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Does exogenous application of glycinebetaine through rooting medium alter rice (*Oryza sativa* L.) mineral nutrient status under saline conditions?

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Summary

An experiment was conducted in growth chamber to assess the role of glycinebetaine (GB) in mineral nutrient status of rice (*Oryza sativa* L.) seedlings under saline conditions. Two rice varieties i.e. NIAB IRR1-9 and Super Basmati were grown in full strength Hoagland's nutrient solution supplied with four levels of GB i.e. Control (without GB), 5, 10 and 15 mM and three salinity treatments i.e. normal Hoagland's nutrient solution (without salt), 60 and 120 mM NaCl. Salt stress markedly reduced shoots and roots fresh and dry biomass, chlorophyll *a*, *b* and total chlorophyll contents, shoot and root K⁺ concentrations while shoots and roots Na⁺ and Cl⁻ contents increased with increase in salinity levels. Exogenous application of GB through rooting medium did not affect shoot fresh and dry weights while root dry weight decreased by exogenously applied GB. Chlorophyll pigments and root Ca²⁺ were increased while shoot and root Na⁺, K⁺ and Cl⁻ decreased by exogenous application of GB in salt stressed plants as compared to non-stressed plants of both varieties. Overall, variety NIAB-IRR-9 was high in plant biomass production as compared to Super Basmati. Glycinebetaine ameliorated the adverse effects of salt by decreasing shoots and roots Na⁺ and Cl⁻ concentrations.

Introduction

Rice is most valuable cereal crop providing one-third of the total carbohydrate source. It is a staple food for three billion people and provides about 50-80% of daily calorie intake. Rice is considered as a salt-sensitive monocot plant (FRANCOIS and MASS, 1994; MAAS and HOFFMAN, 1997). The salt tolerance of rice varies with growth stage i.e. it is tolerant during germination and sensitive at the flowering stage (ALAM et al., 2004). So the response of rice to salinity stress also depends on the stage of salinity application (ALAM et al., 2004). Salinity stress perturbs plant growth by disturbing soil osmotic potential which makes difficult for roots to absorb water from the soil, and secondly, accumulation of high salt levels in the plant body becomes toxic for plants (MUNNS and TESTER, 2008). For example, high amount of Na⁺ reduces the growth of many salt sensitive plants (JALEEL et al., 2007). Inside the plant tissues high Na⁺ causes detrimental effects, the most prominent of them is disturbance in ion homeostasis (GREENWAY and MUNNS, 1980; ASHRAF, 2004). Potassium is essentially required by plants to maintain the activity of many enzymes, and osmoregulation. For this purpose, plants need to acquire K⁺ continuously so as to maintain K⁺-Na⁺ selectivity in plants under saline conditions. Osmoprotectants are important in enhancing the stress tolerance of plants (BOHNERT and JENSEN, 1996; ZHU, 2001). These are involved in protection of plants membranous system scavenging of reactive oxygen species (BOHNERT and JENSEN, 1996), preserve activity of enzymes and thylakoid and plasma membrane from various stresses like salt or heat stress (RHODES and HANSON, 1993), help in maintaining osmotic adjustment (ZHAO et al., 2007). Examples

of osmoprotectants are polyols, proline, trehalose, sucrose and quaternary ammonium compounds (QACs) such as glycinebetaine, hydroxyprolinebetaine, prolinebetaine, alaninebetaine, choline *O*-sulfate, and pipercolatebetaine (RHODES and HANSON, 1993; ASHRAF and FOOLAD, 2007). Compatible solutes are involved in amelioration of salt induced adverse effects. Of these compatible solutes, the most extensively studied organic compound involved in salinity tolerance is glycinebetaine (GB) (KUMAR et al., 2004). Under saline conditions, GB plays an important role in maintaining osmotic adjustment (ZHAO et al., 2007). In addition to its role as osmoprotectant, GB also stabilizes photosynthetic reactions, the structure of extrinsic proteins of the PS II complex, and ATP synthesis, as well as the cell membranes (IOLIVET et al., 1982) and activation of enzymes (GORHAM, 1995). Rice plants neither synthesize nor accumulate glycinebetaine (RATHINASABAPATHI et al., 1993).

