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AVOIDANCE OF SODIUM ACCUMULATION IN THE SHOOT CONFERS TOLERANCE TO SALT STRESS IN CULTIVATED BARLEY

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Abstract

Growth and development of plants is adversely affected by ionic and osmotic stresses in the saline soil. Although barley (*Hordeum vulgare*) is regarded as relatively tolerant to salt stress among the Tririceae, modern barley cultivars suffer severely at salt concentrations encountered in the field constructed in reclaimed tidelands. This study was conducted to elucidate salt tolerance mechanisms of a collection from Tunisia (Tunisia 76; T76) at the germination and early seedling stages. Gwandongpi 41 (G41) was used as a salt-susceptible control variety. Germination was examined in a culture solution containing 0, 150, 300, and 450 mM NaCl, respectively. Mineral content was analyzed for the three-week-old seedlings grown in 0 or 200 mM NaCl solutions for 0, 1, 3, and 5 days, respectively. Both varieties showed drastic differences in root and shoot growth at germination in saline conditions. T76 showed over 97% germination while G41 only 7% at 300 mM NaCl. The root and shoot growth at germination was negatively related to Na^+ contents in the shoots. Sodium ion content was about three times higher in G41 than in T76 in the shoot of the seedlings treated for 5 days at 200 mM NaCl. The K^+/Na^+ ratio, which was mostly affected by Na^+ content, was significantly higher in T76 than in G41. These results indicate that higher salt tolerance of T76 is conferred, at least in part, by the avoidance of salt accumulation in the shoot at high salt conditions.

Introduction

Saline environments adversely affect plant growth and development by the toxic effects of salts in the soil and absorbed by plants. However, plant species respond differently to the saline environments. Halophytes naturally grow even in soils where NaCl concentrations are higher than 250 mM. On the other hand, the large majorities of plant species are glycophytes and are easily damaged by salinity (Ashraf *et al*., 2008). Among the cereals, barley (*Hordeum vulgare*) is most-tolerant to salinity, and is considered as marginal halophytes (Glenn *et al*., 1998).

Barley can tolerate about 5 grams per liter of dissolved salts (Ayers & Wescott, 1989; Glenn & Brown, 1999). However, the levels of tolerance vary by the genotype and the developmental stage. In general, salt tolerance is higher in cultivated barley than wild barley species at seed germination, but vice versa at the seedling stage (Mano & Takeda, 1998). The levels of salt tolerance are variable among varieties of different geographical origins and even among those sharing the same geographical origin (Mano & Takeda, 1998; Lee *et al*., 1997).

Salt tolerance of barley plants is also affected by soil conditions. In dry saline soils, the primary limiting factors for plant growth and development are high pH and sodicity. In case where other abiotic stress factors interact with salinity, plant growth is further adversely affected. For example, in the irrigated saline land and reclaimed tideland where ground water level is high, hypoxic root zone due to excessive water further reduces plant's tolerance to salinity (Barrett-Lennard, 2003; Colmer *et al*., 2005).

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Plants have variable ability to adjust under saline conditions, and the capacity depends on the array of response mechanisms to salinity (Siddiqi *et al*., 2007). Some of the important mechanisms conferring plants tolerance to salinity include ion exclusion (Munns & James, 2003; Ashraf, 2004; Naeem & Muhammad, 2006; Hameed *et al*., 2008), maintenance of K^+ uptake (Gorham, 1993), osmotic adjustment, tissue tolerance of high Na^+ (Yeo & Flowers, 1983), accumulation of Na^+ in older leaves (Wolf *et al*., 1991) and enhanced vigor (Colmer *et al*., 2005).

A large agricultural field is being developed in the reclaimed tideland in the southwestern costal area of the Republic of Korea. As a large portion of the field is going to be used for crop cultivation, we selected a salt-tolerant barley variety T76 (a collection from Tunisia, Tunisia 76) from the previous germplasm screening experiment conducted in a saline paddy field in the reclaimed tideland. In this study we attempted to understand physiological bases of tolerance of T76 to salinity by comparatively investigating its physiological responses to saline conditions with those of a salt-susceptible variety.

Materials and Methods

Experimental materials: In the preliminary study, we screened 1,038 varieties and breeding lines of barley in the paddy field, which was developed in the reclaimed tideland and contained NaCl by 0.4% (w/w). For this study we selected a salt-tolerant variety, Tunisia 76 (T76), and a salt-susceptible variety, Gwandongpi 41 (G41) based on their performance in the saline experimental field (Table 1). T76, a landrace of Tunisia, is six-rowed covered barley and G41, Japanese six-rowed naked barley.

