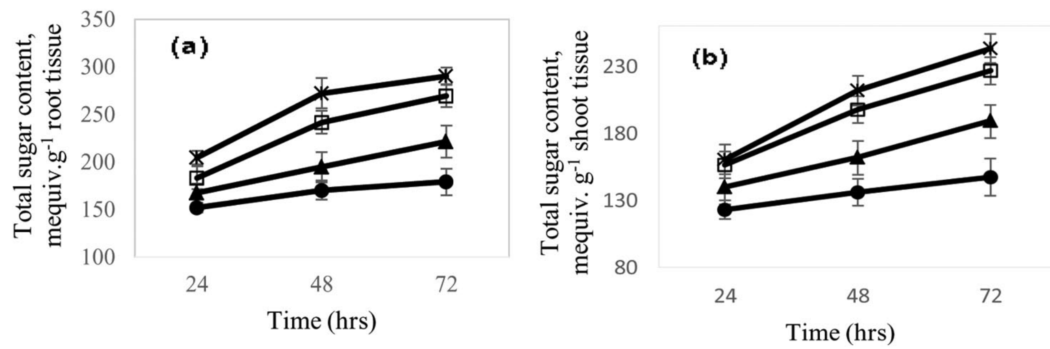

Fig. 7. The effect of different concentrations of water stress on the accumulation of total sugar in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

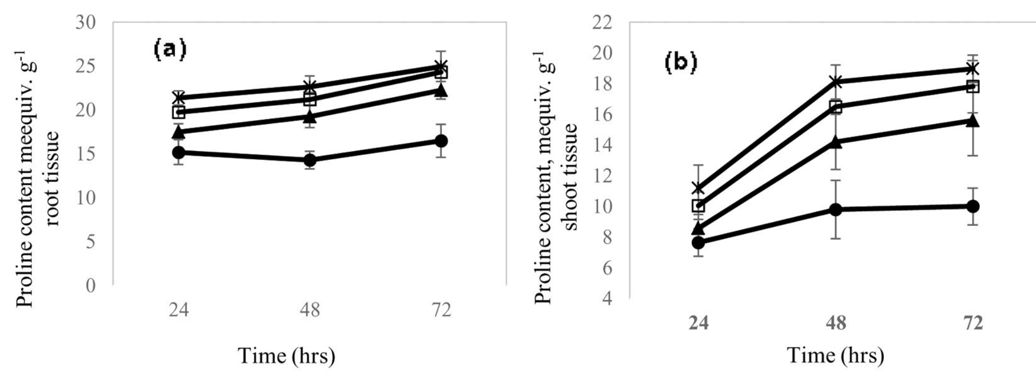

Fig. 8. The effect of different concentrations of water stress on proline content in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

Water deficit caused a reduction in protein content in the root of rice from 5.76 to 39.15% at 24 to 72 hrs following PEG (10-30%) application. It also reduced the accumulation of protein content in the shoot of rice seedlings and the inhibitory effect was sustained up to 72 hrs of treatment. A maximum of 49.18% reduction in protein content in the shoot was recorded at 72 hrs of treatment (Fig. 9a and b). Similarly, an inhibition of protein content was observed in *Lycium ruthenicum* with water deficit treatment (Guo *et al.* 2018).

At the beginning, superoxide dismutase (SOD) activity increased but as the severity of the water stress increased, the SOD activity decreased. The highest increase in SOD activity was 4-fold subjected to the 20% PEG treatment in rice leaves at 48 hrs of treatment (Fig. 10a).

Peroxidase (POD) activity showed the highest increase by 5.5-fold subjected to the 30% PEG while 3.9-fold and 4.3-fold increased in POD activity which was found due to 10 and 20% PEG application, respectively, as compared to control (Fig. 10b). Catalase (CAT) activity was positively related to the degree of water stress experienced by rice seedlings. Application of 10, 20 and 30% PEG stimulated CAT activity by 2-, 3.7- and 4.4-fold, respectively, in the leaves of rice at 48 hrs of treatment (Fig. 10c). Similar magnitude of higher activities of SOD, POD and CAT were recorded in durum wheat (Pour-Aboughadareh *et al.* 2020).

