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INFLUENCE OF EXOGENOUSLY APPLIED BRASSINOSTEROIDS ON THE MINERAL NUTRIENT STATUS OF TWO WHEAT CULTIVARS GROWN UNDER SALINE CONDITIONS

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Abstract

In order to assess the influence of exogenous application of varying concentrations of brassinosterioids as a foliar spray in inducing salt tolerance in wheat (*Triticum aestivum* L.), a seedling experiment was conducted in small plastic pots. A salt tolerant S-24 and a moderately salt sensitive MH-97 were grown under normal or saline conditions (150 mM NaCl) for two weeks. Varying concentrations of 24-epibrassinolide (24-epiBL) were foliarly applied @ 0 (water spray), 0.001, 0.025, 0.050, 0.075, 0.10, 0.125, 0.150, 0.175, and 0.20 mg L^{-1} on two wheat cultivars, S-24 and MH-97. Salt stress reduced the growth of both cultivars. However, this inhibitory effect of salt stress was less on S-24. Foliar application of brassinosteroids improved the growth. However, a maximum increase in growth of both cultivars was observed at 0.125 mg/L of BRs. Furthermore, higher concentration of brassinosteroids did not improve the growth of both wheat cultivars under normal or saline conditions. Foliar spray of brassinosteriods reduced leaf Na^+, and enhanced leaf K^+, leaf Ca^{2+}, and K^+/Na^+ ratios, while Cl^- was inconsistently increased or decreased with increasing level of brassinosteriods. Overall, salt-induced reduction in growth of wheat cultivars was alleviated by foliarly applied brassinosteriods. However, the effectiveness of brassinosteroids in ameliorating the adverse effects of salt stress depends upon the concentration applied. Brassinosteroids improved Ca^{2+}/Na^+ and K^+/Na^+ ratio of wheat cultivars by enhancing uptake of Ca^{2+} and K^+ and reducing that of Na^+, which might have contributed to the salt tolerance of both wheat cultivars.
Keywords: foliar spray, salt tolerance, K^+/Na^+ ratio, Ca^{2+}/Na^+ ratio

Introduction

Salinity is one of the major problems limiting plant growth and development in most parts of the world (Ashraf, 1994; 2004). Salt stress causes many adverse effects on crops because plants may suffer due to osmotically induced water stress, specific ion toxicity, ion imbalance, and oxidative stress, i.e., production of reactive oxygen species (Ashraf, 1994; 2004; Munns *et al*., 2006). Accumulation of excess Na^+ may perturb metabolic processes where low Na^+ and high K^+ or Ca^{2+} are required for optimum function (Marschner, 1995). For example, a decrease in nitrate reductase activity, inhibition of photosystem II (Orcutt & Nilsen, 2000), and chlorophyll breakdown (Krishnamurthy *et al.,* 1987) are all associated with increased Na^+ concentrations. Thus, the regulation of Na^+ uptake by cells and long distance Na^+ transport is considered to be a crucial adaptation of plants to salt stress (Munns *et al*., 2006).

Although several mechanical, chemical and biological approaches are being pursued to overcome the menace of soil salinity, exogenous application of plant growth regulators

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(PGRS), e.g., auxins (Dhingra & Varghese 1985), cytokinins (Ammzallage *et al*., 1992) gibberellins (Amzallog *et al*. 1992), ethylene (Brown & Khan, 1976), abscisic acid (Amzallage *et al*. 1992), and even brassinosteroids (Vardhini & Rao, 1997) is considered to be effective in alleviating the adverse effects of salinity stress.

Brassinosteroids are as potential as other natural plant growth regulators in regulating a number of physiological biochemical processes. For example, Kauschmann *et al*. (1996) reported that BRs are also essential for proper plant development and play an important role in controlling cell elongation. The potential applications of brassinosteroids in agriculture and horticulture are dependent not only on their ability to enhance crop yield, but also to up-regulate other physiological processes (Clouse & Sasse, 1998). As a result, it may become feasible to grow crops under unfavorable (stressful) conditions, such as high salinity, drought or insufficient nutrient supply to plants (Prusakova, *et al*., 1996).

A number of studies show that exogenously applied BRs are effective in controlling plant growth under stressful rather than optimal conditions. For example, shoot dry biomass and seed yield of *Brassica juncea* were considerably increased by the exogenous application of brassinosteroids (Hayat *et al*., 2000) and more seed production was observed in groundnut with exogenous 24-epibrassinolide (Vardhini & Rao, 1998).

