International Journal of Biometeorology (2018) 62:1375–1387
https://doi.org/10.1007/s00484-018-1537-z

ORIGINAL PAPER

Boron application improves yield of rice cultivars under high temperature stress during vegetative and reproductive stages

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Received: 2 July 2017 / Revised: 13 February 2018 / Accepted: 29 March 2018 / Published online: 12 April 2018

Abstract

It is reported that high temperatures (HT) would cause a marked decrease in world rice production. In tropical regions, high temperatures are a constraint to rice production and the most damaging effect is on spikelet sterility. Boron (B) plays a very important role in the cell wall formation, sugar translocation, and reproduction of the rice crop and could play an important role in alleviating high temperature stress. A pot culture experiment was conducted to study the effect of B application on high temperature tolerance of rice cultivars in B-deficient soil. The treatments comprised of four boron application treatments viz. control (B0), soil application of 1 kg B ha^{-1} (B1), soil application of 2 kg B ha^{-1} (B2), and foliar spray of 0.2% B (Bfs); three rice cultivars viz. Annapurna (HT stress tolerant), Naveen, and Shatabdi (both HT stress susceptible); and three temperature regimes viz. ambient (AT), HT at vegetative stage (HTV), and HT at reproductive stage (HTR). The results revealed that high temperature stress during vegetative or flowering stage reduced grain yield of rice cultivars mainly because of low pollen viability and spikelet fertility. The effects of high temperature on the spikelet fertility and grain filling varied among cultivars and the growth stages of plant when exposed to the high temperature stress. Under high temperature stress, the tolerant cultivar displays higher cell membrane stability, less accumulation of osmolytes, more antioxidant enzyme activities, and higher pollen viability and spikelet fertility than the susceptible cultivars. In the present work, soil application of boron was effective in reducing the negative effects of high temperature both at vegetative and reproductive stages. Application of B results into higher grain yield under both ambient and high temperature condition over control for all the three cultivars; however, more increase was observed for the susceptible cultivar over the tolerant one. The results suggest that the exogenous application of boron had a substantial effect on cell membrane stability, sugar mobilization, pollen viability, and spikelet fertility, hence the yield. The cultivars due to their variation in the tolerance level for high temperature stress behaved differently, and at high temperature stress, more response of the application of boron was seen in susceptible cultivars.

Keywords Antioxidant enzymes · Boron uptake · Cell membrane stability · Osmolytes · Pollen viability · Spikelet fertility

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00484-018-1537-z) contains supplementary material, which is available to authorized users.

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Introduction

Rice is the staple food for more than 100 million households in South and Southeast Asia and its cultivation is the main source of income for them (Redfern et al. 2012). Accumulation of greenhouse gases in the atmosphere has warmed the planet. At the end of this century, the surface air temperature probably will increase by around 1.4–5.8 °C (IPCC 2007). The rice is mostly cultivated in regions where temperatures are above the optimal (28/22 °C) for growth (Prasad et al. 2006). Any further increase in mean temperature or episodes of high temperatures during sensitive crop growth stages may reduce rice yields drastically. High temperature is already a major environmental stress in tropical environments that limits rice productivity, and the major impact of relatively higher temperatures is the reductions in grain weight and quality (Prasad et al. 2017). Teixeira et al. (2013) have reported that temperate and sub-tropical agricultural areas might bear substantial crop yield losses due to extreme temperature episodes. Heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development (Wahid et al. 2007). It is reported that heat stress affects all vegetative and reproductive stages to some extent: during the vegetative stage, high day temperature can affect leaf gas exchange properties that can cause significant increases in the abortion of floral buds and opened flowers during the reproductive stage (Guilioni et al. 1997).

High temperature stress affects the plant growth and development and also induces morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity and ultimately the yield. The cell membrane is one of the main cellular targets to different stresses (Levitt 1980). Cell membrane stability is a physiological index widely used for the evaluation of drought and temperature tolerance (Fokar et al. 1998; Maavimani et al. 2014; Mohammed and Tarpley 2009a). High temperature stress can increase cell damage by decreasing membrane thermostability, thereby disrupting water, ion, and organic solute movement across plant membranes, affecting all other metabolic activities (Christiansen 1978). The cell membrane stability, which measures electrolyte leakage from leaf disks at high temperature, is one of the simplest techniques to evaluate the performance of plants under high temperature (Sullivan 1972). High temperature stress also reported to induce the rapid production and accumulation of reactive oxygen species (ROS) (Hasanuzzaman et al. 2013). These high levels of ROS are harmful to all cellular compounds and negatively influence cellular metabolic processes (Mohammed and Tarpley 2009a). Plants grown under abiotic stresses, including salinity, water deficit, and extreme temperatures, have reported to accumulate certain organic compounds of low molecular mass, generally known as compatible osmolytes (Hare et al. 1998; Wahid et al. 2007), and accumulation of such compounds may contribute to enhanced stress tolerance of plants. Similarly, accumulation of soluble sugars under heat stress has been reported in sugarcane, which entails great implications for heat tolerance due to their involvement in the osmotic adjustment under heat stress (Wahid and Close 2007). The reproductive stage is relatively more sensitive than the vegetative stage to high temperature stress in many crop species including rice (Jagadish et al. 2014; Driedonks et al. 2016). Earlier studies have shown that spikelets that are exposed to temperatures > 35 °C at anthesis for about 5 days during the flowering period remained sterile and set no seed (Satake and Yoshida 1978). Poor anther dehiscence and low pollen production and hence low numbers of germinating pollen grains on the stigma may cause sterility (Matsui and Omasa 2002; Prasad et al. 2006; Jagadish et al. 2007). Spikelet sterility at high temperature was also reported to be related with the genotypic variation in rice (Prasad et al. 2006; Jagadish et al. 2007).

