



Paclobutrazol induced physio-biochemical manifestations to ameliorate water deficit stress in rice (*Oryza sativa*)

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ABSTRACT

In this study, the mechanism of paclobutrazol (PBZ) mediated improvement in tolerance to water deficit in rice (*Oryza sativa* L.) genotypes were thoroughly investigated. Rice genotypes were subjected to different doses (0, 30, 60, 90, 120 ppm) of paclobutrazol. Study revealed that relative water content, membrane stability index, total chlorophyll and abscisic acid content significantly increased by application of PBZ in contrasting rice genotypes, i.e. Nagina-22 (drought-tolerant genotype), IR-64 (drought sensitive) and IR-64 DTY1.1 [drought-tolerant, near isogenic line of IR-64 developed by the introgression of a major QTL (*qDTY1.1*)] under polyethylene glycol mediated water deficit stress. The increase was found to be of higher magnitude in 90 ppm dose of paclobutrazol compared to other doses. Our results suggest that PBZ application could significantly improve tolerance in rice genotypes particularly susceptible genotype under limited water availability through selective changes in physio-biochemical parameters.

Key words: ABA, MSI, PBZ, PEG, Rice, RWC, Water deficit stress

Rice (*Oryza sativa* L.) plays a major role as a staple food, supporting more than three billion people and comprising 50–80% of their daily calorie intake (Khush 2005). Drought is a major constraint responsible for decrease in rice production worldwide and to fulfil the demand, efforts are being made to improve crop yields. Water deficit stress (WDS) affects rice at morphological, physiological, biochemical and molecular levels and thereby affects its yield (Ali *et al.* 2011, Mawlong *et al.* 2016, Changan *et al.* 2018).

Plant growth regulators play vital roles in coordination of many growth and behavioural processes in rice, which regulate the amount, type, and direction of plant growth (Anjum *et al.* 2011). Hence, improved tolerance to drought has been an important goal in crop improvement programs. Therefore, enhancing productivity of rice under water deficit stress through novel genetic approaches and exogenous plant growth regulators can be an important strategy. The use of plant growth regulators such as GA₃, PBZ, 6-BA or their

compounds, are becoming more popular now a days (Pan *et al.* 2013). Paclobutrazol [(2*RS*, 3*RS*)-1-(4-chlorophenyl) methyl-4, 4-dimethyl-2-(1*H*-1, 2, 4-triazol-1-yl) penten-3-ol] (PBZ) is one of the members of triazole family which has plant growth regulating properties.

The primary mode of action of PBZ is inhibition of *ent*-kaurene oxidase, which catalyzes the sequential oxidation from *ent*-kaurene to *ent*-kaurenoic acid in the early sequence of GA biosynthesis (Graebe 1987). PBZ has been used to provide plant protection against abiotic stresses such as chilling (Lin *et al.* 2006), water stress (kumar *et al.* 2018), flooding (Webb and Fletcher 1996) and salinity (Sharma *et al.* 2018). However, no report on rice so far is available which reflects PBZ effect in rice grown under water deficit stress.

For drought stress induction, one of the approaches is to use high molecular weight osmotic substances, like polyethylene glycol (PEG) (Landjeva *et al.* 2008). In present study, effect of different doses of PBZ on the physio-hormonal changes in response to PEG induced WDS were investigated, as our understanding of the PBZ induced amelioration of WDS in rice is very limited.

MATERIALS AND METHODS

Seed material: Three rice genotypes, viz. Nagina-22 (N 22) (drought tolerant), IR-64 DTY1.1 (drought-tolerant, near isogenic line (NIL) of IR-64) and IR 64 (drought sensitive) were obtained from Division of Genetics and Plant Breeding, Indian Agricultural Research Institute, New Delhi, India in the year of 2015–2016. The seeds were

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surface sterilized with 0.2% mercuric chloride solution for 5 min with frequent shaking and thoroughly washed with tap water. Seedlings were grown for 21 days and then were transplanted in to 4 × 4 inches pots and grown in National Phytotron Facility, IARI under controlled conditions (12 h light, 30°C day/25°C night, 75% RH).

Water deficit stress and PBZ application: After 20 days of transplanting, PEG (MW: 6000) with concentration of 10 mg/l was used for induction of drought stress. The soil drenching method was used for the application of varying doses of PBZ (0, 30, 60, 90, 120 ppm) and PEG directly to the plants. 50 ml PEG and PBZ was used for each pot.

Plant sampling: The plant samples were collected 5 days after treatment. The uppermost fully expanded flag leaf was used for recording the observations.

