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INFLUENCE OF EXOGENOUS APPLICATION OF BRASSINOSTEROID ON GROWTH AND MINERAL NUTRIENTS OF WHEAT (*TRITICUM AESTIVUM* L.) UNDER SALINE CONDITIONS

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Abstract

Brassinosteroids (BRs) are a novel group of plant growth regulators which regulate growth and development of plants. BRs application is also effective in ameliorating the adverse effects of abiotic stresses including salt stress. A pot experiment was conducted to assess the influence of exogenous application of BRs as a foliar spray on wheat (*Triticum aestivum* L.) growth and pattern of nutrient accumulation under control or saline conditions. 24-epibrassinolide (24-epiBL) was foliarly applied @ 0 (water spray), 0.0125, 0.025, and 0.0375 mg L^{-1} on two wheat cultivars viz., S-24 and MH-97. Two salinity treatments 0 (control) and 150 mmol L^{-1} of NaCl were also applied in full strength Hoagland's nutrient solution. Exogenous application of 24-epiBL increased plant biomass under both saline and non-saline conditions, but it had a non-significant effect on leaf Na^+, K^+, Ca^{2+}, and Cl^- contents or K^+/Na^+ ratios while in roots a similar pattern was observed but only for root K^+ and K^+/Na^+ ratios. Overall, exogenous application of BRs as foliar spray improved growth of wheat plants under saline and non-saline conditions, but it did not have any prominent effect on accumulation of different mineral nutrients in the two cultivars examined.

Introduction

High concentrations of salts in soil inhibit growth by causing specific ion toxicity and water deficits (Epstein, 1980; Greenway & Munns, 1980), and affecting mineral nutrition (Ball *et al*., 1987; Lazof & Läuchli, 1991). Water relation characteristics of plants change with the accumulation of toxic ions such as Na^+ and Cl^- into the leaves (Ball & Farquhar, 1984; Seemann & Critchley, 1985; Sobrado, 1999). High concentrations of Na^+ and Cl^- in the growth medium may perturb the uptake and accumulation of other nutrients such as K^+, N, P, and Ca^{2+} and produce high ratios of Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+} and Cl^-/NO_3^- (Grattan & Grieve, 1999).

Plants possess the ability to synthesize a variety of steroids which play important function as hormone was not confirmed until 1979 (Khripach *et al*., 2000). Research on brassinosteroids began about 36 years ago when Mitchell *et al*., (1970) first reported that organic extracts can be used to promote stem elongation and cell division in plants. Brassinosteroids, first extracted from *Brassica napus* pollen are growth promoter steroid hormones (Clouse & Sasse, 1998). In view of some reports it is evident that the exogenous application of brassinosteroids as a foliar spray improves growth and yield of some plants e.g. tomato (Vardhini & Rao, 2001), and *Arachis hypogaea* (Vardhini & Rao, 1998). Exogenous application of brassinosteroids can also alleviate the adverse effects of various environmental stresses and produces resistance in plants against these stresses e.g., salt stress (Hathout, 1996; Vardhini & Rao, 1997), heat stress (Zhu *et al*., 1998), drought stress (Li & Van Staden, 1998), and chilling stress (Wilen *et al*., 1995). Brassinosteroids are known to act along with auxins to stimulate cell elongation (Katsumi, 1991; Sasse, 1991).

Exogenously applied brassinosteroid has an ameliorating the adverse effects of salt stress on seed germination and growth (Anuradha & Rao, 2001), and root elongation (Amzallag & Goloubinoff, 2003). Similarly, exogenous application of BRs is also effective in seed germination and growth of rice plants under saline conditions (Anuradha & Rao, 2001).

BRs are known to play a vital role in the regulation of ion uptake (Khripach *et al*., 2000). These are also used to reduce the accumulation of heavy metals and radioactive elements (Khripach *et al*., 1996), but very little information is available in the literature on the effect of BRs on accumulation of whether or not toxic elements like Na^+ and Cl^-, so the present study was conducted to assess exogenous application of BRs as foliar spray could reduce the accumulation of Na^+ and Cl^- in wheat plants subjected to saline conditions.