In response to the high temperature stress, various approaches are being used to mitigate its adverse effects on the crops. Among the various methods to reduce high temperature stress in plant, foliar applications of, or pre-sowing seed treatment with, low concentrations of inorganic salts, osmoprotectants, signaling molecules (e.g., growth hormones), and oxidants (e.g., H_2O_2) and preconditioning of plants are common approaches (Wahid et al. 2007) which have been tried and found to be effective to various levels of the stress (Khan et al. 2013). In addition to chemical regulators, proper plant nutrition is one of the good strategies to alleviate the temperature stress in crops (Krishnan et al. 2011; Waraich et al. 2012). Boron (B) is directly or indirectly involved in several physiological and biochemical processes during plant growth and may be helpful in mitigating some of the harmful effects of the high temperature. Boron plays a very important role in the cell wall formation, sugar translocation, and reproduction of plants (García-Hernández and Cassab López 2005). One of the major effects of B in plants is that it helps in the pollen germination and the formation of pollen tube (Loomis and Durst 1992) and improves pollen viability (Garg et al. 1979). Boron can also increase the antioxidant activities of plants and can be useful in alleviating the reactive oxygen species (ROS) damage due to high temperature stress (Cakmak and Romheld 1997). Boron nutrition improves sugar transport in the plant body and decreases the phenolic compounds in leaves, which helps to improve seed germination and seed grain formation (Dell and Huang 1997). Boron deficiency has been identified as one of the most important factors causing sterility in cereals because of poor development of anthers and pollen and failure of pollen germination (García-Hernández and Cassab López 2005). For efficient uptake of B by the plant, application method plays a vital role. Among different practices, soil application is the most prevalent method of B addition in the developed world however, for instant correction of the deficiency, nutrient application through foliar spray is also practiced (Rehman et al. 2014).

We hypothesized that application of B can be helpful in reducing the adverse effects of high temperature stress both at mid-vegetative and reproductive stages in rice and improves the grain yield in B-deficient soil. Therefore, a study was conducted to evaluate the influence of B application on the cell membrane stability, accumulation of osmolytes, antioxidant enzymes, panicle fertility, and grain yield of rice cultivars.

Materials and methods

Soil and plant materials

The experiment was conducted using B-deficient soil with three indica rice cultivars (Annapurna, Naveen, and Shatabdi) under different temperature conditions at the net house of ICAR-National Rice Research Institute, Cuttack, Odisha, India, during the dry season of 2015. Three rice cultivars Annapurna (HT stress tolerant), Naveen, and Shatabdi (both HT stress susceptible) selected for this study are known to have different tolerance to HT stress (CRRI 2012). Seeds of Naveen and Shatabdi were sown on 28 December 2014 and Annapurna on 8 January 2015 because of their different maturity durations. Twenty-one-day-old seedlings were transplanted to plastic pots having dimensions 30 cm in height and 25 cm in diameter and loaded with 12 kg sieved soils. The soil nutrient conditions were as follows: organic carbon of 5.2 g kg^{-1}, available nitrogen of 112.1 kg ha^{-1}, available phosphorus of 12.4 kg ha^{-1}, available potassium of 134.6 kg ha^{-1}, and hot water-soluble boron of 0.38 mg kg^{-1}. Before transplanting, 0.6 g of urea, 0.95 g KH_2PO_4, and 0.52 g KCl were applied to each pot, and another 0.3 g of urea was top dressed at mid-tillering and panicle initiation (dose of fertilizer was kept as 100:50:50 kg ha^{-1} for NPK, respectively). Each pot contained one hill with two seedlings per hill and was the experimental unit. All pots maintained a water layer of 2–3 cm in depth during the whole growth period, except for drainage just before the harvesting. Other crop managements were conducted following the same procedure as the conventional cultivation method.

Boron and high temperature stress treatment

The potted rice cultivars were subjected to four doses of B and grown under three temperature conditions. Four doses of B comprised of control (no B application; B0), 1 kg ha^{-1} B applied to soil (B1), 2 kg ha^{-1} B applied to soil (B2), and 0.2% foliar spray of B (Bfs). Soil applications were done before the transplanting and foliar spray at active tillering and panicle initiation stages. Each time during foliar spray, 50 mL of the 0.2% of boric acid solution in 0.5% urea (adjuvant) was sprayed on a single plant using a manual sprayer. Three conditions of temperature treatments were included for growing of three sets of the treatments. One set is grown throughout at ambient temperature condition (AT) in the net house and other two sets were grown in temperature tunnel at vegetative (mid-tillering to panicle initiation for 30 days; HTV) and reproductive (PI to flowering for 30 days; HTR) stages, respectively. The treatments were replicated thrice. The temperature tunnel was equipped with the temperature and humidity sensors for recording environmental condition of the tunnel. The cooling of the tunnels was carried out by using exhaust fan for air circulation and hanging wet gunny bags at one side of the tunnel to limit its boundary and preventing gradient temperature. Firstly, the plants approaching mid-tillering stage were moved to the tunnel for high temperature treatment from the mid-tillering to panicle initiation stage for 30 days (HTV). Another set of plants were then moved to the tunnel for high temperature treatment from the panicle initiation to flowering stage for 30 days (HTR). After the imposition of the HT stress treatments, the pots were taken out of the tunnel and grown in ambient condition outside the tunnel until maturity stage. The average maximum temperatures of the tunnel during vegetative and reproductive stages were 37.6 and 39.2 °C, respectively, and average relative humidity (RH) was 67.8 and 68.1%, respectively (Fig. 1). The respective average temperature for ambient condition was 33.0 and 34.4 °C and average RH was 42.6 and 52.9%. During the period of high temperature treatments, temperatures in the HTV and HTR treatments were constantly higher than those in the control. The photosynthetically active radiation (PAR) in the tunnel was about 74% of the PAR in the net house. This was measured using line quantum sensor.

