SEED TREATMENT WITH PACLOBUTRAZOL AFFECTS EARLY GROWTH, PHOTOSYNTHESIS, CHLOROPHYLL FLUORESCENCE AND PHYSIOLOGY OF RICE

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Abstract. A pot experiment was conducted to investigate the effects of paclobutrazol seed treatment on early growth, photosynthesis and physio-biochemical attributes on rice. The seeds of two rice cultivars i.e., Basmati-385 and Xiangyaxiangzhan were treated with paclobutrazol at 40 mg per 5 kg of seeds (T) whilst non-treated seeds were taken as control (CK). Result showed that the seedling length of Basmati-385 and Xiangyaxiangzhan was reduced by 32.16 and 26.85% when seeds were treated with paclobutrazol, however, net photosynthetic rate, maximal efficiency of PSII photochemistry (*Fv/Fm*) and electron transport rate (*ETR*) were increased by 25.34 and 7.98%, 4.22 and 7.76% and 30.07 and 11.84% in Basmati-385 and Xiangyaxiangzhan, respectively. The malondialdehyde (MDA) contents were reduced up to 14% in both rice cultivars under paclobutrazol treatment. Furthermore, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were increased by 4.92 and 3.58%, 10.64 and 14.42%, 31.19 and 25.80% in paclobutrazol treated seeds than CK in both Basmati-385 and Xiangyaxiangzhan, respectively. In addition, dry weight per unit seedling length was significantly correlated with SPAD values, root length, surface area, diameter, and root volume, net photosynthetic rate, chlorophyll a, chlorophyll b, carotenoids, *Fv/Fm*, *ETR*, antioxidant enzymes, soluble sugar, and soluble protein of both rice cultivars. However, negative correlations were also recorded between dry weight per unit seedling length and intercellular CO_2, transpirational rates, non-photochemical quenching (*NPQ*) and malondialdehyde (MDA) contents in both Basmati-385 and Xiangyaxiangzhan. Hence, paclobuztrazol seed treatment enhanced photosynthetic and gas exchange attributes, physio-biochemical attributes and root morphological characters in rice.
Keywords: *antioxidants; seed dressing agent; net photosynthetic rate; rice seedlings; root morphology*

Introduction

Paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol -1-yl) pentan-3-ol] is a broad-spectrum gibberellin biosynthesis inhibitor which was developed by Imperial Chemical Industries (ICI) agrochemicals in 1986 (French et al., 1990).

Paclobutrazol being a triazole, regulates the plant growth by antagonizing the hormone gibberellin biosynthesis by inhibiting the ent-kaurene oxidase enzyme which catalyses the oxidation of ent-kaurene to ent-kaurenoic acid in the terpenoid pathway for the production of gibberellins through inactivation of cytochrome P450-dependent monooxygenases (Fletcher et al., 2000). Paclobutrazol hinders gibberellic acid (GA) and endogenous indole acetic acid (IAA), whilst enhances abscisic acid (ABA), cytokinin and ethylene production within the plants (Fletcher et al., 2000; Zhang et al., 1998). Roles of auxins and cytokinins to promote growth and development of lateral and adventitious roots have been well reported (Fletcher et al., 2000; Zhang et al., 1998). The previous work has shown ample effectiveness of paclobutrazol and certain other triazoles for improving proline and soluble proteins, lignin contents, decreasing transpirational rate through the partial closure of stomata in several crops (Gopi et al., 2006; Özmen et al., 2003; Wang et al., 2015; Kamran et al., 2018). It is being widely used in many crops, mainly for producing shorter plant canopies whilst its anti-gibberellic behaviour has been reported in numerous plants (Özmen et al., 2003; Kamran et al., 2018; Upreti et al., 2013). Commonly, paclobutrazol and triazole regulate various plant morpho-physiological functions such as root growth stimulation, reduction in shoot growth (Jaleel et al., 2007; Manivannan et al., 2008), enhancement of chlorophyll contents, net photosynthetic rate, and carbohydrate content (Fletcher et al., 2000; Zhang et al., 1998), reduction of free-radical induced damage, improving antioxidant efficacy (Fletcher et al., 2000; Zhang et al., 1998). It further regulates cytokinin production and hinders abscisic acid biosynthesis (Fletcher et al., 2000).

In rice production system, paclobutrazol has been used to develop semi-dwarf and/or dwarf plant varieties to reduce lodging and to improve rice yield (Street et al., 1986). Furthermore, paclobutrazol treatment improved chlorophyll contents, root morphology plant architectural characters (Yim et al., 1997). Foliar applied paclobutrazol-induced growth regulations has been previously reported (Xiang et al., 2017), however a little is known about the seed treatment with paclobutrazol-induced modulations in morpho-physiological and biochemical attributes of rice. Therefore, present study was conducted to investigate the effects of rice seed treatment with paclobutrazol on the morphological and physio-biochemical traits of rice.

