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Physio-Biochemical Responses of Different Zinc-Rich Rice Varieties Subjected to Drought Stress

Dipa Rani Sarker[1], Bondhon Chakraborty[2], Md. Kamrul Hasan Kazal[1], Mohammad Anowar Hossain[1], Rakhi Chacrabati[3], Chayon Goswami[1*]

[1]Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[2]Department of Crop Botany, Habiganj Agricultural University, Habiganj-3300, Bangladesh.
[3]Interdisciplinary Institute for Food Security, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

*Corresponding author: chayon.goswami@bau.edu.bd

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Abstract

Scarcity of water is one of the main critical constraints to rice production. This study evaluated the drought tolerance of four zinc-enriched rice varieties- BRRI dhan74, BRRI dhan84, BRRI dhan100, and Binadhan-20, along with BRRI dhan66 as a drought-tolerant positive control. Rice seedlings were subjected to controlled drought conditions in earthen pots. After 30 days under drought stress, various physiological and biochemical parameters such as chlorophyll and carotenoid contents, osmolyte accumulation (proline, total soluble sugars), antioxidant enzyme activities (CAT, POD, APX), relative water content, and morphological traits of the rice seedlings were analyzed. Results demonstrated significant varietal differences in drought response. BRRI dhan84 demonstrated superior performance with minimal chlorophyll loss (12.25% reduction compared to respective control group), enhanced antioxidant activity (200% increase in CAT and 97.53% increase in APX), and moderate reductions in shoot and root growth (45.46% reduction in root length and 17.13% reduction in shoot length), closely resembling the effects observed in BRRI dhan66. Principal component analysis supported BRRI dhan84's favorable clustering under stress. The findings suggest BRRI dhan84 is a promising candidate for cultivation in drought-prone regions, offering a dual advantage of micronutrient biofortification and abiotic stress resilience.

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Introduction

A major portion of the world's population consumes rice (*Oryza sativa* L., belonging to the family Gramineae) as a staple grain, and it is essential to food security, particularly in Bangladesh, where its cultivation and variety development are prioritized. It primarily consists of carbohydrates (77.94%), along with protein, fat, moisture, and ash. While micronutrients like zinc (Zn) are essential for human health, many impoverished populations in Bangladesh rely on low-Zn rice, leading to widespread Zn deficiency (Kader *et al.*, 2021). Zinc is essential for cell development, growth, and metabolism. Its deficiency ranks fifth in disease burden in developing countries, causing stunted growth, weakened immunity, and increased mortality, including 400,000 annual deaths from respiratory and diarrheal diseases (Gupta *et al*., 2020b). In Bangladesh, zinc deficiency affects 45% of preschoolers and 36% of children under five, mainly due to malnutrition and poverty. As rice is a dietary staple, biofortified zinc-rich rice varieties such as BRRI dhan74, BRRI dhan84, BRRI dhan100, and Binadhan-20 (providing 24.2–27.6 mg/kg Zn) offer a sustainable solution. BRRI dhan74, BRRI dhan84, and BRRI dhan100 are cultivated in the Boro season (December–May), whereas Binadhan-20, BRRI dhan66, BRRI dhan62, and BRRI dhan71 are cultivated in the Aman season (July–December). The northern region of Bangladesh faces significant food insecurity due to severe drought, primarily caused by reduced rainfall and water scarcity (Adhikary *et al*., 2013). As rice is a drought-sensitive crop and drought is thought to have an impact on 50% of worldwide rice production, developing drought-tolerant cultivars is essential (Lafitte and Bennett, 2002). Drought stress hampers plant growth and physiological processes, affecting germination, root traits, biomass, and photosynthesis through pigment degradation and stomatal dysfunction. It also induces oxidative stress, disrupting enzyme activity and promoting leaf senescence (Allakhverdiev, 2020). Antioxidant enzymes like CAT, APX, and GPOX, along with osmolytes like proline, play critical roles in mitigating the detrimental effects of drought (Uarrota *et al*., 2018).

Drought stress reduces rice yield by impairing cell growth and antioxidant capacity, leading to various morphological, biochemical, physiological, and molecular changes (Bhandari *et al*., 2023). Aus- and indica-type accessions show better drought tolerance, and morphological, physiological, and biochemical screening offers a cost-effective selection method (Gupta *et al*., 2020a). These methods also focus on seedling traits linked to water-use efficiency to select genotypes with improved drought resistance and productivity. Therefore, the present study evaluated the physio-biochemical responses of different zinc-rich rice varieties subjected to drought stress.

Materials and Methods

1. Varieties used for the experiment

Four rice varieties were collected for experimentation: three zinc-rich varieties (BRRI dhan74, BRRI dhan84, and BRRI dhan100) from the Bangladesh Rice Research Institute (BRRI) and Binadhan-20 from the Bangladesh Institute of Nuclear Agriculture (BINA). The drought-tolerant variety BRRI dhan66 was used as a positive control. All seeds were stored in a refrigerated condition until used for research purposes.

2. Experimental location and period

This experiment was conducted in the Department of Biochemistry and Molecular Biology at Bangladesh Agricultural University (BAU) in Mymensingh, during the period from June 2022 to January 2023. This study area is geographically located at 24°43′26.8″N and 90°26′29.6″E. The region experiences a tropical monsoon climate, characterized by high relative humidity ranging from 80% to 84% and also observes an average annual rainfall of approximately 2000 mm.

3. Germination of seeds

Surface sterilization of seeds was done by using 5% sodium hypochlorite solution and then by 70% ethanol. The seeds were washed thoroughly with distilled water. Subsequently, the seeds were kept in

Petri dishes containing moist filter paper and incubation was done in the dark condition for 3 days at 25 °C to facilitate germination (Ervin *et al*., 2002).

4. Preparation of the pot for growing seedlings

Pots for rice seedlings contained 7 kg of soil each. Fertilizers such as 0.052 g Triple Superphosphate (TSP), 0.0667 g Muriate of Potash (MP), and 0.139 g Gypsum per kg of soil were added in each pot. The experiment was arranged in a completely randomized design (CRD) with all pots randomly assigned to treatment combinations of rice varieties and water regimes.

5. Transfer of germinated seeds into the pot

Germinated seedlings were transplanted into individual pots and were grown in well-watered conditions for 21 days. Drought stress was induced by withholding irrigation starting 21 days after transplanting (Saha *et al*., 2020). A well-watered treatment was maintained throughout the experiment and served as the control for comparison with the drought-stressed plants. At the end of the treatment, after 30 days under drought stress, sample collection was done from both control and drought-stressed plants, and different parameters were measured.

