

## IS BORON REQUIRED TO IMPROVE RICE GROWTH AND YIELD IN SALINE ENVIRONMENT?

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### Abstract

The nutritional functions of boron were investigated in improving rice growth and yield, both in solution and soil culture environments. Three rice cultivars [viz., KS-282 (salt-tolerant), BG-402-4 (mixed behavior) and IR-28 (salt-sensitive)] of differential salinity tolerant were used to investigate the ameliorative nutritional aspects of boron. Boron was applied @ 25, 50, 100, 200, 400 and 800ng B mL<sup>-1</sup> in the presence (80 mol m<sup>-3</sup>) and absence (0 mol m<sup>-3</sup>) of NaCl salinity whereas in solution culture, B was applied @ 1.5, 3.0 and 6.0 kg ha<sup>-1</sup> artificially to prepare saline (ECe 9.0 d Sm<sup>-1</sup>, SAR 5.46, pHs 7.8), and saline-sodic soils (ECe 9.0 d Sm<sup>-1</sup>, SAR 28.2, pHs 8.2). Application of B improved all growth parameters i.e., tillering capacity, shoot and root length, and shoot and root weight because of external B application @ 200-400ng mL<sup>-1</sup> in solution culture in the presence and absence of NaCl salinity. In shoot Na<sup>+</sup> and Cl<sup>-</sup> decreased; whereas K<sup>+</sup> concentration and K<sup>+</sup>: Na<sup>+</sup> ratio improved because of B supplied to saline medium. The ameliorative effect on paddy and straw yield and paddy: straw ratio was recorded at all external B supplied as compared to control. The highest improvement was recorded at 1.5kg B ha<sup>-1</sup> in the saline and saline sodic soils. Nevertheless the highest B application @ 6 kg B ha<sup>-1</sup> had shown an adverse affect on paddy and straw production in saline sodic soils in all the three cultivars as compared with all other B rates and control. The beneficial effect of B was due to reduced shoot Na<sup>+</sup> and Cl<sup>-</sup> concentration and better ratio of K<sup>+</sup> and Na<sup>+</sup> in shoot. Seed setting was improved in all the three cultivars because of external B supply to saline and saline sodic soils.

### Introduction

Salinity is one of the most critical constraints and hampers agricultural production in many areas around the world, including Pakistan (Greenway & Munns, 1980; Hasegawa *et al.*, 2000; Ashraf & Foolad, 2007). Approximately 40,000 ha of arable land in Pakistan are lost annually to cultivation due to salinity. It is estimated that about one third of irrigated land has been affected with salinity and saline area is sharply increasing each year (Ahmad *et al.*, 2006; Ashraf *et al.*, 2008). High concentration of soluble salts in the soil moisture of root zone results in reduced plant growth rates, loss of turgor, premature senescence, leaf abscission and petiole epinasty (Ashraf & Harris, 2004; Noreen & Ashraf, 2008).

Over limed and alkaline soils commonly contribute to boron deficiency (Cook & Millar, 1939). In Pakistan, its deficiency is wide spread (Sillanpaa, 1990; Rashid & Raffique, 1992) and prevailed up to 50% in cotton belt (Rashid, 1995; Rashid & Raffique, 1997) and 10-45% in rice fields (Tahir *et al.*, 1990; Zia, 1993). Crop responses to boron application under alkaline calcareous soils are just expected as its availability in soil decreases with increasing pH above 7 (Wang *et al.*, 2001; Berger, 1949). A marked increase in the paddy yield with the application of boron was reported in non-saline soil

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(Chaudhry *et al.*, 1976). Increasing supply of boron increased the accumulation of boron in roots and shoots (Nable *et al.*, 1990; Akram *et al.*, 2006). Vasil (1987) reported that the stigma, style and ovary often contain high concentration of boron and this boron occurs in pollen at about  $0.7 \text{ mg kg}^{-1}$  dry weight. Increased B concentration up to  $1.0 \text{ mg kg}^{-1}$  in salt affected loamy soil resulted in an increased in the yield of Bajra (Lal *et al.*, 1979).

