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Effect of foliar application of boron on growth, yield, chlorophyll, amylose and nitrate reductase activity in rice

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ABSTRACT

A field trial was conducted during wet season 2010 to evaluate the effect of foliar application of boron (B) on the content of chlorophyll, amylose & boron, nitrate reductase activity and yield of rice genotypes namely, IET 20979, IET 21007, IET 21106, IET 21114, IET 21519, IET 21540 and Rasi. Boron was applied at 0.2- 0.8ppm at the time of anthesis. The results revealed that 0.4ppm foliar application of boron evoked best response from all genotypes in terms of total dry matter, biological yield, economic yield and harvest index. The content of chlorophyll, boron, amylose and nitrate reductase activity increased significantly in most of the genotypes as compared to the control. The study suggests that foliar application of boron may enhance performance of rice plant in terms of grain yield and quality.

Key words: *Oryza sativa L, growth, chlorophyll, amylose, boron*

Boron is an essential micronutrient for normal growth of higher plants. Boron deficiency in soil is widespread in several crop growing regions from tropical to temperate zones of the world (Shorrocks, 1997). It is required for better growth, yield of crop and plays important role in cell development and elongation, protein synthesis, pollen tube formation and pollen viability etc (Gupta and Solanki, 2013). Extent of boron deficiency is next to that of Zn. Boron deficiency reduces not only yield but also the quality of crops which include thinner stems, shorter and fewer tillers, and failure to produce viable seeds (Dell and Huang, 1997). The deficient boron level in soil limits plant growth or damage to the photosynthesis system and can causes severe physiological responses, such as the disruption in chlorophyll and auxin biosynthesis, leading to reduction in fruit or crop yield (Hirsch and Torrey, 1980). Boron is also involved in N_2 fixation, the activity of nitrogenase enzyme in boron-deficient cells is inhibited even when other metabolic processes like photosynthesis, respiration etc are not affected. It has been shown that short-term boron deficiency leads to a decline in root and leaf nitrate contents without affecting NR activity or the concentrations of other macronutrients such as magnesium, calcium, potassium or phosphate (Camacho-Cristóbal *et al.*, 2005). Acting on the membrane level, boron deficiency has been reported to inhibit membrane uptake of number of nutrients and also affects nutrient transport capacity due to lowering ATPase and NAD(P)H mediated redox activity (Jabeen and Ahmad, 2011). Boron deficiency affects the expression level of genes related to nitrogen metabolism, oxidative stress, boron uptake, and cell wall synthesis (Camacho-Cristóbal *et al.*, 2008).

Boron phytotoxicity also manifests in a broad range of physiological effects, including decreased shoot and root growth, root cell division and RNA content, reduced leaf chlorophyll, lower photosynthetic rates and stomatal conductance and decreased lignin and suberin levels (Roessner *et al.*, 2006). Leaf toxicity symptoms in barley are characterized by interveinal chlorotic or necrotic patches, generally at the margins and tips of older leaves (Nable and Paull, 1991).

Boron can be provided to plants both by soil as well as foliar application. Foliar applied boron is believed to retain significant phloem mobility to flowering meristems. Thus, foliar sprays of boron provide not only a means to apply boron at a particular growth stage, but it also permits a rapidly-acting remedial action soon after the diagnosis of deficiency (Rashid *et al.*, 2004). Therefore, this study was aimed at characterizing the effect of foliar application of boron on various physiological and biochemical parameters in different rice genotypes to workout variability in yield and quality of grains for further improvement.

MATERIALS AND METHODS

A field experiment was conducted in three separate independent split plot design with three replications, 10 × 20 cm spacing with normal recommended package and practices that are being adopted during kharif season 2010 in Norman E. Borologue Crop Research Centre, G.B. Pant University of Agriculture and Technology Pantnagar, U.S. Nagar (Uttrakhand), India. Geographically, the site lies in Tarai plains about 30 km southwards of foothills of Shivalik range of the Himalayas at 29° N latitude, 79° 29' E longitude and at an altitude of 243.8 meter above the mean sea level and experiences humid subtropical climate with hot dry summers and cool winters. Winter season extends from November to March. The monsoon sets during second or third week of June and continues till September end. The soil of the transplantation site belonged to Typic Happlodoll with pH 8.0, EC 0.63, organic carbon 1.05% and 0.6 ppm boron content. It had gentle to moderate slope and was fine, loamy and mixed hyperthermic type. The seeds of seven rice genotypes, namely, IET 20979, IET 21007, IET 21106, IET 21114, IET 21519, IET 21540 and Rasi were obtained from the Directorate of Rice Research, Rajendranagar, Hyderabad, India. At anthesis boron was applied as foliar spray in the morning (10-11AM) with conc. 0.2 (T_1), 0.4 (T_2) and 0.8 (T_3) ppm while control was sprayed with water alone.