Materials and Methods

To assess the effect of the exogenous application of brassinosteroid as foliar spray in alleviation of the adverse effects of salinity on plants of two spring wheat cultivars, S-24 and MH-97, an experiment was conducted in the Botanic Garden of the University of Agriculture, Faisalabad in a net-house supplied with natural sunlight during November-April, 2004-2005. Two salinity treatments were: control (0 mmol L^{-1} NaCl + full strength Hoagland's nutrient solution) and 150 mmol L^{-1} of NaCl in full strength Hoagland's nutrient solution. There were four 24-epibrassinolide levels (control (water spray), 0.0125, 0.025 and 0.0375 mg L^{-1}) which were applied exogenously as foliar spray@ 25 mL/pot. In addition, Tween-20 (0.1%) was added to BRs solutions as a surfactant to ensure the penetration of brassinosteroid into the leaf tissue.

Seeds of two wheat cultivars were obtained from the Department of Botany, University of Agriculture, Faisalabad. Twenty seeds were sown per plastic pot (24.5 cm diameter and 28 cm deep) each containing 10 kg well washed dry river sand. Each pot was supplied with two liters of full strength Hoagland's nutrient solution on alternate days. The volume was enough to flush through all the salts previously present in the sand. After 14 days of growth, plants were thinned to six plants per pot. Salinity (NaCl) treatment (150 mmol L^{-1}) in full strength Hoagland's nutrient solution was initiated after 41 days of sowing. The NaCl concentration was increased step-wise in aliquots of 50 mmol L^{-1} every day until the appropriate concentration was attained. Each time, two liters of appropriate treatment solution were applied in the evening. Brassinosteroid (24-epibrassinolide (M. wt. = 480.7) of SIGMA-ALDRICH CHEME Gmbh, Germany) was sprayed two days after the start of salt treatment. The data for the following parameters were recorded after 45 days of BRs application.

Plant biomass: Two plants from each pot were uprooted carefully and washed with distilled water and fresh weights of both shoots and roots recorded. Then, the plant samples were oven-dried at 65ºC for one week and their dry weights recorded.

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). Na^+, K^+ and Ca^{2+} cations in the digests were determined with a flame photometer (Jenway, PFP-7). For the determination of Cl^-, the ground shoot and root material (100 mg) was extracted in 10 ml distilled water at 80 °C for 4 h. The Cl^- content in the extracts was determined with a chloride analyzer (Model 926, Sherwood Scientific Ltd., Cambridge, UK).

Statistical analysis: Analysis of variance of the data from each attribute was computed using the MSTAT Computer Program (MSTAT Development Team, 1989).The Duncan's New Multiple Range test at 5% level of probability was used to test the differences among mean values (Steel & Torrie, 1986).

Results

Shoot fresh and dry biomass of both S-24 and MH-97 cultivars decreased significantly due to salt stress i.e. 150 m*M* of NaCl. However, exogenous application of 24-epiBL was effective in improving growth, because 24-epiBL increased shoot fresh and dry biomass under both non-saline and saline conditions, although varying levels of 24-epiBL had differential effect on both cultivars. Under non-saline conditions, the effective level of 24-epiBL for S-24 was 0.0125 and for MH-97 0.025 mg L^{-1}. In contrast, under saline conditions BR caused an increase in shoot fresh and dry mass only in S-24, while it did not alter the fresh and dry biomass of MH-97 under saline conditions (Table 1; Fig. 1). Overall, cultivar S-24 was superior in plant biomass as compared to MH-97 under saline conditions.

Saline growth medium caused a significant increase in leaf or root Na^+ of both cultivars of hexaploid wheat (Table 1; Fig. 2). Application of 24-epiBL as a foliar spray did not alter the leaf Na^+ contents of both cultivars under saline or non-saline conditions. However, root Na^+ was significantly high at 0.0375 mg L^{-1} level of 24-epiBL in both cultivars under saline conditions, but the cultivar response was different under non-saline conditions, where 0.025 mg L^{-1} of 24-epiBL was found responsible for slightly increasing root Na^+ contents. Variation in both cultivars for leaf or root Na^+ was not prominent under both saline and non-saline conditions.

A significant reduction in leaf and root K^+ contents of both cultivars occurred due to the addition of NaCl to the growth medium (Table 1; Fig. 2). Exogenous application of EBL did not alter leaf or root K^+ of the salt stressed and non-stressed plants of both cultivars. Difference in both cultivars was also non-significant in the above mentioned attributes.