In view of the above reports the primary objective of the present study was to assess whether the exogenous application of 24- epibrassinolide could alleviate the damaging effects of salt stress on wheat at early growth stages and how far it could regulate ion homeostasis in wheat plants under saline conditions. Despite this, determination of the optimal concentration at which BR can help the wheat plant to maintain its growth under saline conditions was also one of secondary objectives of the study.

Material and Methods

The present study was conducted to investigate the influence of different levels of 24-epibrassinolide (EBR, Sigma, USA) on growth and ion homeostasis of two wheat genotypes S-24 (salt tolerant) and MH-97 (moderately salt sensitive) under salt stress. The seeds of both the genotypes were obtained from the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Before experimentation, all the seed samples were surface sterilized with 10 % sodium hypochlorite solution for five minutes and washed three times with sterilized distilled water. The experiment was conducted in the Botanic Garden of the University of Agriculture Faisalabad during 2005-2006, where the average PAR measured at noon ranged from 848 to 1254 µmol $m^{-2}.s^{-1}$, day/night relative humidity 58/74 %, and temperature 24/8 ^{o}C. Sodium chloride was used as a source of salt. Fifteen seeds of each of two wheat cultivars were sown in plastic pots filled with washed sand supplied with Hoagland's nutrient solution. The experiment comprised ten levels of 24-epibrassinolide (0, 0.001, 0.025, 0.050, 0.075, 0.10, 0.125, 0.150, 0.175, and 0.20 mg/L) as a foliar spray and two levels of salt (0 and 150 mM NaCl) with four replications and two wheat genotypes. Plants were allowed to establish for eight days before the start of salt treatment. After the establishment of seedlings plants were then thinned to 8 equal sized plants in each pot. Two days after thinning, salt concentrations were gradually increased by increments of 50 m*M* NaCl at 2 day intervals to attain the desired salinity level of 150 m*M* NaCl after 8 days. The day salt treatment was completed the seedlings were sprayed at 14:00 h with varying concentrations of 24-

epibrassinolide prepared in distilled deionized water (1.2 mL/plant of BRs solutions were sprayed). The plants were harvested after 10 days of the foliar application of 24-epibrassinolide and separated into shoots and roots. After measuring fresh masses of shoots, the samples were dried at 65°C for one week so as to measure dry masses.

Determination of Na^+, K^+, and Ca^{2+}: The oven-dried ground material (0.1 g) of leaves or roots was digested with 2 ml of sulfuric acid - hydrogen peroxide mixture according to the method of Wolf (1980). Potassium and sodium in the digests were determined with a flame photometer (PFP-7, Jenway, Felsted, Dunmow, Essex, U.K). Shoot or root samples of 100 mg each were ground and extracted in 10 ml of distilled water, heated at 80°C till the volume of the water became half. The volume was again maintained 10 mL with distilled water. Cl^- content was determined with a chloride meter (Jenway, PCLM 3).

Statistical analysis: Analysis of variance of all data sets was computed using a Costat 6.33 computer package (Cohort Software, California, USA). The mean values were compared with the least significant difference test (LSD) following Snedecor & Cochran (1980).

Results

Fresh and dry weights of shoots and roots of both wheat cultivars were markedly reduced due to salt stress. However, exogenous application of all levels of 24-epibrassinolide significantly ($P \leq 0.01$) enhanced the shoot fresh and dry weights of both cultivars under control and saline conditions. But maximum improvement in terms of shoot fresh and dry weights was recorded at 0.125 mg/L of 24-epibrassinolide under control and saline conditions (Table 1). A slight improvement in root fresh weight of both cultivars occurred due to exogenously applied BRs, but this was not true for root dry weight. Maximum improvement in root fresh weight was found at much lower level (0.025 mg/L) than that for shoot fresh or dry weights (0.125 mg/L).

Sodium (Na^+) in the shoots and roots of both cultivars was significantly increased with the imposition of salt stress. Both cultivars differed significantly in accumulating Na^+ in the shoots or roots. Exogenous application of 24-epibrasinolide significantly reduced shoot and root Na^+ in both cultivars under saline conditions, and 0.125 mg/L level of 24-epibrasinolide proved to be the most effective in decreasing shoot Na^+ in both cultivars (Fig. 1; Table 1). However, the decreasing trend of Na^+ in the roots of both cultivars with foliar application of 24-epibrassinolide was not consistent, because the maximum reduction in root Na^+ of salt stressed plants in S-24 was observed at 0.025 mg/L BRs, whereas, that in MH-97 at 0.075 mg/L BRs.

Although shoot or root K^+ was reduced significantly in both cultivars with the imposition of salt stress in the rooting medium, foliar spray of 24-epibrassinolide significantly increased the shoot or root K^+ in the stressed and non-stressed plants of both cultivars. A maximum increase in shoot or root K^+ was observed when 24-epibrasinolide was applied @ 0.125 mg/L followed by 0.10 mg/L (Fig. 2; Table 1).