Sampling and measurements

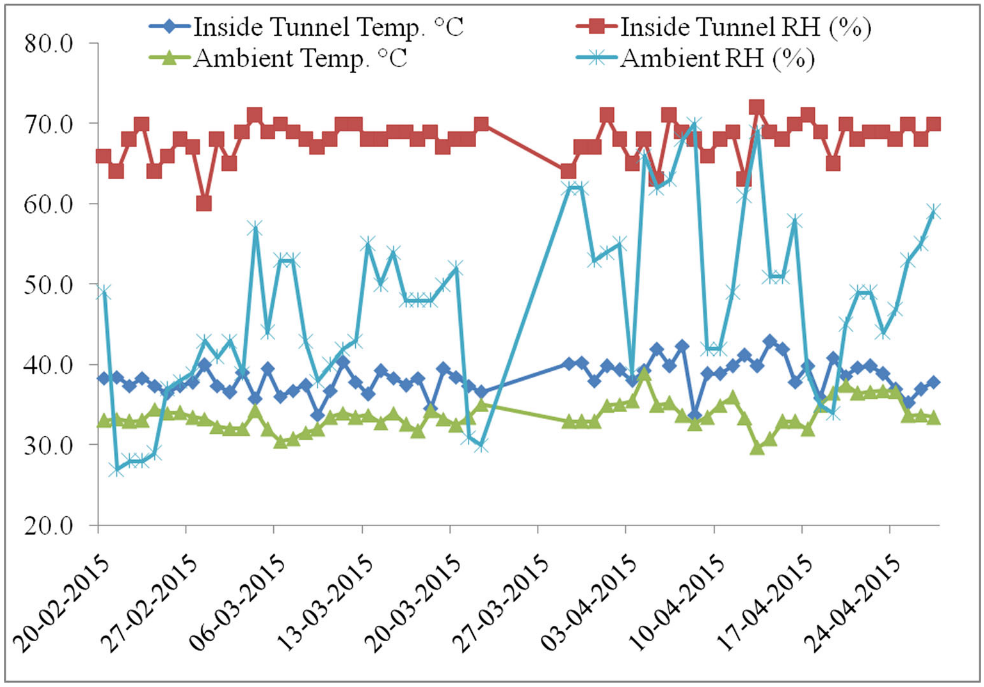
Plant sampling

Flag leaves of the rice plants were collected at flowering stage at the time of first flowering in neighboring panicle for physiological parameter measurements. Part flag leaves were dried at 105 °C for 1 h and then at 70 °C until constant weight in a forced-air oven, subsequently milled to powder for measurements of soluble sugar content. The remainder (fresh sample) was used for assay of cell membrane stability and catalase, peroxidase, and superoxide dismutase enzymes activities. For pollen viability study, three individual florets from three different panicles were collected from each cultivar during flowering in ambient and high temperature treatments. Generally third or fourth spikelets on the branch were used to estimate pollen viability.

Pollen viability

Pollen viability was estimated using 1% iodine potassium iodide (IKI) stain. Pollen grains stained uniformly were considered viable. For pollen viability, three anthers from three different panicles were collected early in the morning before anthesis, stored in 70% ethanol, and brought to laboratory for further analysis. Anthers were opened with a needle and pollen grains were immediately brushed on glass slide and covered with a drop of IKI. Pollen viability was estimated as the ratio of number of stained pollen to total number of pollen grains and expressed as percentage (Prasad et al. 2006).

Fig. 1 Daily maximum temperatures and relative humidity in ambient and inside tunnel treatments for high temperature at vegetative (20.02.2015 to 22.03.2015) stage and high temperature at reproductive (29.03.2015 to 27.04.2015) stage during the crop-growing season in 2015



Cell membrane stability

Leaf membrane thermal stability was measured by an electrolytic leakage technique (Agarie et al. 1995) which is a common method of evaluating damage to membranes and expressed as cell membrane stability (CMS) (Fokar et al. 1998). Briefly, to measure CMS, leaf samples are collected and cut into small pieces (3.5 cm long). These pieces are then taken in a closed tube with 1 mL distilled water. The treatment tubes are placed in a water bath at 52 °C for 1 h (T1), while the controls are kept at 10 °C (C1). After treatment, 9 mL of distilled water is added to all tubes (treatment and control) and incubated at 10 °C for 24 h. The tubes are brought to room temperature, and the conductance of the solution is measured with a conductivity meter (T1, C1). After the measurements are taken, the tubes are autoclaved at 100 °C for 15 min and their conductance is measured again (T2, C2). CMS is then calculated as by following formula:

$$\text{Cell membrane thermostability (CMS)\%} = [\{1-(\mathrm{T1}/\mathrm{T2})\}/\{1-(C1/C2)\}] \times 100$$

Soluble sugar content

For the determination of the reducing and non-reducing sugars, 1 g of leaf was crushed with 80% ethanol and centrifuged at 2000 rpm for 20 min and supernatant is collected. Total soluble sugar content was measured based on the anthrone method (Dubois et al. 1951). The supernatant obtained was separated into another test tube and 12.5 mL of 80% alcohol was added to it. One milliliter of the solution was taken and 1 mL of 0.2% anthrone was added. The mixture was heated in a water bath at 100 °C for 10 min. The reaction was terminated by incubating the mixture on ice for 5 min. Total soluble sugar content was determined using a spectrophotometer at 620 nm. Estimation of reducing sugar was done by the method of Miller (1959). To 1.0 mL of alcoholic extract, 3 mL of 3,5-dinitrosalicylic acid (DNSA) reagent was added and boiled in water bath for 5 min. The absorbance was measured at 515 nm. From a standard curve of glucose, the quantity of reducing sugar was calculated and expressed as mg g^{-1} leaf. The amount of non-reducing sugar was measured by subtracting the value of reducing sugar from the value of total soluble sugar.

Antioxidant enzymes activities

A 500-mg sample of leaves was homogenized in 10 mL of grinding medium prepared for each enzyme. Catalase (CAT) and peroxidase (POD) activities were estimated following the method of Cakmak and Marschner (1992). The sample is ground with 50 mM phosphate buffer of pH 7.0 and centrifuged and the supernatant is utilized as enzymes source. The reaction mixture for CAT contained 25 mM phosphate buffer (pH 7.0) and 10 mM H_2O_2, and for PER, the reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol (O-methoxyphenol), and 10 mM H_2O_2. The decomposition of H_2O_2 for CAT was followed at 240 nm and POD activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation activity.

Superoxide dismutase assay is based on the capacity of the extracts to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) in the presence of the riboflavin–light-NBT system (Beauchamp and Fridovich 1971). Briefly, samples are extracted with 1.5 mM HEPES buffer (pH 7.6) containing 0.1 mM EDTA and the supernatant obtained after centrifugation is used as enzyme source. The reaction mix contains phosphate buffer, methionine, NBT, EDTA, riboflavin, and enzyme extract. The reaction is started by placing the reaction mix under a light source. After 10 min, the reaction is stopped by switching off the lights and the absorbance is read at 560 nm. A non-irradiated reaction mixture will have zero absorption at 560 nm, while the reaction mixture lacking enzyme will develop the most intense color. The volume of enzyme extract producing 50% inhibition of the reaction has to be calculated. One unit of superoxide dismutase activity is defined as the enzyme which causes 50% inhibition of the initial rate of reaction in the absence of enzyme.

