

## INFLUENCE OF WATERLOGGING STRESS ON NUTRIENT UPTAKE AND BIOCHEMICAL PARAMETERS IN RICE

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### Abstract

This study aimed to investigate the effect of waterlogging on nutrient uptake and its association with biochemical parameters, including reducing and total sugars, proline content, and antioxidant enzyme activities in *Oryza sativa* L. grown under hydroponic culture. Following up to 21 days of waterlogging induced by a continuous nitrogen gas supply,  $K^+$  accumulation significantly decreased by up to 35.6% in roots and 40% in shoots, while  $Na^+$  accumulation notably increased by 64% in roots and 58% in shoots at 21 days after treatment, which reflects an imbalance in  $K^+/Na^+$  homeostasis.  $NO_3^-$  content in roots and shoots decreased up to 23 and 27.8%, respectively. Furthermore,  $Ca^{2+}$  and  $Mg^{2+}$  levels in roots decreased by 9.7 and 25%, respectively. On the other hand,  $Fe^{2+}$  accumulation increased by 30% in roots after 21 days of exposure to waterlogging. Waterlogging stress triggered a notable rise in reducing sugar (55.5%), total sugar (37.6%), and proline (92.2%) content particularly in roots, reflecting a clear metabolic response to prolonged hypoxia. Furthermore, a 3.3-fold increase in superoxide dismutase 2.1-fold rise in peroxidase and a 2.7-fold increase in catalase activity in leaves indicated an activation of primary enzymatic antioxidant responses. Although these nutrient and biochemical changes are immediate responses to hypoxia, they are ultimately insufficient to prevent declines in overall growth. Comprehending these primary physiological and biochemical alterations provides essential baseline insights for developing waterlogging tolerant rice cultivars.

### Introduction

Global climate change is closely linked to changes in precipitation and floods. In recent decades, waterlogging stress, has affected crop productivity in agricultural regions and influenced plant distribution in natural ecosystems. In waterlogged soil, all pores are filled with water, which modifies the ideal solid : pore material (50 : 50) and soil : air volume (75 : 25) ratios, which affect the physiological and biochemical functions of the plant (Morales-Olmedo *et al.* 2015). Nowadays, waterlogging has been increasing worldwide due to inadequate drainage systems, increased soil degradation, and the impacts of climate change (Manghwar *et al.* 2024). In recent years, the frequency and severity of waterlogging have increased across Bangladesh, due to extreme weather conditions associated with climate change, insufficient drainage capacity, poorly designed flood-control systems, and unplanned infrastructure development. In Bangladesh, the north-east, south-west, and south-central regions experience some of the most severe waterlogging (Tareq *et al.* 2018, Akter *et al.* 2023).

Waterlogging reduced nutrient absorption, leading to deficiencies of essential nutrients like potassium and calcium (Zhang *et al.* 2025a). On the other hand, waterlogging increased the accumulation of  $Na^+$  in the root, stem and leaves of maize (Samad 2025). Inhibitory effect of waterlogging on nitrate accumulation was observed in wheat (Herzog *et al.* 2015). Waterlogging posed a major global threat to agriculture by inducing ion toxicities in roots due to soil redox changes (Zhang *et al.* 2025b). Elevated soluble sugar content was observed in cucumber under waterlogging stress (Barickman *et al.* 2019). Proline content significantly enhanced in *Helianthus annuus* under waterlogging condition (Dalai and Sardar 2021). In *Arabidopsis thaliana*, flooding

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triggered a marked increase in reactive oxygen species (ROS) accumulation (Peláez-Vico *et al.* 2023). Waterlogging significantly altered enzymatic antioxidant activities (SOD, CAT and POD) in peanut (Sharma *et al.* 2024).

