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IMPROVING POLLEN VIABILITY AND STIGMA RECEPTIVITY BY BORON APPLICATION AS A PHYSIOLOGICAL APPROACH FOR HIGH YIELDS IN RICE

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ABSTRACT
A field experiment was conducted to know the effect of boron (B) on reproductive growth which showed positive influence on yield attributes viz., higher number of spikelets and grain number was recorded at 0.8 ppm and 0.4 ppm B sparyin IET 21519. At 0.4 and 0.8 ppm B sprayin the genotypes of IET 20979 and IET 21106 recorded higher values for spikelet number, grain number, panicle fresh and dry weights as well as grain yields. Mean maximum pollen viability of 86 % was recorded at 0.4 ppm B spray, among the genotypes, maximum value was recorded in Rasi (92.1 %), IET 21106 (87.5 %), IET 21114 (86.5 %) and IET 20979 (85 %). Stigma receptivity followed almost similar pattern to pollen viability. Higher grain yields were recorded in IET 20979 at 0.2 ppm B spray (777 g m^{-2}) and at 0.4 ppm B spray (772 g m^{-2}), but mean maximum grain yield was (691 g m^{-2}) recorded at 0.4 ppm B treatment. This study has revealed that IET 20979 and IET 21519 responded more positively to boron application at 0.4 ppm, this increase in grain yield was mainly due increase in pollen viability and stigma receptivity.

INTRODUCTION

Rice (*Oryza sativa* L.) is most important food crop in the world and feeds more than half of the global population. Around one hundred and seventeen countries grow rice, hence called as "global grain". World rice producing area achieved 160.6 million hectares in the year 2014-2015, India stands the second largest producer of rice next to china (Anonymous, 2014). To feed the ever increasing population of India, it becomes necessary to increase the production of rice. Rice cultivation is spread in various agro climatic situations of the country where it is subjected to various biotic and abiotic stresses.

Yield can be controlled by polygenes and it is an complex character and highly influenced by genotype and environment interactions.Several factors including drought, low soil pH,calcareous nature of soil and B leaching and fixation have been considered as the possible reasons of B deficiency (Rehman *et al*., 2014). In rice, B content is up to 10-time lower than those of dicot plants.The effects of B deficiency are much apparent on reproductive growth than on early vegetative growth in rice.

Among the micronutrients boron deficiency is found to affect plant growth and reduce yields and is one of the major constraints limiting the production of crops. Boron has been found to play a key role in reproductive processes affecting anther development, pollen germination, pollen tube growth, floret fertility and seed development. Boron application has a positive influence on growth, yield and quality of the crop (Ganie *et al*., 2014).

Many of the studies focus on development and function of the staminate flower, especially the pollen. Pollen viability and stigma receptivity are prerequisite for successful pollination and seed set in flowering plants. Pollen viability is considered as a phase in which pollen remains able to germinate on a receptive or compatible stigma which varies from species to species, ranging from minutes after shedding to days. Stigma receptivity is nothing but stigmatic capacity to support pollen germination. B deficiency decreases the growth of pollen tube and fertilization thus causing failure of grain setting (Khan *et al.,* 2011). In spite of the significant decrease in reproductive growth under the B deficient conditions, importance of B transport within floral organs was still unknown until very recently (Tanaka *et al.,* 2014). Foliar spray of boron promotes cell growth, development of panicle, enhanced pollination and translocation of sugars which increased the panicle length, seeds/panicle and test weight of rice grains (Singh *et al*., 2015).

Understanding the physiological mechanism of yield improvement by foliar applied B and its grain formation potential is lacking. It was hypothesized that foliar applied B can meet the B requirement of rice and improves the grain yield, by decreasing the spikelet sterility.The major objective of this study was to evaluate the influence of foliage applied B on the panicle fertility, pollen viability, stigma receptivity and grain yield of selected rice geno types.Hence, the present paper deals with improving of grain yield through increasing the pollen viability and stigma receptivity of cultivated rice genotypes by B foliar application.

MATERIALS AND METHODS

A field experiment was laid out in Randomized Block Design (factorial concept) with three replications and spacing of 10 x 20 cm was adopted in an 8.0 x 7.0 m^2 plots to know the effect of boron (B) on reproductive growth and yield attributes of seven genotypes (IET 20979, IET 21007, IET 21106, IET 21114, IET 21519, IET 21540 and Rasi (check)) against four levels of B spray (control, 0.2 ppm (0.375 kg B ha^{-1}), 0.4 (0.75 kg B ha^{-1}) and 0.8 ppm (1.5 kg B ha^{-1}) given at flowering stage at Indian Institute of Rice Research farm, Rajendra nagar, Hyderabad. Number of panicles, panicle fresh and dry weight was measured using three randomly selected plants at harvesting stage by Yang *et al*. (2007). Number of spikelets and filled grains per panicle was measured with grain counter by Yang *et al*. (2007).