Materials and methods

Experimental details

A pot experiment was conducted at Experimental Research Farm, College of Agriculture, South China Agricultural University, Guangzhou (23°09′ N, 113°22′ E and 11 m from mean sea level) China in September 2016. Seeds of two popularized aromatic rice cultivars i.e., Basmati-385 and Xiangyaxixangzhan were soaked in water for 12 h at room temperature and then put into the dark incubator at constant temperature (35 °C) for 12 h for germination. The following treatments were applied to germinated seed before sowing i.e., CK (no paclobutrazol treatment, taken as control) and T (paclobutrazol treatment (40 mg per 5 kg seeds). On 12th September, 2016, 50 seeds per pot were sown according to the layout (*Fig. 1*). The average temperature was 28.5 °C to 29.0 °C during experimental period, while the average humidity was about 68-70%. The experimental soil was collected from the paddy field and containing 1.14 g kg^{-1} total N, 0.92 g kg^{-1} total P, 16.65 g kg^{-1} total K, 77.35 mg kg^{-1} available N, 61.34 mg kg^{-1} available P, 127.04 mg kg^{-1} available K, and 23.34 g kg^{-1} organic matter.

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Before pot filling, the soil was kept under shade, air-dried, crushed and passed through 2 cm sieve.

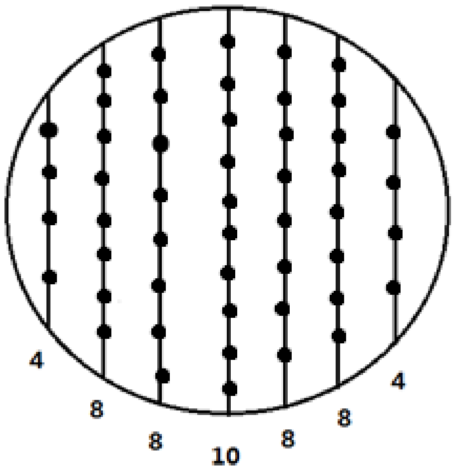

Figure 1. *Layout of the sowing of seeds in pots*

Seedling growth parameters

On 15th October, seedlings were collected for determination of seedling growth parameters and physio-biochemical indices. The samples were uprooted manually, washed with tap water to clean deposits in the roots. The morphological attributes i.e., seedling length, stem base width, roots morphology, SPAD values and dry weight per seedling were recorded. Leaf SPAD values were recorded with a SPAD meter "SPAD-502" and represented as relative chlorophyll contents. Roots morphology was determined by WinRHTZO Root System Analysis through scanning the roots of rice seedlings (Gu et al., 2010).

Photosynthesis and chlorophyll fluorescence

Photosynthesis and chlorophyll fluorescence were determined on the 32th day after emergence. Portable photosynthesis system (LI-6400, LI-COR, USA) was used to determine net photosynthetic rate and gas exchange attributes i.e., stomatal conductance, intercellular CO_2, and transpirational rates at 09:00–10:30 a.m. according to the standard method (Pan et al., 2015). Maximal efficiency of PSII photochemistry (*Fv/Fm*), the quantum yield of PSII, non-photochemical quenching (*qN*), electron transport rate (*ETR*) were measured with an integrating fluorescence fluoro-meter (LI-6400–40 leaf chamber fluorometer, Li–Cor, USA) under dark conditions.

Physio-biochemical parameters

Fresh leaves were separated from the plant for each treatment, double washed with distilled water and stored at −80 °C till physio-biochemical analysis. The measurements were repeated in quadruplicate and mean values were calculated.

The photosynthetic pigments were determined by using 95% alcohol to extract contents (Lichtenthaler et al., 1987). The absorption was read at 665 nm, 652 nm, 649 nm and 470 nm.

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The malondialdehyde (MDA) contents were measured by reacting with thiobarbituric acid (TBA) (Schmedes et al., 1989). The absorbance of the reaction solutions were recorded at 532 nm, 600 nm, and 450 nm. The reaction solutions were calculated as: MDA content ($\mu mol \cdot L^{-1}$) = 6.45 (OD_{532} − OD_{600}) − 0.56 OD_{450} and expressed as $\mu mol \cdot g^{-1}$ FW (fresh weight).

Nitro blue tetrazolium (NBT) method was used to measure the superoxide (SOD, EC 1.15.1.1) activity (Li, 2000). The reaction mixture contained 1.75 ml of buffer (pH 7.8), 0.3 ml of 130 $mM \cdot l^{-1}$ methionine buffer, 0.3 ml of 750 $\mu mol \cdot l^{-1}$ NBT buffer, 0.3 ml of 100 $\mu mol \cdot l^{-1}$ EDTA-Na_2 buffer, 0.3 ml of 20 $\mu mol \cdot l^{-1}$ lactoflavin and 0.05 ml of enzyme extract. After reaction, the change in color was measured at 560 nm. One unit of SOD activity is equal to the volume of extract needed to cause 50% inhibition of the color reaction.