6. Investigated Parameters

6.1. Physiological and biochemical characters of rice genotypes for drought tolerant at seedling stage

6.1.1. Determination of Chlorophyll Content

The content of Chlorophyll was measured according to the previously described method (Long *et al*., 1985). Briefly, fresh rice leaves were collected from healthy plants grown in the rooftop garden of the Department. The uppermost fully expanded rice leaves were carefully washed with distilled water, blotted dry using sterile paper towels, and then cut into small segments using a sterile scalpel. A 0.05g rice leaf sample was added to 25ml of 80% acetone and was kept in the dark for 7 days for complete extraction of chlorophyll. Chlorophyll content was measured with the help of a spectrophotometer at 663 nm (A_{663}) and 645 nm (A_{645}). Using these values, chlorophyll a and chlorophyll b contents were calculated, and the total chlorophyll content was obtained from the sum of chlorophyll a and chlorophyll b:

Chlorophyll a (mg/g) = $\{(12.7 \times A_{663}) - (2.79 \times A_{645})\} \times$ Dilution Factor (DF)

Chlorophyll b (mg/g) = $\{(21.5 \times A_{645}) - (5.1 \times A_{663})\} \times$ Dilution Factor (DF)

Total chlorophyll (mg/g) = (Chlorophyll a + Chlorophyll b)

6.1.2. Determination of beta carotene content

Beta-carotene content was measured according to the method developed by Sarker and Oba (2019). A 500 mg fresh leaf sample was extracted with 80% acetone, centrifuged, and diluted. Absorbances at 510 nm (A_{510}) and 480 nm (A_{480}) were measured to calculate beta-carotene content in mg per gram of fresh weight.

Beta-carotene (mg/g) = $\{7.6(A_{480}) - 1.49(A_{510}) \times$ Final volume/ (1000 × fresh weight of leaf)$\}$

6.1.3. Determination of ascorbate content of leaf

The ascorbate (vitamin C) content of rice leaves was measured using the 2,6-dichloroindophenol (DCIP) method (Nielsen, 2024). In the assay, ascorbic acid acts as a reducing agent, converting the colored indicator dye into a colorless form. During titration, the endpoint is reached when excess indicator dye remains unreduced, producing a rose-pink coloration in the acidic medium. The titer value of the dye was determined using a standard ascorbic acid solution. Briefly, a 5 g leaf sample was blended with 100 ml meta-phosphoric acid and filtered. 5 ml of extract was titrated with DCIP to measure vitamin C, which reduces the dye until a pink endpoint appears. Ascorbate content was then calculated.

6.1.4. Determination of total soluble sugar

The concentration of total soluble sugar was estimated by the anthrone-sulfuric acid method (Franscistt *et al*., 1971). Fresh leaves were ground with 5 ml of hot 80% ethanol, and the extract was properly centrifuged to remove debris. A 0.2 ml aliquot of the prepared supernatant was diluted with 0.8 ml of distilled water. Standard glucose solutions were prepared, and 4 ml of anthrone reagent was added. Incubation of the samples was done in a boiling water bath for 10 minutes. Then the samples were allowed to cool to room temperature, and the absorbance of the samples was recorded at 620 nm using a spectrophotometer. The concentration of total soluble sugar in the diluted samples was determined using a glucose standard curve, and multiplication of the obtained values by the dilution factor resulted the sugar content in the original extracts.

6.1.5. Determination of relative water content

Fresh weight (FW), dry weight (DW), and turgid weight (TW) were measured from random samples of 5 flag leaves per group. TW was obtained after 24-hour water immersion, and DW after 48-hour oven-drying at 70°C. Relative Water Content (RWC) was obtained using the following formula (Barrs and Weatherley, 1962):

$$\text{RWC (\%)} = (\text{FW}-\text{DW})/\ (\text{TW}-\text{DW}) \times 100$$

6.1.6. Determination of proline

To measure proline content according to the previously described method (Bates *et al*., 1973), 50 mg of fresh leaf tissue was properly mixed with 10 mL of 3% sulfosalicylic acid, followed by homogenization, centrifugation, and filtration of the extract. Then, 2 ml of the filtrate were mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin, followed by shaking. Incubation of leaf samples was done at 100°C for 1 hour, cooled, and treated with toluene. Absorbance (at 520 nm) of the solution was measured with the help of a spectrophotometer, and proline content was obtained from a standard curve, and the result was expressed as mg/100g fresh leaves.

6.1.7. Measurement of antioxidant enzyme activity

a. Determination of catalase activity

A 50 mg fresh leaf sample was homogenized in 3 ml of 50 mM potassium phosphate buffer (pH 8.0), centrifuged at 12,000 rpm for 10 min at 4°C, and the collected supernatant was used to assay catalase activity. Catalase activity of the samples was measured by following the method of Aebi (1984). Catalase activity was measured by adding enzyme extract to a reaction mixture with potassium phosphate buffer, EDTA, and H_2O_2. Absorbance at 240 nm was recorded every 30 seconds for 2 minutes, and enzymatic activity was calculated based on the decrease in the values of absorbance per minute using the extinction coefficient (40 $M^{-1}cm^{-1}$ for H_2O_2).

$$\text{CAT (mM min}^{-1}\text{ g}^{-1}\text{ F.W.)} = \{(\text{Absorbance difference/min}) \times \text{DF} \times 1000\}/\ (40 \times 1000)$$

b. Determination of peroxidase activity

Peroxidase (POD) activity was determined following the method described by Nakano and Asada (1981). A reaction mixture was prepared that contains potassium phosphate buffer, EDTA, H_2O_2, and Guaiacol. Peroxidase activity was measured by adding the enzyme extract and tracking absorbance at 470 nm for 2 minutes. Enzyme activity was estimated from the absorbance change per minute using the H_2O_2 extinction coefficient (26.6 $M^{-1}cm^{-1}$).

POD (mM min^{-1} g^{-1} F.W.) = {(Absorbance difference/min) × DF × 1000}/ (26.6 × 1000)

c. Determination of ascorbate peroxidase activity

Ascorbate peroxidase (APX) activity was determined using the method developed by Aebi (1984). A solution containing potassium phosphate buffer, EDTA, H_2O_2, and ascorbate was prepared, and absorbance at 290 nm was measured for two minutes after adding the extract. Using the extinction coefficient of 2.8 $M^{-1}cm^{-1}$ for H_2O_2, ascorbate peroxidase activity was measured using the following formula-

APX (mM min^{-1} g^{-1} F.W.) = {(Absorbance difference/min) × DF × 1000}/ (2.8 × 1000)

6.1.8. Determination of total antioxidant capacity

The total antioxidant capacity in leaves was measured using a method developed by Sarker and Oba (2019). A 5 g leaf sample was extracted with ethanol, evaporated, and oven-dried. Antioxidant activity was measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, where absorbance at 517 nm was recorded after reacting the extract with DPPH. Antioxidant activity was determined by calculating the percentage of DPPH inhibition relative to the control, using the following equation:

Antioxidant activity (%) = {(Abs. blank- Abs. sample)/Abs. blank} × 100

6.2. Morphological characters of rice genotypes for drought tolerance at the seedling stage

From the shoot initiation to the root tip, the length of the root was measured. The measurement of shoot length was obtained by subtracting the root length from the plant length. The area of the leaves was measured by using a leaf area meter (LI-3100, LI-COR Inc., Nebraska, USA).

Statistical Analysis

R (version 4.2.2) software was used to perform the statistical analysis, with one-way ANOVA followed by Tukey's post hoc test ($P<0.05$). The means ± standard error mean (SEM) of the data were displayed. The "ggplot2," "factoextra," "FactoMineR," and "corrplot" packages were used to perform principal component analysis (PCA).