Table 1. Mean squares from analysis of variance (ANOVA) of data for shoot and root biomass and different nutrients of salt stressed and non-stressed plants of two spring wheat cultivars differing in salinity tolerance when grown for 10 days under varying levels of 24-epibrassinolide applied foliarly.

		Shoot			
SOV	**df**	**F.w**	**D.w**	**Na^+**	**K^+**
Main effects					
Salinity	1	99.12 ***	0.55 ***	2095.85***	235.75 ***
Variety	1	30.18 ***	0.20 ***	35.75 ***	8.27 ns
Spray	9	3.70 **	0.012 **	30.32 ***	34.31 ***
Interactions					
Salinity X Variety	1	0.0013 ns	0.00011 ns	24.30 **	8.80 ns
Salinity X Spray	9	0.646 ns	0.015 *	26.15 ***	12.59 **
Variety X Spray	9	0.544 ns	0.005 *	15.35 ***	14.64 **
Salinity X Variety X Spray	9	0.674 ns	0.012 **	14.84 ***	20.85 ***
Error	80	1.211	0.015	2.67	4.46

		Root			
SOV	**df**	**fw**	**dw**	**Na^+**	**K^+**
Main effects					
Salinity	1	177.57***	0.63 ***	2783.84***	380.38 ***
Variety	1	43.96***	0.64 ***	45.81 ***	10.24 ns
Spray	9	1.01 ***	0.002 ns	33.64 ***	34.85 ***
Interactions					
Salinity X Variety	1	0.20 ns	0.028 *	31.66 **	61.28 ***
Salinity X Spray	9	0.26 ns	0.002 ns	28.68 ***	19.94 ***
Variety X Spray	9	0.17 ns	0.005 ns	9.60 **	10.48 **
Salinity X Variety X Spray	9	0.39 ns	0.005 ns	10.96 ***	12.34 ***
Error	80	0.29	0.0007	3.10	2.31

		Shoot			
SOV	**df**	**Ca^{2+}**	**Cl^-**	**K^+/Na^+**	**Ca^{2+}/Na^+**
Main effects					
Salinity	1	5.52 **	3381.41 ***	194.85 ***	2.82 ***
Variety	1	0.71 ns	222.49 ***	0.75 *	0.013 ns
Spray	9	1.71 *	15.47 ns	1.22 ***	0.025**
Interactions					
Salinity X Variety	1	0.32 ns	56.31 *	0.39 ns	0.035 *
Salinity X Spray	9	2.16 **	38.31 ***	0.52 **	0.033 ***
Variety X Spray	9	0.85 ns	9.79 ns	0.88 ***	0.022 *
Salinity X Variety X Spray	9	0.76 ns	5.95 ns	0.17 ns	0.015 ns
Error	80	0.76	9.31	0.17	0.008

		Root			
SOV	**df**	**Ca^{2+}**	**Cl^-**	**K^+/Na^+**	**Ca^{2+}/Na^+**
Main effects					
Salinity	1	5.48 **	3290.72 ***	199.08 ***	3.71 ***
Variety	1	0.27 ns	197.63 ***	0.71 *	0.017 ns
Spray	9	2.10 ***	16.16 ns	1.06 ***	0.040 ***
Interactions					
Salinity X Variety	1	0.73 ns	69.62 **	0.66 *	0.009 ns
Salinity X Spray	9	0.79 ns	36.24 ***	0.35 *	0.021 **
Variety X Spray	9	1.05 *	8.91 ns	0.39 *	0.028 ***
Salinity X Variety X Spray	9	0.52 ns	6.04 ns	0.12 ns	0.018 **
Error	80	0.52	9.94	0.16	0.006