Biosynthesis of organic solutes such as GB is energetically costly, so the exogenous application of these compounds has been suggested as an alternative approach to improve crop productivity under stress. Exogenous application of GB to leaves or roots has shown to increase the tolerance to different stresses of many species of plants, including both natural accumulators and non accumulators (HAYASHI et al., 1998). There are reports that the addition of glycinebetaine to plants counteracts the unfavorable effects of salinity on seedling growth (GADALLAH, 1999). Keeping in view all these reports, the main objective of this study was to assess the role of GB in amelioration of adverse effects of salt stress on rice at seedling stage with respect to accumulation of more or less mineral nutrients especially K⁺ as beneficial and Na⁺ or Cl⁻ as toxic elements.

Materials and methods

The experiment was conducted to assess the influence of exogenous application of glycinebetaine on seedling growth and mineral nutrients of rice (*Oryza sativa* L.) under saline conditions. The experiment was conducted in growth room (12-h day length, light intensity 150 μmol m⁻² s⁻¹ PAR, growth room temperature 28 ± 2 °C) at Department of Botany, University of Agriculture, Faisalabad.

Seeds of two rice varieties i.e. NIAB-IRR1-9 and Super Basmati were obtained from Ayub Agriculture Research Institute, Faisalabad. Seeds were surface sterilized with 5 % sodium hypochloride for 10-15 minutes and then washed with water. Fifty seeds of each variety were germinated on double layer of filter paper moistened with full strength Hoagland's nutrient solution with or without salinity and/or glycinebetaine. The experiment was laid out in completely randomized design with three salinity treatments (Control (non-saline), 60 and 120 mM of NaCl) and four glycinebetaine (GB) levels (Control (without GB), 5, 10, and 15 mM of GB) with four replications. Salinity was supplied with Hoagland's nutrient solution. After six days of seed sowing, 30 seedlings of comparable size were transplanted into each small plastic pot containing 225 ml of each treatment of salinity and/or GB applied through rooting medium. The plants were allowed to establish for 10 days (16 days after sowing). Twenty five plants were harvested 16 days after sowing.

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After measuring seedling's fresh weight, plant samples were oven dried at 65 °C to constant dry weight.

Chlorophyll pigments: The chlorophyll contents were determined from green leaves following ARNON (1949). Fresh leaf material (0.2 g) was extracted in 80% acetone and centrifuged at 10,000 x g for 5 minutes. Absorbance of the supernatants of all samples was measured at 663 and 645 nm using UV-Visible spectrophotometer (Hitachi-U2001, Tokyo, Japan).

Determination of Mineral Elements in Plant Tissues

The dried ground shoot and root material (0.1 g) was digested with sulphuric acid and hydrogen peroxide according to the method of WOLF (1982). Sodium, potassium, and calcium cations were determined with a flame photometer (Jenway, PFP-7), while for determination of Cl⁻ ion, chloride analyzer (Sherwood chloride analyzer, 926 Japan) was used. The dried ground material was digested in distilled water for Cl⁻ determination.

Statistical Analysis

An analysis of variance using the COSTAT v 6.3 was applied to analyze the data by using statistical software (Cohort Software, Berkeley, California). Comparison of mean values was done by using SNEDECOR and COCHRAN (1980) least significance difference.

Results

Data for shoot fresh and dry weights of 25 rice seedling, when plants were supplied with different levels of glycinebetaine in rooting medium under control or saline conditions showed that salinity had highly reducing effect (Tab. 1; Fig. 1) on above mentioned attributes. Rooting medium glycinebetaine (GB) did not increase or decrease shoot fresh and dry biomasses. Shoot fresh and dry weights were low at highest level of salinity i.e. 120 mM NaCl as compared to other levels (Fig. 1).