Results and Discussion

1. Effect of drought stress on chlorophyll and beta-carotene content

Drought stress reduced chlorophyll content in rice leaves, with varying impacts across different varieties (Figure 1). The reduction in chlorophyll a was lowest in BRRI dhan66 (5.39%), followed by BRRI dhan84 (12.25%), Binadhan-20 (26.61%), BRRI dhan74 (31.55%), and BRRI dhan100 (41.07%). Chlorophyll b content showed the highest decrease in BRRI dhan100 (48.57%) and the lowest in BRRI dhan66 (6.53%). Total chlorophyll reduction was minimal in BRRI dhan66 (5.72%) and BRRI dhan84 (13.11%), while it was higher in Binadhan-20 (27.67%), BRRI dhan74 (28.51%), and BRRI dhan100 (42.86%). Drought stress impairs photosynthesis by reducing carbon fixation, disrupting chloroplast structure, and causing stomatal closure (Hu *et al*., 2023). It leads to chlorophyll degradation, yellowing of leaves, and reduced growth, while carotenoids help in photoprotection (Jarin *et al*., 2024). Water stress limits CO_2 availability, decreases chlorophyll content, and alters pigment ratios (Yang *et al*., 2024). Additionally, it reduces root growth and nutrient absorption, increasing oxidative damage (Geng *et al*., 2024). In zinc-rich rice varieties, drought stress insignificantly decreased the content of chlorophyll in the leaves. However, the reduction was comparatively lower in BRRI dhan84 than in the other varieties, except BRRI dhan66.

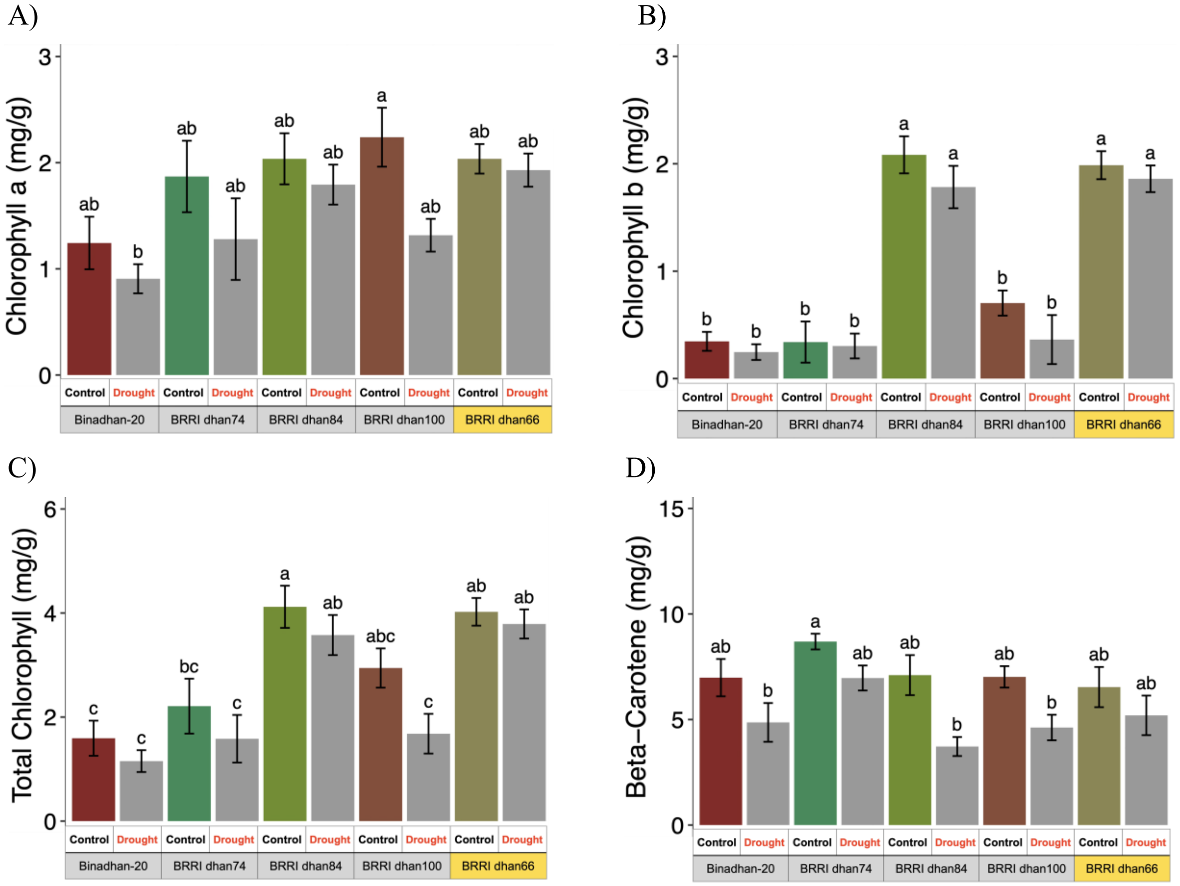


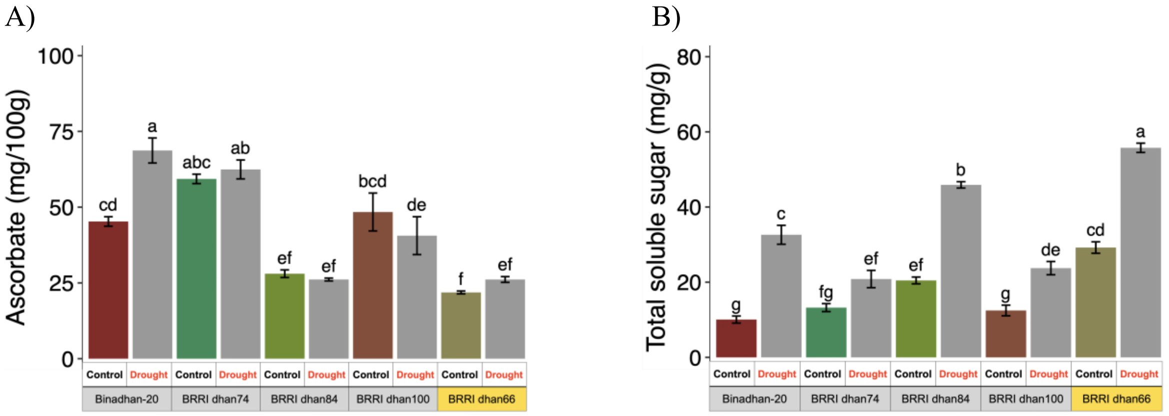
Figure 1. Effect of drought stress on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and beta-carotene (D) content in rice varieties. Vertical bars in the graph represent mean values ± SEM (n≥3)

Drought stress reduced beta-carotene content in all zinc-rich rice varieties studied (Figure 1D). Among the rice varieties, the highest decrease was observed in BRRI dhan84, which was 47.61%, whereas the lowest reduction was found in BRRI dhan74 (19.79%). However, the values were statistically insignificant when compared to their respective control groups. Drought stress also reduced the beta-carotene level of Binadhan-20 and BRRI dhan100 by 30.37% and 34.19%, respectively. However, these changes were statistically insignificant compared to the control groups. Drought stress reduces photosynthesis by lowering relative water content, causing stomatal closure, decreased CO_2 intake, and impaired electron transport, which affects photosystem efficiency and ATP production. Carotenoids, important for photosynthesis and stress response, are cleaved into bioactive molecules like β-cyclocitral and β-cyclocitric acid, enhancing stress tolerance. In rice, carotenoid levels decreased under drought stress, with remarkable but insignificant reductions in Binadhan-20, BRRI dhan100, and BRRI dhan84, while slight declines were observed in BRRI dhan74 and BRRI dhan66.