More than half of the world's population depends on rice as a staple crop, and Asia accounts for almost 90% of its production and consumption (FAO 2021). Despite being adapted to flooded conditions, the length and severity of waterlogging stress severely limit rice growth and yield, unlike controlled paddy flooding; it disrupts plant metabolism and physiological functions. Hence, studying waterlogging-induced alterations in nutrient uptake and essential biochemical processes in rice is essential for developing strategies to improve stress management and maintain crop productivity. This study aimed to investigate the effect of waterlogging on the accumulation and distribution of key ions and their association with biochemical parameters (reducing and total sugars, proline and different antioxidant enzymes) in rice grown under hydroponic culture.

### Materials and Methods

The experiments were conducted in the Department of Botany, University of Dhaka, Bangladesh. Rice (*Oryza sativa* L. cv. BRRI Dhan 75) was selected as the experimental plant material. Seeds of rice were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur. The seeds were surface sterilized with 5.25% sodium hypochlorite solution and then the seeds were spread over cotton gauze on a perforated plastic lid and were placed upon the beaker filled with half strength Hoagland solution (Hoagland and Arnon 1950). Following germination the seedlings were transferred to a light bank. Rice seedlings were grown throughout the experiment at a day/night temperature of  $30^{\circ}\text{C} \pm 1^{\circ}\text{C}/25^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and day/night length of 14 hrs/10 hrs. Fourteen-day-old seedlings were subjected under anaerobic (treatment) or aerobic conditions (control) for 21 days. For the control, the nutrient solution was continuously aerated using an air pump to maintain optimal dissolved oxygen levels. Conversely, for the waterlogging condition, nitrogen gas was supplied continuously into the hydroponic culture to remove dissolved oxygen from the nutrient solution and induce anaerobic conditions. Dissolved oxygen levels were regularly monitored using a DO meter. The plants were grown in plastic buckets with perforated lids, where each hole received a single seedling, which was plugged with spongy foam for support. Solution was replaced after every 48 hrs. Roots and shoots were collected in triplicate after 3, 7, 14 and 21 days of waterlogging stress.

$\text{K}^+$ ,  $\text{Na}^+$  and  $\text{NO}_3^-$  were extracted from dry tissue by boiling in a water bath with two changes of 10 ml of distilled water. Potassium and sodium ions were estimated using flame photometry following the method of Jackson (1973). Nitrate was determined following the method of Cataldo *et al.* (1975).  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Fe}^{2+}$  were extracted by digestion in a mixture of nitric and perchloric acid (4 : 1) using a hot sand bath. The amount of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Fe}^{2+}$  in the extract was determined by an atomic absorption spectrophotometer (Yoshida *et al.* 1976). Reducing and total sugar were extracted by boiling fresh tissue in 80% ethanol and were determined by Somogyi-Nelson method (Nelson 1944 and Somogyi 1952) and Dubois *et al.* (1956) method, respectively. Determination of proline was done according to the method of Bates *et al.* (1973). Activities of different antioxidant enzymes, including SOD, POD and CAT, were measured according to the protocol described by Zhang *et al.* (2005).

### Results and Discussion

Accumulation of  $\text{K}^+$  in the root of rice plants was gradually decreased by 7 to 35.6% from 3 to 21 days of waterlogging treatment. In the shoot, waterlogging caused a gradual inhibition of  $\text{K}^+$  transport and the inhibitory effect was sustained up to 21 days of treatment (Fig. 1a, b). Similar waterlogging-induced inhibition of  $\text{K}^+$  accumulation was reported in rapeseed (Wang *et al.* 2022).

Waterlogging increased  $\text{Na}^+$  content in the root of rice plants by 12 to 64% from 3 to 21 days of treatment. Accumulation of  $\text{Na}^+$  in the shoot was increased upto 58% at 21 days of waterlogging treatment (Fig. 1c, d). Correspondingly, Chen *et al.* (2021) reported that flooded conditions significantly increased  $\text{Na}^+$  concentration and altered  $\text{Na}^+/\text{K}^+$  balance in rice tissues.

The inhibition of  $\text{NO}_3^-$  content in the root of rice plant ranged from 5 to 23% under waterlogged treatment from 3 to 21 days of application. Similar magnitude of inhibition of  $\text{NO}_3^-$  accumulation was also observed in the shoot. A maximum inhibition of 27.8% in the shoot occurred at 14 days of treatment (Fig. 1e, f). Shiono *et al.* (2024) reported a marked decline in the uptake of nitrate in rice when exposed to waterlogging stress.