Application of low levels of GB i.e. 15 mM was slightly effective in increasing root fresh and dry biomasses of NIAB-IRRI-9 under non saline or 60 and 120 mM NaCl for super Basmati. Both root fresh and dry biomasses decreased with increase in salt stress (Tab. 1; Fig. 1) except at 120 mM of NaCl where 15 mM GB increased root biomass. Among both varieties, NIAB-IRRI-9 was high while Super Basmati low in root fresh and dry biomasses.

Chlorophyll pigments i.e. chlorophyll *a*, *b* and total chlorophylls were also decreased markedly under saline conditions as compared to non-saline. Application of GB in rooting medium was more effective under non-saline or at 60 mM NaCl where chlorophyll *a* and *b* pigments were high. NIAB-IRRI-9 was superior in chlorophyll pigmentation as compared to super basmati (Tab. 1; Fig. 1).

Shoot and root Na⁺ contents increased prominently under saline conditions in both rice varieties i.e. NIAB-IRRI-9 and Super Basmati. Exogenous application of GB through rooting medium slightly decreased Na⁺ level in shoot and root in both varieties under

Tab. 1: Mean squares from analysis of variance of data for growth attributes and mineral nutrients of rice (*Oryza sativa* L.) when plants were supplied with different levels of glycinebetaine in rooting medium for 16 days under control or saline conditions.

Source of variance	Degrees of freedom	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
Glycinebetaine (GB)	3	0.004ns	7.713**	0.288***	0.002***
Salinity (S)	2	4.151***	0.003***	1.129***	0.002***
Varieties (V)	1	0.275***	0.010***	4.438***	0.006***
GB x S	6	0.017***	0.001***	0.0649**	1.709ns
GB x V	3	0.005ns	3.156ns	0.258***	7.470***
S x V	2	0.007ns	8.567**	0.730***	0.002***
GB x S x V	6	0.027***	0.001***	0.045ns	5.576***
Error	72	0.003	1.602	0.020	8.430
		Shoot Na ⁺	Shoot K ⁺	Shoot Ca ²⁺	Shoot Cl ⁻
Glycinebetaine (GB)	3	39.58**	72.83***	3.892**	1350.8***
Salinity (S)	2	6418.0***	783.5***	29.94***	74231.2***
Varieties (V)	1	261.7***	22.04ns	11.34***	2289.0***
GB x S	6	14.30ns	16.04*	1.103ns	528.4***
GB x V	3	15.27ns	15.14ns	0.225ns	366.3*
S x V	2	25.35*	2.072ns	1.148ns	940.32***
GB x S x V	6	11.54ns	9.489ns	2.603**	372.5**
Error	72	7.504	6.265	0.824	96.183
		Root Na ⁺	Root K ⁺	Root Ca ²⁺	Root Cl ⁻
Glycinebetaine (GB)	3	43.39ns	82.65***	166.7***	57.14*
Salinity (S)	2	1669.8***	403.6***	277.9***	6201.7***
Varieties (V)	1	885.8***	41.34***	126.0**	802.5***
GB x S	6	140.7***	27.18***	59.47**	121.0***
GB x V	3	62.03ns	4.899ns	89.12**	204.4***
S x V	2	2154.5***	11.62*	207.4***	94.86**
GB x S x V	6	45.07ns	6.513ns	11.38ns	339.8***
Error	72	26.60	3.454	16.28	15.47