Beside deficiency, boron toxicity has also been recognized in Australia (Cartwright *et al.*, 1984) and Pakistan (Sillanpaa, 1982). Boron toxicity is often compared with the associative problems of excessive salts accumulation (Gupta, 1993) and usually found at toxic levels in saline and sodic soils (Hutchison & Viets, 1969).

The present study was conducted to investigate the effect of boron supply under salt stress environment on the growth, yield and ionic composition of three rice cultivars differing in their salinity tolerance behavior.

## Materials and Methods

**Solution culture experiment:** In solution culture experiment, 14 days old seedlings of 3 rice cultivars i.e., KS-282 (salt-tolerant), BG-402-4 (mixed behavior) and IR-28 (salt-sensitive) raised in B free medium were transplanted to foam-plugged holes in polystyrene sheet floating over 25L of B free Yoshida nutrition solution (Yoshida *et al.*, 1976) in plastic tub ( $52 \times 35.5 \times 17.5 \text{ cm}$ ). There were 9 equidistant holes in each sheet; 3 holes were used randomly for each cultivar. Two seedlings were transplanted hole<sup>-1</sup>. In 18 tubs NaCl salinity of  $80 \text{ mol m}^{-3}$  was developed in three splits while other 18 were without salt. Boron concentrations of 25, 50, 100, 200, 400 and  $800 \text{ mg mol}^{-1}$  were developed with  $\text{H}_3\text{BO}_3$  in the growth tubes with and without salt. In tub trial, there were 36 treatments. Plants were harvested 20 days after salt stress (DASS). Data regarding tillering capacity shoot and root dry weights were recorded.

**Soil culture experiment:** The experiment was conducted in a net house in glazed pots (29 cm deep, 27 cm diameter) to study the effect of B supply on the growth and yield of rice in salt affected soils. Each pot was filled with 10 kg pre-treated soil. A non-saline soil ( $\text{ECe } 2.5 \text{ dSm}^{-1}$ , pH 7.8 and SAR 1.21) was collected and passed through 2mm sieve after drying. The saline soil ( $\text{ECe } 9.0 \text{ dS m}^{-1}$ ) was developed by adding calculated amount of mixture of salts ( $\text{Na}_2\text{SO}_4 + \text{NaCl} + \text{CaCl}_2 + \text{MgSO}_4$ ) commonly present in salt affected soils of Pakistan in the ratio of 9: 5: 5: 1 (equivalent basis) saline sodic soil ( $\text{ECe } 9.0 \text{ dSm}^{-1}$ ; SAR 28.2) was developed by using the above mentioned salts and by spraying  $\text{NaHCO}_3$  solution and allowing the soil to go through wet and dry cycles to attain equilibrium. The soils were puddled before the transplantation of rice seedling. Nitrogen was applied as urea @  $150 \text{ kg ha}^{-1}$  in three equal splits i.e., at transplantation, 20 days after transplantation and 45(DAT). A basal dose of P ( $18 \text{ kg P}_2\text{O}_5$ ), ( $50 \text{ kg K}_2\text{O}$ ) was also applied as single super phosphate and sulphate of potash, respectively on hectare<sup>-1</sup> basis during puddling. Boron was applied as Borax @ 1.5, 3.0 and  $6.0 \text{ kg B ha}^{-1}$ . The experiment was laid out in completely randomized design (CRD). Six seedlings (30 day old) of each cultivar were transplanted in each pot and thinned to three seedlings pot<sup>-1</sup> after a week interval. Soil was kept submerged by adding canal water daily. At panicle initiation stage, mother shoot was sampled for analysis of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ . At maturity, data regarding different agronomic parameters were recorded. Statistical procedures were used to analyze the data (Steel & Torrie, 1980).