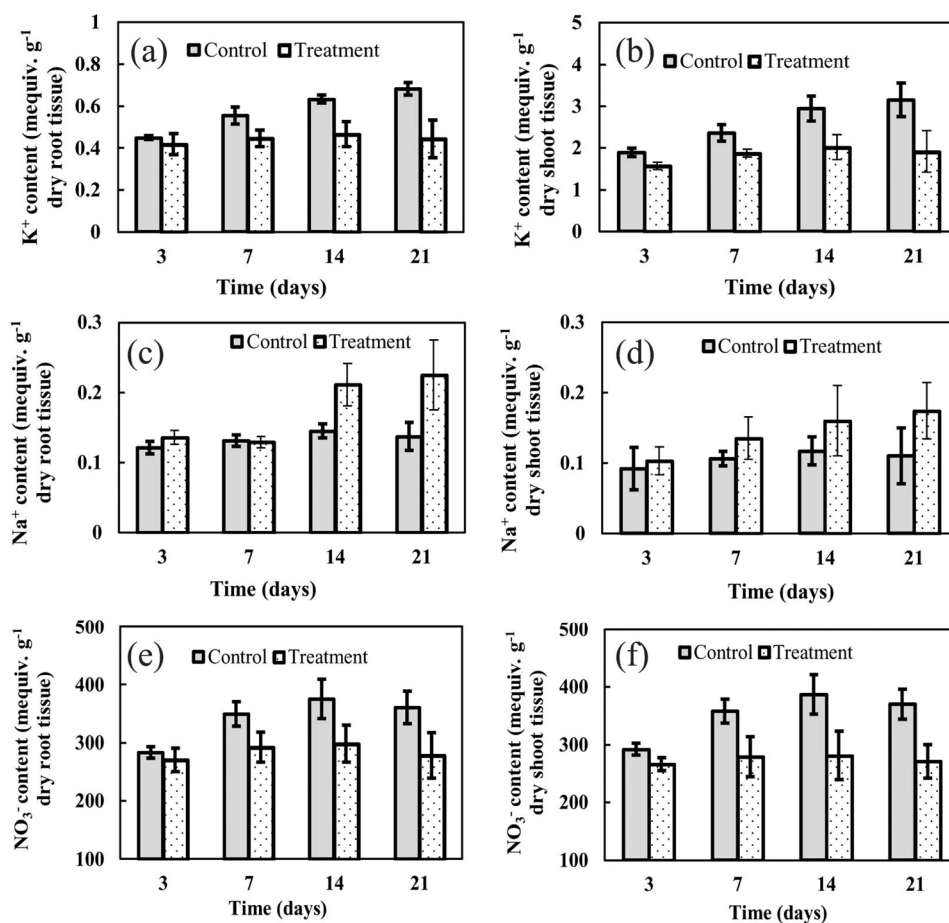


Fig. 1. Effect of waterlogging on the accumulation of  $\text{K}^+$  (a, b),  $\text{Na}^+$  (c, d) and  $\text{NO}_3^-$  (e, f) of rice plants grown in solution culture. Each value is the mean of three replicates  $\pm$  standard error.

Waterlogging stress reduced  $\text{Ca}^{2+}$  accumulation in the root of rice plants except an initial stimulation. In the root, waterlogging reduced  $\text{Ca}^{2+}$  content by 9.7% at 21 days of treatment. In the shoot,  $\text{Ca}^{2+}$  accumulation was also decreased following waterlogging application except an initial stimulation (Fig. 2a, b). According to Beegum *et al.* (2023), the concentration of  $\text{Ca}^{2+}$  in cotton was decreased with an increase in the number of days of waterlogging.