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively
ns = non-significant

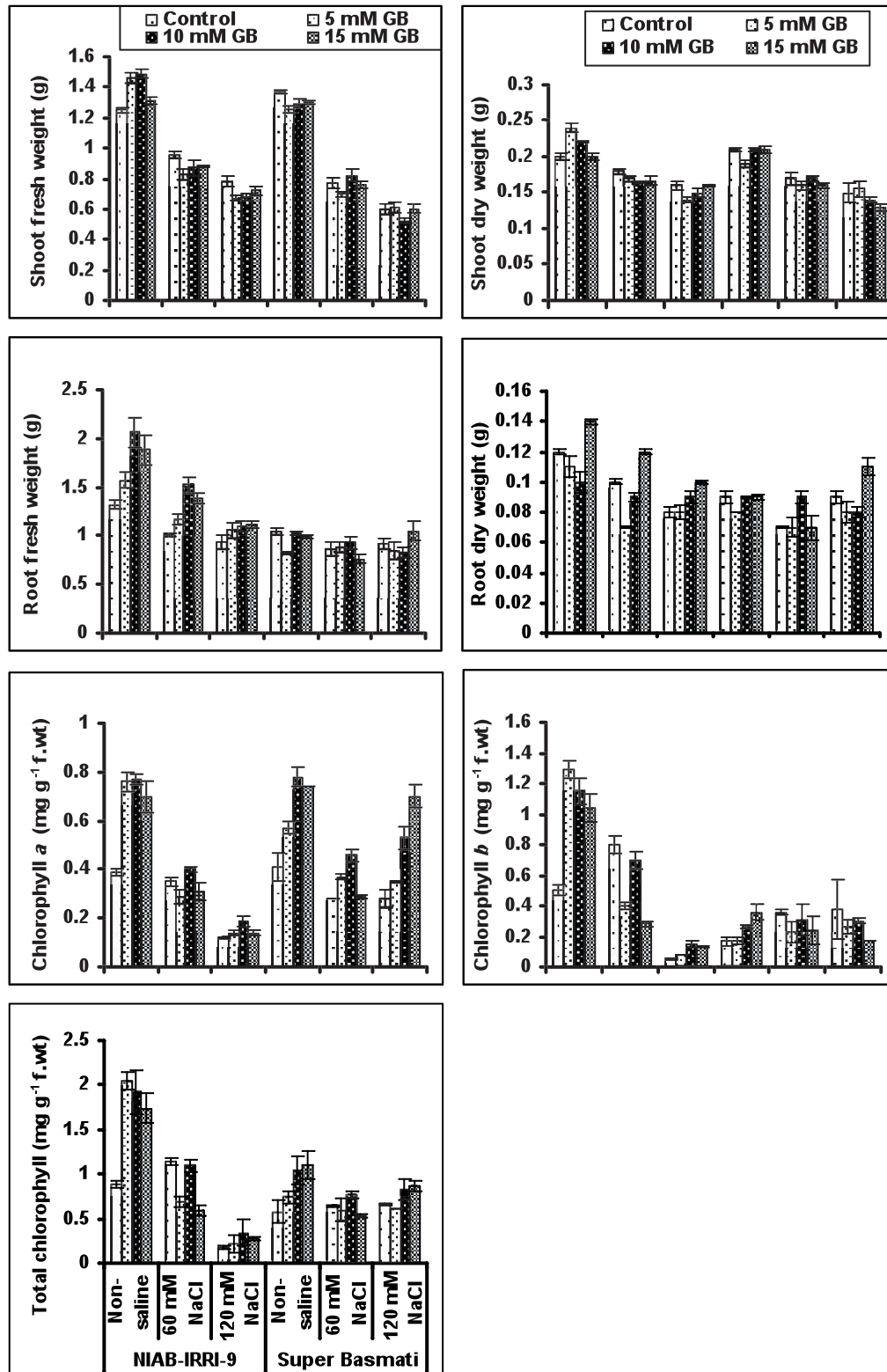


Fig. 1: Shoots and roots biomass and chlorophyll pigments of rice (*Oryza sativa* L.) when plants were supplied with different levels of glycinebetaine in rooting medium for 16 days under control or saline conditions.

non-stressed or salt stressed conditions except the variable behavior of Super Basmati at 120 mM of NaCl (Tab. 1; Fig. 2).