2. Effect of drought stress on ascorbate, total soluble sugar, relative water, and proline content of rice plant

Results of the present study found that drought stress significantly influenced ascorbate content in rice plants, with varietal differences observed (Figure 2A). The highest increase was 51.72% in Binadhan-20. A 19.53% increase was noted in BRRI Dhan66, but it was not statistically significant. Decreases in ascorbate content were recorded in BRRI dhan84 (6.81%) and BRRI dhan100 (16.14%), while BRRI dhan74 showed a slight increase of 5.26%. Ascorbate is an essential non-enzymatic antioxidant in plants, aiding in photosynthesis, cell division, and stress adaptation by scavenging reactive oxygen species (ROS) under abiotic stresses. It regulates key signaling pathways, including ROS, ABA, and auxin, promoting plant growth and metabolism (Wang *et al*., 2019). Elevated ascorbate levels enhance oxidative tolerance, especially in rice. Based on the findings of the current investigation, drought stress increased ascorbate content in Binadhan-20, BRRI dhan66, and BRRI dhan74. On the contrary, BRRI dhan84 and BRRI dhan100 exhibited a slight decrease in ascorbate concentration when compared to the other varieties.

Drought stress significantly increased the total soluble sugar content in rice varieties (Figure 2B). The increase was highest in Binadhan-20 (224.25%), followed by BRRI dhan84 (124.18%), BRRI dhan100 (90.22%), BRRI dhan66 (90.79%), and BRRI dhan74 (57.48%). These results suggest that the most soluble sugar accumulation under drought stress was found in Binadhan-20, followed by BRRI dhan84, BRRI dhan100, and BRRI dhan74. Under drought stress, plants accumulate osmoprotectants like soluble sugars, proline, and amino acids to maintain turgor and cell function. Soluble sugars (sucrose, glucose, fructose) aid osmotic adjustment, especially in susceptible rice varieties (Živanović *et al*., 2020). The highest increase in total soluble sugars was observed in BINA dhan-20, and the least increase was observed in BRRI dhan74. BRRI dhan84 showed the second-highest increase, which was close to the positive control BRRI dhan66. During drought conditions, plants can maintain their leaf turgor (stiffness) by adjusting their osmotic pressure by accumulating solutes such as proline, glycine betaine, sucrose, nitrate reductase activity, and others in their cytoplasm, which helps them to absorb more water from the dry soil.



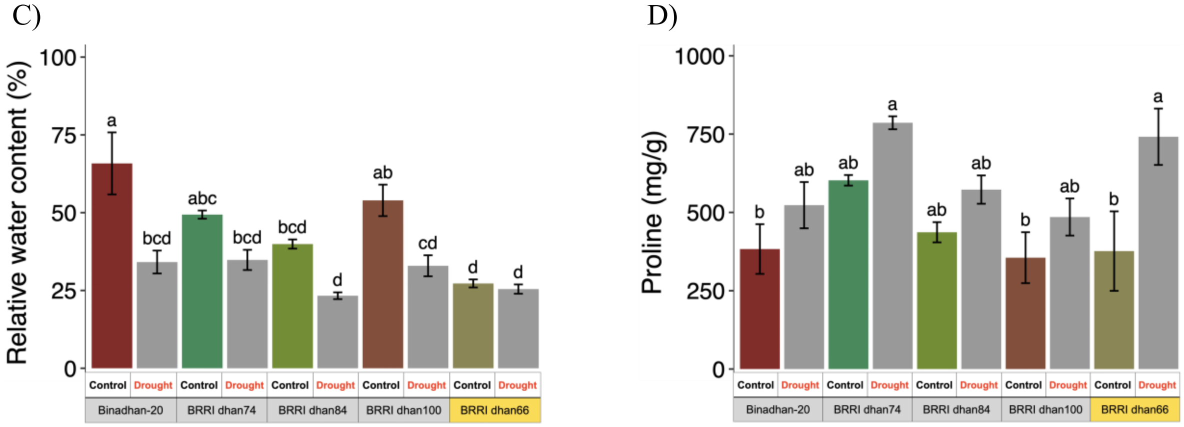


Figure 2. The effect of drought stress on ascorbate (A), Total soluble sugar (B), Relative water content (C), and Proline content (D) in different types of rice varieties. Vertical bars represent the mean values ± SEM (n≥3)

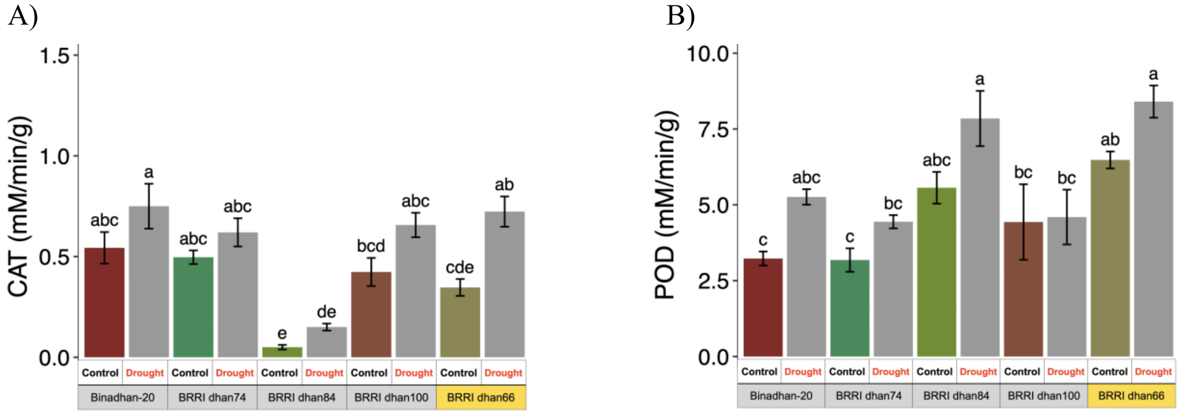
Under drought stress, all rice varieties showed a decrease in relative water content (Figure 2C). The drought-tolerant BRRI dhan66 had the smallest reduction at 6.64%. Among zinc-enriched varieties, Binadhan-20 experienced the largest decrease (48.15%), followed by BRRI dhan84 (41.59%), BRRI dhan100 (38.98%), and BRRI dhan74 (29.51%). Overall, zinc-enriched varieties showed higher reductions in RWC compared to BRRI dhan66. Under drought stress conditions, the roots of the rice plant absorb comparatively less water than the well-watered conditions. This reduction in water uptake contributes to a water deficit condition in the rice plant, ultimately decreasing the relative water content (RWC). Drought stress reduces RWC, affecting water use efficiency (WUE) and yield in rice (Gupta *et al*., 2020a). Among the varieties studied, Binadhan-20 and BRRI dhan100 exhibited a significant decrease in relative water content, while BRRI dhan84 also showed a reduction in relative water content comparable to that of BRRI dhan66, indicating its ability to tolerate drought stress.

