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SALINITY TOLERANCE IN BARLEY (*HORDEUM VULGARE* L.): EFFECTS OF VARYING NaCl, K^+/Na^+ AND $NaHCO_3$ LEVELS ON CULTIVARS DIFFERING IN TOLERANCE

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Abstract

Although barley (*Hordeum vulgare* L.) is regarded as salt tolerant among crop plants, its growth and plant development is severely affected by ionic and osmotic stresses in salt-affected soils. To elucidate the tolerance mechanism, growth and ion uptake of three barley cultivars, differing in salt tolerance, were examined under different levels of NaCl, K^+/Na^+ and $NaHCO_3$ in the root medium. The cultivars differed greatly in their responses to varying root medium conditions. Plant growth was more adversely affected by $NaHCO_3$ than NaCl. In general, biomass yields were comparable under control and 100 mM NaCl. However, growth of all three cultivars was significantly inhibited by $NaHCO_3$ even at low concentration (10 mM). Improved K^+ supply in saline medium increased K^+ uptake and growth of less tolerant cultivars. K^+ uptake was more adversely affected by $NaHCO_3$ than NaCl salinity. Selective K^+ uptake and lower Cl^- in shoots seemed to be associated with the growth responses. K application would help better growth of these cultivars on K-deficient saline-sodic soils and under irrigation with poor quality water having high Residual Sodium Carbonate (RSC) and/or Sodium Adsorption Ratio (SAR).

Introduction

Salinisation of agricultural soils is one of the major processes resulting in low crop productivity. One of the possible management options to the salinity problem, namely Saline Agriculture, aims at utilization (rather than reclamation) of salt-affected land to achieve economic crop production by growing suitable salt tolerant plants that provide a range of choices depending on the environmental conditions (Qureshi & Barrett-Lennard, 1998). Barley (*Hordeum vulgare* L.) is rated as salt tolerant among the crop plants; however, a great genetic variation exists for salt tolerance in its cultivars (Niazi *et al*., 1987, 1992). Salt tolerance in *Triticeae* is generally considered to be associated with Na^+ ion exclusion and plant's ability to sustain acquisition and maintain adequate levels of K^+ during growth under saline conditions (Kader & Lindberg, 2005; Colmer *et al*., 2005). Tavakoli *et al*., (2010) reported that salt tolerant barley genotype `Afzal' produced higher dry mass compared to salt sensitive genotype under salt stress conditions (200 mM NaCl) and higher tolerance in genotype Afzal was associated with a higher K^+/Na^+ ratio of the shoots. NaCl toxicity is largely attributed to the effects of Na^+ and only rarely to those of Cl^- (Tester & Davenport, 2003). Under saline field conditions, the plants may be subjected to different salt levels and ionic stresses. The objective of the present study was to investigate the effects of varying K^+/Na^+ ratios and Cl^- or bicarbonate levels in root medium on the growth of barley cultivars differing in salt tolerance.

Materials and Methods

Three cultivars of barley (*Hordeum vulgare* L.) were selected for these studies on the basis of their relative salt tolerance [i.e., electrical conductivity (EC) of root medium causing a 50% relative-to-control reduction in yield]: PK-30130 = 18.4 dS m^{-1}, PK-30163 = 14.6 dS m^{-1} and PK-30046 = 10.6 dS m^{-1} (Niazi *et al*., 1987). Seedlings were established and allowed to grow for a week in ½ strength Hoagland nutrient solution in pots filled with inert quartz gravel. Thereafter, the plants were exposed to equi-molar concentrations of NaCl (100 mM) and NaCl+KCl (95+5 mM) in two steps of 50 mM NaCl. In another experiment, one-week old seedlings were exposed to different levels of $NaHCO_3$ (10 or 20 mM) and $NaHCO_3$+NaCl (10+10 mM). Each treatment had four replicate pots, each with four seedlings. The salinity levels were maintained and solutions were aerated daily. The root medium solutions were completely replaced with fresh solutions having respective salt levels after every two weeks during the growth period. The plants were grown in the respective salinity treatments for about 7 weeks (42-45 days), and were harvested. Biomass of roots and shoots was determined separately. Plant parts (shoot/root) were analysed for Na^+, K^+ by flame photometry after wet digestion and for Cl^- by titration after extraction in hot water (80°C) for 15 minutes.

To investigate the effects of water quality on plant growth, barley cultivars grown in pots filled with normal soil were irrigated with canal water (EC = 0.25 – 0.30 dS m^{-1}) or with saline groundwater [EC = 4.8 dS m^{-1}, Sodium Adsorption Ratio (SAR) = 40, Residual Sodium Carbonate (RSC) = 21], each in 8 replicate pots. The plants were grown to maturity; total biomass and grain yield were recorded.