Pollen viability and Stigma receptivity was observed with Binocular Microscope by adopting the following procedures, Pollen viability was counted based on IKI staining pattern on pollen (Prasad *et al*., 2006) and Stigma receptivity was measured with aniline blue-lactophenol stain (Teryokhin *et al*., 2002).

RESULTS AND DISCUSSION

Panicle number per m^{-2}

Panicle number per m^{-2} at harvest showed significant variation among the genotypes but B treatments and its interaction did not showed any significant variation (Table 1). Similar non significant correlation with B application for panicle number was reported by Gunes *et al*. (2003). In contrast to our results higher number of panicles with the application of 2 kg B ha^{-1} was observed in rice by Kabir *et al*. (2007), Khan *et al*. (2006) and both wheat and rice at 1 kg B ha^{-1} observed by Khan *et al*. (2011). Higher number of panicles were recorded in the genotype IET 20979 (408) and IET 21106 (370) which were on par. Panicle production depends on the genotype and also on effective tillers per plant [Kabir *et al*. (2007), Rashid *et al*. (2009) and Saleem *et al*. (2010)].

Spikelet number per panicle

Spikelet number per panicle at harvest showed significant variations among the genotypes, B treatments and its interaction (Table 1). Higher spikelet number per panicle was recorded at 0.4 ppm (133) and 0.8 ppm (130) B treatment which were on par. Application of 1 kg B ha^{-1} increases spikelet number (Shah *et al*., 2011), panicle number m^{-2}, number of spikelets and number of grains (Saleem *et al*., 2010). High number were recorded in IET 21519 (194) followed by IET 21540 (167), IET 21114 (109) and IET 20979 (106) which showed significant difference. Higher number of spikelets was recorded in IET 21519 at 0.8 ppm (219) and 0.4 ppm (204) and with no B sprays (191).Yields of rice grains were severely impaired under low boron levels, because of inhibited panicle formation and followed by reduced spikelet numbers (Tanaka *et al*., 2014).

Pollen viability or Pollen fertility

Pollen viability forms an important component for grain set. Significant variations were recorded with different concentrations of B spray, genotypes and interactions also (Table 2). Application of 0.4 ppm B significantly increased the pollen viability. Mean maximum value of 86 % pollen viability was recorded when B was sprayed at 0.4 ppm (Figure 1). Similar to our result Lordkaew *et al*. (2010) noticed that more pollen viability in B applied plants than in control plants. Guar *et al*. (2014) noticed that foliar applied Borax response was more positive due to boron which play an important role in translocation of carbohydrates, auxin synthesis and increased pollen viability and fertilization.

Mean maximum value was recorded in Rasi (92.1 %) which was significantly higher than other genotypes namely IET 21106 (87.5 %), IET 21114 (86.5 %) and IET 20979 (85 %) and showed significant differences. In Rasi pollen viability at 0.4 ppm B spray (86.8%) and 0.2 ppm B spray (93.4%) were on par. Under B availability pollen viability increases resulted in higher grain yield (Rehman *et al*., 2014). B application leads to increase in the pollen viability as well as reduction in pollen sterility which resulted in higher grain yields in rice (Yu and Bell, 2002). Adequate B supply ensures efficient pollen tube and root growth (Tanaka *et al*., 2014). Ahmad *et al*. (2009) observed that the boron nutrition in different rice cultivars resulted in better pollination and seed setting, low spikelet sterility and more grain formation. Dark stained Pollen grain

Table 1: Effect of B application on Panicle number per m^{-2} and Spikelet number per panicle

Panicle number per m^{-2}						Spikelet number per panicle				
Treatments	B spray concentrations (ppm)					B spray concentrations (ppm)				
Genotypes	Control	0.2	0.4	0.8	Mean	Control	0.2	0.4	0.8	Mean
IET 20979	392	440	410	392	408	101	104	117	102	106
IET 21007	312	312	287	373	321	99	101	114	95	102
IET 21106	390	383	405	303	370	88	106	93	136	106
IET 21114	292	273	295	332	298	109	103	120	103	109
IET 21519	237	302	247	223	252	191	162	204	219	194
IET 21540	317	317	285	302	305	164	163	189	151	167
Rasi (Check)	353	340	348	358	350	97	84	95	102	94
Mean	327	338	325	326		121	118	133	130	
	SEm		CD (p = 0.05)			SEm		CD (p = 0.05)		
Treatments(T)	10.18		NS			2.30		6.52		
Genotypes (G)	13.48		38.22			3.04		8.62		
Interaction	26.96		NS			6.08		17.25		