The peroxidase (POD, EC 1.11.1.7) activity was measured by using enzyme extract. An aliquot of 50 μl of extract was added to the reaction solution containing 1 ml of 0.3% H_2O_2, 0.95 ml of 0.2% guaiacol and 1 ml of 50 $mmol \cdot L^{-1}$ buffer (pH 7.0). The absorbance change of the brown guaiacol was recorded at 470 nm to calculate POD activity. One POD unit of enzyme activity was defined as the absorbance increase due to guaiacol oxidation by 0.01 ($U \cdot g^{-1}$) (Luo et al., 2017). For catalase (CAT, EC 1.11.1.6) activity, an aliquot of 50 μl of enzyme extract was added to the reaction solution containing 1 ml of 0.3% H_2O_2 and 1.95 ml of H_2O. The change in absorbance was recorded at 240 nm. One unit of enzyme activity was defined as the absorbance decrease by 0.01 ($U \cdot g^{-1}$ FW) (Aebi et al., 1983).

The protein contents of leaves were estimated by using Coomassie Brilliant Blue G250 Reagent, and the absorbance was recorded at 595 nm and expressed as $\mu g \cdot g^{-1}$ of fresh weight (Bradford et al., 1976). The soluble sugar contents were determined by using anthrone-sulfuric acid method (Sun et al., 2010). The absorbance was recorded at 620 nm and expressed as $mg \cdot g^{-1}$ of fresh weight. Proline contents were estimated by using ninhydrin (Bates et al., 1973). The absorbance of the red chromophore in the toluene fraction was recorded at 520 nm and the amount of proline was estimated by comparing with a standard curve ($y = 0.0531 x - 0.0054$) and expressed as $\mu g\ g^{-1}$ FW.

Experimental design and statistical analyses

There were 10 pots per treatment and all pots were arranged in completely randomized design (CRD). Data were analysed by a statistical software “Statistix 8.1” (Analytical Software, Tallahassee, FL, USA) whilst treatment means were compared by using least significant difference (LSD) test at 5% probability level. Computer software “Origin 8.1” (Origin Lab Co., Northampton, MA, USA) was used for graphical representation.

Results

Seedling growth parameters

Seed treatment with paclobutrazol (T) significantly ($P \leq 0.05$) affected seedling length, base stem width, SPAD values and dry weight per unit seedling length (*Table 1*). Comparing T with CK, both Basmati-385 and Xiangyaxianzhan had lower seedling length whilst paclobutrazol enhanced SPAD values, base stem width and dry weight per unit seedling length. For example, 32.16 and 26.85% reduction in seedling length were

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recorded in Basmati-385 and Xiangyaxianzhan, respectively, whereas an increase of 34.46 and 17.36% in base stem width, 25.59 and 16.41% in SPAD values, and 38.81 and 51.41% in dry weight per unit seedling length were recorded in paclobutrazol seed treatment in treated seeds than control for Basmati-385 and Xiangyaxianzhan, respectively. Negative correlations were recorded between dry weight per unit seedling length in Basmati-385 and Xiangyaxiangzhan but significant positive correlations were observed between dry weight per unit seedling length and stem base width, SPAD values and root length (*Table 2*). The morphological appearance of treated and non-treated rice seedlings was presented in *Figure 2*.

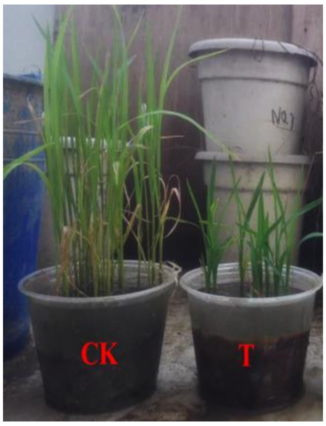

Figure 2. *The physical appearance treated and non-treated rice seedlings. CK (non-treated); T (seed treatment with paclobutrazol at 40 mg per 5 kg seeds)*

Paclobutrazol seed treatment also affected root morphology in terms of root length, surface area, diameter and volume (*Table 1*). Root length was 6.26 and 15.22% higher than CK in Basmati-385 and Xiangyaxiangzhan, respectively. Seedlings of Basmati-385 and Xiangyaxiangzhan in paclobutrazol seed treatment had 8.24 and 11.28% higher root diameter and 15.54 and 71.83% higher root volume, respectively than CK. Moreover, significant and positive correlations were observed between dry weight per unit seedling length, root surface area, root volume in response to paclobutrazol seed treatment (*Table 2*).