Leaf Ca^{2+} in both cultivars was significantly reduced as a result of imposition of salt stress (Table 1; Fig. 2). However, this reducing effect of salt stress on leaf Ca^{2+} was more in cv. S-24 than that in cv-MH-97. Changes in leaf Ca^{2+} were found to be non-significant due to exogenous application of EBL. However, application of 0.025 mg L^{-1} EBL slightly reduced the leaf Ca^{2+} contents of both cultivars under non-saline conditions while other levels of EBL did not alter leaf Ca^{2+} in both saline or non-saline conditions.

Although both cultivars did not differ in accumulation of root Ca^{2+}, foliarly applied EBL significantly changed the root Ca^{2+} of both cultivars. For example, root Ca^{2+} was higher in the non-stressed plants of MH-97 at 0.025 mg L^{-1} EBL than that in S-24, but the two cultivars did not differ significantly at other levels of 24-epiBL. Under saline conditions, 0.0125 mg L^{-1} of EBL caused a significant reduction in root Ca^{2+} of both cultivars.

Table 1. Mean squares from analyses of variance of data for shoot biomass and mineral nutrients of two wheat cultivars when their salt-stressed and non-stressed plants were sprayed with brassinosteroid (BR).

Source of variations	Degrees of freedom	Shoot fresh weight	Shoot dry weight	Leaf Na^+	Root Na^+
Cultivars (Cvs)	1	160.70***	18.814***	0.001ns	4.41ns
Salinity (S)	1	1960.2***	64.000***	1265.1***	765.9***
Brassinosteroid (BR)	3	72.12***	5.016***	2.430ns	8.751**
Cvs x S	1	17.308ns	3.045*	3.084ns	0.007ns
Cvs x BR	3	21.835*	0.908ns	2.257ns	1.541ns
S x BR	3	37.753**	1.003ns	4.527*	12.52**
Cvs x S x BR	3	39.91***	1.935**	4.200*	1.602ns
Error	48	6.183	0.453	1.385	1.980
Source of variations	**Degrees of freedom**	**Leaf K^+**	**Root K^+**	**Leaf Ca^{2+}**	**Root Ca^{2+}**
Cultivars (Cvs)	1	29.16ns	0.078ns	3.115**	0.151ns
Salinity (S)	1	2740.5***	227.90***	57.30***	46.84***
Brassinosteroid (BR)	3	7.629ns	1.421ns	0.240ns	0.605*
Cvs x S	1	51.12*	12.81*	0.770ns	0.301ns
Cvs x BR	3	15.06ns	2.195ns	0.037ns	0.277ns
S x BR	3	4.585ns	1.233ns	0.366ns	0.224ns
Cvs x S x BR	3	9.374ns	2.350ns	0.2529ns	0.682**
Error	48	11.410	2.671	0.290	0.151
Source of variations	**Degrees of freedom**	**Leaf Cl^-**	**Root Cl^-**	**Leaf K^+/Na^+ ratio**	**Root K^+/Na^+ ratio**
Cultivars (Cvs)	1	6.269ns	2.139ns	7.101*	0.952***
Salinity (S)	1	2254.2***	1048.3***	793.2***	20.153***
Brassinosteroid (BR)	3	19.498**	23.60***	3.195ns	0.045ns
Cvs x S	1	45.95**	0.002ns	5.989*	1.350***
Cvs x BR	3	1.925ns	4.568*	0.039ns	0.084ns
S x BR	3	3.877ns	7.903**	4.409*	0.277**
Cvs x S x BR	3	9.426ns	4.177*	0.641ns	0.123ns
Error	48	4.406	1.457	1.404	0.056

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

Salt stressed plants of both cultivars had significantly higher leaf Cl^- than that in the non-stressed plants (Table 1; Fig. 2). The effect of exogenously applied 24-epiBL with respect to leaf Cl^- was also positive. For example, the higher level (0.0375 mg L^{-1}) of 24-epiBL caused a significant increase in leaf Cl^- of the stressed plants of S-24, whereas it had no significant effect on leaf Cl^- of the salinized plants of MH-97. However, the reverse was true in the non-stressed plants of the two cultivars.