Relative water content

Leaf relative water content (RWC) was estimated as per method of Weatherley 1950.

$$\text{RWC} = [(\text{Fresh wt.} - \text{Dry wt.}) / (\text{Turgid wt.} - \text{Dry wt.})] \times 100$$

Membrane stability index: Membrane stability index (MSI) was estimated according to the method described by Prema Chandra *et al.* (1990). For estimation of membrane stability index 100 mg leaf material, in two sets, was taken in test tubes containing 10 ml of double distilled water. One set was heated at 40°C for 30 min in a metabolic water bath, and the electrical conductivity of the solution was recorded on a conductivity bridge (C1). Second set was boiled at 100°C on a boiling water bath for 10 min, and its conductivity was measured on a conductivity bridge (C2). Membrane stability index (MSI) was calculated as:

$$\text{MSI} = [1 - (C1/C2)] \times 100$$

Total chlorophyll content: Total chlorophyll content (TCC) was estimated as per the method described by Hiscox and Israelstam (1979). Thirty mg fresh leaf sample was added to the test tubes containing 4 ml DMSO. Tubes were kept in dark for 4 h at 6°C. Then the tubes were taken out, cooled at room temperature and the absorbance was recorded at 663 and 645 nm using DMSO as blank. The result was expressed as mg/g dry wt.

$$\text{Total chlorophyll} = (20.2 \times A_{645} + 8.02 \times A_{663}) \times V/W \times 1000$$

where, A₆₆₃, Absorbance values at 663 nm; A₆₄₅ = Absorbance values at 645 nm

ABA estimation: ABA was estimated by HPLC method using RP-HPLC C18 column (Zeevaert *et al.* 1999, Ali *et al.* 2011). 2 g of leaf sample was powdered in liquid nitrogen and 2 ml of 80% acetone was added for ABA extraction. The homogenate was passed through Whatman No.1 filter paper. The filtrate was vacuum dried to remove acetone. The lipid soluble material deposited on the walls of the conical flask was dissolved in 1% acetic acid. Before HPLC, the samples were filtered through a sterilized 22 µm Millipore filter, using a disposable syringe.

Preparation of ABA standard solution: Standard ABA

solution was prepared by dissolving it in 95% methanol. 100 ppm, 200 ppm and 300 ppm solutions were prepared and their corresponding concentrations were measured using RP-HPLC. C18 column was used as the stationary phase. Methanol: H₂O (60:40) was used as mobile phase at a flow rate of 1 ml/min. Run time was set at 6 min.

Statistical analysis: All the experiments were carried out in triplicate. The data were analyzed with the help of pre-loaded software in Excel, programmed for statistical calculations. Duncan's multiple range tests (DMRT) were performed to determine significant difference between means at a significance level of $P \leq 0.05$ and reported as the mean $\pm$ standard error (SE).

RESULTS AND DISCUSSION

Paclobutrazol increased relative water content under water deficit stress: RWC in the leaves generally ranged between 88–95% in fully turgid transpiring leaves under normal conditions and to about 30–40% in severely stressed conditions, depending on the species (Schlemmer *et al.* 2005). In most of the cases in plants typical leaf RWC is at around 60–70% under initial stage of water deficit stress. RWC is also considered as a useful indicator of water status of plant cells under drought stress (Mawlong *et al.* 2015, Meher *et al.* 2018) and showed relationship with yield parameters. PBZ treatment kept relative leaf water content higher than the non-treated plants, when subjected to WDS. It showed the ability of PBZ to maintain leaf water content under water deficit stress. The water deficit stress in the plants was imposed by the use of polyethylene glycol and samples were collected 5 days after treatment. The RWC recorded was 76.11, 76.93, 77.60 at 30, 83.63, 82.09, 82.97 at 60, 86.63, 85.24, 86.14 at 90 and 83.19, 83.67, 82.44 at 120 ppm PBZ for three genotypes N 22, IR 64 and IR 64 DTY1.1 respectively. In all the cases, RWC was higher as compared to the plants grown under WDS without PBZ application, as RWC was 75.21, 72.83 and 74.10 for three genotypes N 22, IR 64 and IR 64 DTY1.1 respectively. The RWC for all the three genotypes under controlled conditions was in the range of 91–94%. Observations revealed about 15% decreases in water deficit stress almost in all the genotypes as compared to control. Among the four doses of PBZ (30, 60, 90, 120 ppm) used for water deficit stress amelioration, 90 ppm PBZ showed maximum tolerance to stress. RWC was 15.17%, 17.04% and 16.24% higher in N 22, IR 64 and IR 64 DTY1.1 respectively as compared with water deficit stress observed in other doses of PBZ. The genotype IR 64 was more responsive towards the PBZ application as compared to other genotypes. Out of four concentrations of PBZ applied 90 ppm found to be more effective under WDS in rice plants. Tolerant genotypes (N 22 and IR 64 DTY1.1) maintained higher RWC (75.21–74.21%) as compared to susceptible IR 64 (72.83%) under water-stress conditions (Fig 1). Reports are also there that PBZ induced the water stress tolerance of the plants by maintaining fresh weight and RWC and other physiological and biochemical parameters of plants species (Jungklang *et al.* 2017). PBZ