Proline accumulation increased in all rice varieties under drought stress, with the drought-tolerant BRRI dhan66 showing the highest increase at 96.98% (Figure 2D). The other varieties, such as BRRI dhan74, BRRI dhan84, BRRI dhan100, and Binadhan-20 exhibited comparatively lower proline accumulation than the BRRI dhan66. Among these four varieties, in comparison to the control groups, the highest proline accumulation increase was observed in Binadhan-20 (36.59%), followed by BRRI dhan100 (36.52%), BRRI dhan84 (31.17%), and BRRI dhan74 (30.5%), respectively. However, these differences were not statistically significant. Proline enhances drought tolerance in rice by acting as an osmoprotectant, antioxidant, and signaling molecule (Jarin *et al*., 2024). In this study, an enhancement in proline content was observed in all cultivars. Binadhan-20 exhibited the highest increase, while BRRI dhan74 showed the lowest increment compared to the control plants. Proline accumulation also increased in the case of BRRI dhan84.

3. Effect of drought stress on enzymatic antioxidant activity and DPPH radical scavenging ability

The impact of drought stress on reactive oxygen species (ROS) management in rice genotypes is investigated by measuring the activities of key antioxidant enzymes, such as catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD). These enzymes are essential for reducing superoxide and hydrogen peroxide levels, which help to mitigate oxidative damage under stress. Drought stress resulted in a significant increase in catalase (CAT) activity across all rice types, with the largest increase in BRRI dhan66 (105.71%). Among zinc-enriched cultivars, BRRI dhan84 showed the highest increase (200%), followed by BRRI dhan100 (57.14%), Binadhan-20 (38.89%), and BRRI dhan74 (24%). BRRI dhan84 exhibited the highest overall tolerance due to its increased ROS scavenging capacity, characterized by increased CAT activity (Fig. 3A). Under drought stress, POD activity increased in all rice varieties (Fig. 3B). BRRI dhan66 showed the highest increase (29.63%), followed by zinc-enriched Binadhan-20 (62.85%), BRRI dhan84 (41.19%), BRRI dhan74 (39.62%), and BRRI dhan100 (3.84%). Differences were not statistically significant. The present study found increased APX activity in all drought-treated rice varieties (Fig. 3C). BRRI dhan84 showed the highest increase (97.53%), followed by Binadhan-20 (61.49%), BRRI dhan74 (58.77%), BRRI dhan100 (55.04%), and BRRI dhan66 (89.73%), though the latter was not statistically significant. BRRI dhan74 had a significant increase when compared with the control group. The ability of a plant extract to scavenge DPPH radicals can be used to measure its overall antioxidant activity. Our results demonstrated that DPPH radical scavenging ability increased in all rice varieties under drought stress (Fig. 3D). BRRI dhan66 showed an increase in DPPH radical scavenging ability at 30.4%, while the zinc-rich varieties, including BRRI dhan74, BRRI dhan84, BRRI dhan100, and Binadhan-20, also exhibited an increase in DPPH radical scavenging ability under drought stress. Among the four zinc-rich rice varieties, the highest DPPH radical scavenging ability was found in BRRI dhan74 (45.62%), followed by Binadhan-20 (34.88%), BRRI dhan84 (16.02%), and BRRI dhan100 (15.42%). However, these changes were not statistically significant when compared with the control groups.

Plants respond to abiotic stresses through oxidative stress, generating reactive oxygen species (ROS). Plants can escape the destructive effects of ROS by developing a strong defense system of antioxidant enzymes, like APX, CAT, and POD (Jarin *et al*., 2024). Under drought stress, BRRI dhan84 exhibited a stronger increase in CAT activity compared to the control group, which was found to be similar to the positive control, BRRI dhan66. In drought-tolerant rice like BRRI dhan66, higher catalase (CAT) activity effectively scavenges H_2O_2, enhancing drought tolerance. Binadhan-20 showed the highest rise in POD activity, while BRRI dhan84 showed the second-highest values in POD activity. APX activity increases under drought stress in rice, especially at the tillering stage, aiding H_2O_2 detoxification via the AsA-GSH pathway (Wang *et al*., 2019). Among the rice varieties, BRRI dhan84 showed the highest increase in APX activity under water deficit conditions, and its value was closer to that of the positive control (BRRI dhan66). Production of antioxidants and their action to prevent free radicals are determined by DPPH radical scavenging activity and are expressed as inhibition percentage. When subjected to drought stress, BRRI dhan74 showed an increase in percent inhibition, while other varieties also exhibited a slight increase when compared to their growth under normal conditions.



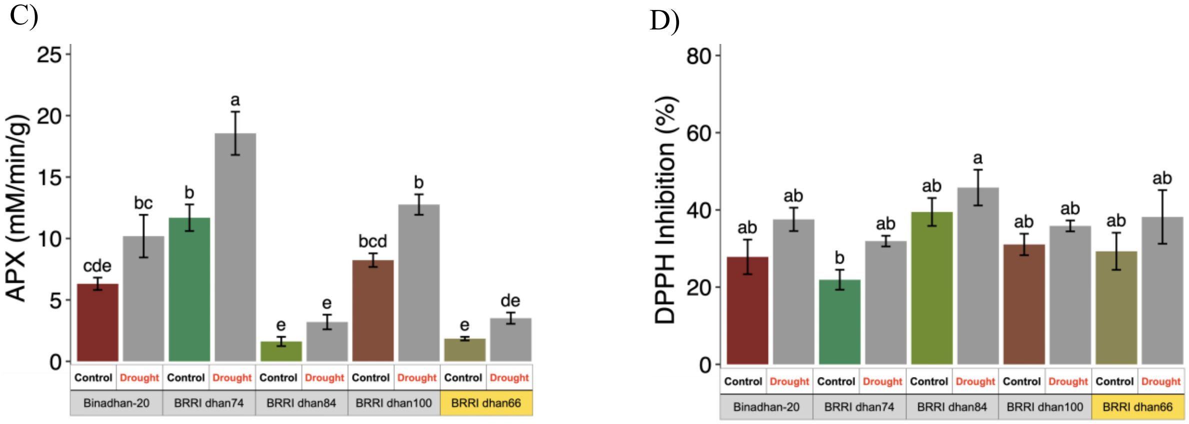
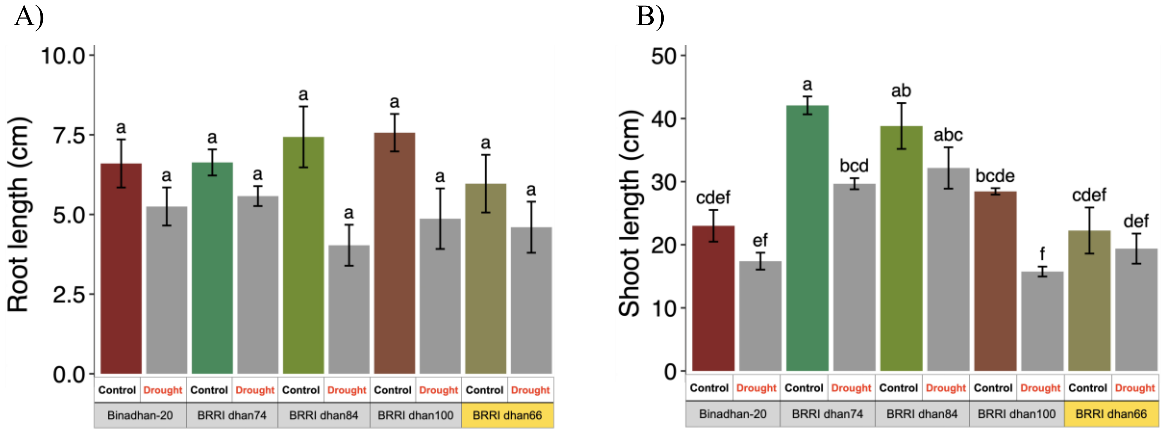