Table 1. *Effect of seed dressing with paclobutrazol on rice seedling quality*

		Shoot				**Root**			
		Height (cm)	**Base stem width (mm)**	**SPAD values**	**Dry weight per unit seedling length (mg cm^{-1})**	**Length (cm)**	**Surface area (cm^2)**	**Diameter (mm)**	**Volume (cm^3)**
Basmati	CK	20.93a	6.21b	20.07b	5.86b	228.66b	16.60b	0.23b	0.10b
	T	14.13b	8.44a	24.79a	7.84a	242.97a	18.48a	0.25a	0.12a
Xiangyaxiangzhan	CK	17.90a	5.34b	17.53b	3.27b	142.26b	9.83b	0.23b	0.05b
	T	13.17b	6.14a	20.57a	4.97a	163.90a	13.77a	0.25a	0.10a

Values sharing a common letter within a column don’t differ significantly at P ≤ 0.05 according to least significant difference (LSD) test. CK (non-treated); T (seed treatment with paclobutrazol at 40 mg per 5 kg seeds)

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Table 2. *Correlation coefficients among dry weight per unit seedling length, height, stem base width, SPAD, length, surf area, average diameter and root volume*

	Basmati	***P* value**	**Xiangyaxiangzhan**	***P* value**
Height	-0.9622	0.0021	-0.9865	0.0003
Stem base width	0.9923	0.0001	0.9500	0.0037
SPAD values	0.9877	0.0002	0.9487	0.0039
Length	0.9461	0.0043	0.9821	0.0005
Surf area	0.8870	0.0184	0.9741	0.0010
Average diameter	0.9479	0.0040	0.8414	0.0357
Root volume	0.7036	0.1187	0.9755	0.0009

Net photosynthesis, gas exchange and chlorophyll contents

Net photosynthetic rate and gas exchange attributes were substantially affected by paclobutrazol seed treatment. Both Basmati-385 and Xiangyaxiangzhan had 24.47 and 7.55% higher net photosynthetic rate (respectively) in paclobutrazol seed treatment compared with CK (*Fig. 3a*), whereas the both cultivars had decreased intercellular CO_2 concentration (6.60 and 6.69% reduction in Basmati and Xiangyaxiangzhan, respectively) in paclobutrazol seed treatment as compared with CK (*Fig. 3c*).

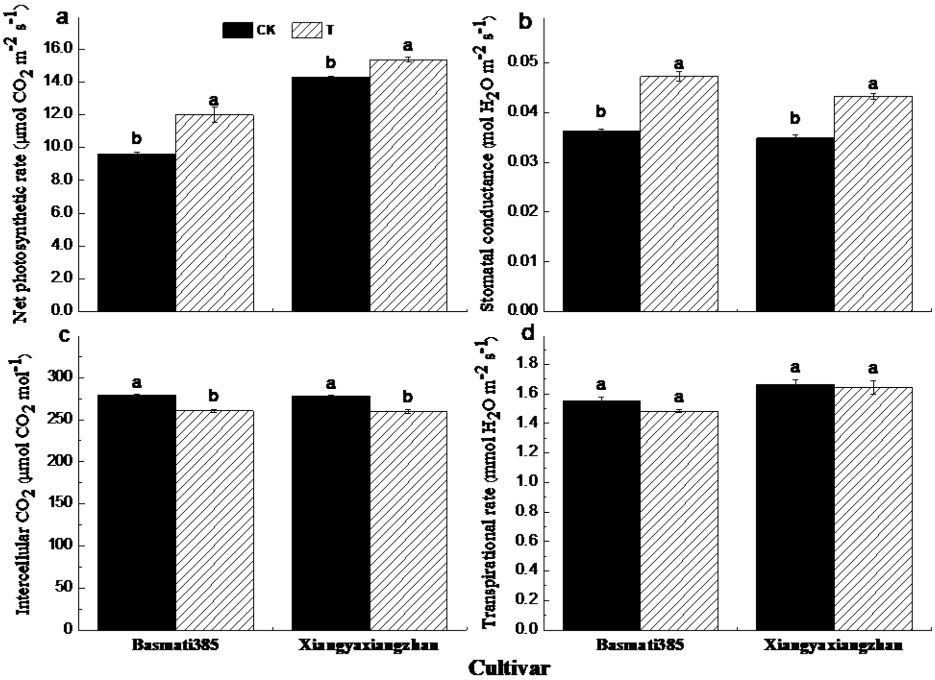

Figure 3. *Effect of seed treatment with paclobutrazol on a) net photosynthesis, b) stomatal conductance, c) intercellular* CO_2*, d) transpirational rate. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at* $P \leq 0.05$ *according to least significant difference (LSD) test. CK (non-treated); T (seed treatment with paclobutrazol at 40 mg per 5 kg seeds)*