Magnesium accumulation in the root of waterlogged rice was decreased by 6 to 25% from 3 to 21 days of treatment. In the shoot, the accumulation of  $Mg^{2+}$  was gradually declined by 10.8 to 27.5% from 3 to 21 days of waterlogging application (Fig. 2c, d). Waterlogging caused acute deficiencies of magnesium in cotton (Zhang *et al.* 2021).

Exposure of the roots of rice plants to waterlogged condition caused 7.4, 19.6, 24.6 and 30.0% stimulation of  $Fe^{2+}$  content at 3, 7, 14 and 21 days of treatment, respectively. In the shoot, waterlogging increased accumulation of  $Fe^{2+}$  from 3 to 21 days of treatment leading to the highest increase by 51.4% at 21 days of treatment (Fig. 2e, f). This increase in  $Fe^{2+}$  accumulation under waterlogged conditions might be attributed to the reduction of ferric iron ( $Fe^{3+}$ ) to the more soluble ferrous form ( $Fe^{2+}$ ) in anaerobic soils, which enhanced Fe uptake by plants. This result is supported by Liu *et al.* (2020) who found that anaerobic soil conditions increased  $Fe^{2+}$  in plants.

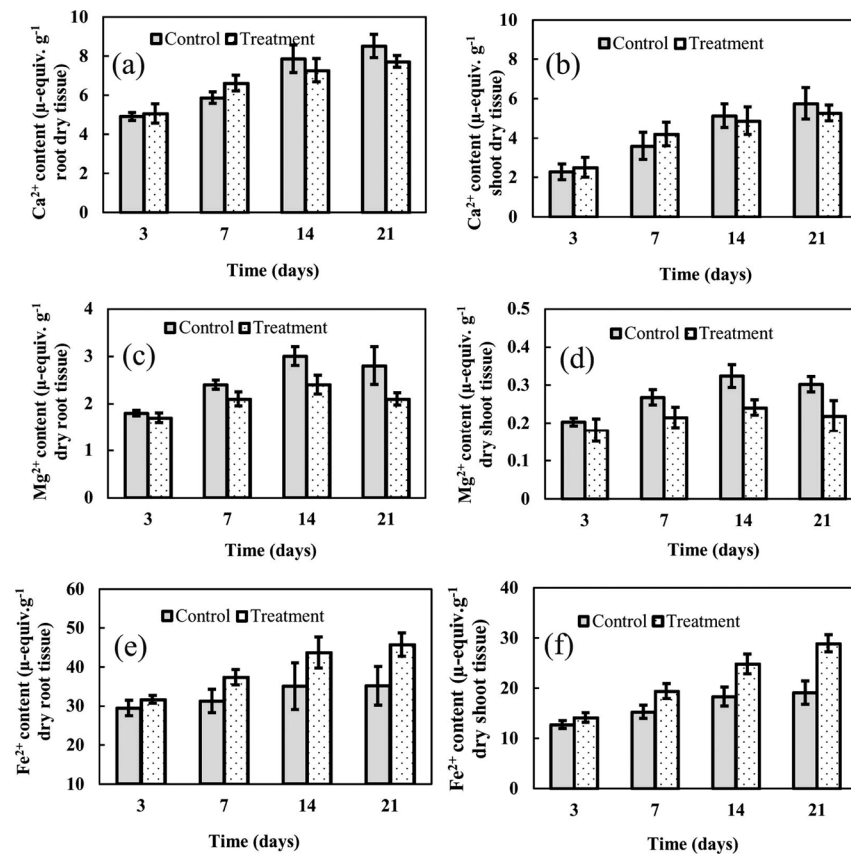


Fig. 2. Effect of waterlogging on the accumulation of  $Ca^{2+}$  (a, b),  $Mg^{2+}$  (c, d) and  $Fe^{2+}$  (e, f) of rice plants.

The accumulation of reducing sugar was increased in the root of rice from 3 to 21 days of waterlogging treatment. The maximum stimulation of reducing sugar accumulation in the root of rice was 55.5% at 14 days following waterlogging application. In the shoot of rice, similar stimulation of the accumulation of reducing sugar was also observed due to waterlogging treatment (Fig. 3a, b). This result is in agreement with the work of Ji and Hyun (2023) who reported that waterlogging resulted in an increase in the accumulation of reducing sugar in balloon flower.