Spikelet sterility

Rice panicles are harvested during the maturity stage of the crop and numbers of filled and unfilled grains per panicle were recorded. Spikelet fertility was estimated as the ratio of number of filled grains to total number of reproductive sites (florets) and expressed as percentage. Each floret was pressed between the forefinger and thumb to determine if the grain was filled or not (Prasad et al. 2006). Number of filled grains included both completely and partially filled grains. Dry weights of filled and unfilled spikelets were recorded.

Yield and harvest index

At maturity, rice plants from each pot were sampled for the determination of grain and straw yield. Grain yields were estimated at 14% moisture content. Harvest index was calculated as a ratio of grain weight to total above ground crop dry weight.

Statistical analysis

Data were analyzed by SAS statistical package 9.3 (SAS 2008). Least significant difference (LSD) at $p < 0.05$ probability level was used to distinguish among treatments. The results reported here are from a single experiment.

Results

Cell membrane stability

The results revealed the cell membrane stability (CMS) was greater in plants grown under ambient condition (62.1%) compared with the plants grown under high temperature either at vegetative (49.0%) or reproductive stages (56.0%) (Table 1). On an average, plants grown under high temperature showed 9% (at reproductive stage) and 21% (at vegetative stage) increase in electrolytic leakage compared with plants grown under ambient temperature. Application of boron at 1 and 2 kg ha^{-1} and 0.2% foliar spray decreased electrolytic leakage of rice leaves by 23.0, 23.8, and 13.9%, respectively, compared with the control treatment. A significant difference ($p < 0.05$) was observed among the rice cultivars, in which maximum electrolyte leakage was observed for Shatabdi followed by Naveen and Annapurna. The interaction effects of TxV and BxV were also significant suggesting differential effects of temperature on the rice cultivars (tolerant and susceptible) and their responses to the B applications. At high temperature stress, highest reduction in CMS (28.0%) was observed for Naveen at HTV and lowest (2.7%) for Annapurna at HTR treatment. Tolerant cultivar Annapurna recorded less response to the soil application of B, whereas the Shatabdi recorded the highest (39.0%) in B2 treatment.

Reducing and non-reducing sugars

Reducing and non-reducing sugar contents in rice plant increased significantly ($p < 0.05$) under high temperature both at vegetative (HTV) and reproductive stages (HTR); however, more increase was observed at vegetative stage (Table 1). Application of B at 2 kg ha^{-1} (B2) led to about 17.7 and 31.0% decrease in reducing and non-reducing sugar contents, respectively, as compared to the no application of B (B0). Application of B at 1 kg ha^{-1} and 0.2% foliar spray also significantly decreased the reducing and non-reducing sugar contents over the control (B0). Among the rice cultivars, Shatabdi had significantly higher reducing (48.3 mg kg^{-1}) and non-reducing (29.9 mg kg^{-1}) sugar contents and lowest values for these sugars were recorded in Annapurna (42.5 and 24.6 mg kg^{-1}, respectively). The interaction effects of temperature vs. cultivar for reducing and non-reducing sugars and boron vs. cultivar for non-reducing sugars were also significant. Highest values of reducing and non-reducing sugar contents were observed under HTV for all the three cultivars which were significantly higher than HTR and AT. At AT, no significant difference was observed among the rice cultivars, whereas at HTV and HTR, significantly lower values of reducing and non-reducing sugars were observed in Annapurna as compared to both Naveen and Shatabdi.

Catalase, peroxidase, and superoxide dismutase enzyme activities

The specific activity of catalase (CAT) was 34.6 U g^{-1} FW min^{-1} in AT, which increased to 47.9 U g^{-1} FW min^{-1} in HTV and 57.8 U g^{-1} FW min^{-1} in HTR (Table 2). The difference in

Table 1 Cell membrane stability, reducing and non-reducing sugar contents of the leaf of rice cultivars under different temperature and boron application treatments

	Cell membrane stability (%)				Reducing sugar (mg kg^{-1})				Non-reducing sugar (mg kg^{-1})			
	Annapurna	Naveen	Shatabdi	Mean	Annapurna	Naveen	Shatabdi	Mean	Annapurna	Naveen	Shatabdi	Mean
AT	64.0	63.0	59.2	62.1	39.5	40.0	40.1	39.9	22.0	23.1	23.3	22.8
HTV	56.5	45.3	45.2	49.0	47.9	53.3	56.2	52.5	27.6	31.7	35.4	31.6
HTR	62.3	52.6	53.1	56.0	40.1	44.9	48.8	44.6	24.1	27.6	31.2	27.6
B0	55.5	46.7	42.9	48.3	44.9	50.6	54.2	49.9	28.7	32.6	36.6	32.6
B1	65.2	55.7	57.5	59.5	41.7	43.6	45.8	43.7	22.2	24.3	25.5	24.0
B2	59.2	60.6	59.6	59.8	38.9	41.6	42.7	41.1	21.9	22.1	23.5	22.5
Bfs	63.8	51.5	50.0	55.1	44.5	48.5	50.7	47.9	25.5	31.0	34.1	30.2
Mean	60.9	53.6	52.5		42.5	46.1	48.3		24.6	27.5	29.9	
CD ($p < 0.05$)												
T				2.6				1.7				1.0
B				3.0				2.0				1.1
C				2.6				1.7				1.0
TxB				NS				NS				NS
TxC				4.6				3.0				1.7
BxC				5.3				NS				2.0
TxBxC				9.1				NS				NS

AT ambient temperature, *HTV* high temperature at vegetative stage, *HTR* high temperature at reproductive stage, *B0* no application of boron, *B1* soil application of boron at 1.0 kg ha^{-1}, *B2* soil application of boron at 2.0 kg ha^{-1}, *Bfs* foliar spray of boron at 0.2%, *T* temperature treatments, *B* boron application treatments, *C* cultivars