The data were subjected to statistical analysis and significance of differences between treatments was determined by *ANOVA* using PC package *CoStat* (*CoHort Software*, Berkeley, USA).

Results

All three cultivars had maximum biomass yield in control; fresh and dry masses of shoots decreased significantly in salt treatments (Table 1). However, the cultivars showed varied responses to salinity. Growth of cultivar PK-30163 was significantly decreased at 100 mM NaCl and addition of KCl in the root medium increased its growth. Other cultivars had comparable dry mass under both salinity treatments irrespective of K^+/Na^+ ratio in the medium. Root growth was less adversely affected by salinity than shoot growth in all cultivars. Water uptake did not appear to be affected under saline conditions as only minor differences were observed in the dry/fresh mass ratio, a measure of tissue water content (Table 1).

Na^+ contents in shoots of all cultivars increased significantly under NaCl treatment. Addition of KCl in root medium solution had little effect on shoot Na^+ contents of PK-30130 but decreased Na^+ content in varieties PK-30163 and PK-30046. K^+ contents in shoots of all cultivars increased and K^+/Na^+ ratios in shoots were higher in NaCl+KCl treatment than in NaCl alone (Table 1). Cl^- contents in shoots increased under saline treatments and addition of KCl tended to reduce Cl^- uptake in cultivars having lower tolerance (PK-30163 and PK-30046). Cl^- contents in shoots of PK-30130 grown in NaCl alone or NaCl+KCl were similar (Table 1).

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Table 1. Effect of NaCl (100 mM) and NaCl+KCl (95+5 mM) on growth and concentrations of sodium, potassium and chloride in shoots of barley cultivars.
Values are means of four replicates, each with four plants. Electrical conductivity of the root medium solution was maintained as: Control = 2.3±0.2 dS m^{-1}, NaCl (100 mM) = 12.0±0.5 dS m^{-1}, and NaCl+KCl (95+5 mM) = 12.5±0.5 dS m^{-1}.

Cultivar	Control	NaCl (100 mM)	NaCl+KCl (95+5 mM)
		Shoot fresh mass (g pot^{-1})	
PK-30130	56.97a*	36.02b	30.70b
PK-30163	42.95a	20.85c	30.90b
PK-30046	41.55a	31.55b	35.60ab
		Shoot dry mass (g pot^{-1})	
PK-30130	4.65a (0.081)**	3.43a (0.095)	2.71a (0.088)
PK-30163	3.33a (0.077)	1.49b (0.071)	2.67ab (0.086)
PK-30046	3.74a (0.090)	3.14a (0.099)	3.19a (0.089)
		Root dry mass (g pot^{-1})	
PK-30130	4.66a	4.44a	3.85b
PK-30163	4.61a	3.77b	4.11ab
PK-30046	4.97a	4.13a	4.31a
		Sodium concentration (mg g^{-1} dry weight)	
PK-30130	10.92b	39.96a	39.26a
PK-30163	10.06c	44.27a	38.71b
PK-30046	9.2c	47.15a	38.81b
		Potassium concentration (mg g^{-1} dry weight)	
PK-30130	44.16a	19.01c	25.40b
PK-30163	40.50a	21.65c	26.52b
PK-30046	40.57a	20.47c	27.20b
		K^+/Na^+ ratio	
PK-30130	4.04a	0.475b	0.646b
PK-30163	4.02a	0.489b	0.686b
PK-30046	4.41a	0.434c	0.701b
		Chloride concentration (mg g^{-1} dry weight)	
PK-30130	25.20b	57.58a	60.14a
PK-30163	25.81c	77.49a	59.42b
PK-30046	24.42b	67.82a	63.73a

*Values followed by different letters in a row differ significantly at $p \leq 0.05$ level
**Values in parentheses are dry/fresh mass ratios

Growth of all cultivars was adversely affected by $NaHCO_3$ in the root medium. The effect was more pronounced in less tolerant cultivars (Table 2). $NaHCO_3$ (20 mM) proved more toxic than equi-molar level of $NaHCO_3$+NaCl (10+10 mM). Na^+ contents in shoots and roots of barley cultivars increased while K^+ contents decreased under $NaHCO_3$. Shoot K^+ contents, particularly in cultivar PK-30130, were higher under $NaHCO_3$+NaCl (10+10 mM) than in $NaHCO_3$ (20 mM) treatment. In contrast to comparable Na^+ contents in roots and shoots, K^+ concentrations in shoots were higher than in roots of all cultivars, in general. This was very clear from invariably higher K^+/Na^+ ratios in shoots than respective roots, indicating selective transport of K^+ from root to shoot (Table 2).