Total plant dry matter of three plant samples from each replication was recorded at active tillering and flowering by uprooting the plant and placing the samples in an oven at 65^0C for three days. Thereafter, weight of each sample was recorded. Each plant was uprooted from ground level at maturity and thereafter dried, the weight of intact plant was determined before threshing and the total weight of the above ground biomass was recorded and expressed as biological yield in tons/ha. Grain yield (economic yield) from each replication was recorded and finally expressed in tons/ha after harvesting.

Chlorophyll content was determined in fresh leaves at flowering by using method as described by (Hiscox *et al.*, 1979).

Nitrate Reductase (NR) activity was estimated *in vivo* in freshly harvested flag leaves at flowering by using method as described by (Hageman and Hucklesby, 1971).

Amylose content was estimated in rice grains by using method as described by (McCready *et al.*, 1950).

Boron content in shoots and grains at maturity was determined according to the method given by (Wolf, 1974).

The data recorded in triplicate was analyzed using ANOVA (analysis of variance) in accordance with using the SPSS-16 statistical package to quantify and evaluate the analysis of variation and means were tested using Duncan's test. The treatment means were compared at a significance level of 5% and the ranking of treatments denoted by alphabets.

RESULTS AND DISCUSSION

It is well known that rice is sensitive to micronutrient deficiency. Boron application and its deficiency lead to less growth and ultimately decreased yield. As it is associated with the development of cell wall and cell differentiation and hence, helps in root elongation, shoot growth and finally total dry matter of plant. In our investigation the total dry matter of rice crops at the time of flowering was recorded after foliar application of boron. A significant increase was found in total dry matter. The overall total dry matter was recorded maximum (853.09 g m^{-2}) at 0.8ppm boron level. A maximum 65.26% increase in total dry matter was recorded in IET 20979 however, in other genotypes it was ranges between 1.38-59.74% (Table 1). The improvement in total dry matter as a result of B application might be due to the enhanced photosynthetic and metabolic activity during flowering stage that leads

Table 1. Effect of different boron levels (0.2-0.8ppm) on total dry matter (g m^{-2}) at flowering in different genotypes of rice

Genotypes	Total dry matter (g m^{-2}) at Flowering stage			
	(0 ppm)	(0.2 ppm)	(0.4 ppm)	(0.8 ppm)
IET 20979	532.67^{d}	780.67^{bcd}	634.33^{c}	880.33^{b}
IET 21007	574.00^{d-}	556.67^{d}	909.00^{ab}	796.67^{bc}
IET 21106	677.33^{c}	882.33^{abc}	604.00^{c}	1082.00^{a}
IET 21114	851.67^{b}	723.67^{cd}	694.00^{bc}	734.00^{d}
IET 21519	1124.00^{a}	1073.00^{a}	736.67^{bc}	921.33^{a}
IET 21540	1095.33^{a}	966.67^{ab}	1006.67^{a}	911.00^{b}
Rasi	676.00^{c}	685.33^{cd}	916.67^{ab}	646.33^{d}

Means followed by a common letter in the columns are not significantly different

to an increase in various plant metabolic pathways responsible for cell division and elongation. Similarly, an application of boron also increased leaf area, which might be responsible for the increase in total dry matter. (Hatwar *et al.*, 2003). Boron deficient castor bean plants had less amount of dry matter per plant as compared to those treated with 0.27 mg/l of boron (Silva and Ferreyra, 1998). The external supply of boron in rice also improved the mean shoot dry weight significantly at 50-800 ng B mL^{-1} (Mehmood *et al.*, 2009).

The biological yield of rice genotypes was affected by the application of boron. At 0.4 ppm boron, maximum biological yield was found in genotype IET 21106 (20 tons/ha) with 13.68% increase as compared with control (Table 2). The biological yield increased due to the availability of boron which enhanced the plant height, number of tillers, leaf weight, weight and number of leaves per plant. Overall mean showed maximum economic yield (5.10 t/ha) at 0.4ppm boron. The maximum 11.6% increase in grain yield as compared with control was observed in IET 21106 (6.33 t/ha) at same level of boron (Table 2).