Table 2: Effect of B application on Pollen viability (%) and Grain number per panicle

	Pollen viability (%)					Grain number per panicle				
Treatments	B spray concentrations (ppm)					B spray concentrations (ppm)				
Genotypes	Control	0.2	0.4	0.8	Mean	Control	0.2	0.4	0.8	Mean
IET 20979	84.0	84.2	88.5	83.2	85.0	86	87	93	83	87
IET 21007	70.9	73.0	76.6	74.0	73.6	69	73	84	69	74
IET 21106	87.2	87.7	92.2	82.9	87.5	77	92	82	104	89
IET 21114	82.7	85.5	89.8	86.9	86.2	94	87	99	87	92
IET 21519	75.4	76.4	80.3	80.8	88.2	144	122	157	173	149
IET 21540	77.7	73.5	78.0	77.8	86.8	128	119	141	114	125
Rasi (Check)	86.9	93.4	96.8	91.4	92.1	84	77	86	91	85
Mean	80.7	82.0	86.0	82.4		97	94	106	103	
	SEm	CD (p = 0.05)				SEm	CD (p = 0.05)			
Treatments (T)	0.37	1.05				1.20	3.41			
Genotypes (G)	0.49	1.39				1.59	4.52			
Interaction	0.98	2.79				3.18	9.03			

Table 3: Effect of B application on Panicle fresh weight (g m^{-2}) and Panicle dry weight (g m^{-2})

	Pollen viability (%)					Grain number per panicle				
Treatments	B spray concentrations (ppm)					B spray concentrations (ppm)				
Genotypes	Control	0.2	0.4	0.8	Mean	Control	0.2	0.4	0.8	Mean
IET 20979	842	955	972	834	900	732	830	845	725	783
IET 21007	676	713	716	794	725	588	620	623	690	630
IET 21106	813	943	870	899	881	707	820	757	782	766
IET 21114	859	748	950	877	858	747	650	825	758	745
IET 21519	807	840	866	906	855	702	730	753	788	743
IET 21540	856	817	857	748	819	743	710	745	650	712
Rasi (Check)	855	768	826	891	835	743	668	718	775	726
Mean	815	826	865	850		709	718	752	738	
	SEm	CD (p = 0.05)				SEm	CD (p = 0.05)			
Treatments (T)	0.56	1.59				0.39	1.11			
Genotypes (G)	0.74	2.10				0.52	1.48			
Interaction	1.48	4.21				1.04	2.96			

Table 4: Effect of B application on grain yield (g m^{-2}) in different rice genotypes

	Grain yield (g m^{-2})				
Treatments	Boron concentration (ppm)				
Genotypes	Control	0.2	0.4	0.8	Mean
IET 20979	675	777	772	665	722
IET 21007	522	558	558	620	565
IET 21106	665	758	715	710	712
IET 21114	693	602	758	705	690
IET 21519	643	665	690	720	680
IET 21540	673	637	670	583	641
Rasi (check)	685	605	677	738	676
Mean	651	657	691	677	
	SEm	CD (p =0.05)			
Treatments (T)	1.24	3.52			
Genotypes (G)	1.64	4.66			
Interaction	3.29	9.33			

was viable and weekly stained or unstained pollen grain was nonviable

Stigma receptivity

Significant variations were recorded with different levels of B spray. Application of 0.4 ppm B significantly increased the stigma receptivity and recorded maximum receptivity of stigma. Maximum value was recorded in Rasi (check) which was significantly higher than other genotypes namely IET 20979, IET 21519 and IET 21114 which showed significant differences. In Rasi at 0.4 ppm B spray gave maximum stigma receptivity depicted in Figure 2.