When grown in normal soil, all the three cultivars had comparable growth under irrigation with canal water or saline groundwater (Table 3). However, overall biomass yield was decreased while grain yield was not affected under irrigation with saline water. Salinity and sodicity (EC = 5.55±0.33 dS m^{-1}, SAR = 42.0±1.45) of soil irrigated with saline water were higher than soil irrigated with fresh water (EC = 0.86±0.03 dS m^{-1}, SAR = 3.24±0.06) indicating accumulation of salts in the former treatment.

Discussion

Growth of all cultivars was decreased to varying degrees in saline treatments as compared to control (Table 1). Reduced growth under saline conditions is a common response of many plant species including barley (Mahmood *et al*., 1996; Niazi *et al*., 1987, 1992). Garthwaite *et al*. (2005) reported that among *Hordeum* spp., growth of *H. vulgare* was more adversely affected by salinity (150-450 mM) compared to wild species. In other studies, growth of barley seedlings was inhibited at 150 mM NaCl, but not at 75 mM (Cramer *et al*., 1989). The dry/fresh mass is a measure of water uptake, the ratio being inversely related to water content (Naidoo, 1985). Water uptake did not appear to be related with growth reduction under saline conditions as the most affected plants (PK-30163) in 100 mM NaCl treatment had lower dry/fresh mass ratio indicating higher tissue water content (Table 1).

Salt tolerance is generally considered to be associated with Na^+ ion exclusion during growth under saline conditions (Tester & Davenport, 2003; Colmer *et al*., 2005; Kook *et al*., 2009). Ion concentrations in shoots of plants grown in control were similar for different barley cultivars. Na^+ and Cl^- in plant shoots increased while K^+ concentration decreased significantly under saline (100 mM NaCl or NaCl+KCl) treatments (Table 1). Na^+ toxicity is strongly linked to plant's ability to maintain uptake and within plant distribution of K^+ (Kader & Lindberg, 2005). In the present studies, a similar trend was observed as indicated by lower K^+/Na^+ ratios in plant shoots in 100 mM NaCl treatment. Further, the K^+/Na^+ ratios improved with addition of KCl in the root medium, i.e., NaCl+KCl (95+5 mM) treatment. In wheat, grain yield was correlated with Na^+ exclusion and associated enhanced K^+/Na^+

discrimination, although this relationship does not hold across all genotypes (El-Hendawy *et al*., 2005), showing that Na^+ exclusion (i.e., higher K^+/Na^+ ratio) is not the only mechanism of salt tolerance (Munns *et al*., 2006). Kronzucker *et al*., (2006) reported that growth response of a cultivar can be identical in the presence of cytosolic Na^+/K^+ ratios that differ by as much as five-fold. NaCl toxicity is largely attributed to the effects of Na^+ and rarely those of Cl^- (Tester & Davenport, 2003). Contrary to this, growth of barley cultivar PK-30163 was lowest in the saline treatment (100 mM NaCl) where shoots accumulated the highest Cl^- (Table 1). Wild *Hordeum* species maintained lower concentrations of Cl^- in leaves than *H. vulgare* even at high salinity, and such restricted entry of Cl^- and Na^+ into shoots was related to salt tolerance (Garthwaite *et al*., 2005).

Table 2. Effect of different levels of $NaHCO_3$ (10 or 20 mM) and $NaHCO_3$+NaCl (10+10 mM) on growth and sodium and potassium concentrations in barley cultivars. Values are means of four replicates, each with four plants.

Cultivar	Control	$NaHCO_3$ (10 mM)	$NaHCO_3$ (20 mM)	$NaHCO_3$+NaCl (10+10 mM)
	Shoot fresh mass (g pot^{-1})			
PK-30130	94.45a*	88.05ab	47.02c	70.52b
PK-30163	108.4a	51.62c	44.62c	79.62b
PK-30046	114.6a	72.95b	40.95c	72.17b
	Shoot dry mass (g pot^{-1})			
PK-30130	15.62a	13.37ab	7.22c	11.10b
PK-30163	14.87a	7.47b	7.45b	13.12a
PK-30046	15.57a	10.67b	6.75c	11.17b
	Root dry mass (g pot^{-1})			
PK-30130	5.77a	4.57ab	2.80c	4.25b
PK-30163	6.30a	2.95b	2.75b	4.32ab
PK-30046	5.09a	4.45a	2.67b	4.15a
	Shoot sodium (mg g^{-1} dry weight)			
PK-30130	6.61c	19.26b	25.87a	25.30a
PK-30163	8.43c	19.55b	24.72a	23.00a
PK-30046	7.76c	18.68b	25.30a	23.00ab
	Shoot potassium (mg g^{-1} dry weight)			
PK-30130	37.44a	34.02ab	27.10c	30.12bc
PK-30163	45.63a	36.85b	24.53c	27.01c
PK-30046	47.38a	32.17b	24.86c	26.52c
	Shoot K^+/Na^+ ratio			
PK-30130	5.672 (4.603)**	1.766 (3.514)	1.047 (2.164)	1.190 (2.957)
PK-30163	5.411 (4.684)	1.885 (2.295)	0.992 (2.055)	1.174 (2.505)
PK-30046	6.106 (4.117)	1.722 (3.010)	0.982 (1.972)	1.153 (2.468)
	Root sodium (mg g^{-1} dry weight)			
PK-30130	11.78b	23.86a	24.98a	26.16a
PK-30163	12.93c	21.85b	25.30a	21.85b
PK-30046	13.41b	22.13a	24.72a	23.57a
	Root potassium (mg g^{-1} dry weight)			
PK-30130	14.52a	11.99a	12.09a	10.53a
PK-30163	14.95ab	17.94a	12.18bc	10.23c
PK-30046	19.89a	12.67b	12.28b	11.01b
	Root K^+/Na^+ ratio			
PK-30130	1.232	0.502	0.483	0.402
PK-30163	1.155	0.821	0.481	0.468
PK-30046	1.483	0.572	0.497	0.467