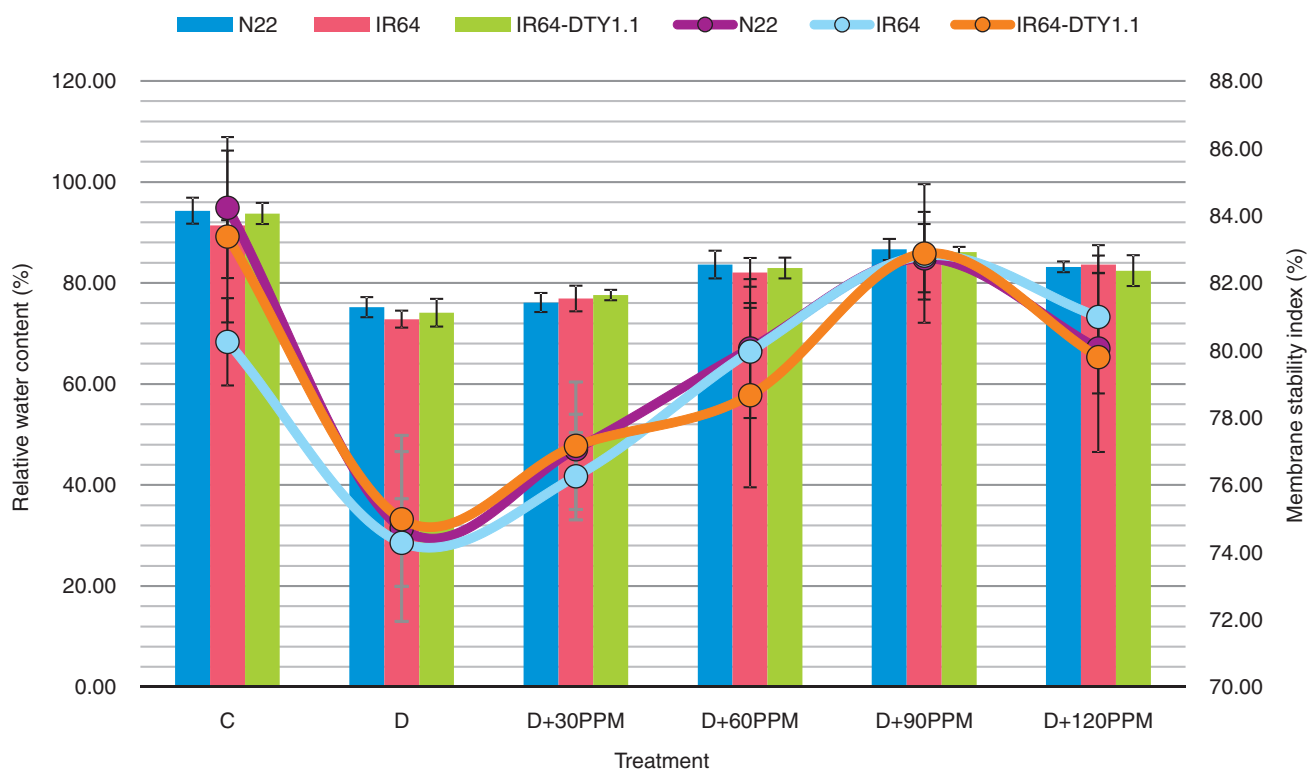


Fig 1 Effect of paclobutrazol on relative water content and membrane stability index in shoot of the contrasting rice genotypes under water deficit stress conditions. Where, C, Control; D, water deficit stress; PPM, PBZ concentration; Bar Graphs depict RWC, while line graphs depict MSI.

treatment showed significant increase in RWC (10–17%) in shoots under WDS as compared to control.

The tolerant genotypes exhibited higher values of RWC indicating their greater efficiency to acquire water from the soil than susceptible ones. This could be due to adaptive features of tolerant genotypes under WDS.