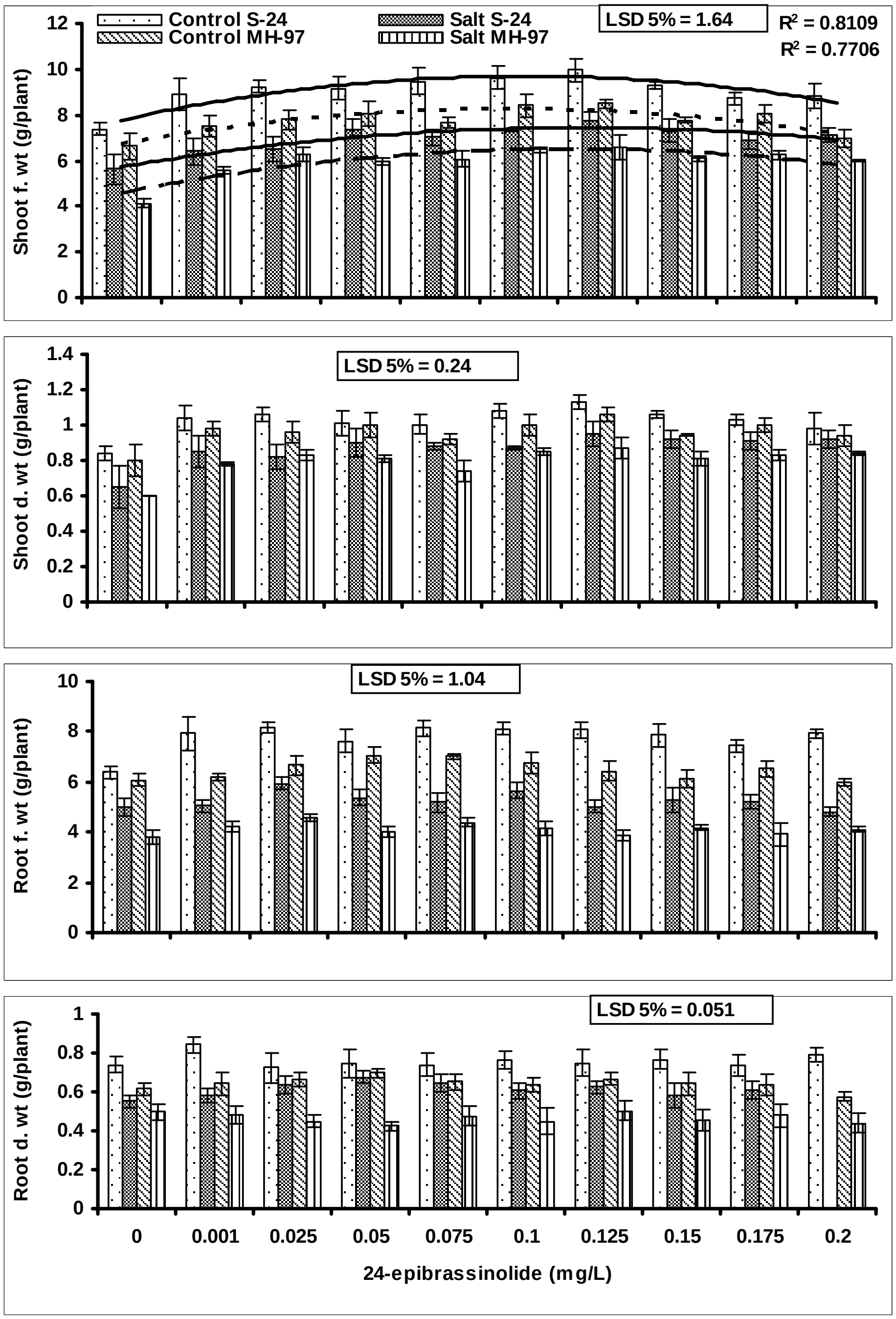

Fig. 1. Shoot and root fresh and dry weights (g) of salt stressed and non-stressed plants of two spring wheat cultivars differing in salinity tolerance when grown for 10 days under varying levels of 24-epibrassinolide applied foliarly.