However, there was no significant difference between T and CK for transpirational rates in both cultivars as shown in *Figure 1d*. In addition, the leaves of seedlings in

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paclobutrazol seed treatment had higher contents of chlorophyll a, chlorophyll b and carotenoid. Compared with CK, paclobutrazol seed treatment increased 50.19 and 11.55% chl a, 47.22 and 12.23% chl b contents, 56.53 and 9.45% in carotenoids and 44.53 and 12.48% in total chl contents of Basmati and Xiangyaxiangzhan, respectively (*Fig. 4a-d*). Furthermore, significant positive correlations were recorded between dry weight per unit seedling length and net photosynthetic rate, stomatal conductance, chl a, chl b, carotenoids and total chl contents in both rice cultivars, however, negative correlations were found among dry weight per unit seedling length and intercellular CO_2 as well as transpiration rates in Basmati and Xiangyaxiangzhan (*Table 3*).

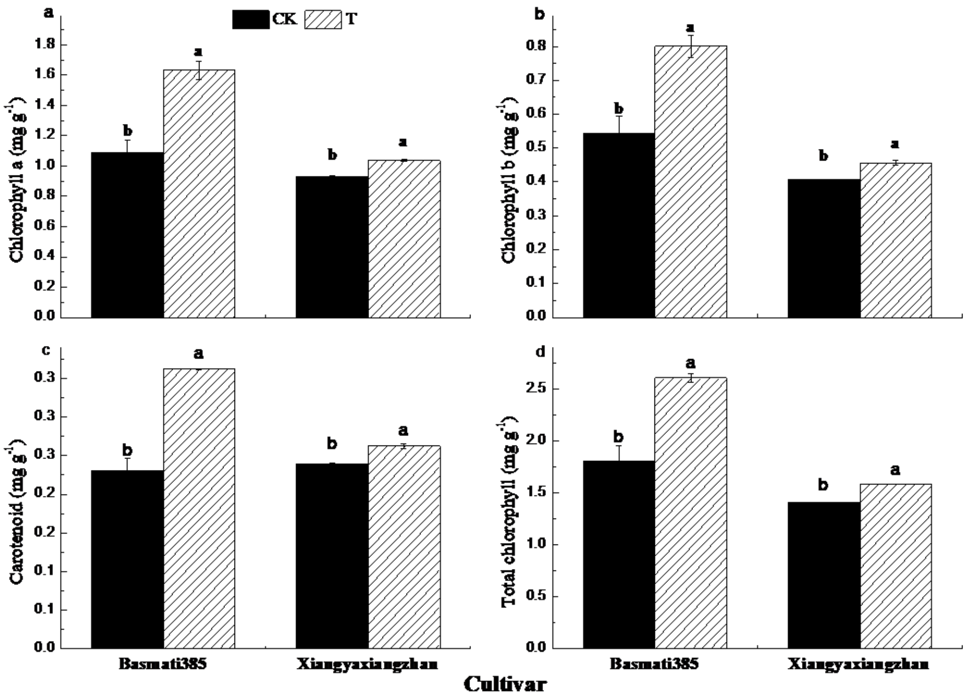

Figure 4. *Effect of seed treatment with paclobutrazol on a) chlorophyll a, b) chlorophyll b, c) carotenoid, d) total chlorophyll. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at P ≤ 0.05 according to least significant difference (LSD) test. CK (non-treated); T (seed treatment with paclobutrazol at 40 mg per 5 kg seeds)*

Table 3. *Correlation coefficients among dry weight per unit seedling length, net photosynthetic rate, stomatal conductance, intercellular CO_2, transpirational rates, chlorophyll a, chlorophyll b, carotenoids and total chlorophyll*

	Basmati	***P* value**	**Xiangyaxiangzhan**	***P* value**
Net photosynthetic rate	0.9191	0.0096	0.9484	0.0039
Stomatal conductance	0.9936	0.0001	0.9712	0.0012
Intercellular CO_2	-0.9722	0.0012	-0.9473	0.0041
Transpirational rates	-0.7883	0.0625	-0.1930	0.7141
Chlorophyll a	0.9126	0.0111	0.9826	0.0004
Chlorophyll b	0.8906	0.0173	0.9717	0.0012
Carotenoids	0.9343	0.0063	0.9558	0.0029
Total chlorophyll	0.9011	0.0142	0.9951	0.0000

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Chlorophyll fluorescence

Significant differences in chlorophyll fluorescence parameters i.e., maximal efficiency of PSII photochemistry (*Fv/Fm*), photochemical quenching (*qP*), electron transport rate (*ETR*), F_0 and F_m. Compared with CK, an increase of 4.08 and 5.39% in *Fv/Fm*, 15.72 and 6.13% in *qP*, 21.76 and 31.36% in *F0*, 21.45 and 18.48% in *Fm*, 32.50 and 10.39% in *ETR* was recorded for both Basmati-385 and Xiangyaxuangzhan, respectively (*Fig. 5a-f*). Moreover, significant decrease was recorded in non-photochemical quenching (*NPQ*) with the application paclobutrazol as compared with CK (*Fig. 5e*). Additionally, positive correlations were observed between dry weight per unit seedling length and *Fv/Fm, ETR, qP*, F_0 and F_m whilst dry weight per unit seedling length was negatively associated with *NPQ* (*Table 4*).