Waterlogging caused an increase in total sugar content in the root of rice from 28 to 37.6% at 3 to 21 day following waterlogging application. It also increased the accumulation of total sugar in the shoot of rice plants and the stimulatory effect was sustained up to 21 days of treatment. A maximum of 43.4% increase in total sugar content in the shoot was recorded at 14 days of treatment (Fig. 3c, d). Similarly, an increase in total soluble sugar content was observed in pigeon pea with waterlogging treatment (Duhan *et al.* 2017).

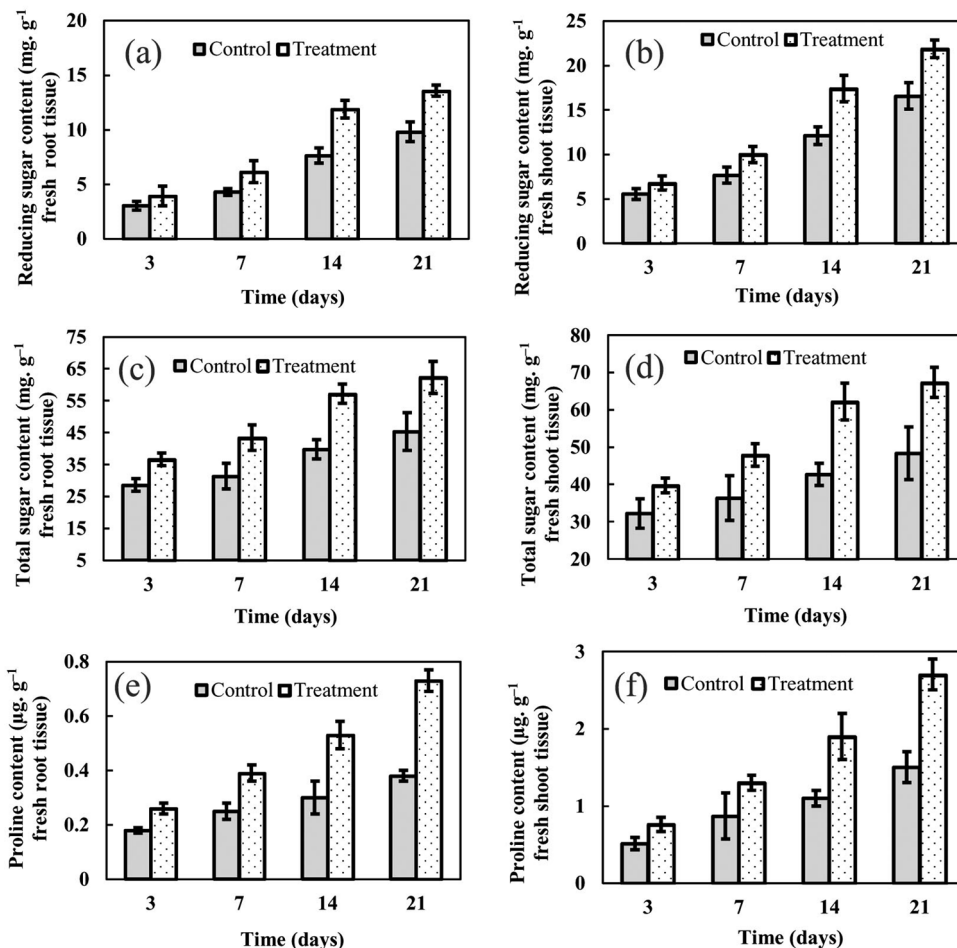


Fig. 3. Effect of waterlogging on the accumulation of reducing sugar (a, b), total sugar (c, d) and proline (e, f) of rice plants.