## Results and Discussion

**Solution culture:** Results showed that number of tillers plant<sup>-1</sup> as affected by external boron application under saline (80 mol m<sup>-3</sup>) NaCl and non-saline (3 mol m<sup>-3</sup>) NaCl environment are present (Table 1). A steep reduction in number of tiller plant<sup>-1</sup> was observed because of salinity (80 mol m<sup>-3</sup> NaCl) under non-saline environment (0 mol m<sup>-3</sup> NaCl salinity), number of tillers plant<sup>-1</sup> increased significantly at 100-400 ng B mL<sup>-1</sup> as compared to lower and higher supply of external boron but the differences among these ratio were statistically non-significant under saline environment (80 mol m<sup>-3</sup> NaCl salinity), external boron rates of 200-400 ng mL<sup>-1</sup>, gave significantly higher number of tillers as compared to the other boron rates. The lowest tillering was found where boron was applied @ 25 and 800 ng mL<sup>-1</sup> both under saline and saline sodic conditions. As far as cultivars were concerned at 0 mol m<sup>-3</sup> NaCl salinity, all the three cultivars produced significantly higher and similar number of tillers plant<sup>-1</sup> at external boron application of 200-400 ng mL<sup>-1</sup> as compared to lower and higher rates of boron. At 80 mol m<sup>-3</sup> NaCl salinity, KS-282 and BG-402-4 produced maximum and significantly higher number of tiller at 200-400 ng B mL<sup>-1</sup> whereas, IR-28 did not show any response to external boron supply. Significant differences exist among the three cultivars when compared on the basis of mean number of tillers per plant, BG204-4 was significantly superior to the other two cultivars by producing higher number of tillers plant<sup>-1</sup> at 0 mol m<sup>-3</sup> NaCl salinity. At 80 mol m<sup>-3</sup> NaCl salinity, KS-282 and BG 402-4 had statistically similar tillering capacity. IR-28 at both 0 and 80 mol m<sup>-3</sup> NaCl salinity had the lowest tillering.

Data regarding the root and shoot dry weight revealed that a steep reduction in shoot dry weight was observed because of salinity (Table 1). External supply of boron, although improved mean shoot dry weight significantly at 50-800 ng B mL<sup>-1</sup> over its minimum supply under non-saline conditions, statistically it was maximum being at 400ng B mL<sup>-1</sup> while boron rates of 50-200 ng B mL<sup>-1</sup> had statistically the similar shoot dry weight. Under saline conditions no significant improvement in shoot dry weight was observed at 25-200 ng B mL<sup>-1</sup>. However, boron @ 400 ng mL<sup>-1</sup> produced statistically the highest shoot dry weight as compared to lower and higher rates of external boron. Shoot dry weight in case of KS-282 improved significantly at 0 mol m<sup>-3</sup> NaCl salinity because of external supply of boron @ 100-400 ng B mL<sup>-1</sup> whereas in case of BG-402-4, although all external boron concentration significantly improved shoot dry weight over its minimum and maximum external boron supply (25, 800 ng B mL<sup>-1</sup>) maximum shoot dry weight was obtained at 50-400 ng B mL<sup>-1</sup>. Regarding IR-28, external boron supply of 50 ng B mL<sup>-1</sup> was the only rate found beneficial in improving dry weight over all the external rates of boron supply (25-800 ng B mL<sup>-1</sup>) gave significant improvement in shoot dry weight of KS-28 under saline environment was observed at external supply of 200 ng B mL<sup>-1</sup>. In case of IR-28 a significant improvement was observed at external boron supply of 100-400 ng B mL<sup>-1</sup>. Whereas, external supply of boron did not cause any significant improvement in shoot dry weight of BG-402-4. Comparison of cultivars revealed that KS-282 was at par at 0 mol m<sup>-3</sup> NaCl salinity but was found superior to the other two cultivars in term of shoot dry weight at 80 mol m<sup>-3</sup> NaCl salinity. The lowest shoot dry weight at both salinities was recorded in case of IR-28. Further, at 80mol m<sup>-3</sup> NaCl salinity, IR-28 could not survive at highest boron supply (800 ng B mL<sup>-1</sup>).