Rooting medium salinity significantly reduced shoot and root K⁺ concentration of both rice varieties. Decreasing trend was almost similar in both varieties. Pattern for GB action was highly reducing

under saline conditions while it was consistent with almost similar values under saline conditions. NIAB-IRRI-9 was slightly high in shoot and root K⁺ as compared to Super Basmati in this variable (Tab. 1; Fig. 2).

Salt stress significantly increased shoot Ca²⁺ concentration in

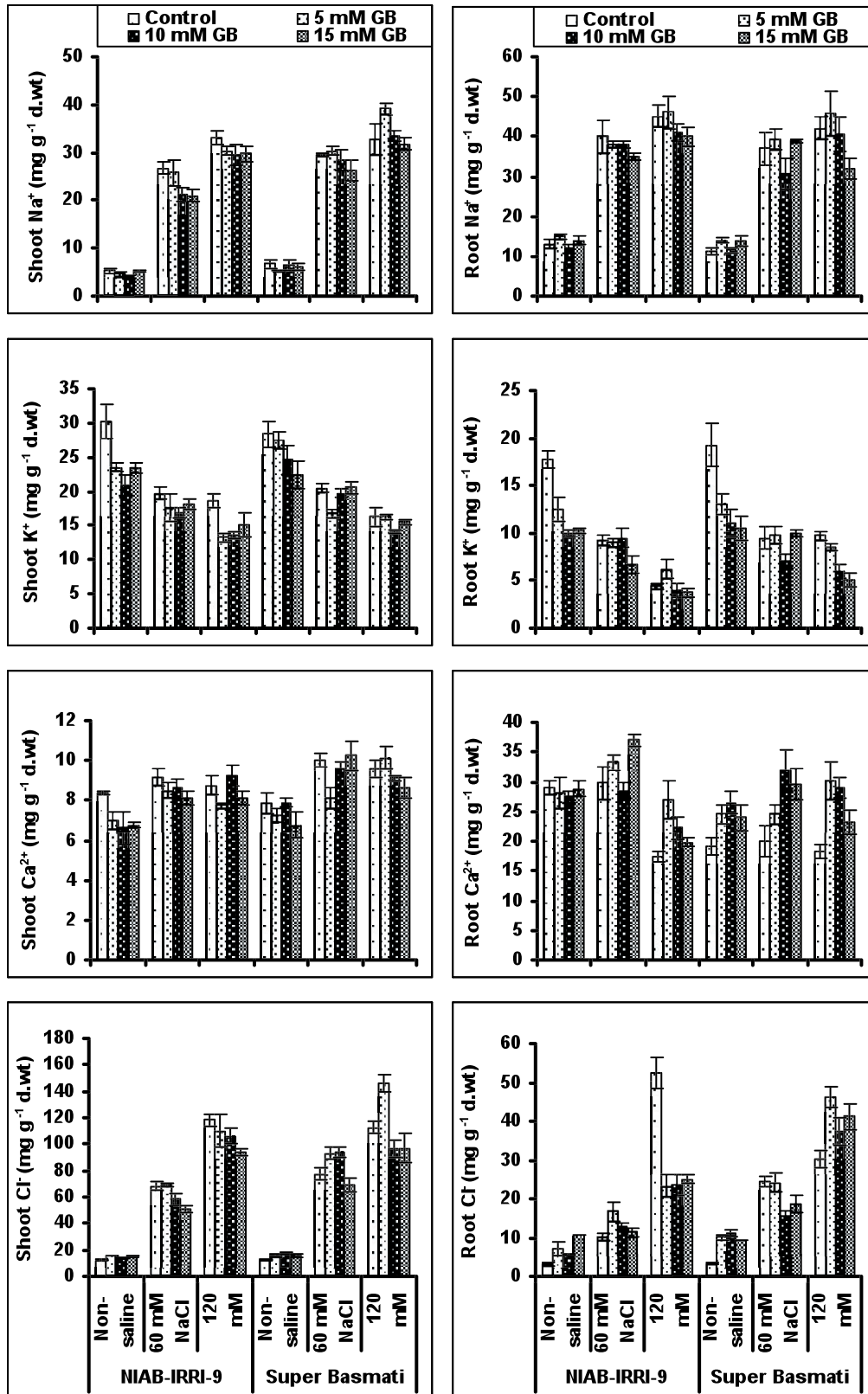


Fig. 2: Mineral nutrients in shoots and roots of rice (*Oryza sativa* L.) when plants were supplied with different levels of glycinebetaine in rooting medium for 16 days under control or saline conditions.

both varieties. However, this increase was almost similar at 60 or 120 mM NaCl. Effect of exogenous application of GB is neither decreasing nor increasing. Rooting medium GB application

significantly enhanced the root Ca²⁺ concentration while due to salinity stress low root Ca²⁺ concentration was observed only at 120 mM NaCl in NIAB-IRRI-9 where as in Super Basmati root Ca²⁺

was almost similar under both non-saline and saline conditions. At 120 mM NaCl the most effective level of GB was 5 mM while 10 or 15 mM was effective at other levels of salt stress (Tab. 1; Fig. 2). Shoot or root Cl⁻ contents increased tremendously with increase in salinity levels. While application of GB in rooting medium was quite effective in reducing the Cl⁻ contents in both shoots and roots of NIAB-IRRI-9 while reverse was true Super Basmati. Of various salt levels, 120 mM NaCl caused highest increase in shoot or root Cl⁻ contents while 5 mM GB was most effective in enhancing shoot or root Cl⁻ in Super Basmati at 120 mM NaCl level (Tab. 1; Fig. 1).

Discussion

Osmoprotectants are widely used to alleviate the adverse effects of salt stress on plant (ASHRAF and FOOLAD, 2007). Of various osmoprotectants glycinebetaine (GB) is widely reported to be effective when applied exogenously under various stress conditions (HARINASUT et al., 1996; RAJASEKARAN et al., 1997; ASHRAF and FOOLAD, 2007). Salt stress is one of the major abiotic stresses perturbing the plant growth and ion