Root Cl^- of both cultivars was significantly increased due to salt stress imposed in the growth medium (Table 1; Fig. 2). Cultivars did not differ significantly in root Cl^-. However, exogenous application of 24-epiBL caused a significant decrease in root Cl^- of the stressed plants of both cultivars, whereas it did not cause any change in root Cl^- of the non-stressed plants of both cultivars.

K^+/Na^+ ratios of leaves and roots were significantly reduced in both cultivars due to imposition of salt stress ($p \leq 0.05$ and 0.001, respectively). Both cultivars had similar values of leaf or root K^+/Na^+ ratios under saline conditions (Table 1; Fig. 3). Although exogenous application of 24-epiBL did not cause a significant change in leaf or root K^+/Na^+ ratios, the increasing levels of 24-epiBL applied at all different growth stages caused increase or decrease in leaf or root K^+/Na^+ ratios only in the non-stressed plants. However, 0.0125 mg L^{-1} 24-epiBL caused a slight increase in leaf or root K^+/Na^+ ratios in the salt stressed plants of MH-97.

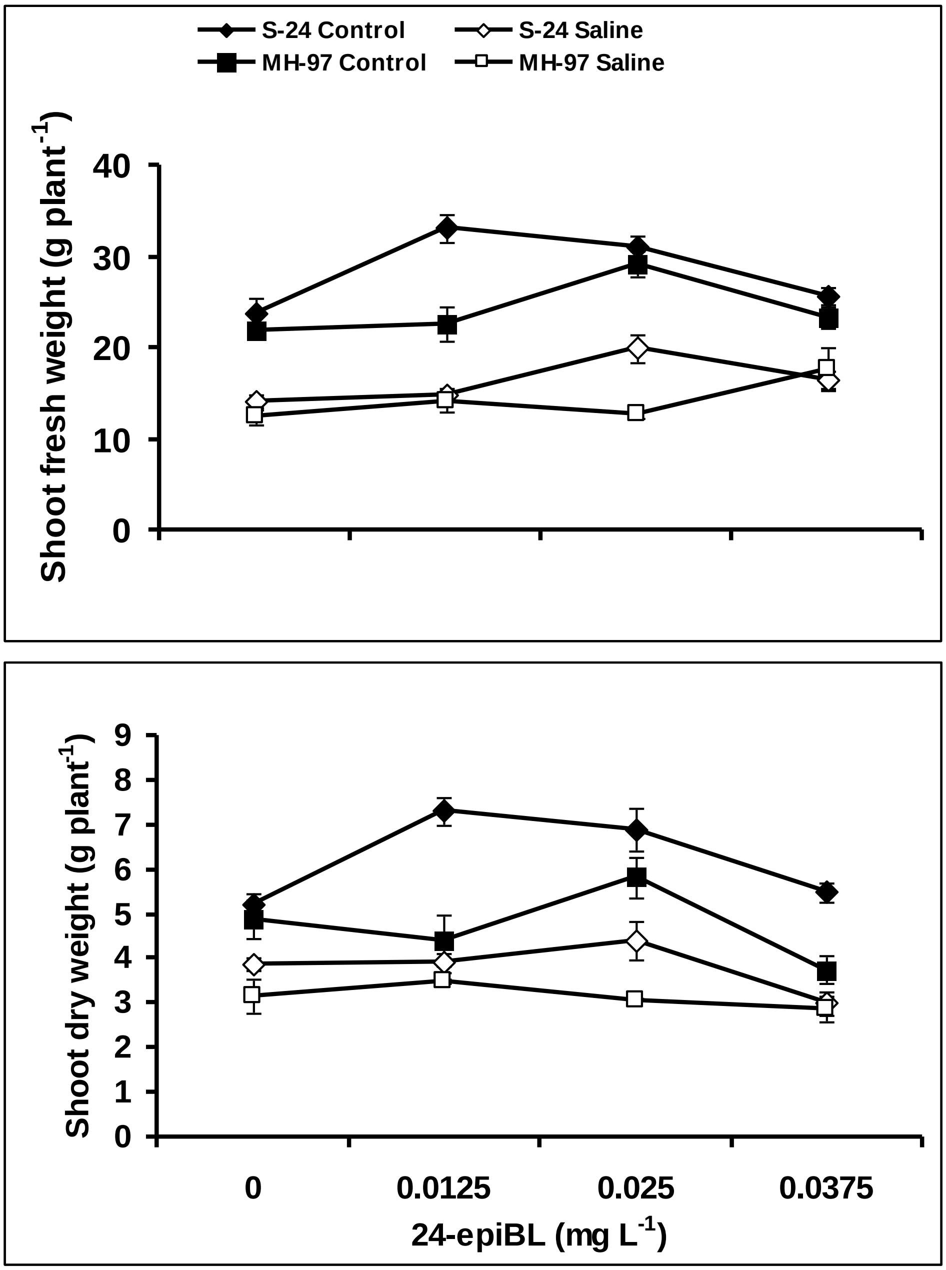

Fig. 1. Shoot biomass of two wheat cultivars when their salt-stressed and non-stressed plants were sprayed with brassinosteroid (BR).