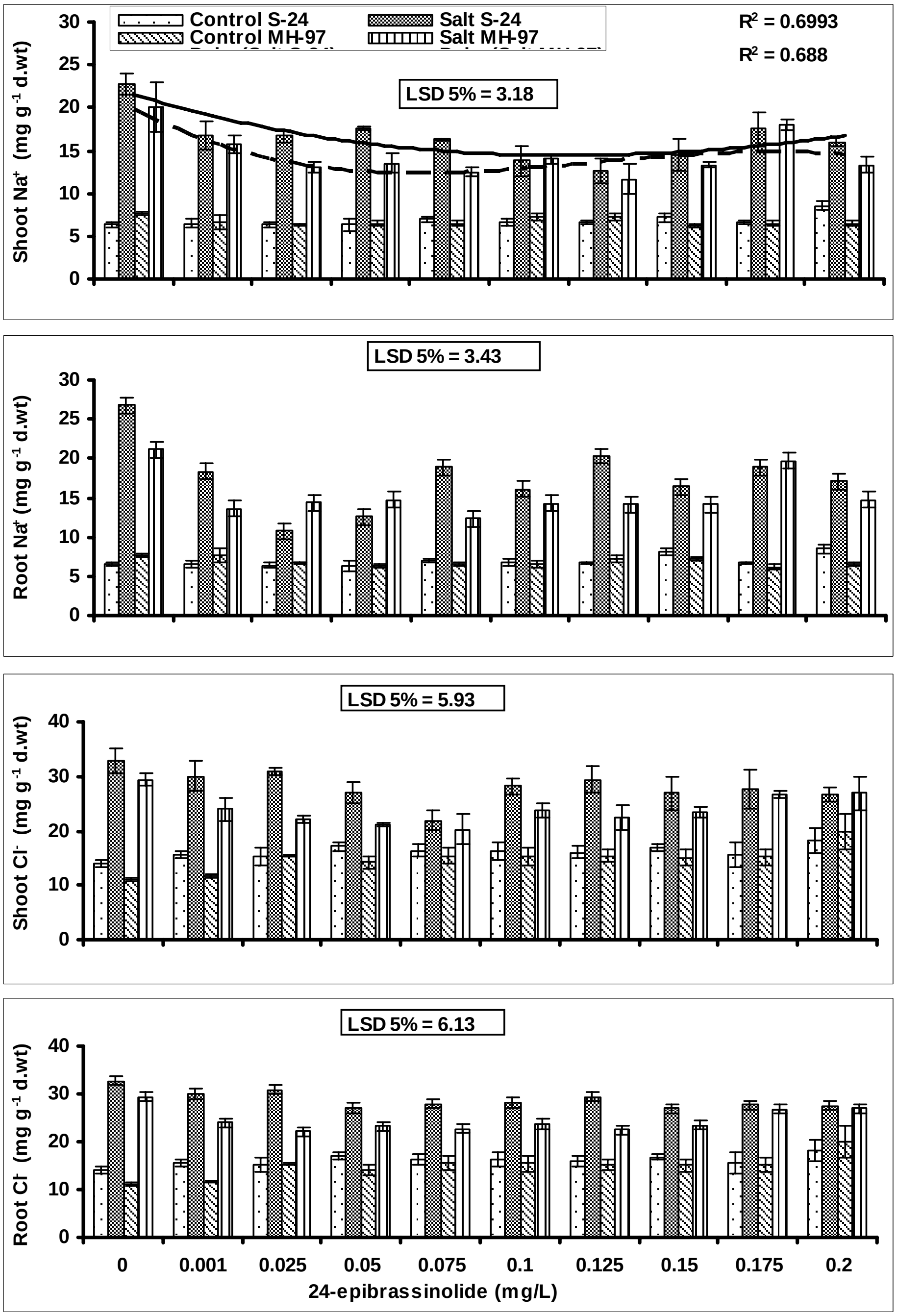

Fig. 2. Shoot and root Na^+ and Cl^- (mg g^{-1} d.wt) of salt stressed and non-stressed plants of two spring wheat cultivars differing in salinity tolerance when grown for 10 days under varying levels of 24-epibrassinolide applied foliarly.