The harvest index indicates the total biomass at time of seed maturity. It depends upon economic and biological yield of the crop plants. When the grain and biological yield increase by the application of boron the harvest index ultimately increases. In our study, the effect of different boron levels on harvest index was also observed. There was not much difference at 0.2 and 0.4 ppm boron as harvest index was found to be 32.5% and 31.52% respectively. IET 21519 showed maximum (43.51%) and Rasi showed minimum (20.08%) harvest index at 0.4ppm level of foliar boron application. (Table 2). Similarly, Hussain and Yasin (2004) also observed that grain and straw yield was affected by 3 kg/ha boron fertilizers in BRRI Dhan 30 and that low grain and straw yield was observed in control condition. Application of 1.0 kg/ha of boron, reduced the panicle sterility, substantially increased grain yield in two cultivars of rice i.e. Basmati 385 and Super Basmati (25% and 20% over the control,

Table 2. Effect of different boron levels (0.2-0.8ppm) on total biological yield (t ha^{-1}), economic yield (t ha^{-1}) and harvest index (%) in different genotypes of rice

Genotypes	Biological yield (t ha^{-1})				Economic yield (t ha^{-1})				Harvest index (%)			
	(0 ppm)	(0.2 ppm)	(0.4 ppm)	(0.8 ppm)	(0 ppm)	(0.2 ppm)	(0.4 ppm)	(0.8 ppm)	(0 ppm)	(0.2 ppm)	(0.4 ppm)	(0.8 ppm)
IET 20979	13.33^{b}	14.00^{cd}	15.67^{cd}	13.33^{d}	4.33^{cd}	4.83^{ab}	4.83^{cd}	5.08^{b}	32.74^{b}	34.59^{a}	31.04^{a}	38.39^{a}
IET 21007	15.33^{ab}	14.67^{bcd}	18.67^{ab}	17.33^{a}	4.92^{bc}	5.50^{a}	5.67^{ab}	5.33^{b}	32.29^{b}	37.57^{a}	30.42^{bc}	30.84^{bc}
IET 21106	17.67^{a}	17.33^{a}	20.00^{a}	16.00^{ab}	5.67^{a}	5.17^{ab}	6.33^{a}	6.17^{b}	32.28^{b}	29.86^{a}	31.83^{a}	38.54^{a}
IET 21114	15.00^{ab}	16.00^{abc}	16.67^{bc}	16.00^{ab}	5.25^{ab}	5.08^{ab}	5.67^{ab}	4.50^{c}	35.10^{b}	32.10^{a}	34.14^{ab}	28.13^{bcd}
IET 21519	14.00^{b}	15.00^{abcd}	13.00^{d}	15.33^{bc}	4.08^{d}	4.33^{b}	5.50^{ab}	4.08^{d}	29.66^{ab}	28.94^{a}	43.51^{a}	26.71^{cd}
IET 21540	13.33^{b}	16.67^{ab}	14.67^{cd}	14.00^{cd}	3.94^{d}	5.25^{ab}	4.33^{c}	3.58^{e}	29.73^{ab}	31.60^{a}	29.61^{bc}	25.77^{d}
Rasi	14.00^{b}	13.00^{d}	16.67^{bc}	13.67^{d}	3.08^{e}	4.25^{b}	3.33^{d}	4.33^{cd}	22.42^{b}	32.84^{a}	20.08^{c}	31.73^{b}

Means followed by a common letter in the columns are not significantly different respectively) (Rashid *et al.*, 2004; Dunn *et al.*, 2005). The straw yield of wheat was also increased by applying 2 to 4 kg Bha^{-1}. Boron deficiency in wheat causes relatively more reduction of straw than grain yield (Rashid *et al.*, 2011). The soil application was also effective in many studies. The cumulative effect of boron on rice grain yield was recorded by the application of 2 kg B ha^{-1} (Khan *et al.*, 2006). Boron influences the harvest index significantly with application of 2 kg B ha^{-1} at ray floret stage in sunflower at par with the same dose applied at button stage. The harvest index in sunflower was more than control in all the treatments of boron (Zahoor *et al.*, 2011).