Rice plants subjected to waterlogging stress for 21 days accumulated significantly higher amounts of proline in the root compared to that of control plants. Waterlogging increased accumulation of proline in the root by 44.4 to 92.2% from 3 to 21 days of application. Due to waterlogging stress, 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. Waterlogging caused 49.0 to 80.3% increase in proline content in the shoot from 3 to 21 days of treatment (Fig. 3e, f). Waterlogging caused an increase in the amount of proline content in cucumber plants (Barickman *et al.* 2019).

Waterlogged stress resulted in a 3.3-fold increase in superoxide dismutase (SOD) activity in the leaves of rice plants at 15 days of treatment. Similarly, under the same circumstances, peroxidase (POD) activity showed a 2.1-fold rise and catalase (CAT) activity was found to increase in by 2.7-fold (Fig. 4). In waterlogging tolerant soybean variety, waterlogging stress led to enhanced activities of POD, CAT, and SOD, whereas the sensitive variety exhibited a decline in these enzyme activities. This indicated that improved waterlogging tolerance in soybean was linked to increased antioxidant enzyme activity (Sharmin *et al.* 2024).

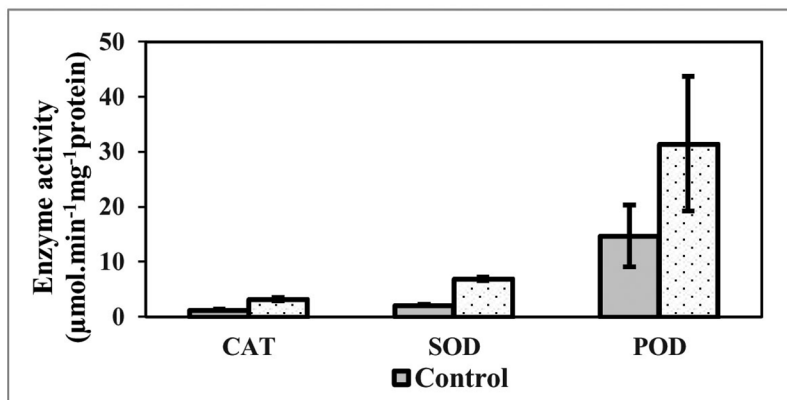


Fig. 4. Effect of waterlogging on the activity of catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) in leaves of rice plants at 14 days of treatment. Each value is the mean of three replicates  $\pm$  standard error.

Waterlogging stress resulted in a considerable increase in accumulation of  $\text{Na}^+$  with a concomitant decrease in that of  $\text{K}^+$  in rice plants. It was apparent from this result that waterlogging altered  $\text{K}^+/\text{Na}^+$  selectivity. Lower amount of  $\text{K}^+$  might disrupt different metabolic activities of plants. On the other hand, increase in  $\text{Na}^+$  accumulation might increase metabolic toxicity. Waterlogging application inhibited  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  accumulation in rice plants. The inhibition of  $\text{Ca}^{2+}$  accumulation might affect the permeability characteristic of plasma membrane because  $\text{Ca}^{2+}$  is necessary for maintaining this unique characteristic of plasma membrane. As  $\text{Mg}^{2+}$  is a constituent of chlorophyll, therefore, waterlogging-induced decrease in  $\text{Mg}^{2+}$  accumulation would reduce the synthesis of chlorophyll. This resulted in a nutrient imbalance in the plant. Due to waterlogged condition, reducing and total sugar was increased in rice plants. This rise in reducing and total sugar level might be crucial in maintaining the osmotic potential of cell sap during this stress. Waterlogging stress stimulated proline content in rice plants. Proline has a high positive impact on plants exposed to various stress conditions. Besides serving as an excellent osmolyte, proline performs three other crucial functions during stress, including metal chelation, antioxidant defense and signaling. Waterlogging-induced increase in proline content in rice plants indicated that the plant species was stressed as a result of exposure to waterlogging. The upregulation of SOD, POD and CAT under waterlogging stress in rice (BRRI Dhan 75) indicated an enhanced antioxidant defense response, which may help in maintaining cellular redox balance under hypoxic conditions and potentially mitigate oxidative stress.

#### Acknowledgements

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