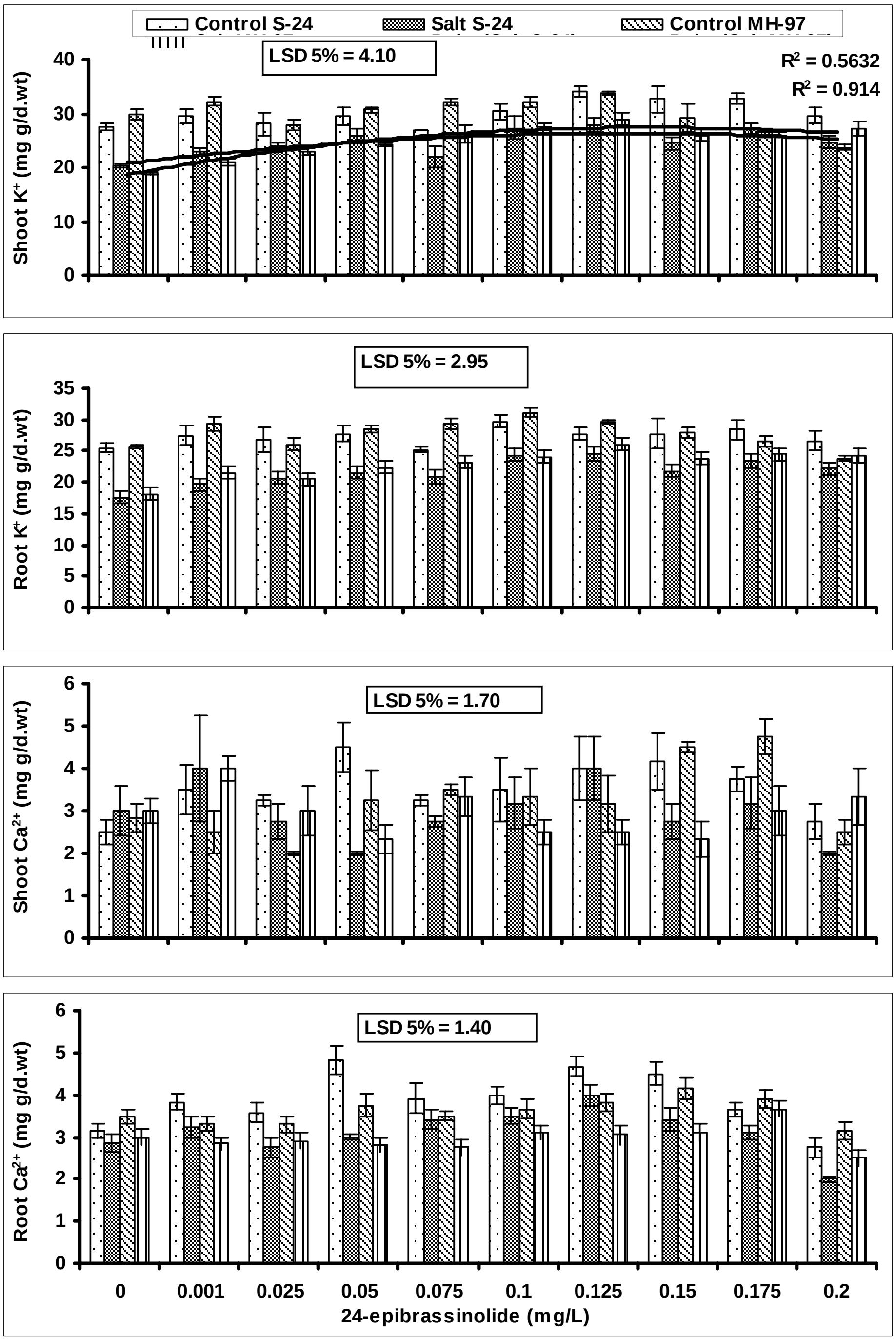

Fig. 3. Shoot and root K^+ and Ca^{2+} (mg g^{-1} d.wt) of salt stressed and non-stressed plants of two spring wheat cultivars differing in salinity tolerance when grown for 10 days under varying levels of 24-epibrassinolide applied foliarly.