The response of growth and yield attributes ultimately depends upon the photosynthetic rate which in turn is dependent on chlorophyll contents. Results of present study revealed that chlorophyll a, b and total chlorophyll of most of the rice genotypes increased in response boron. At the time of flowering, the total chlorophyll content was affected by different levels of boron. The overall mean of treatment showed maximum chlorophyll content (2.26 mg g^{-1} fr. wt) at 0.4ppm boron. In Rasi, 0.4 and 0.8 ppm of boron resulted the maximum total chlorophyll content (2.67 mg g^{-1} fr. wt) and minimum in IET 21540 (1.74 mg g^{-1} fr. wt) in the control (Fig. 1). It might be due to the optimum amount of boron availability. However, boron deficiency reduces the chlorophylls which results in reduction in the photochemical reactions and net photosynthetic rate. Excess amount of boron can damage the photosynthesis system and the ultra structure of chloroplast. These results are in agreement with the results of a study on super basmati rice, in which a significant increment in chlorophyll contents (a, b and total chlorophyll) was recorded in response to soil as well as foliar application of boron. The highest chlorophyll contents (a, b and total) was recorded in Zn + B at 6 + 3 kg/acre treated plants (Arif *et al.*, 2012). Similarly in case of broad bean, chlorophyll a, b and total chlorophylls increased by foliar application (Sharaf *et al.*, 2009). In another study, 10, 20 and 250 mg/ l boron and zinc spray on *Vigna sinensis* caused significant increase in the contents of chlorophyll a and b (Hassanein *et al.*, 2000).

The amylose content of rice usually ranges between 15-35%. However, in contrast to high amylose content rice, low amylose content rice shows high volume expansion and high degree of flakiness so intermediate amylose rice are preferred in most rice growing areas of the world (Fasahat *et al.*, 2012). Proper supply of essential nutrients help to maintain

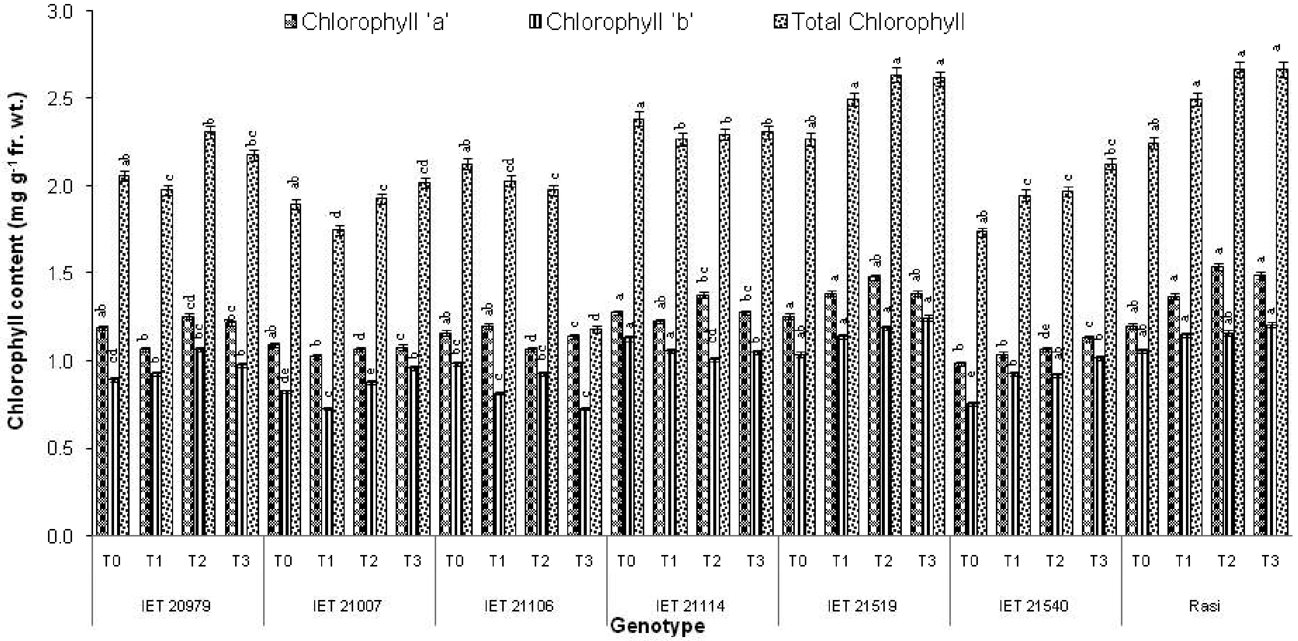

Fig.1. Effect of different B levels control (T_0) , 0.2 (T_1) , 0.4 (T_2) and 0.8ppm (T_3) on Chlorophyll content at flowering stage in different genotypes of rice.