Table 2 Antioxidant enzyme activities of the rice cultivars under different temperature and boron application treatments

	Catalase activity (U g^{-1} min^{-1})				Peroxidase activity (U g^{-1} min^{-1})				Superoxide dismutase activity (U g^{-1})			
	Annapurna	Naveen	Shatabdi	Mean	Annapurna	Naveen	Shatabdi	Mean	Annapurna	Naveen	Shatabdi	Mean
AT	30.5	35.1	38.3	34.6	32.0	34.9	34.8	33.9	49.7	59.4	51.6	53.6
HTV	51.1	46.6	46.2	47.9	43.8	42.1	37.5	41.1	76.6	70.1	68.7	71.8
HTR	60.4	57.3	55.9	57.8	53.8	42.6	49.8	48.7	84.9	79.2	79.4	81.2
B0	41.1	33.1	33.0	35.7	39.8	33.1	31.6	34.8	65.5	59.0	47.4	57.3
B1	48.4	51.9	51.0	50.4	44.0	41.9	46.1	44.0	69.7	71.2	72.8	71.2
B2	56.5	59.1	58.3	58.0	51.0	45.3	48.1	48.1	77.6	81.7	89.5	82.9
Bfs	43.3	41.1	44.9	43.1	38.0	39.1	36.8	38.0	68.7	66.3	56.6	63.9
Mean	47.3	46.3	46.8		43.2	39.9	40.7		70.4	69.5	66.6	
CD ($p < 0.05$)												
T				1.8				1.5				2.5
B				2.1				1.8				2.9
C				NS				1.5				2.5
TxB				3.6				3.1				5.0
TxC				3.1				2.7				4.3
BxC				3.6				3.1				5.0
TxBxC				6.2				5.3				8.6

AT ambient temperature, *HTV* high temperature at vegetative stage, *HTR* high temperature at reproductive stage, *B0* no application of boron, *B1* soil application of boron at 1.0 kg ha^{-1}, *B2* soil application of boron at 2.0 kg ha^{-1}, *Bfs* foliar spray of boron at 0.2%, *T* temperature treatments, *B* boron application treatments, *C* cultivars the CAT activity under the temperature treatments was significant. With the application of boron, the CAT activity increased significantly as compared to the control (B0). The highest increase (62.4%) was observed in B2 which was significantly higher as compared to all other treatments of B application. Foliar spray of B (Bfs) also resulted in significantly higher values of CAT activity (43.1 U g^{-1} FW min^{-1}) as compared to control (B0), but this value was significantly lower than the soil application of 1 and 2 kg ha^{-1} B (B1 and B2). In the rice cultivars, no significant difference was observed w.r.t. CAT activity. Higher response of B application on CAT activity was observed under both HTV and HTR as compared to AT.

The peroxidase activity (POD) was significantly lower (33.9 U g^{-1} FW min^{-1}) in AT as compared to the 41.1 U g^{-1} FW min^{-1} in HTV and 48.7 U g^{-1} FW min^{-1} in HTR (Table 2). Under the temperature stress at vegetative and reproductive stages, the POD activity increased by 21.4 and 43.8%, respectively, as compared to ambient temperature. Among the B application treatments, the POD activity was lowest in B0 (34.8 U g^{-1} FW min^{-1}) and increased significantly in B1, B2, and Bfs. Highest increase was noted in the B2 which was significantly higher than all other treatments. Foliar spray of B (Bfs) also resulted in significantly higher values of POD activity (38.0 U g^{-1} FW min^{-1}) as compared to control (B0), but this value was significantly lower than the soil application of 1 and 2 kg ha^{-1} B (B1 and B2). Among the rice cultivars, lowest value of POD activity (39.9 U g^{-1} FW min^{-1}) was measured for Naveen, which was significantly lower than the Annapurna. Higher response of B application on POD activity was observed under both HTV and HTR as compared to AT. Under AT, application of B at 1 and 2 kg ha^{-1} in soil and 0.2% foliar spray resulted in 8.4, 12.8, and 6.7% increase, respectively, in POD activity as compared to the B0, whereas at HTV and HTR, the increase was 20.8, 32.5, and 7.1% and 46.9, 65.3, and 12.7%, respectively, over B0 (data not shown).

The superoxide dismutase (SOD) enzyme showed significantly higher activity of 81.2 U g^{-1} FW (Table 2) in HTR as compared to AT (53.6 U g^{-1} FW) and HTV (71.8 U g^{-1} FW). Under the temperature stress at vegetative and reproductive stages, the SOD activity increased by 34.0 and 51.5%, respectively, as compared to ambient temperature. With the application of boron, the SOD activity increased significantly as compared to the control (B0). The highest increase (44.8%) was observed in B2 which was significantly higher as compared to all other treatments of B. Foliar spray of B (Bfs) also resulted in significantly higher values of SOD activity (63.9 U g^{-1} FW) as compared to control (B0), but this value was significantly lower than the soil application of 1 and 2 kg ha^{-1} B (B1 and B2). The rice cultivars also varied in the SOD activity and among them, highest SOD activity (70.4 U g^{-1}FW) was measured for Annapurna, which was at par with the SOD activity in Naveen (69.5 U g^{-1} FW) and significantly higher than the SOD activity in Shatabdi (66.6 U g^{-1} FW). At the same level of temperature treatments, significantly higher SOD activities were observed in HT stress tolerant cultivar Annapurna as compared to HT stress susceptible cultivars Naveen and Shatabdi. Boron application had a significant effect on increasing SOD activity in the studied cultivars, and highest increase was observed under B2 (44.8%). Under AT, application of B at 1 and 2 kg ha^{-1} in soil and 0.2% foliar spray resulted in 16.0, 30.5, and 4.9% increase, respectively, in SOD activity as compared to the B0, whereas at HTV and HTR, the increase was 22.6, 44.3, and 10.6% and 32.3, 55.7, and 17.3%, respectively, over B0 (data not shown).