homeostasis (ALAM et al., 2004; SARA et al., 2004). In current study salt stress adversely affected the shoots and roots biomass of rice which are in agreement with CABALETA and CORDERO (1991) who also reported decrease in fresh and dry biomass of rice under salt stress conditions. It might be due to limited supply of metabolites to young growing tissue (MASS and NIEMAN, 1978) or interference of NaCl with production of proteins or damage to enzyme proteins exposed to low water potential (WEIMBERG et al., 1982). Rooting medium application of GB mitigated the adverse effects of salinity. Application of GB is effective in improving plant growth and related mechanisms for both accumulators and/or non-accumulators (HARINASUT et al., 1996; RAJASEKARAN et al., 1997; ASHRAF and FOOLAD, 2007). However, in our study role of GB was neither increasing nor decreasing. These results are contradictory with HAYASHI et al. (1998) who reported tolerance of plants with GB application. Our findings are in agreement with (LIN and KAO, 1995) that GB has not mitigated the inhibitory effect of NaCl. The application of GB to rice seedling under non saline conditions did not substantially affect the shoot and root fresh weight (LUTTS, 2000; RAHMAN et al., 2002). Response of Super Basmati was in accordance with this statement but NIAB-IRRI-9 not.

Chlorophyll *a*, *b* and total chlorophyll contents decreased in rice under saline conditions and this reducing effect is already reported in other crops like wheat (IQBAL et al., 2002), rice (MITSUYER et al., 2003), maize (ASHRAFUZZAMAN et al., 2000) and cotton (LEIDI and SAIZE, 1997). This decrease in chlorophyll *b* may be due to depletion of O₂ in standing water. Exogenous application of GB through rooting medium increased chlorophyll pigments under non-saline or moderate saline (60 mM NaCl) conditions. Increase in chlorophyll contents by exogenously applied GB was also reported under non-saline conditions in maize and other crops (ROBINSON and JONES, 1986; HARINASUT et al., 1995; YAUG et al., 1996; HARINASUT et al., 1996; GADALLAH, 1999). Of various level of GB, 10 mM was effective in enhancing chlorophyll pigments under saline conditions.