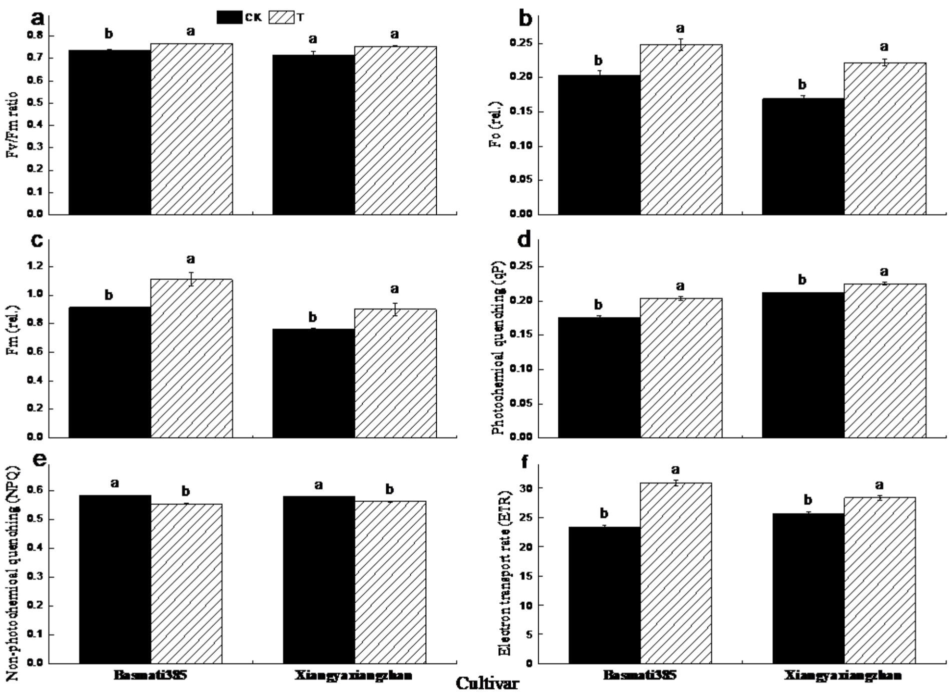

Figure 5. *Effect of seed treatment with paclobutrazol on a) Fv/Fm, b) F0, c) Fm, d) photochemical quenching (qP), e) Non-photochemical quenching (NPQ), f) electron transport rate (ETR). Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $P \leq 0.05$ according to least significant difference (LSD) test. CK (non-treated); T (seed treatment with paclobutrazol at 40 mg per 5 kg seeds)*

Table 4. *Correlation coefficients among Fv/Fm, ETR, NPQ, qP, F_0, F_m*

Parameters	Basmati	*P* value	Xiangyaxiangzhan	*P* value
Fv/Fm	0.9695	0.0014	0.7341	0.0967
ETR	0.9832	0.0004	0.9244	0.0084
NPQ	–0.9511	0.0035	–0.9688	0.0014
qP	0.9618	0.0022	0.9477	0.004
F_0	0.8980	0.0151	0.9591	0.0025
F_m	0.8849	0.0191	0.8543	0.0303

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Anti-oxidant responses, malodialdehyde, and soluble protein and sugar

Paclobutrazol regulated anti-oxidative enzymatic activities in terms of SOD, POD and CAT, but lowered lipid per-oxidation (in terms of MDA production) and also induced changes in both protein and sugar contents in both rice cultivars (*Fig. 6a-f*). The POD, SOD and CAT activities were enhanced by 10.79 and 15.71%, 4.44 and 3.57%, and 31.30 and 26.48% in both Basmati-385 and Xiangyaxiangzhan, respectively compared with CK. On the other hand, Basmati-385 and Xiangyaxiangzhan showed a decrease of 16.43 and 14.33% in MDA contents, respectively with the application of paclobutrazol as compared with CK. Paclobutrazol seed treatment led to substantial improvements in soluble protein and soluble sugar contents of both rice cultivars. For instance, an increase of 8.87 and 28.38% in soluble protein and 19.57 and 25.44% in soluble sugar contents was recorded for Basmati-385 and Xiangyaxianzhan, respectively with the application of paclobutrazol. Furthermore, positive correlations were recorded among dry weight per unit seedling length and CAT, SOD, POD, soluble protein, proline and soluble sugar. However, negative correlation was recorded between dry weight per unit seedling length and MDA contents (*Table 5*).