the amylose-amylopectin ratio, boron may be one of such nutrient which may be used to maintain the percentage of amylose-amylopectin. In our study the amylose content was influenced by the foliar application of boron in all the genotypes and the overall mean of treatment showed that maximum amylose (17.88%) content in grains at 0.4 ppm level whereas, it was 14.96% in control. Maximum amylose content was recorded in IET 21106 and IET 21114 (17.88%) at 0.8ppm and minimum in IET 21007 (13.37%) in contro (Fig. 2). In all cases, amylose content increased with all the treatments; 0.4ppm boron was most effective to increase the amylose content. It might be due to the borate ion reacts chemically with the sugar molecule, and the resulting complex is transported across the membrane (Herrera-Rodriguez *et al.,* 2010). Actually syntheses of simple sugars or photosynthates are required for increasing the amylose, amylopectin and hence starch. Boron increases the rate of transport of sugars for the photosynthetic organs. However, boron does not play direct role in the synthesis of amylose or in activating the enzymes related to amylose synthesis (Lemoine *et al.*, 2013).

Nitrate assimilation in higher plants is greatly influenced by various essential nutrients and boron is one of them (Camacho-Cristobal and Gonzalez-Fontes, 1999). It has been shown that short-term boron deficiency lead to a decline in root and especially, leaf nitrate contents by decreasing NR activity (Kastori and Petrovic, 1989). In our investigation, the nitrate reductase (µmol NO_2^- g^{-1} fresh wt. h^{-1}) activity, measured at flowering, was affected by different levels of boron. Boron at 0.4 to 0.8 ppm was more effective in all the genotypes. Maximum NR activity was observed in IET 21007 (0.211 µmol NO_2^- g^{-1} fresh wt. h^{-1}) and minimum in IET 21540 (0.111 µmol NO_2^- g^{-1} fresh wt. h^{-1}) at 0.8 and 0.4 ppm boron respectively (Fig. 3). This might be due to the adequate and quick supply of boron through phloem tissues to the floral meristems which can influence the uptake and metabolism of nitrogen (Camacho-Cristobal and Gonzalez-Fontes, 2007). Interestingly, it was also reported that both a deficiency and high (toxic) levels of boron decreased the total N content and the activity of nitrate reductase and thus resulted in increased content of nitrates in roots and shoots of the sunflower plants. (Kastori and Petrovic, 1989). Similar to our study solution culture and pot experiment of *Brassica napus* indicated that nitrate reductase activity in the leaves of rape plants (Ningyou No. 7 and Ningyou No. 8) grown on nutrient solutions increased markedly with increasing boron concentration (from 0.025 micrograms B/ml to 0.1 micrograms B/ml). Nitrate reductase activity in leaves increased from the basal to the top parts of the plant when boron was supplied (Shen *et al.*, 1993). Although it cannot be discounted that excess boron concentration directly inhibits NR activity in root and leaf tissues of barley and wheat, it seems unlikely that it was the sole cause of the reduced NR activity observed in both species (Mahboobi *et al.,* 2002). Interactive effect of nitrogen and boron may also be responsible for such effects, since 2.5 to 40 mg kg^{-1} boric acid and 75 to 300 mg kg^{-1} N showed the

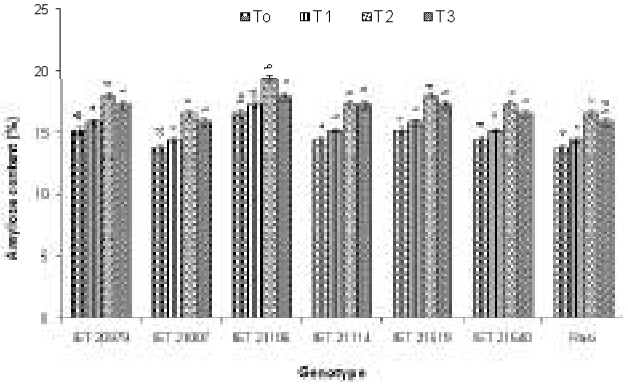

Fig. 2 . Effect of different B levels control (T_0) , 0.2 (T_1) , 0.4 (T_2) and 0.8ppm (T_3) on grain amylose content (%) in different genotypes of rice.