Grain number per panicle

Grain number per m^{-2} at harvest showed significant variations among the genotypes, B treatments and its interaction (Table 2). High number of grains was recorded at 0.4 ppm (106) and 0.8 ppm (103) B treatment which was on par. High number of grains were recorded in IET 21519 (149) followed by IET 21540 (125), IET 21114 (92), IET 21106 (89) and IET 20979 (87) which showed significant difference. Interaction revealed that maximum grains were recorded at 0.8 ppm (173) and 0.4 ppm (157) B spray in IET 21519 which were significantly different. Maximum values for number of panicles m^{-2}, number of spikelets per panicle and number of grains per panicle were recorded at 0.4 ppm B spray (DRR annual progress report 2009).

Panicle weight

At harvest panicle fresh and dry weights recorded mean maximum values at 0.4 ppm B concentration (865 and 752 g m^{-2}) followed by 0.8 ppm (850 and 738 g m^{-2}) which was significantly different. Among genotypes evaluated IET 20979 recorded the mean maximum value (900 and 783 g m^{-2}) followed by IET 21106 (881 and 766 g m^{-2}) and IET 21114 (858 and 745 g m^{-2}) which were significantly different from IET 21114. Interaction revealed that 0.4 ppm B in IET 20979 (972 and 845 g m^{-2}) and 0.2 ppm IET 20979 (955 and 830 g m^{-2}) were maximum and differed significantly (Table 3). In rice 6, 4

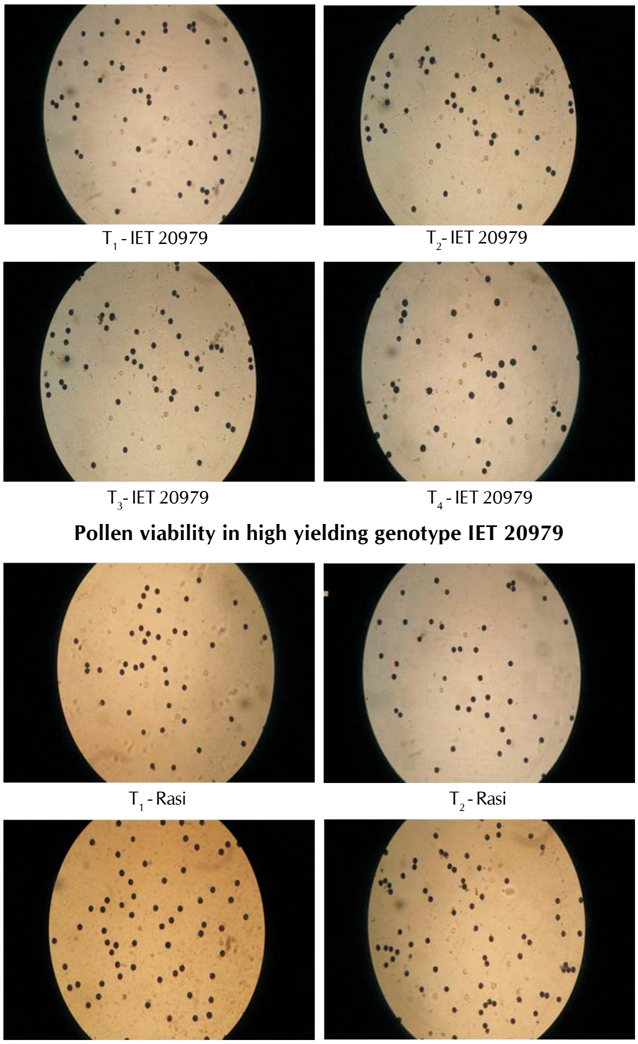

Figure 1: Pollen viability (T_1 - Control, T_2 - 0.2 ppm, T_3 - 0.4 ppm, T_4 - 0.8 ppm B spray)