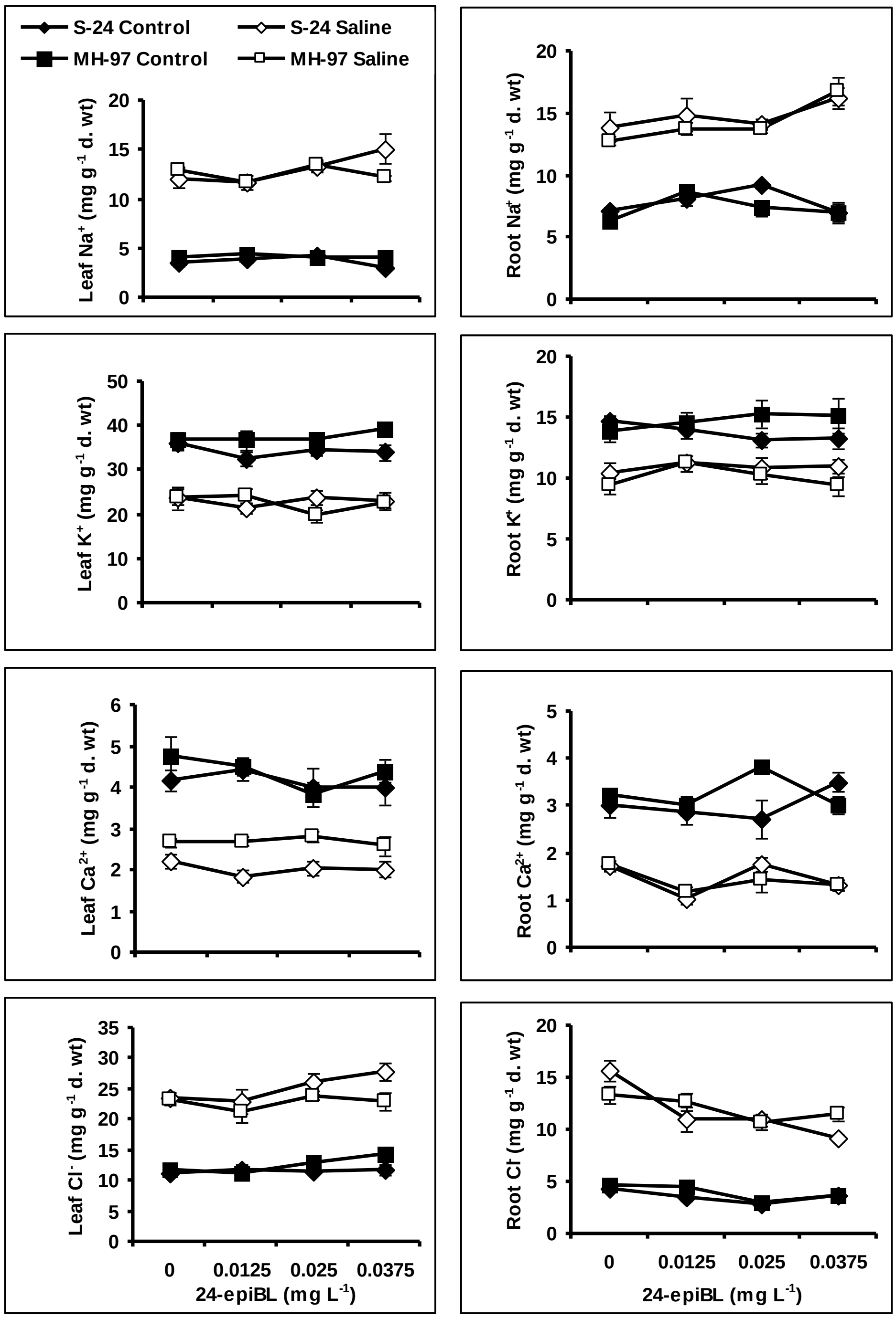

Fig. 2. Mineral nutrients of two wheat cultivars when their salt-stressed and non-stressed plants were sprayed with brassinosteroid (BR).