Similarly to shoot dry weigh, root dry weight also improved because of external B supply both under 0 and 80 mol m<sup>-3</sup> NaCl salinity (Table 1) and the increase was maximum at 400 ng B mL<sup>-1</sup>. Under 80mol m<sup>-3</sup> NaCl salinity, the mean root dry weigh although increased consistently up to 400 ng B mL<sup>-1</sup>, statistically improvement was the same from 50-400 ng B mL<sup>-1</sup>.



**Pot culture:** In pot culture results showed that panicle length reduced significantly in saline sodic soil as opposed to saline soil (Table 2). External supply of boron significantly improved panicle length over control up to 6.0 kg B ha<sup>-1</sup> in saline and up to 3.0 kg B ha<sup>-1</sup> in saline sodic soil. Instead, boron @ 6.0 kg B ha<sup>-1</sup> in saline sodic soil had adverse affect on this parameter and produced statistically less length panicle as compared to control. As regard cultivars in saline soil, boron application of 1.5 and 3.0 kg B ha<sup>-1</sup> caused significant improvement in the panicle length of KS-282 and Bg-402-4 over control (0 kg B ha<sup>-1</sup>). However, the differences between treatments of 3.0 and 6.0 and 0 & 6.0 kg B ha<sup>-1</sup> were statistically not sharp. In case of IR-28, although panicle length improved significantly at external boron rates (1.5 to 6.0 kg B ha<sup>-1</sup>) compared to control, the differences between 1.5, 6.0 and 3.0, 6.0 kg B ha<sup>-1</sup> were statistically alike under saline sodic soil, in KS-282 significant improvement of panicle length was observed up to 3.0 kg B ha<sup>-1</sup> over control. However, boron rates have 1.5 and 3.0 kg B ha<sup>-1</sup> were statistically similar. In case of BG-402-4, panicle length improved significantly because of each incremental rate of boron from 0-3.0 kg B ha<sup>-1</sup>. The highest rate of external boron supply (6.0 kg B ha<sup>-1</sup>), however, had statistically the same panicle length as was measured in case of control (0 kg B ha<sup>-1</sup>). As regards IR-28, significant improvement in panicle length was only at 1.5 kg B ha<sup>-1</sup> compared with all the other boron rates. Boron application @ 6.0 kg B ha<sup>-1</sup> resulted in less lengthy panicles compared to all the other boron rates including control. Overall KS-282 had significantly the longest and IR-28 had the shortest panicle in both the soils.

Results revealed that Na<sup>+</sup> concentration in shoot is decreased by external application of boron under saline and saline-sodic soils (Table 3). A significant decreased in Na<sup>+</sup> concentration was observed as a result of boron application up to 1.5 kg ha<sup>-1</sup> in saline as well in saline-sodic soils over control. While, cultivars in saline and saline-sodic soils, boron application of 1.5 kg B ha<sup>-1</sup> caused significant reduction in Na<sup>+</sup> concentration in the shoot of KS-282, BG-402-4 and IR-28, respectively over untreated control. However, the differences between treatments of 0, 1.5, 3.0 and 6.0 kg B ha<sup>-1</sup> were statistically more sharp in saline-sodic as compared to saline soils. But in case of IR-28, showed less reduction in Na<sup>+</sup> concentration in shoot at external boron rates (1.5, 6.0 kg B ha<sup>-1</sup>) compared to KS-282 and BG-402-4 under saline and saline-sodic soils. Overall KS-282 had significantly the lowest Na<sup>+</sup> concentration in shoot and IR-28 had the maximum Na<sup>+</sup> concentration in shoot in case of both the soils.

Data regarding K<sup>+</sup> concentration in shoot is shown in Table 3. Overall means of different levels of boron application revealed that K<sup>+</sup> uptake was statistically more in saline soil as compared to saline-sodic soil. But the most suitable level of external boron application for both soil conditions was 1.5kg ha<sup>-1</sup>, followed by 3.0 and 6.0 kg ha<sup>-1</sup>, respectively as compared to control. However, KS-282 performed statistically better under saline soil at 1.5 kg ha<sup>-1</sup> as compared to BG-402-4 and IR-28 while in case of saline-sodic soil, BG-402-4 showed maximum uptake at 1.5kg ha<sup>-1</sup>, followed by KS-282 and IR-28 respectively.