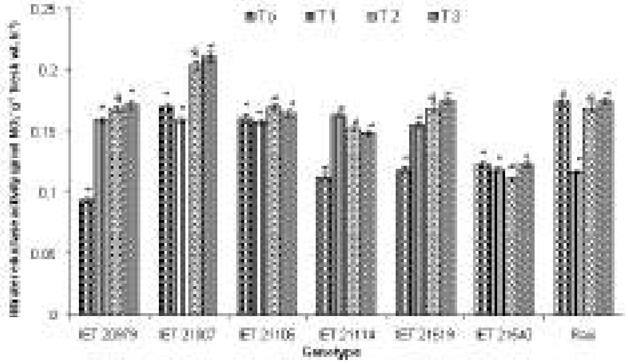

Fig. 3 . Effect of different B level on Nitrate reductase activity (µmol NO 2^- g^{-1} fresh wt.h^{-1}) at flowering stage in different genotypes of rice.

Table 3. Effect of different levels of boron (0.2-0.8ppm) on boron content (mg kg $^{-1}$) in rice grains and rice shoots in different genotypes of rice at maturity

Genotype	Boron content (mg kg^{-1}) in rice grains				Boron content (mg kg^{-1}) in rice shoots			
	(0 ppm)	(0.2ppm)	(0.4ppm)	(0.8ppm)	(0 ppm)	(0.2ppm)	(0.4ppm)	(0.8ppm)
IET 20979	12.84^{bc}	15.73^{a}	15.09^{a}	16.02^{a}	13.76^{a}	14.54^{c}	15.44^{a}	17.35^{b}
IET 21007	13.66^{abc}	15.42^{a}	16.46^{a}	17.12^{a}	15.59^{a}	15.56^{abc}	16.45^{a}	19.26^{ab}
IET 21106	15.36^{a}	15.94^{a}	16.87^{a}	17.66^{a}	15.55^{a}	16.93^{a}	17.46^{a}	19.50^{a}
IET 21114	14.64^{ab}	15.63^{a}	17.00^{a}	17.20^{a}	15.64^{a}	16.83^{ab}	17.37^{a}	19.16^{ab}
IET 21519	13.03^{bc}	14.73^{a}	16.84^{a}	17.50^{a}	14.75^{a}	15.50^{abc}	16.85^{a}	19.10^{ab}
IET 21540	14.24^{abc}	15.07^{a}	16.56^{a}	17.49^{a}	14.98^{a}	15.61^{abc}	16.00$_{a}$	18.81^{ab}
Rasi	13.39^{c}	14.77^{a}	16.16^{a}	16.72^{a}	14.70^{a}	15.35^{bc}	16.10^{a}	18.91^{ab}

Means followed by a common letter in the columns are not significantly different

interactive effect on growth in rice. Increasing level of nitrogen significantly affect the NR activity (Koohkan *et al.,* 2008).

Boron content in grains and shoots increased significantly with the levels of boron application in most of the genotypes. The overall mean showed that boron content in grains increased with the foliar spray of boron from 13.88 to 17.10 mg kg^{-1} irrespective of genotypes. The highest increase in boron content as compared to control was recorded in rice grains (4.47 mg kg^{-1}) and shoots (4.35 mg kg^{-1}) of IET 21519 at 0.8 ppm boron. However, at same treatment level, lowest increase (2.3 mg kg^{-1}) in grains of IET21106 and in shoots (3.52 mg kg^{-1}) of IET21114 was observed. In case of shoot, the overall mean ranged from15.0 to 18.87ppm boron irrespective of genotype and maximum 19.50 mg kg^{-1} was observed in IET21106 at 0.8ppm (Table 3). The absorption of boron is pH dependent and the uptake of boron in plants is found in the form of boric acid (Tanaka and Fujiwara, 2008). The soil or foliar application also increased boron content in different parts of rice plants. This is supported by Rashid *et al.,* 2004 who found that 1 kg boron ha^{-1} increased the concentration of boron in rice shoots as well as grains. Boron application also increased wheat grain yield significantly over check (No boron). The highest grain yield of 3490 kg ha^{-1} was recorded by the application of 1 kg B ha^{-1}, followed by 3287 kg ha^{-1} with application of 2 kg B ha^{-1} (Kizilgoz and Erda, 2010). From the present study it can be concluded that the effect of foliar application of boron on various physiological and biochemical parameters in different genotypes of rice showed genetic variability in their threshold limits of boron utilization i.e., most of the genotypes were influenced by 0.4 ppm boron however in some genotypes, biological yield was increased at 0.8 ppm boron but this increase in biological yield did not much influence grain yield which also showed that for better translocation of photosynthates from source to sink, 0.4 ppm of foliar applied boron seems to be sufficient for better yield.AcknowledgementsAuthors are thankful to DRR (ICAR), Hyderabad,India for extending financial support under the All India coordinated Rice Improvement programme.

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