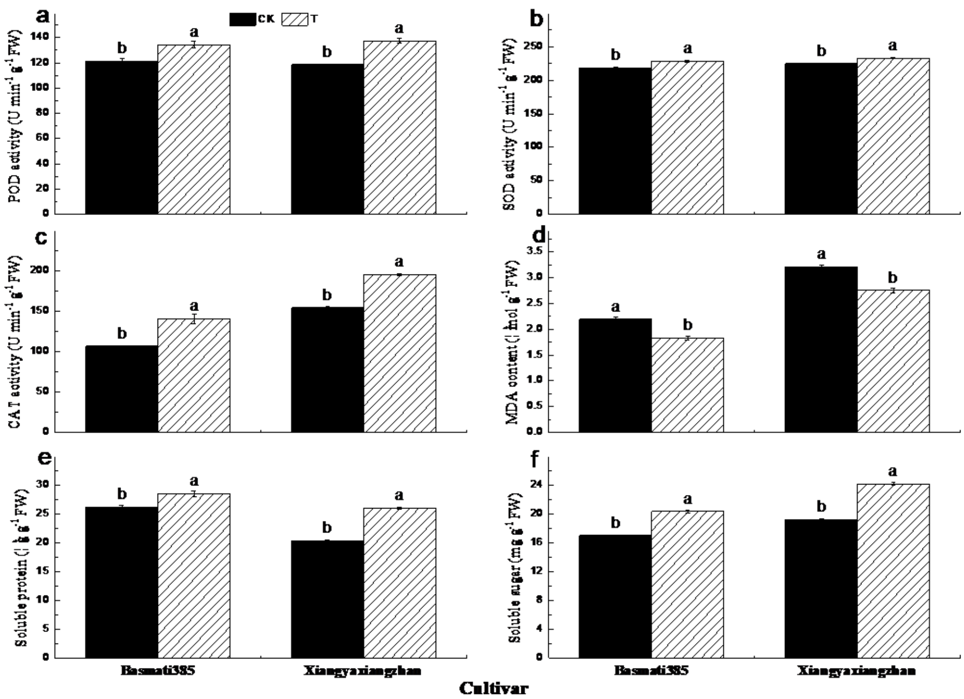

Figure 6. *Effect of seed treatment with paclobutrazol on activity of a) POD, b) SOD, c) CAT, content of d) MDA, e) soluble protein, f) soluble sugar. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at P ≤ 0.05 according to least significant difference (LSD) test*

Discussion

Application of growth retardants modifies plant growth, development, root architecture, and physio-biochemical traits in crop plants. Previously, various troazoles have been studied as growth retardants and as anti-lodging agent of which paclobutrazole is a potential anti-gibberellic triazole (Peng et al., 2014). It inhibits the

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biosynthesis of endogenous GA, hence can be used for canopy management in various crops. It also provides relief against various abiotic stresses by regulating photosynthetic process, osmolyte production and activities of various enzymatic and non-enzymatic antioxidants (Hu et al., 2016). In this study, it was observed that seed treatment with paclobutrazol (T) substantially reduced seedling length whilst improved base stem width, SPAD values and dry weight per unit seedling length and root morphology (*Table 1*). Moreover, significant and positive correlations were observed between dry weight per unit seedling length, root surface area, root volume in response to paclobutrazol seed treatment (*Table 2*). The result of reduction in seedling length and increase in the dry weight per unit seedling in paclobutrazol seed treatment are in agreement with Fletcher et al. (2000) who have indicated that paclobutrazol could induce semi-dwarf phenotype due to decreased production in growth promoting hormones which would help to reduce unnecessary vertical growth while promote productive growth. Previously, it was observed that semi-dwarf plants were able to avoid the serious grain losses from lodging. The roots help in the acquisition of plant nutrients from soil, so its morphological characteristics play an important role in plant growth (Yang et al., 2004). In present study, seed treatment with paclobutrazol stimulated root growth and thus improved the early growth of both rice cultivars. Increased root growth by paclobutrazol is associated with an increased level of endogenous cytokinin that promotes plant growth and development and delays senescence in plants (Fletcher et al., 2000). Seed treatment with paclobutrazol improved photosynthetic and gas exchange attributes and chlorophyll contents of both rice cultivars (*Figs. 3* and *4*), whereas dry weight per unit seedling length was positively correlated with net photosynthetic rate, stomatal conductance, chl a, chl b, carotenoids and total chl contents in both rice cultivars (*Table 3*). Photosynthesis is the process of converting light energy into chemical energy by synthesizing some organic compounds. Chlorophyll absorbs energy from the light, and energy is then used to convert carbon dioxide into carbohydrates (Connelly et al., 1997). The increased chl and carotenoid contents by paclobutrazol treatment may be due to improved root growth, which is the major site for cytokinin biosynthesis (Fletcher et al., 2000). They further reported that high levels of cytokinins may stimulate chlorophyll biosynthesis, and hence photosynthetic capacities of plants (Fletcher et al., 2000). Enhancement of photosynthetic pigment in plants by paclobutrazol has also been reported in barley (Özmen et al., 2003) and wheat (Hajihashemi et al., 2006). Trizole-induced modulations in leaf greenness, photosynthesis, chlorophyll biosynthesis and root morphological traits were noted in agronomic and horticultural crops (Hajihashemi et al., 2007).