Figure 3. The impact of drought stress on catalase (A), Peroxidase (B), and Ascorbate Peroxidase (C) activities in rice varieties and the influence of drought stress on the ability of various rice varieties to combat DPPH radicals (D). Vertical bars represent mean ± SEM (n≥3)

4. Effect of drought stress on root length, shoot length, and leaf area of rice varieties

Drought stress reduced root length in all rice varieties. BRRI dhan66 (22.95%), BRRI dhan84 (45.46%), BRRI dhan74 (15.84%), BRRI dhan100 (35.67%), and Binadhan-20 (20.45%) showed reductions, with no significant statistical differences (Figure 4A). Drought stress reduced shoot length in rice varieties due to lower osmotic potential, decreased wall extensibility, and limited cellular expansion (Figure 4B). The reduction varied among varieties i.e., BRRI dhan66 had the least decrease (12.85%), followed by BRRI dhan84 (17.13%), and the highest decline was observed in BRRI dhan100 (44.64%). Water deficit reduced leaf area in all zinc-rich rice varieties. Compared to the respective control group, the lowest decrease in leaf area due to the drought stress was observed in Binadhan-20 (32.66%), followed by BRRI dhan84 (42.26%), BRRI dhan74 (58.87%), and BRRI dhan100 (69.56%). However, significant decreases were only observed in BRRI dhan74, BRRI dhan84, and BRRI dhan100 (Figure 4C).



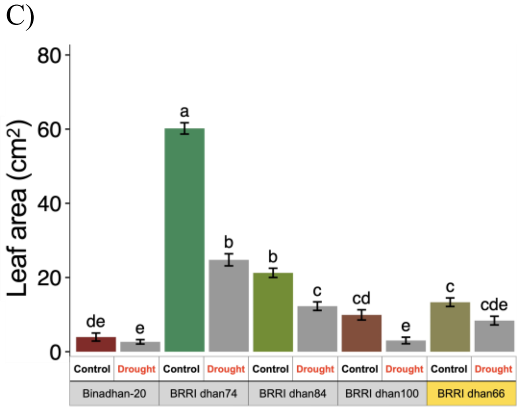


Figure 4. Effect of drought stress on root length (A), shoot length (B), and leaf area (C) of different rice varieties. Vertical bars represent mean ± SEM (n≥3)

Under drought, root traits are altered, but adaptive growth in deeper zones improves water uptake. Primary roots use osmolytes, hormones, and antioxidants, while lateral roots enhance absorption. Higher root hydraulic conductivity supports yield and efficiency (Kalra *et al*., 2024), though soil shrinkage lowers conductance (Yang *et al*., 2024). In the present study, BRRI dhan84 showed the least root length reduction (15.84%), indicating moderate tolerance. On the other hand, the root length in BRRI dhan74, Binadhan-20, and BRRI dhan100 was reduced by 15.84%, 20.45%, and 35.67%, respectively. Thus, it can be speculated that the BRRI dhan84 has moderate potential to withstand the effect of drought as characterized by prohibiting the reduction of root length under water deficit conditions. Drought exposure reduces shoot length in plants due to decreased cell growth and water stress, with the reduction linked to drought severity and duration (Gedam *et al*., 2021). BRRI dhan84, among four zinc-rich rice varieties, showed the least shoot length reduction, indicating better drought tolerance than BRRI dhan74 and BRRI dhan100. Drought stress reduces leaf area by hindering cell development, causing leaf shrinkage and thicker cell walls (Jarin *et al*., 2024). Leaf rolling, regulated by bulliform cells, conserves water and aids photosynthesis, but severe rolling impedes growth (Jarin *et al*., 2024). In this study, rice varieties showed varying levels of reductions in leaf area, with Binadhan-20 exhibiting the least reduction and BRRI dhan100 the most. BRRI dhan84's leaf area reduction due to drought stress was similar to that of BRRI dhan66, suggesting that BRRI dhan84 may have some level of tolerance to drought.

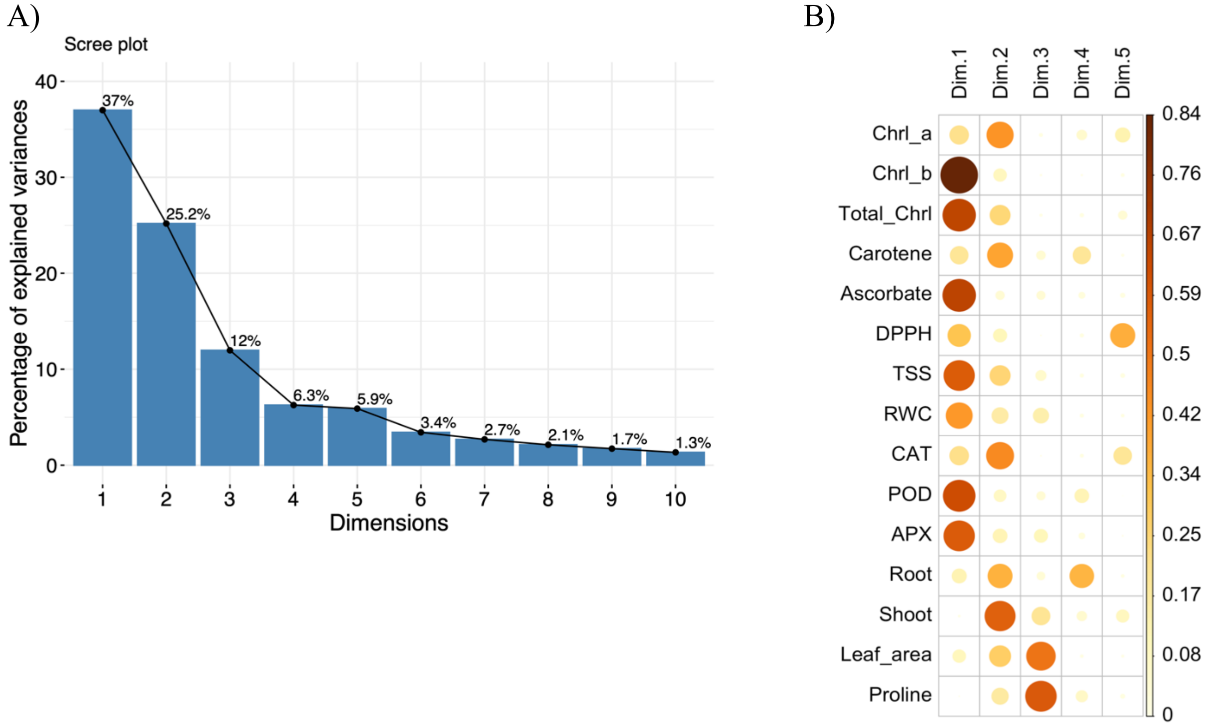
5. Principal Component Analysis (PCA)

The explained variance of ten primary components is shown on the Scree plot (Figure 5A). The first five dimensions (Dim. 1 to 5) extracted from PCA (Fig. 5B). Circle size and color intensity indicate the contribution (ranging from 0 to 0.84) of each variable to a given dimension. Dimension 1, which accounts for 37% of the variance, is determined by chlorophyll B, total chlorophyll, ascorbate, TSS, RWC, POD, and APX. Dimension 2, contributing 25.2% of the variance, is defined by chlorophyll A, beta-carotene, CAT, and shoot length. Dimensions 3 and 4 account for 6.3% and 12% of the variance, respectively, with leaf area and proline contributing to dimension 3 and root length to dimension 4. Dimension 5 accounts for 5.9% of the variance. Dimensions 6 through 10 have variances of 3.4%, 2.7%, 2.1%, 1.7%, and 1.3%, respectively.