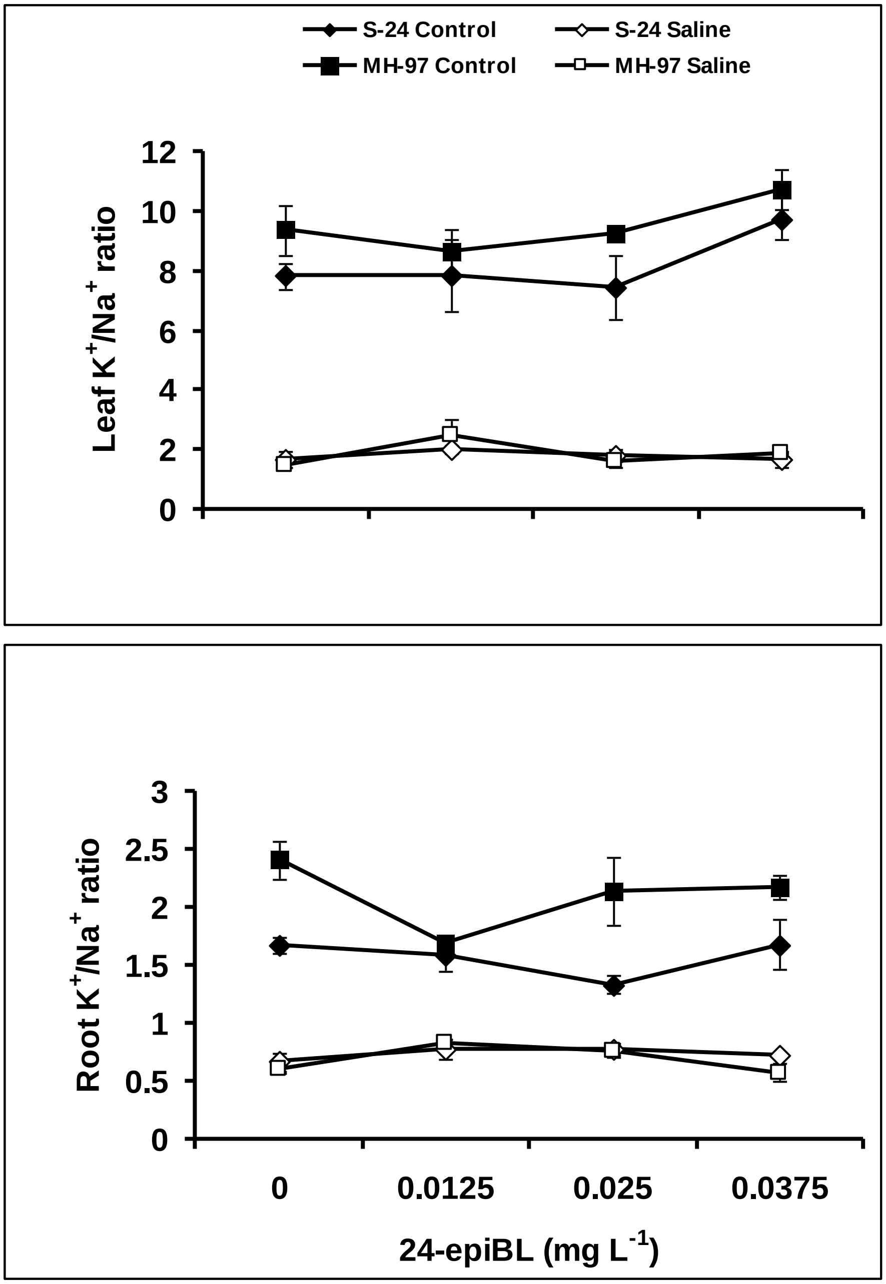

Fig. 3. Leaf and root K^+/Na^+ ratios of two wheat cultivars when their salt-stressed and non-stressed plants were sprayed with brassinosteroid (BR).