Table 5. *Correlation coefficients among dry weight per unit seedling length, CAT, MDA, POD, SOD, protein, proline and sugar*

Parameters	Basmati	*P* value	Xiangyaxiangzhan	*P* value
POD	0.8576	0.0290	0.9802	0.0006
SOD	0.9500	0.0037	0.9651	0.0018
CAT	0.9581	0.0026	0.9968	0.0000
MDA	-0.9787	0.0007	–0.9698	0.0014
Soluble protein	0.8275	0.0421	0.9908	0.0001
Soluble sugar	0.9879	0.0002	0.9958	0.0000

Seed treatment with paclobutrazol enhanced *Fv/Fm, qP, F0, Fm and ETR* but decreased *NPQ* in both Basmati-385 and Xiangyaxuangzhan as compared to CK (*Fig. 5*). These parameters were also positively correlated with dry weight per unit seedling length except *NPQ* which showed negative relations (*Table 4*). The improvement of *Fv/Fm*, *ETR* and *qP* manifested that paclobutrazol treatment have improved the activity of PSII reaction center and enhanced the energy efficiency. The results are in agreement with Hajihashemi et al. (2006) who have reported that high levels of cytokinins may stimulate chlorophyll biosynthesis which may result in improved activity of PS-II reaction center.

Paclobutrazol seed treatment further improved the antioxidant enzyme activities i.e., POD, SOD and CAT, as well as soluble protein and soluble sugars whilst reduced MDA contents (*Fig. 6*). Furthermore, positive correlations were recorded among dry weight per unit seedling length and CAT, SOD, POD, soluble protein, proline and soluble sugar whereas negative correlation was recorded between dry weight per unit seedling length and MDA contents. MDA production is an important indicator of oxidative stress. Lower MDA contents in paclobutrazol treatment indicated that its application may reduce the rate of lipid peroxidation. The results are similar to the previous studies because it can react with free amino acids and produce ethylene in cellular membranes (Rakwal et al., 2003) whilst it could imparts the characteristics of cellular membranes and results in increased ion leakage through cell membranes (Dash et al., 2002). When plants are subjected to external stress, reactive oxygen species (ROS) will accumulate inside which may cause oxidative damage to cellular membranes and organelles. On this occasion, proteins and sugar would help to maintain cellular structures and functions whilst anti-oxidants help for quenching ROS. For instance, SOD scavenges superoxide radical whereas POD and CAT involve in scavenging H_2O_2 (Pan et al., 2013). Thus paclobutrazol induced regulations in protein and sugar accumulation and anti-oxidative defence responses. This indicated that application of paclobutrazol will increase resistance ability of seedlings against stress. The fundamental mechanism of paclobutrazol for improving antioxidant defence system has not been fully understood (Sankar et al., 2007). Possibly, paclobutrazol might increase antioxidant efficiency via enzymatic and non-enzymatic antioxidants and the results are similar to former reports that paclobutrazol enhances antioxidant defence system by increasing the activities of different anti-oxidant enzymes i.e., SOD, CAT, APX, and POX and the contents of ascorbate, glutathione, and α-tocopherol (Jaleel et al., 2007; Manivannan et al., 2008; Sankar et al., 2007). Enhancing antioxidant efficiency in plants by paclobutrazol has also been reported in *Catharanthus roseus* (Jaleel et al., 2007) and barley (Özmen et al., 2003). It is also possible that high antioxidant efficiency may result to prevent degradation of chlorophylls and carotenoids due to their higher ability to scavenge and trap ROS before damaging cells (Kong et al., 2017).

Conclusion

This study showed that seed treatment with paclobutrazol improved the shoot and root characters i.e., seedling length, base stem width, dry weight per unit seedling length, root length, root surface area, root diameter and root volume. Paclobutrazol treatment further modulated the photosynthetic and gas exchange traits, promoted the activities of antioxidants i.e., POD, SOD, and CAT whilst reduced MDA contents. Improved photosynthesis and anti-oxidant activities could lead to improved early

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growth of both rice cultivars; nonetheless optimization of paclobutrazol concentration for seed treatment of different crops is needed in future.

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