The PCA results for five rice cultivars under drought conditions indicated that principal components 1 (PC1) and 2 (PC2) explained 62.2% of the variance in the data (Figure 5C). Key parameters such as drought score and chlorophyll levels were clustered positively for BRRI dhan66 and BRRI dhan84, indicating a positive correlation. Conversely, chlorophyll a, carotene, and catalase were grouped negatively for control varieties BRRI dhan74, Binadhan-20, and BRRI dhan100. It was suggested that greater drought tolerance was exhibited by BRRI dhan84, similar to the drought-tolerant control variety, BRRI dhan66.

In the PCA Biplot, it was clear that the drought score, Chlorophyll b, total chlorophyll, ascorbate, TSS, RWC, POD, and APX of BRRI dhan66 and BRRI dhan84 were grouped with positive loadings on the right side of the biplot. However, BRRI dhan66 (control) and BRRI dhan84 (control) were observed in the upper right quadrant, while BRRI dhan66 (drought) and BRRI dhan84 (drought) were located in the lower right quadrant of the biplot. These observations suggest that these parameters exhibited a positive correlation among themselves. It can be speculated that BRRI dhan84 is more tolerant to drought than other kinds of zinc-rich rice varieties studied due to its similarity in response under drought stress with the tolerant variety, BRRI dhan66.

The study identifies key traits contributing to drought tolerance in rice, including reduced chlorophyll and carotenoid levels, increased ascorbate content, higher free radical scavenging ability, elevated soluble sugars, low relative water content, higher antioxidant activity, and high proline accumulation. The drought tolerability of a rice variety is also supported by the profound root and shoot performance under the stress condition. Among four Zn-rich rice varieties tested, BRRI dhan84 was found to be the most suitable for drought-prone areas.



C)

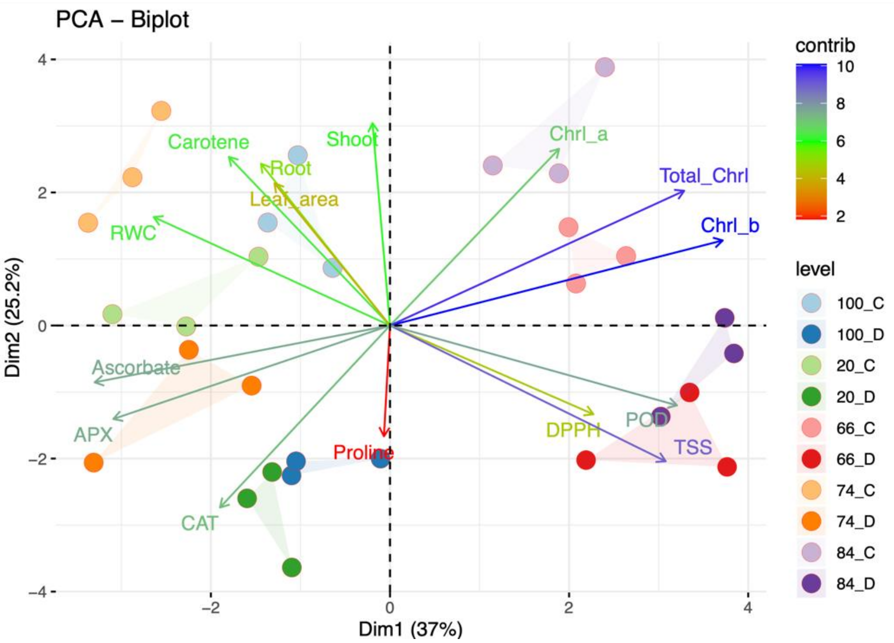


Figure 5. Scree plot of principal component analysis (PCA) showing the variance explained by each dimension (A), Corrplot representing the correlations (color and size of circles) of each measured parameters with the first five principal components derived from PCA (B), and PCA biplot showing the distribution of rice samples and contributions of measured parameters (C)

Chrl_a= Cholorophyll a; Chrl_b= Chlorophyll b; Total_Chrl= Total Chlorophyll; Carotene = Beta-Carotene; Ascorbate= Vitamin C; Proline= Proline content; TSS= Total soluble sugar; DPPH= DPPH scavenging capacity (% inhibition); CAT= Catalase activity; APX= Ascorbate Peroxidase activity; POD= Peroxidase activity; Root= Root length; Shoot= Shoot length; RWC= Relative water content; Leaf_area= Leaf area. 100_C and 100_D represent BRRI dhan100 under control and drought conditions, respectively; 20_C and 20_D represent Binadhan-20; 66_C and 66_D represent BRRI dhan66; 74_C and 74_D represent BRRI dhan74; 84_C and 84_D represent BRRI dhan84 under control and drought conditions, respectively.

Conclusions

Rice production is severely affected by drought stress, which causes significant monetary losses. This study showed that drought stress adversely affected photosynthesis, pigment stability, antioxidant balance, osmolyte accumulation, and growth traits in zinc-rich rice varieties. However, BRRI dhan84 consistently demonstrated moderate resilience, showing responses that were similar to those of the tolerant control BRRI dhan66. These responses included higher antioxidant enzyme activities (CAT, POD, APX), increased soluble sugars and proline, and stable relative water content, along with relatively lower reductions in chlorophyll, carotenoids, root and shoot length, and leaf area of the rice seedlings. According to these results, BRRI dhan84 is the most suitable zinc-rich variety to grow in drought-prone areas since it may possess stronger adaptive mechanisms against water deficit condition.

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Conflicts of Interest

The authors hereby declare that they have no financial, personal, or professional conflicts of interest.

Funding Declaration

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Author Contribution

Dipa Rani Sarker: investigation; formal analysis; Bondhon Chakraborty: resources writing-review and editing; investigation; data curation; formal analysis; Md. Kamrul Hasan Kazal: writing-original draft; visualization; writing-review and editing; Mohammad Anowar Hossain: supervision; formal analysis; Rakhi Chacrabati: conceptualization, software; Methodology; validation; data curation; Chayon Goswami: conceptualization; methodology; supervision; funding acquisition; project administration; software; resources writing- review and editing. All authors have read and approved the final version of the manuscript for submission to the journal.