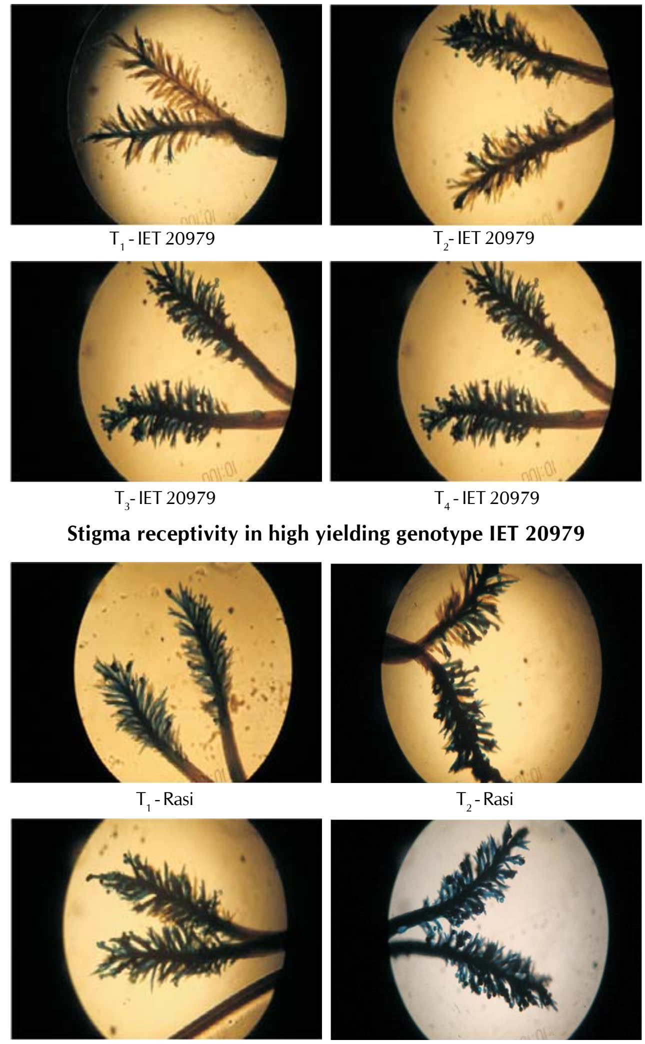

Figure 2: Stigma receptivity (T_1 - Control, T_2 - 0.2 ppm, T_3 - 0.4 ppm, T_4 - 0.8 ppm B spray)

and 10 per cent increase in culm, leaf and panicle weights were recorded due to 0.4 ppm B spray at flowering stage (DRR annual progress report 2009). Rashid *et al.* (2009) observed a substantial increase in grain yield of rice cultivars, due to reduced panicle sterility after B application. However, adequate supply of B ensures grain setting as indicated by decrease in panicle sterility (Rehman *et al.*, 2014). Reduced panicle sterility indicates more number of grains and high panicle weight.

Grain yield

Grain yield (g m^{-2}) significantly varied with different B concentrations, genotypes and also with interaction (Table 4). Maximum grain yield (691 g m^{-2}) recorded at 0.4 ppm B treatment. Among the genotypes, IET 20979 recorded maximum grain yield (722 g m^{-2}) followed by IET 21106 (712 g m^{-2}). Interaction showed significantly higher grain yields at 0.2 ppm B spray in IET 20979 (777 g m^{-2}) and at 0.4 ppm B spray in IET 20979 (772 g m^{-2}) which were on par.

The genotype IET 20979 showed more number of panicles, maximum panicle fresh and dry weight at 0.4 ppm B spray which contributes to production of high yields followed by IET 21519 genotype possessed highest number of spikelets, number of filled grains at 0.4 ppm B spray. IET 21106 was recorded second highest panicle number and filled grain per cent. Effect of B application on IET 21114 genotype increased number of filled grains in turn produced high yields. The percentage of stigma receptivity increased steadily from low to high B concentrations showing minor differences between them compared to pollen viability. Though Rasi used as check and showed maximum pollen viability and stigma receptivity at 0.4 ppm B spray, it cannot produce higher yields compared to other genotypes due to its low spikelet number. IET 21007 genotype was a low yielder due to its lowest number of spikelets per panicle. Principal reason of increase in grain yield by foliar application of B was due to substantial decrease in panicle sterility and increase in grain size (Rehman *et al.*, 2014).

Foliar spray of boron may also improved the yield attributing characters reported by Rashid *et al*. (2009), Singh *et al*. (2015) and Ganie *et al*. (2014). Application of 3 kg Borax ha^{-1} significantly increased yield attributes (Saleem *et al*., 2010). Kabir *et al*. (2007) reported that highest increments in rice grain yield were recorded at 2 kg B ha^{-1}, similar results reported by Khan *et al*. (2006) and Hussain *et al*. (2012). Application of 1.5 kg B ha^{-1} in saline and saline sodic soil improves the grain yields (Mehmood *et al*., 2009). Application of 1.0 kg B ha^{-1}enhanced grain yields by increase in number of productive tillers $plant^{-1}$ and number of grains $panicle^{-1}$of rice (Shah *et al*., 2011). This study has revealed that application of boron had resulted in the increase in grain yield of rice genotypes. IET 20979 and IET 21519 responded more positively to boron application. Increase in grain yield was recorded when B applied at 0.4 ppm as pollen viability and stigma receptivity showed positive correlation with yield.

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