Pollen viability and spikelet fertility

Our results indicated significant differences between the high temperature stress at vegetative and reproductive stages as compared to ambient temperature on pollen viability (Table 3). Rice plants grown under HTV and HTR showed reduced pollen viability by 3.5 and 16.5%, respectively, as compared to the AT treatment, which recorded highest (82.4%) pollen viability. Under the B application treatments, significantly higher values of pollen viability were recorded for B2 (80.7%) and B1 (79.1%), which were statistically at par and significantly higher than the B0 (71.6%) and Bfs (76.4%). Among the rice cultivars, highest pollen viability (79.8%) was observed in Annapurna, which was at par with that observed in Naveen (78.3%) and significantly higher than that observed in Shatabdi (72.7%). The interaction effect of temperature vs. cultivar was also significant, and highest value of pollen viability was observed under AT for all the three cultivars which were significantly higher than HTR. At AT, no significant difference was observed among the rice cultivars, whereas at HTR, significantly lower value of pollen viability was observed in Shatabdi (59.2%) as compared to both Annapurna (74.4%) and Naveen (72.9%). The HTV treatment also differs significantly with HTR treatment for all the three rice cultivars, and higher values of pollen viability for each cultivar were observed under HTV. Pollen viability of Annapurna at HTV was not significantly different that at AT; however, for Naveen and Shatabdi, significantly lower pollen viability was observed under HTV as compared to AT.

There were differences between the high temperatures, among the boron treatments, and among the cultivars with respect to spikelet fertility (Table 3). On an average, plants grown under HTV and HTR showed 5.7 and 19.7% decrease in spikelet fertility, compared to AT. Plants treated with B either as soil application or foliar spray resulted in 9.4 to 16.9% increase in the spikelet fertility. Among the rice cultivars, Annapurna had significantly higher spikelet fertility (82.2%) which was at par with the spikelet fertility in Naveen (80.3%) and lowest value was observed in Shatabdi

Table 3 Pollen viability and spikelet fertility of the rice cultivars under different temperature and boron application treatments

	Pollen viability (%)				Spikelet fertility (%)			
	Annapurna	Naveen	Shatabdi	Mean	Annapurna	Naveen	Shatabdi	Mean
AT	83.7	82.9	80.7	82.4	87.4	89.3	82.5	86.4
HTV	81.4	79.1	78.2	79.6	85.1	82.7	76.5	81.4
HTR	74.4	72.9	59.2	68.8	74.1	69.0	65.1	69.4
B0	75.0	71.2	68.5	71.6	75.9	73.6	65.7	71.8
B1	83.8	80.0	73.5	79.1	84.4	82.6	79.3	82.1
B2	84.0	82.8	75.4	80.7	86.9	84.5	80.2	83.9
Bfs	76.6	79.1	73.4	76.4	81.5	80.7	73.4	78.5
Mean	79.8	78.3	72.7		82.2	80.3	74.7	
CD ($p < 0.05$)								
T				3.0				2.2
B				3.4				2.6
C				3.0				2.2
TxB				NS				NS
TxC				5.1				NS
BxC				NS				NS
TxBxC				NS				NS

AT ambient temperature, *HTV* high temperature at vegetative stage, *HTR* high temperature at reproductive stage, *B0* no application of boron, *B1* soil application of boron at 1.0 kg ha^{-1}, *B2* soil application of boron at 2.0 kg ha^{-1}, *Bfs* foliar spray of boron at 0.2%, *T* temperature treatments, *B* boron application treatments, *C* cultivars

(74.7%). The effects of HT stress on spikelet fertility varied among cultivars. High temperature stress tolerant cultivar Annapurna received relative small influence under the HT stress with spikelet fertility reductions by 2.6 and 15.1%, at HTV and HTR, respectively, as compared with that under AT. In the HT stress sensitive cultivar Shatabdi, the reductions were 7.2 and 21.1%, at HTV and HTR, respectively, when compared with the AT. The fertilization rates of all the studied cultivars reduced under HT stress. The HT stress sensitive cultivars had greater reduction than the HT stress tolerant cultivar. Genotypic variations were also observed within the same HT stress type. For instance, Annapurna had smaller reduction in fertilization rate (15.1%) than Shatabdi (21.1%) and Naveen (22.7%) at HTR as compared to AT. If the reduction of fertilization rate under HT stress was considered as an indicator to evaluate the HT stress tolerance, the tolerance to HT of the three cultivars was in the order of Annapurna > Naveen > Shatabdi.

Boron content and uptake

The boron content in grain did not show any significant difference under different temperature treatments; however, application of B either through soil application or foliar spray resulted in significant increase in the B contents of the grain as compared to the no application of B (Table 4). Cultivars differed significantly in grain B content, and highest value was observed in Naveen (2.07 mg kg^{-1}) followed by Annapurna (1.82 mg kg^{-1}) and Shatabdi (1.61 mg kg^{-1}). The interaction effects of TxB, BxC, and TxBxC were also significant, which suggests differential response of the B application under HT stress and cultivars with B applications. The straw B content varied significantly under different temperature and B application treatments. Contrary to the grain yield, no significant difference was observed among different temperature treatments in total B uptake by rice plant. However, a significant increase in total B uptake was observed in B2 (17.2%) as compared to all other B application treatments.

Yield and harvest index

The grain yield of rice under different temperature conditions varied significantly and a higher grain yield was recorded under AT (25.5 g pot^{-1}), which decreased significantly both under HTV (23.1 g pot^{-1}) and HTR (17.8 g pot^{-1}) (Table 5). On an average, plants grown under HT showed 9.4% (at vegetative stage) and 30.4% (at reproductive stage) decrease in the rice grain yield compared with plants grown under AT. Application of boron at 1 and 2 kg ha^{-1} and 0.2% foliar spray increased the rice grain yield by 16.7, 20.6, and 8.1%, respectively, compared with the B0. A significant difference ($p < 0.05$) was observed among the rice cultivars, in which maximum rice grain yield was observed for Naveen followed by Shatabdi and Annapurna. High yield of Naveen cultivar may be attributed to the higher yield potential of this cultivar because of its higher growth duration as compared to

Table 4 Grain and straw boron contents and total boron uptake of the rice cultivars under different temperature and boron application treatments