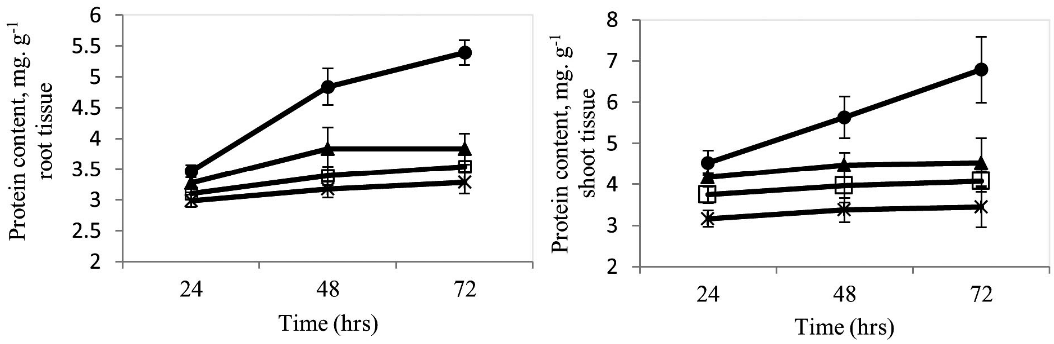

Fig. 9. The effect of different concentrations of water stress on protein content in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

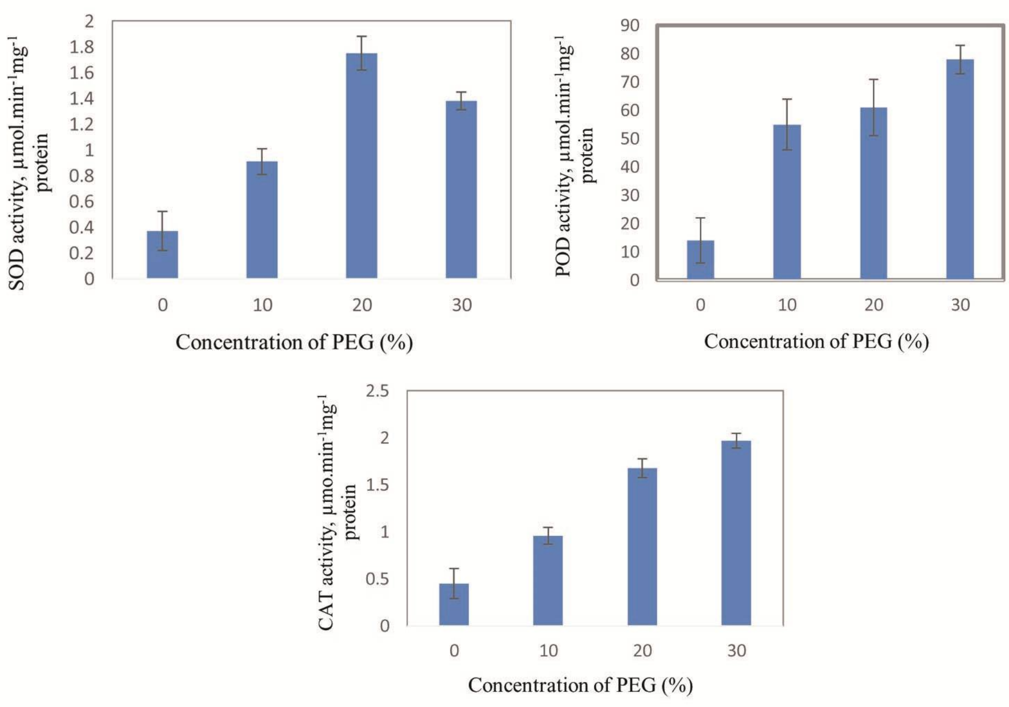

Fig. 10. The effect of different concentrations of water stress on the activity of (a) superoxide dismutase, (b) peroxidase and (c) catalase in the leaves of rice seedlings grown in solution culture. Each value is the mean of three replicates ± standard error.

From the present results, it may be concluded that water stress significantly altered the internal water status by decreasing different physiological and biochemical characteristics. In the present study, rice plants activate some biochemical and antioxidant defense mechanisms when subjected to various water stress levels, which helps in maintaining the structural integrity of the cell components and presumably alleviates oxidative damage. These antioxidant enzymes and proline act as protective mechanisms and represent the first line of defense against reactive oxygen species (ROS). All these characters account for its highest productivity under shortage of water supply. These adaptive mechanisms will assist in resolving water stress issue in Bangladesh. These characteristics can be utilized as screening techniques for water stress tolerance, leading to the future development of new varieties.

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