Absorption of mineral elements from soil is a key process for plants to survive and grow. In the present study, salinity shows significant increase on shoot and root Na⁺. High Na⁺ level in shoots and root might be due to high concentration of Na⁺ in the rooting medium which ultimately resulted in the increased uptake of sodium by plant (ASLAM and MUHAMMAD, 1972). Specific ion toxicities result due to penetration of injurious concentrations of Na⁺ in protoplasts which may lead to an inactivation of enzymes, inhibition of protein synthesis, changes in membrane permeability and damage to cell organelles. Ionic imbalance results in salt stressed plants due to

competition of salt ions with nutrients (ASHRAF et al., 1992; ASHRAF and SARWAR, 2002). Rooting medium GB decreased the shoot and root Na⁺ in both cultivars. RAGHAVENDRA and REDDY (1987) also reported that exogenous application of GB markedly reduced the accumulation of Na⁺ under saline condition. Same behavior was also observed by HEUER (2003) in tomato. One of the effective roles of GB under saline conditions is to lower the level of Na⁺ which might be toxic if accumulated in high concentration. Similar findings were reported for rice in which plants supplemented with GB accumulate low shoot Na⁺ and high K⁺ as compared to untreated plants (LUTTS, 2000; RAHMAN et al., 2002). In our study, results for shoot and root K⁺ are contradictory as GB did not play any effective role in reducing the K⁺ contents of plants. Pattern of K⁺ accumulation is variable for salt sensitive and tolerant varieties (LUTTS, 1999). Our findings are also reverse of GADALLAH (1995) results in which he showed high accumulation of K⁺ with exogenous GB application. Only Super Basmati accumulated more K⁺ with exogenous GB application which is also in accordance with (GREGORIO and SENADHIRA, 1993).

In plants Ca²⁺ is an essential element functioning as second messenger also. It is also involved in membrane integrity. Behavior of crop plants under saline conditions varies from species to species. In our findings shoot or root Ca²⁺ concentrations increased under saline conditions. High Ca²⁺ accumulation is beneficial for membrane stability and is already reported for rice (AHMAD, 1980), who observed that Ca²⁺ concentration increased in rice with increasing salinity level. Exogenous application of GB did not alter the Ca²⁺ concentrations in rice which are in agreement with YOSHIDA et al. (1976) who reported that exogenous GB had no effect on calcium accumulation of stressed plants.

Shoots and roots Cl⁻ concentrations of both rice varieties increased with increasing salinity levels. Increase in Cl⁻ contents is already reported in literature for rice, maize, wheat and many others (ASLAM, 1992; ASLAM et al., 1996; SHAHBAZ and ASHRAF, 2007; NAHEED et al., 2008). Exogenous application of GB decreased the shoot and root Cl⁻ contents under saline conditions as compared to non-saline. Glycinebetaine interferes with osmotic adjustment by reducing Cl⁻ contents in both shoots and roots (HEUER, 2003) as observed in our experiment.

In conclusion, salt stress markedly reduced the plant fresh and dry biomass, chlorophyll contents, shoots and roots K⁺ contents while increased shoots and shoots Na⁺, Cl⁻ and Ca²⁺ contents in both cultivars. Exogenous application of GB significantly ameliorated the adverse effects of salt stress by increasing chlorophyll pigments and decreasing accumulation of toxic elements i.e. Na⁺ and Cl⁻ in both cultivars. Overall, performance of NIAB-IRRI-9 was better as compared to Super Basmati.

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