	Grain boron content (mg kg^{-1})				Straw boron content (mg kg^{-1})				Total boron uptake (mg pot^{-1})			
	Annapurna	Naveen	Shatabdi	Mean	Annapurna	Naveen	Shatabdi	Mean	Annapurna	Naveen	Shatabdi	Mean
AT	1.81	2.05	1.60	1.82	5.7	6.2	5.6	5.8	0.201	0.244	0.202	0.216
HTV	1.84	2.10	1.62	1.85	5.6	6.2	5.8	5.9	0.197	0.230	0.195	0.207
HTR	1.89	2.06	1.61	1.85	6.8	7.1	6.5	6.8	0.209	0.223	0.185	0.206
B0	1.54	1.89	1.59	1.67	6.1	6.9	5.5	6.2	0.189	0.228	0.171	0.196
B1	1.81	2.16	1.67	1.88	5.9	6.3	5.6	5.9	0.201	0.239	0.188	0.209
B2	2.07	2.09	1.70	1.95	6.6	6.8	6.3	6.5	0.226	0.249	0.214	0.230
Bfs	1.96	2.13	1.48	1.86	5.6	6.0	6.4	6.0	0.194	0.215	0.203	0.204
Mean	1.85	2.07	1.61		6.0	6.5	5.9		0.202	0.232	0.194	
CD ($p < 0.05$)												
T				NS				0.2				NS
B				0.09				0.3				0.016
C				0.08				0.2				0.014
TxB				0.15				0.5				NS
TxC				NS				NS				NS
BxC				0.15				0.5				NS
TxBxC				0.27				0.8				NS

AT ambient temperature, *HTV* high temperature at vegetative stage, *HTR* high temperature at reproductive stage, *B0* no application of boron, *B1* soil application of boron at 1.0 kg ha^{-1}, *B2* soil application of boron at 2.0 kg ha^{-1}, *Bfs* foliar spray of boron at 0.2%, *T* temperature treatments, *B* boron application treatments, *C* cultivars

Table 5 Grain yield, straw yield, and harvest index of the rice cultivars under different temperature and boron application treatments

	Grain yield (g pot^{-1})				Straw yield (g pot^{-1})				Harvest index			
	Annapurna	Naveen	Shatabdi	Mean	Annapurna	Naveen	Shatabdi	Mean	Annapurna	Naveen	Shatabdi	Mean
AT	24.2	27.0	25.3	25.5	27.5	30.4	28.9	29.0	0.47	0.47	0.47	0.47
HTV	22.6	24.1	22.7	23.1	27.4	28.8	27.3	27.8	0.45	0.46	0.45	0.45
HTR	18.3	18.7	16.3	17.8	25.5	26.2	24.5	25.4	0.42	0.41	0.40	0.41
B0	19.6	21.2	18.8	19.9	26.0	27.5	25.7	26.4	0.43	0.43	0.42	0.43
B1	22.6	24.5	22.4	23.2	27.2	29.3	26.9	27.8	0.45	0.45	0.45	0.45
B2	23.3	25.0	23.6	24.0	27.2	29.1	28.0	28.1	0.46	0.46	0.46	0.46
Bfs	21.2	22.4	20.9	21.5	26.9	27.9	27.0	27.3	0.44	0.44	0.43	0.44
Mean	21.7	23.3	21.4		26.8	28.5	26.9		0.45	0.45	0.44	
CD ($p < 0.05$)												
T				0.8				1.1				0.01
B				0.9				1.3				0.01
C				0.8				1.1				NS
TxB				NS				NS				NS
TxC				1.4				NS				NS
BxC				NS				NS				NS
TxBxC				NS				NS				NS

AT ambient temperature, *HTV* high temperature at vegetative stage, *HTR* high temperature at reproductive stage, *B0* no application of boron, *B1* soil application of boron at 1.0 kg ha^{-1}, *B2* soil application of boron at 2.0 kg ha^{-1}, *Bfs* foliar spray of boron at 0.2%, *T* temperature treatments, *B* boron application treatments, *C* cultivars

Annapurna. The interaction effect of TxC was also significant suggesting differential effects of temperature on the rice cultivars (tolerant and susceptible) and their responses to the B applications. At HT stress, highest reduction in grain yield (35.5%) was observed for Shatabdi at HTR and lowest (6.5%) for Annapurna at HTV treatment. Tolerant cultivar Annapurna recorded less reduction in grain yield under HTV and HTR as compared to the susceptible cultivars Naveen and Shatabdi. Similar to grain yield, the straw yield also varied significantly with the temperature and boron treatments; however, interaction effects were not significant. The harvest index (HI) was also significantly affected with the HT treatments, and highest reduction in harvest index (12.4%) was observed under HTR followed by 3.3% reduction under HTV as compared to AT. Application of B through soil application either B1 or B2 helps to improve the HI by about 6.0%.

Discussion

Cell membranes are the first structures involved in sensing and transmission of external stress signals. High temperature stress may have adverse effect on the membranes that may disrupt cellular activity or cause death. A sudden heat stress event may cause injury to membrane, which may be a result of denaturation of the membrane proteins or melting of membrane lipids, which subsequently leads into rupture of membrane and loss of cellular content, and is measured by ion leakage (Krishnan et al. 2011). Electrolyte leakage has been used as an index of thermostability of cell membrane to study tolerance mechanism in heat-tolerant genotypes in rice (Mohammed and Tarpley 2009a) and for identifying heat-tolerant genotypes for rice (Maavimani et al. 2014) and in different other crops (Blum 1988). In the present study, reduction in the cell membrane stability was observed with the HT stress either at vegetative or flowering stage; however, higher reduction was observed at vegetative stage. It has been earlier reported that the membrane stability of the rice plants decreases under HT. The decreased rice yields due to high temperatures were attributed to high cell electrolytic leakage (Mohammed and Tarpley 2009a); however, Prasad et al. (2006) observed no relationship between electrolytic leakage and yield. The negative effect of HT stress on the cell membrane stability was alleviated by the application of B either through soil application or foliar spray. Boron seems to participate in membrane structure since it can form complexes with membrane constituents such as glycolipids and/or glycoproteins (Shkolnik 1984; Cakmak and Romheld 1997). Soil application of B was found to be more effective as compared to the foliar spray. Among different practices, soil application is the most prevalent method of B addition (Farooq et al. 2012) as the B is readily absorbed by roots and is rapidly translocated to the growing points and actively transpiring tissues and, as a result, becomes well distributed throughout the plant (Shorrocks 1997).