Discussion

In view of a number of studies it is evident that exogenous application of BRs induces abiotic stress tolerance in plants, e.g., exogenous application of BRs increased salinity tolerance in rice (Anuradha & Rao, 2001), tomato (Prakash *et al*., 1999), and chickpea (Ali *et al*., 2007). Exogenous application of BRs increased shoot biomass as is evident from the data presented in Table 1. In growth and development, two phenomena cell division and cell enlargement play a vital role. However, BRs are known to affect both cell division and cell enlargement (Steffens, 1991). Stimulation in cell division due to BRs has already been observed in cultured parenchyma cells of *Helianthus tuberosus* (Clouse & Zurek, 1991), and in protoplasts of Chinese cabbage and petunia (Nakajima *et al*., 1996; Oh & Clouse, 1998). Cell expansion is controlled by different processes like coordinated alterations in wall mechanical properties, biochemical processes and gene expressions (Cosgrove, 2000). Primary cell wall in most of the dicotyledonous and non-Poaceae monocotyledonous plants is thought to be composed of cellulose microfibrills. However, in soybean BRs are thought to be involved in wall loosening of epicotyls (Zurek *et al*., 1994) and hypocotyls of *Brassica chinensis* and *Cucurbita maxima* (Wang *et al*., 2001). Thus, the increase in shoot growth in the two wheat cultivars due to exogenous BRs may have been due to stimulation of processes responsible for the cell division and cell elongation.

Mineral nutrients such as K^+ and Ca^{2+} are essentially required for the activities of enzymes, protein synthesis and integrity of cell wall and plasma membrane (Taiz & Zeiger, 2006). The results reported here for tissue ion concentration show an increase in accumulation of Na^+ and Cl^- coupled with a decrease in K^+ and Ca^{2+} in both cultivars, which are in agreement with the view that plants growing under saline conditions suffer ionic imbalance, nutrient deficiency and specific ion toxicity (Ashraf, 1994; 2004; Munns, 2002; 2005). However, exogenous application of EBL did not alter the accumulation of Na^+ and K^+ in the leaves of both cultivars. These results support the findings of Hayat *et al*. (2000) that BRs did not have a significant effect on accumulation of K^+ in mustard plants. Moreover, K^+/Na^+ ratios decreased due to salt stress in both wheat cultivars. These results can be explained in view of some earlier findings in which it was reported that high Na^+ concentration can block high affinity K^+ transporters resulting in decreased K^+ influx and increased Na^+ influx (Gassman *et al*., 1996; Amtmann & Sanders, 1999). However, foliar spray with 0.0125 mg L^{-1} EBL increased K^+/Na^+ ratio in salinized plants of MH-97 plants, whereas the ionic ratio remained almost unchanged in the shoots of salinized S-24 plants. The results for relative increase in K^+ (as calculated by K^+/Na^+ ratio) in the leaves of salt stressed plants of MH-97 due to EBL application do not support the findings of Haubrick *et al*. (2006) in which it was reported that exogenous application of brassinolide inhibited the inward rectifying K^+ channel of *Vicia faba* guard cell protoplasts. However, EBL-induced increase in K^+/Na^+ ratio was not accompanied with an increase in growth of salt stressed MH-97 at higher level of EBL. Similarly, EBL-induced increase in growth was not accompanied with corresponding increase in K^+/Na^+ ratio in the salt stressed plants of S-24. Thus, improvement in growth with epibrassinolide under saline conditions cannot be related to K^+/Na^+ discrimination.

In conclusion, salt stress reduced the growth of both wheat cultivars. This salt induced reduction in growth was ameliorated by the exogenous application of EBL in both cultivars. Although EBL application caused changes in K^+/Na^+ ratios, it did not have a prominent role in accumulation of different nutrients which contribute to salt tolerance in both wheat cultivars.

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