References

Adhikary, S. K., Das, S. K., Saha, G. C., and Chaki, T. (2013). Groundwater drought assessment for Barind irrigation project in Northwestern Bangladesh. In: *20th International Congress on Modelling and Simulation (MODSIM 2013)*, 01-06 December 2013, Adelaide, South Australia. https://vuir.vu.edu.au/id/eprint/24436

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology, 105*, 121-126. https://doi.org/10.1016/s0076-6879(84)05016-3

Allakhverdiev, S. I. (2020). Optimising photosynthesis for environmental fitness. *Functional Plant Biology, 47*(11), 1-5. https://doi.org/10.1071/FPv47n11_FO

Barrs, H. D. and Weatherley, P. E. (1962). A re-examination of the relative turgidity techniques for estimating water deficits in leaves. *Australian Journal of Biological Sciences*, 15, 413-428. http://dx.doi.org/10.1071/BI9620413

Bates, L. S., Waldren, R. P. A., and Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, *39*, 205-207. https://doi.org/10.1007/BF00018060

Bhandari, U., Gajurel, A., Khadka, B., Thapa, I., Chand, I., Bhatta, D., Poudel, A., Pandey, M., Shrestha, S., and Shrestha, J. (2023). Morpho-physiological and biochemical response of rice (*Oryza sativa* L.) to drought stress: A review. *Heliyon*, *9*(3), 1-10. https://doi.org/10.1016/j.heliyon.2023.e13744

Ervin, G. N., and Wetzel, R. G. (2002). Effects of sodium hypochlorite sterilization and dry cold storage on germination of Juncus effusus L. *Wetlands*, *22*(1), 191-195. https://doi.org/10.1672/0277-5212(2002)022[0191:EOSHSA]2.0.CO;2

Franscistt, W., David, F. B., and Robert, M. D. (1971). *The estimation of the total soluble carbohydrate in cauliflower tissue*. Experiments in Plant Physiology. Van Nostrand Reinhold Camp. New York, NY, USA. pp:16. https://surli.cc/biyciy

Gedam, P. A., Thangasamy, A., Shirsat, D. V., Ghosh, S., Bhagat, K. P., Sogam, O. A., and Singh, M. (2021). Screening of onion (*Allium cepa* L.) genotypes for drought tolerance using physiological and yield based indices through multivariate analysis. *Frontiers in Plant Science*, *12*, 1-16. https://doi.org/10.3389/fpls.2021.600371

Geng, A., Lian, W., Wang, Y., Liu, M., Zhang, Y., Wang, X., and Chen, G. (2024). Molecular mechanisms and regulatory pathways underlying drought stress response in rice. *International Journal of Molecular Sciences, 25*(2), 1-37. https://doi.org/10.3390/ijms25021185

Gupta, A., Rico-Medina, A., and Caño-Delgado, A. I. (2020a). The physiology of plant responses to drought. *Science, 368*(6488), 266-269. https://doi.org/10.1126/science.aaz7614

Gupta, S., Brazier, A. K. M., and Lowe, N. M. (2020b). Zinc deficiency in low- and middle-income countries: prevalence and approaches for mitigation. *Journal of Human Nutrition and Dietetics*. 33, 624-643. https://doi.org/10.1111/jhn.12791

Hu, F., Zhang, Y., and Guo, J. (2023). Effects of drought stress on photosynthetic physiological characteristics, leaf microstructure, and related gene expression of yellow horn. *Plant Signaling & Behavior*, *18*(1), 1-10. https://doi.org/10.1080/15592324.2023.2215025

Jarin, A. S., Islam, M. M., Rahat, A., Ahmed, S., Ghosh, P., and Murata, Y. (2024). Drought stress tolerance in rice: Physiological and biochemical insights. *International Journal of Plant Biology, 15*(3), 692-718. https://doi.org/10.3390/ijpb15030051

Kader, M., Shalahuddin, A. K. M., Hore, T. K., Majumder, R. R., Haq, M. E., Fatema, K., Biswas, P. S., and Iftekharuddaula, K. M. (2021). BRRI Dhan100: A zinc-enriched rice variety suitable for irrigated ecosystems in Bangladesh. *Asian Plant Research Journal, 8*(1), 1-8. https://doi.org/10.9734/APRJ/2021/v8i130164

Kalra, A., Goel, S., and Elias, A. A. (2024). Understanding the role of roots in plant response to drought: Way forward to climate-resilient crops. *The Plant Genome, 17*(1), 1-31. https://doi.org/10.1002/tpg2.20395

Lafitte, H. R., and Bennett, J. (2002). *Requirements for aerobic rice: Physiological and molecular considerations*. In: Bouman, B.A.M., Hengsdijk, H., Hardy, B., Bindraban, P.S., Tuong, T.P. and Ladha, J.K., Eds., Water-Wise Rice Production, International Rice Research Institute, 8-11 Apr 2002. Los Baños, Philippines, 259-274. https://agris.fao.org/search/en/providers/122442/records/6472412208fd68d546002eea

Long, S. P., Coombs, J., and Hall, D. O. (1985). *Techniques in bioproductivity and photosynthesis*. Pergamon Press. BABA, Reading, USA. https://share.google/lJZ06arRq79d7cidB

Nakano, Y., and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology, 22*(5), 867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Nielsen, S. S. (2024). Vitamin C determination by indophenol method. In *Nielsen's food analysis laboratory manual* (pp. 153-156). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-031-44970-3_18

Saha, S., Begum, H. H., Nasrin, S., and Samad, R. (2020). Effects of drought stress on pigment and protein contents and antioxidant enzyme activities in five varieties of rice (*Oryza sativa* L.). *Bangladesh Journal of Botany, 49*(4), 997-1002. https://doi.org/10.3329/bjb.v49i4.52516

Sarker, U., and Oba, S. (2019). Nutraceuticals, antioxidant pigments, and phytochemicals in the leaves of *Amaranthus spinosus* and *Amaranthus viridis* weedy species. *Scientific Reports,* 9, 1-10. https://doi.org/10.1038/s41598-019-50977-5

Uarrota, V. G., Stefen, D. L. V., Leolato, L. S., Gindri, D. M., and Nerling, D. (2018). Revisiting carotenoids and their role in plant stress responses: from biosynthesis to plant signaling mechanisms during stress. In *Antioxidants and antioxidant enzymes in higher plants* (pp. 207-232). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-75088-0_10

Wang, X., Liu, H., Yu, F., Hu, B., Jia, Y., Sha, H., and Zhao, H. (2019). Differential activity of the antioxidant defence system and alterations in the accumulation of osmolyte and reactive oxygen species under drought stress and recovery in rice (*Oryza sativa* L.) tillering. *Scientific Reports,* 9: 8543. https://doi.org/10.1038/s41598-019-44958-x

Yang, Y., Ma, X., Yan, L., Li, Y., Wei, S., Teng, Z., Zhang, H., Tang, W., Peng, S., and Li, Y. (2024). Soil-root interface hydraulic conductance determines responses of photosynthesis to drought in rice and wheat. *Plant Physiology, 194*(1), 376-390. https://doi.org/10.1093/plphys/kiad498

Živanović, B., Milić Komić, S., Tosti, T., Vidović, M., Prokić, L., and Veljović Jovanović, S. (2020). Leaf soluble sugars and free amino acids as important components of abscisic acid-mediated drought response in tomato. *Plants*, 9(9), 1-17. https://doi.org/10.3390/plants9091147