Under high temperature stress, rice plants tend to accumulate certain organic compounds of low molecular mass, generally referred as compatible osmolytes (Krishnan et al. 2011). The osmolytes are of diverse nature (sugars, etc.) and their accumulation in plant cells can reduce cell osmotic potential that maintains water absorption and cell turgor pressure (Krishnan et al. 2011).The susceptible plants are unable to maintain adequate cell turgor pressure because of more accumulation of reducing and non-reducing sugars. Application of B helps in proper utilization of soluble sugars due to its active role in the sugar metabolism (Cakmak and Romheld 1997; Blevins and Lukaszewski 1998) in plant tissue which helps in maintaining water balance under high temperature stress. Ito et al. (2009) concluded that high temperatures depress starch accumulation and also the translocation of photoassimilates from the shoot to the ear and that they also increase the level of free sugar and particularly that of sucrose. Cheng et al. (2005) have reported that at high temperature, concentration of sucrose increases, while starch accumulation and sucrose synthase activity decrease.

In earlier studies, HT stress has reported to produce excessive reactive oxygen radicals and low activities of antioxidant enzymes (Mohammed and Tarpley 2009a). In this study, we found that the application of B at higher dose helps to overcome the oxidative stress of the increased free radicals under HT stress condition due to high activity of protective enzymes (POD, CAT, and SOD) in the plants. With no application of B, higher activities of antioxidant enzymes were observed at HTR and in the tolerant cultivar, which indicates that under increased temperature, oxidative stress increases, whereas tolerant cultivar is less affected by this oxidative stress due to increased activity of antioxidant enzymes in them. With increase in the doses of B, the activity of antioxidant enzymes increased and higher increase was observed for the susceptible cultivar as compared to the tolerant one. The result indicates that HT stress tolerant rice cultivar alleviates HT damages by increasing activities of protective enzymes in the antioxidant system of plant, which helps to remove free radicals in plant. Gupta et al. (1993) have reported that increased antioxidant levels in the cell can protect the enzymes against heat-induced reactive oxygen species (ROS)-mediated degradation.

Pollen viability and spikelet fertility were found to be negatively affected under HT stress. High temperature at the reproductive stage (HTR) was found to have higher negative effect on these parameters. It is earlier reported that temperatures higher than the optimum hamper pollen viability (Mohammed and Tarpley 2009b) and induce spikelet sterility and thus decrease rice yield (Jagadish et al. 2007). It has been earlier reported that spikelet sterility of rice greatly increased at temperatures higher than 35 °C (Matsui et al. 1997). Rice cultivars in general show decreases in pollen germination, pollen activity, and floret fertility at HT, with tolerant cultivars showing a slower rate of decrease than susceptible cultivars (Tang et al. 2008). Some of the reasons that could be attributed for increasing spikelet sterility at HT are lack of ability of the floral buds to mobilize carbohydrates under heat stress (Dinar and Rudich 1985), disturbance in the availability and transport of photosynthates to the kernel (Afuakwa et al. 1984), and changes in the activities of starch and sugar biosynthesis enzymes (Keeling et al. 1994; Singletary et al. 1994). Because B plays an important role in the carbohydrate metabolism and sugar translocation, so, it might help in improving the pollen viability and the spikelet fertility. The mechanism of increased pollen viability due to B application at HT stress is not studied here, and hence, ascribing any particular mechanism for the increased pollen viability and spikelet fertility with the application of B under HT stress as observed in this experiment needs further investigation. There were cultivar differences in response to HT; higher pollen viability and spikelet fertility were observed in the tolerant cultivar at HTR.

Results from the present study clearly indicated decreased rice yields as a result of HT stress in accordance with earlier work on rice (Cao et al. 2009; Mohammed and Tarpley 2009a). The response of grain yield to HT stress imposed at vegetative or flowering stages is related with the rice cultivars. The reduction in the yield of the HT stress sensitive cultivars was significantly higher as compared to the HT stress tolerant cultivar, which mainly resulted from the poor pollen viability and low spikelet fertility. Application of boron helps to increase the grain yield in all the rice cultivars; however, more response was observed in the susceptible cultivar as compared to the tolerant one. Earlier studies on effects of B fertilizer on rice concluded that decreases in grain yield were related with the increase in spikelet sterility (Rashid and Yasin 2004; Lordkaew et al. 2013).Our results clearly showed that rice crops under HT had less number of filled grains per unit land area and this was the yield component which contributed most in the lowering of yield at HT during vegetative and reproductive stages. Consequently, the harvest index, which is the ratio of grain yield to the total biomass yield, is also lower at HTV and HTR. Lower harvest index at HTR was mainly due to lower grain yield caused by decreased spikelet fertility. The decrease in spikelet fertility and differential response of cultivars at HT was mainly associated with decreased pollen viability. The tolerant cultivar Annapurna had smallest decreases in spikelet fertility, grain yield, and harvest index at elevated temperature during reproductive stage, while the susceptible cultivar Shatabdi had larger decreases. In general, harvest index is highly correlated with grain yield, which in turn is mainly related to spikelet fertility, a function of number of percent filled grains. Under the HT stress, a higher grain and straw B content was noticed that might also be a reason for increasing the cell membrane stability, pollen viability, spikelet fertility, antioxidant enzymes, and sugar translocation; however, such relationships need further investigations.

Conclusions

High temperature stress during vegetative or flowering stage reduced grain yield of rice cultivars mainly because of low pollen viability and spikelet fertility. Under high temperature stress, the tolerant cultivar displays higher cell membrane stability, less accumulation of osmolytes, more antioxidant enzyme activities, and higher pollen viability and spikelet fertility than the susceptible cultivars. In the present work, soil application of boron was effective in reducing the negative effects of high temperature both at vegetative and reproductive stages. Exogenous application of boron had a substantial effect on cell membrane stability, sugar mobilization, pollen viability, and spikelet fertility, hence the yield. The cultivars due to their variation in the tolerance level for high temperature stress behaved differently and more response of the application of boron was seen in susceptible cultivars at high temperature stress. The exact mechanism behind such variations in physiological responses of plants with boron under high temperature stress needs further investigation.

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