Results showed that concentration of K<sup>+</sup>: Na<sup>+</sup> ratio in shoot is more under saline soil than saline-sodic soils with external supply of boron (Table 4). While the most suitable level of boron application for K<sup>+</sup>: Na<sup>+</sup> ratio in shoot was 1.5kg ha<sup>-1</sup> under both soils environment, followed by 3.0 and 6.0 kg ha<sup>-1</sup>, respectively as compared to control. Over all, KS-282 showed statistically better K<sup>+</sup>: Na<sup>+</sup> ratio in shoot under saline soil at 1.5kg ha<sup>-1</sup> as compared to BG-402-4 and IR-28 whereas, BG-402-4 gave better K<sup>+</sup>: Na<sup>+</sup> ratio at 1.5kg ha<sup>-1</sup>, followed by KS-282 and IR-28 respectively under saline-sodic soil.









Results relating to  $\text{Cl}^{-1}$  concentration in shoot dry weight are shown in Table 4. Overall means indicated that  $\text{Cl}^{-1}$  uptake was statistically more in saline-sodic soil as compared to saline soil by external application of boron. A steep reduction in  $\text{Cl}^{-1}$  concentration was observed @ 1.5 kg B  $\text{ha}^{-1}$  under saline and non-saline environment that was statistically different than control. However, both levels of boron @ 3.0 and 6.0 kg B  $\text{ha}^{-1}$  statistically increased uptake of  $\text{Cl}^{-1}$  in shoot under saline and non-saline conditions respectively that soil had adverse affect on plant growth as compared to control.

## Discussion

Plants grown in saline soils have to face high osmotic stress, high concentration of potentially toxic ions such as  $\text{Na}^{+}$  and  $\text{Cl}^{-}$  which ultimately cause reduction in growth (Martinez & Lauchli, 1993). Ion absorption though facilitates osmotic adjustment but may lead to ion toxicity and nutritional imbalance (Aslam *et al.*, 1993a, 1996; Lutts *et al.*, 1996). Growth inhibition because of salt induced nutritional imbalance can be minimized with the judicious supply of plant nutrients (Aslam *et al.*, 1996). In the present study, salinity reduced all the growth parameters such as tillering capacity, shoot and root dry weight in all the three rice (Table 1). Spike length, paddy and straw yield were also negatively affected due to salinity (Table 2). The reduction in growth varied with cultivars indicating their genetic ability to tolerate salinity (Aslam *et al.*, 1993b). Evidences indicate the response of boron to wheat (Kausar *et al.*, 1990) and rice (Kausar *et al.*, 1991; Ali *et al.*, 1996; Aslam & Qureshi, 1998) under Pakistan agricultural conditions. An increase in productive tillers, straw and paddy yield in rice has also been observed with the application of boron up to 2 kg B  $\text{ha}^{-1}$  (Ali *et al.*, 1996). In the present study, growth improvement in non-saline and saline solution culture has been observed due to boron supply of 200-400 ng B  $\text{mL}^{-1}$  to the growth medium. The increase in number of tillers per plant, shoot and root dry weight in KS-282 and BG 402-4 in saline and non-saline growth medium might be due to the fulfillment of boron deficiency whereas, reduction in growth of IR-28 might be due to low requirements of IR-28 for boron as well as salt induced boron toxicity under saline conditions. The concentration above external boron of 200-400 ng B  $\text{mL}^{-1}$  adversely affected the plant growth in all the three rice (Table 1) and could be attributed to the physiological interference of excessive boron with other plant nutrients. Boron application @ 1.5 kg B  $\text{ha}^{-1}$  to salt affected soils (Saline and saline-sodic), improved number of tillers  $\text{plant}^{-1}$ , panicle length, straw and paddy yield of all rice (Tables 1, 2). High rates of boron (3.0 to 6.0 kg B  $\text{ha}^{-1}$  in both type of salt affected soils inhibited growth and reduced yield. The severe reduction at higher levels of boron might be because of efficient uptake of boron along with higher concentration of  $\text{Na}^{+}$  in salt affected soil. According to Gupta (1993), boron toxicity is often confounded with the associated problem of excessive salt accumulation and usually found at toxic level in saline and saline-sodic soils (Hutchison & Viets, 1969; Ilin & Anikina, 1974). Nevertheless, reduction in growth and yield was more severe in saline-sodic soil as opposed to saline soil specifically at the highest rate of 6.0 kg B  $\text{ha}^{-1}$ . The most adverse effect of boron in saline-sodic soil than saline soil may be that boron uptake by plant is governed by variety of factors, one of which is the effect of associated ions; and the role of  $\text{Ca}^{2+}$  is very important in this regard (Berger, 1949; Eck & Cambell, 1962) and it appears that high levels of  $\text{Ca}^{2+}$  accentuate boron deficiency, but tend to relieve boron toxicity. Excess boron is assumed to combine with  $\text{Ca}^{2+}$  to form compound no longer toxic to the plant (Werkhoven, 1964). The less reduction in paddy yield in saline soil might be due to the better  $\text{Ca}^{2+}/\text{Na}^{+}$  ratio as high level of  $\text{Ca}^{2+}$  in saline soil tended to provide relieve to boron toxicity (Eck & Cambell, 1962; Lal *et al.*, 1979). Amongst