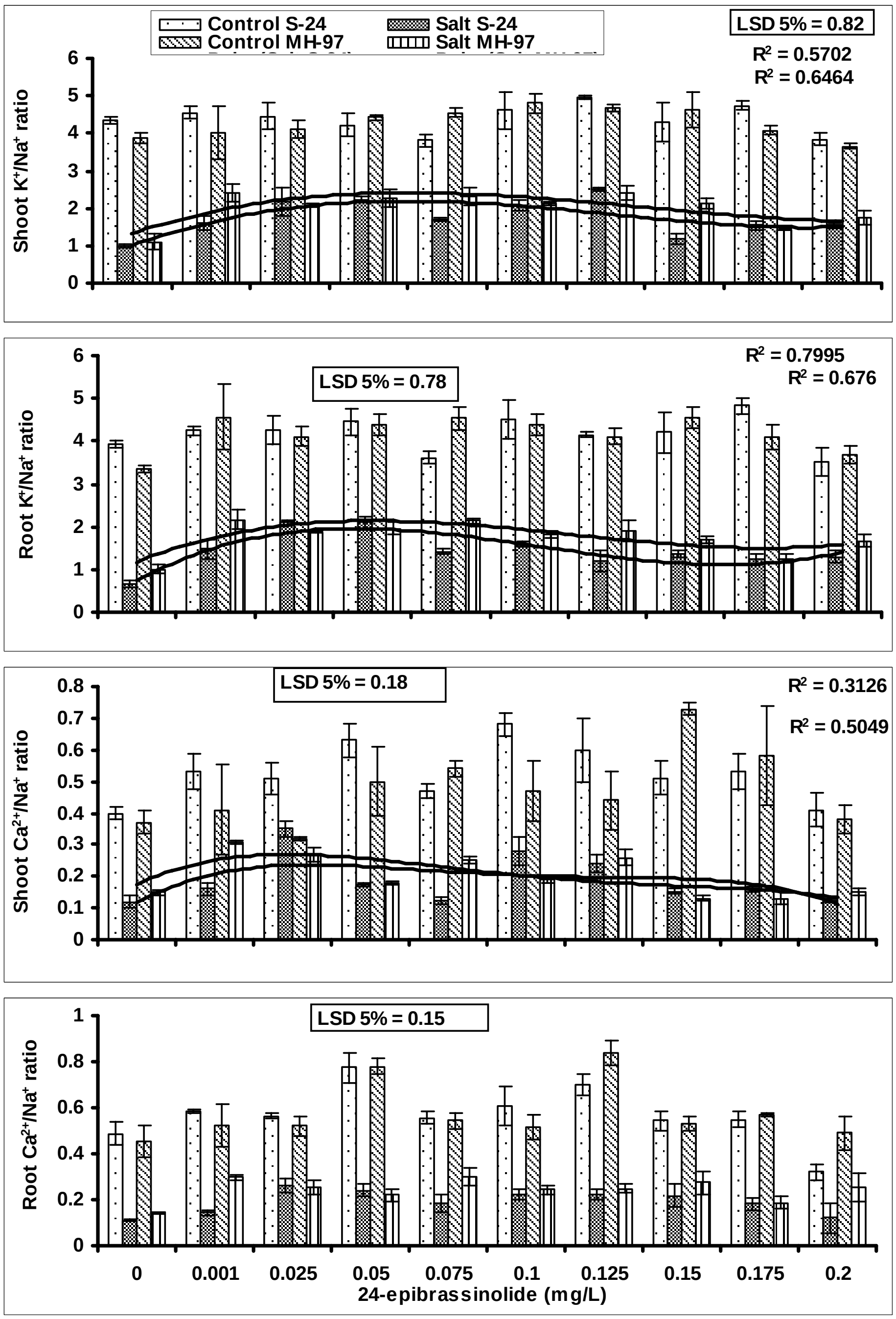

Fig. 4. Shoot K^+/Na^+ and Ca^{2+}/Na^+ ratios of salt stressed and non-stressed plants of two spring wheat cultivars differing in salinity tolerance when grown for 10 days under varying levels of 24-epibrassinolide applied foliarly.

A significant effect of salinity was observed on shoot or root Ca^{2+} in both wheat cultivars. Exogenous application of 24-epibrassinolide as a foliar spray significantly affected shoot Ca^{2+} of both cultivars under saline or non-saline conditions, but the increasing or decreasing effect on shoot Ca^{+} was not consistent at varying external levels of 24-epibrasinolide (Fig. 2; Table 1). However, the root Ca^{2+} was found maximum in the salt stressed plants of S-24 with the exogenous application of 0.125 mg/L 24-epibrassinolide under saline conditions, but in MH-97 it was true at 0.175 mg/L BRs.

Salt stress significantly increased shoot or root Cl^{-} in both cultivars. Overall, exogenous application of all levels of 24-epibrasinolide caused a significant reduction in shoot Cl^{-} in the salt stressed plants of both cultivars, but a maximum decrease was observed at 0.075 mg/L BRs (Fig. 2; Table 1). In contrast, the cultivars responded differently to exogenous BRs, because a maximum reduction in root Cl^{-} of S-24 was found at 0.05 mg/L BRs and in MH-97 at 0.025 mg/L BRs.

Although, salt stress caused a significant reduction in shoot or root K^{+}/Na^{+} ratios in both cultivars, exogenous application of BRs enhanced shoot K^{+}/Na^{+} ratios in both cultivars, and a maximum increase in shoot K^{+}/Na^{+} was observed at 0.125 mg/L BRs. In contrast, although a significant increase in root K^{+}/Na^{+} was observed in both cultivars under salt stress or non stress conditions, the increase in this attribute was not consistent at varying levels of BRs (Fig. 3; Table 1).

Although, shoot or root Ca^{2+}/Na^{+} ratios in both cultivars was reduced due to salt stress, the foliar application of 24-epibrasinolide had a significant increasing effect on shoot Ca^{2+}/Na^{+} ratios in both cultivars under saline and non-saline conditions. The maximum shoot Ca^{2+}/Na^{+} ratio was observed at 0.001, 0.025 and 0.150 mg/L levels of 24-epibrasinolide in MH-97 and S-24, respectively. However, a maximum increase in root Ca^{2+}/Na^{+} ratio in the salt stressed plants of S-24 and MH-97 was observed at 0.025 and 0.075 mg/L BRs (Fig. 3; Table 1).