*Values followed by different letters in a row differ significantly at $p \leq 0.05$ level.

**Values in parentheses are K^+/Na^+ ratio in shoot divided by K^+/Na^+ ratio in root, a measure of selectivity for K^+ transport from root to shoot.

The growth of all barley cultivars was inhibited by $NaHCO_3$ even at low concentration (10 mM) in the root medium (Table 2). Cultivar PK-30163 was the most severely affected as the magnitude of its growth reduction in 10 mM $NaHCO_3$ treatment was much higher than other cultivars. Addition of NaCl in the root medium did not alter the toxic effects of $NaHCO_3$ on cultivars PK-30130 and PK-30046. However, growth of PK-30163 was significantly improved in $NaHCO_3$+NaCl (10+10 mM) treatment than in 10 mM $NaHCO_3$ (Table 2). Selective K^+ uptake has been reported to be associated with salt tolerance in many species (Mahmood *et al*., 1996). However, higher K^+/Na^+ ratio does not always correlate with salt tolerance. No K^+-Na^+ selectivity occurred in sugar beet (Hasegawa & Yoneyama, 1995), a salt tolerant species whereas *Sesbania rostrata* having medium salt tolerance exhibited high discrimination for K^+ uptake (Mahmood, 1998). In the present studies, clear differences were noted among the cultivars for K^+ uptake and within plant distribution under $NaHCO_3$ treatment (Table 2). K^+/Na^+ ratios in roots and shoots of more salt tolerant cultivar (PK-30130) were higher than those in the less tolerant cultivars. Further, selective transport of K^+ from root to shoot was more efficient in cultivar PK-30130 as indicated from higher K^+/Na^+ ratios in shoots than roots. In both experiments, growth responses were related to the efficiency of cultivars to maintain K^+ uptake under stress conditions; the salt tolerant cultivar PK-30130 performed better under $NaHCO_3$.

When grown in normal soil, all the three cultivars had comparable growth under irrigation with canal water or saline groundwater (Table 3). However, overall biomass yield was decreased while grain yield was not affected under saline irrigation water. Salinity and sodicity (EC = 5.55±0.33 dS m^{-1}, SAR = 42.0±1.45) of soil irrigated with saline water were higher than soil irrigated with fresh water (EC = 0.86±0.03 dS m^{-1}, SAR = 3.24±0.06) indicating accumulation of salts in the former treatment.

The present studies have shown that toxic effects of $NaHCO_3$ are more detrimental than NaCl for growth of barley; even low levels of $NaHCO_3$ in root medium impaired uptake of K^+. Thus K application would help improve growth of, particularly less salt tolerant, barley cultivars on K-deficient saline-sodic soils and under irrigation with poor quality (high RSC) water. Further, screening tests using cations and anions combinations closer to the target soils rather than NaCl in solution culture may be more relevant to the field conditions.

Table 3. Effect of canal water (EC = 0.25 – 0.30 dS m^{-1}) and saline water (EC = 4.8 dS m^{-1}, SAR = 40, RSC = 21) irrigation on barley cultivars grown in normal soil.

Values are means ± SD of 8 replicate pots, each with four plants.

Cultivar	Total biomass (g pot^{-1})		Grain yield (g pot^{-1})	
	Fresh water	Saline water	Fresh water	Saline water
PK-30130	22.78 ± 1.92	20.95 ± 1.20	8.85 ± 1.50	8.38 ± 1.37
PK-30046	23.00 ± 1.32	20.57 ± 1.57	8.52 ± 0.95	8.57 ± 1.07
PK-30163	22.93 ± 0.98	20.83 ± 1.64	8.42 ± 0.92	8.26 ± 1.49
Mean (water treatment)	22.90	20.78*	8.59	8.40

*Saline water irrigation had significant ($p \leq 0.05$) effect on biomass yield.

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