Paclobutrazol increased membrane stability index of shoot under water deficit stress: MSI or electrolyte leakage was measured to assess the stress injury to the cell membrane. Leakage reflects changes during stress induction in membrane potential and membrane permeability on the basis of osmotic adjustment under stress. The MSI recorded was 77.07, 76.26, 77.17 at 30 ppm, 80.05, 79.96, 78.67 at 60 ppm, 82.73, 82.73, 82.88 at 90 ppm and 80.05, 81.00, 79.80 at 120 ppm PBZ for three genotypes N 22, IR 64 and IR 64 DTY1.1 respectively. Leaf MSI was maximum (82.88%) at the 90 ppm PBZ in IR 64-DTY1.1 genotype, which indicated minimum electrolytes leakage. PBZ application led to the increase in mean MSI by 10.74%, 11.47% and 10.52% in the N 22, IR 64 and IR 64 DTY1.1 genotype respectively as compared to PEG treated plants. The tolerant genotypes were able to maintain significantly higher mean MSI (84.24%, 83.28%) compared to susceptible ones (80.25%) under control conditions. PBZ application showed increase in drought tolerance in all the genotypes but the effect was more significant in case of susceptible genotypes (IR 64) (Fig 1). These findings indicated that PBZ application helped in maintaining cell membrane stability under WDS.

Membrane stability is a widely used criterion to assess drought tolerance in plants as it seriously impaired both membrane structure and function. Drought induced decrease in MSI has also been reported by Mawlong *et al.* (2015) and Changan *et al.* (2018). These results are also in agreement with the conclusion of Martin *et al.* (1987) in leaf tissues of six woody angiosperm species. Moreover, Almeselmani *et al.* (2011) correlated electrolyte leakage with drought tolerance in *Phaseolus* and *Vigna* species.

Paclobutrazol increased total chlorophyll content under water deficit stress: Leaf chlorophyll content is one of the most important factors in determining the photosynthesis rate and dry matter production. Total chlorophyll content, was estimated under WDS in all the three genotypes differing in their tolerance to water stress at different dose of PBZ in rice. Results revealed that exogenous application of PBZ helped in maintaining the chlorophyll content and hence mitigated the adverse effects of drought stress. The total chlorophyll content was 7.84, 5.71, 7.25 at 30 ppm, 8.14, 5.97, 7.59 at 60 ppm, 9.30, 7.50, 8.53 at 90 ppm and 8.85, 6.85, 8.04 mg/g at 120 ppm PBZ for all the three genotypes N 22, IR 64 and IR 64 DTY1.1 respectively. Total chlorophyll content significantly declined by 26.53% in IR 64 compared to N 22 (19.61%) and IR 64 DTY1.1 (18.33%) as compared to control. PEG treated plants with 90 ppm PBZ showed an increment in the chlorophyll content by 40.22% in IR 64 in comparison to water-stressed plants without PBZ. The genotypes N 22 and IR 64-DTY1.1 showed enhancement in total chlorophyll content by