Discussion

Imposition of salt stress reduces overall growth of most mesophytes as an outcome of many changes in the normal physiology and biochemistry. However, this inhibitory effect of salt stress is related to the particular potential of the plant to cope with the stressful environment based on its genetic potential for tolerance or adaptability through the entire process of development of the species (Munns *et al*., 2006). In the present study, difference in growth of both spring wheat cultivars under saline conditions was expected in view of the differential genetic potential of the two cultivars for salinity tolerance, because line S-24 is already known for its high salt tolerance (Ashraf, 2002), and MH-97 is moderately salt sensitive. However, this negative effect of salt stress on growth is reported to be minimized by the exogenous application of 24-epibrassinolide (Clouse, 1996, Pullman *et al*., 2003). Anuradha & Rao (2001) observed an increase in salinity tolerance in rice at germination and seedling stage when brassinosteroids were applied exogenously under saline conditions. Similarly, while working with embryogenic calli of different species of loblolly pine and rice, Pullman *et al*. (2003) found that exogenous application of brassinosteroids increased the growth and abiotic stress resistance. Since brassinosteroids have a role in cell elongation by promoting transverse orientation of microtubules, growth improvement due to exogenous foliar spray of brassinosteroids might have been due to its accelerating effect on cell elongation (Mayumi & Shibaoka, 1995).

In the present study, higher concentration of brassinosteroids did not improve the growth of both wheat cultivars. Amzallag (2002) emphasized that effectiveness of epibrassinolide on the growth of salt stressed plants depends on the type of species, plant developmental stage, concentration of epibrassinolide, and mode of application. For instance, most effective concentration of epibrassinolide as pre-soaking seed treatment was 0.01 µ*M* in promoting seedling growth of tobacco (Leubner-Metpre, 2001), 10 µ*M* for mungbean (*Vigna radiata*) (Fariduddin *et al*., 2003), 0.005-0.25 µ*M* for loblolly pine (*Psueudosuga menziesii*) and Norway spruce (*Picea abies*) (Pullman *et al*., 2003), 0.9 mg L^{-1} (1.8 µ*M*) for tomato seedlings under non-saline conditions and 0.8 mg L^{-1} (1.6 µM) under saline conditions (Prakash *et al*., 1999). Similarly, exogenous application of 100 µ*M* epibrassinolide as a foliar spray improved the growth and yield of rice (Krishnan *et al*., 1999), while effective concentration of epibrassinolide in improving growth and yield of tomato was 3 µ*M* (Vardhini *et al*., 2001). However, addition of 0.1 µ*M* epibrassinolide in the rooting medium inhibited the growth and nodulation of soybean (*Glycine max*) (Hunter, 2001). In the present study, maximum growth improvement in terms of shoot fresh was recorded at 0.1 and 0.125 mg/L of 24-epibrassinolide under control and saline conditions.

It is well established that synergism, like competition, is an important feature of ion interaction during uptake whereby stimulation of cation uptake by anions and vice versa takes place for maintaining charge balance within the cells (Marschner, 1995). From the results for accumulation of Na^+ in shoots and roots of both wheat cultivars at varying levels of epibrassinolide, it is evident that salt stress induced increase in the Na^+ uptake was reduced by epibrassinolide application, particularly 0.125 mg/L level of 24-epibrasinolide proved to be the most effective in both cultivars. Antagonistic effect of Na^+ on K^+ was also visible, as foliar spray of 24-epibrassinolide reduced Na^+ and enhanced the K^+ uptake in the shoots which resulted in higher shoot K^+/Na^+ ratios in both cultivars with a maximum increase in shoot K^+/Na^+ at 0.125 mg/L BRs. Similarly, shoot Ca^{2+}/Na^+ ratios was also increased due to foliar application of 24-epibrasinolide under saline conditions. However, maximum shoot Ca^{2+}/Na^+ ratio was observed at 0.001, 0.025 mg/L (MH-97) and 0.150 mg/L (S-24). If we draw the relationship between mineral nutrients status (shoot Na^+, K^+, Ca^{2+}, shoot K^+/Na^+ ratio, shoot Ca^{2+}/Na^+ ratio) and growth of both cultivars under saline conditions, it is clear that growth improvement due to foliar spray with brassinosteroids was positively associated with brassinosteroids induced reduction in Na^+ and enhancement in K^+ in the shoots. It is well documented that high Na^+ concentration blocks high affinity K^+ transporters resulting in decreased K^+ influx and increased Na^+ influx (Gassman *et al*., 1996; Amtmann & Sanders, 1999). In view of these findings and the results from the present study, it is suggested that improvement in K^+/Na^+ ratio with brassinosteroids spray might have been due to its some positive effect on the activity of high affinity K transporters.

In conclusion, salt-induced reduction in growth of wheat cultivars was alleviated by foliar spray of brassinosteriods. However, the effectiveness of brassinosteroids in ameliorating the adverse effects of salt stress depends upon the concentration applied. Brassinosteroids improved the Ca^{2+}/Na^+ and K^+/Na^+ ratios of the wheat cultivars by enhancing Ca^{2+} and K^+ uptake, and reducing Na^+ uptake, which may have contributed to enhance salt tolerance of both wheat cultivars.

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