cultivars, the reduction in yield (shoot weight or paddy weight) was more pronounced in IR-28 as compared to KS-282 and BG 402-4 (Table 2). This may be due to genetic ability of cultivar to tolerate boron. Boron toxicity is most common in plants growing in salt affected soils (Nable & Paull, 1991) and plant species, and to some extent cultivars with in the species, differ much in their ability of boron tolerance. Data of experiment reveal that boron @ 200-400 ng B mL<sup>-1</sup> in solution culture and 1.5 kg B ha<sup>-1</sup> in salt affected soils reduced Na concentration and improve yield. Strikingly, tissue Na<sup>+</sup> concentration at this level of external boron was statistically the lowest in all three cultivars specifically in KS-282, and BG 402-4. Further in saline-sodic soil, K<sup>+</sup>: Na<sup>+</sup> ratio improved at an external boron concentration of 1.5 kg B ha<sup>-1</sup> clearly shows that moderate boron dressing is important even in salt affected soil to improve growth and finally the yield by facilitating and enhancing ion exclusion process in plants. The other possible reason of better growth and yield at 1.5 kg B ha<sup>-1</sup> in soil culture and 200-400 ng B mL<sup>-1</sup> in solution culture accompanied with low concentration of saline ions (Na<sup>+</sup>, Cl<sup>-</sup>) by the dilution effect (faster rate of growth) in cultivars at these external boron concentration. Paddy varieties were quite tolerant to high boron concentration (40 mg Kg<sup>-1</sup>) at the germination stage but were sensitive at seedling stage. Nevertheless, a varieties difference exists in their relative resistance to boron, salinity and SAR for germination as well as for the growth rate, up to seedling stage (Paliwal & Mehta, 1973). Salt sensitive rice line IR-28 had the highest concentration of boron in its tissue. According to Paull *et al.*, (1987), boron sensitive cultivars had higher shoot boron concentration than did resistant cultivars. This reflects that IR-28 is not only a salt sensitive but also boron sensitive cultivar. Moderate level of boron improved growth and yield under all set of conditions (solution culture and soil culture). It appears that improvement in growth and yield is because of the fulfillment of plant boron requirements, which in turn help in improving salt tolerance of rice through enhanced ion exclusion mechanisms and a resultant better pollination, seed setting and grain formation.

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(Received for publication 12 January 2008)