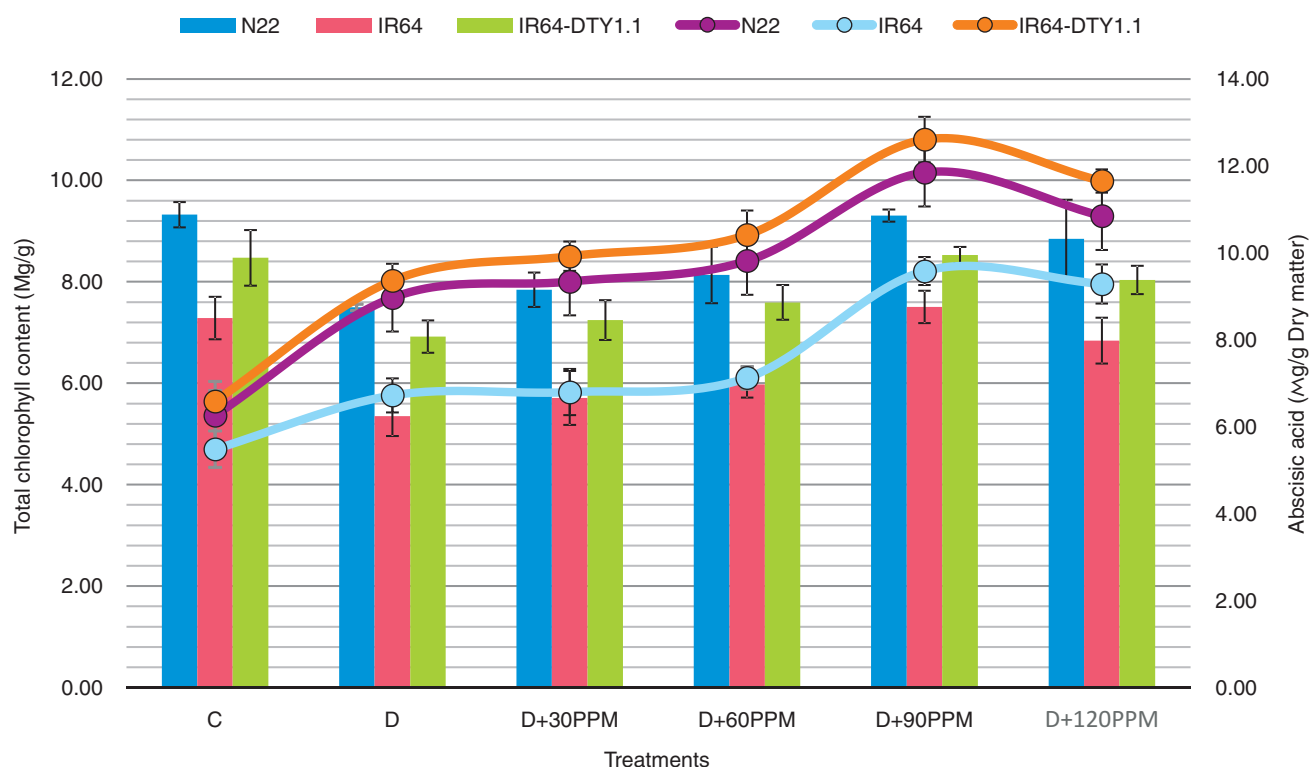


Fig 2 Effect of paclobutrazol on total chlorophyll content and abscisic acid content in shoot of the contrasting rice genotypes under water deficit stress conditions. Where C, Control; D, water deficit stress; PPM, PBZ concentration; Bar Graphs depict total chlorophyll content while line graphs depict ABA.

24.14% and 23.20% as compared with PEG treated plants, respectively (Fig 2). The differences in the total chlorophyll content were observed in rice plants treated with PBZ at 90 ppm compared to other treatment (Fig 2). At 90 ppm PBZ, and D+PBZ plants had chlorophyll content equal to irrigated plants, which suggest that PBZ compensated reduction in chlorophyll content by maintaining higher chlorophyll content in drought stressed plants and thus afforded them better photosynthesis similar to irrigated plants. These findings are in line with some earlier reports in okra (Amin *et al.* 2009) and in wheat (Azzedine *et al.* 2011). The photosynthetic efficiency of plants affects many kinds of growth indices, e.g. chlorophyll content (Feng *et al.* 2014). Loss in chlorophyll content is often observed under water stress; however, it is considered as an adaptive feature in plants grown under water deficit stress (Munne-Bosch and Alegre 1999).

Paclobutrazol increased total abscisic acid content under water deficit stress: The ABA content was 9.34, 6.80, 9.92 at 30 ppm, 9.81, 7.12, 10.41 at 60 ppm 11.85, 9.58, 12.61 at 90 ppm and 10.85, 9.28, 11.65 at 120 ppm PBZ doses of all the three genotypes N 22, IR 64 and IR 64 DTY1.1 respectively. IR 64 genotype showed maximum increase (42.55%) in ABA content at 90 ppm among all the genotypes and treatments, while N 22 and IR 64 DTY1.1 showed 32.31% and 34.70% increase in ABA, respectively. Nonetheless, we have demonstrated that a relative difference in ABA levels occurred between different doses of paclobutrazol-treated with stressed plants.

Water deficit stress induces several physiological responses in plants through ABA signalling which helps them to adapt in adverse conditions. Therefore, abscisic acid (ABA) gets accumulated under drought stress and mediates many stress responses which play versatile functions in regulating many developmental and adaptive stress processes (Cutler *et al.* 2010). The ABA in both control (Non stress) as well as water stress could be increased by paclobutrazol treatment (Fig 2). In untreated rice leaves, when water loss reached 20% of the initial fresh weight, ABA accumulated 22–43%, whereas in the paclobutrazol-treated leaves, ABA was increased up to 32–42% as compared to stressed plants in all genotypes. Pal *et al.* (2016) reported similar kind of results in tomato plants.

A progressive increase in all biochemical parameters was observed under paclobutrazol treatment. The increase was found to be more substantive at 90 ppm as compared to other doses of paclobutrazol. This indicates that paclobutrazol may play important role in mitigating abiotic stress in plants, particularly in the sensitive genotypes. However, there is need to validate the optimum dose of paclobutrazol for improving yield of rice under drought stress in the field conditions. The present findings would facilitate unravelling the paclobutrazol induced physio-biochemical manifestations of drought tolerance in crop plants to improve the yield.

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