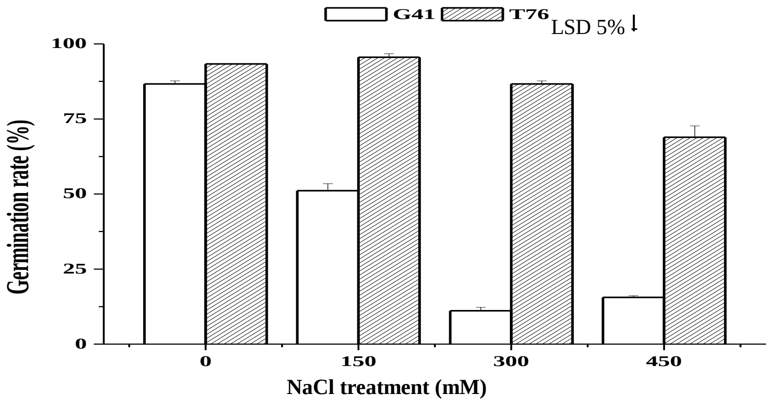
Measurement of germination and early seedling growth: Germination experiments were conducted in Petri dishes containing two layers of filter papers which were soaked in 0, 150, 300, or 450 mM NaCl solutions. Seeds were surface-sterilized in 1% sodium hypochloride solution for 20 min., and washed with sterile distilled water five times. Then seeds were imbibed at 25°C in the dark for 24 h. Ten seeds were placed on each plate and the plates were incubated in a growth chamber set at 25°C with a 16 h/8 h day/night cycle. Seed germination rate was calculated from number of seeds with roots longer than 2 mm which were counted at five days after germination. Shoot growth rate was determined by the number of seedlings whose shoot length was longer than 2 mm at five days after germination.

Mineral ion analysis: In separate experiments, Na^+ and K^+ concentrations were determined in the seedlings at the three-leaf stage. Seedlings were raised up to the three-leaf stage in a solution culture system. Culture solution was formulated according to Kimura (Park *et al*., 2003) and was constantly aerated. The seedlings were treated with the culture solution containing 200 mM NaCl for up to five days. Sodium chloride concentration of the solution was increased up to 200 mM by the daily increment of 50 mM. Samples were collected every day after the initiation of NaCl treatment and dried in an oven at 70°C for 3 days. Dried samples were wet-digested using the mixture of H_2SO_4 and H_2O_2 (Lee & Kim, 2001), and Na^+ and K^+ content was analyzed using an inductively coupled plasma spectrophotometer (ICPS-7500, Shimadzu Corp., Japan). All experiments were replicated three times and data were analyzed using the statistical analysis program, Statistix (Statistix 9 Analytical Software, USA).

Table 1. Performance of Gwandongpi 41 (G41) and Tunisia 76 (T76) in a saline field containing 0.4% (w/w) of NaCl.

	Seedling establishment (%)*		Effective tiller (ea)		Plant height (cm)	
	G41	**T76**	**G41**	**T76**	**G41**	**T76**
Control	80.00	85.00	6.67	4.86	47.50	51.93
Saline soil (0.4% (w/w)	50.00	80.00	1.00	1.00	15.80	24.36

*[number of seedlings established after two months from planting/80 seeds planted]*100



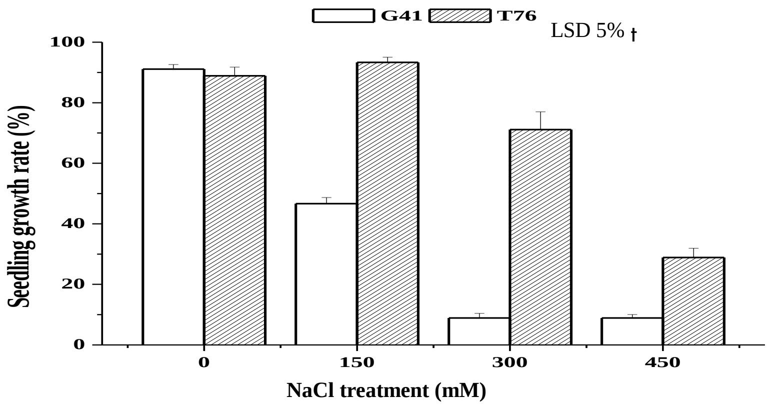


Fig. 1. Germination rate and seedling growth rate of Gwandongpi 41(G41) and Tunisia 76 (T76) in various saline conditions. Seeds were germinated at 0, 150, 300, or 450 mM NaCl solutions.

Results

Seed germination: Seed germination and seedling growth under the saline conditions were drastically contrasting between the selected salt-tolerant variety, T76, and the salt-susceptible variety, G41. At 150 mM NaCl, germination rate of T76 was maintained over 95% whereas that of G41 was decreased to 51%. Germination rate of T76 was 69% but that of G41 was 16% at 450 mM NaCl. Similarly, shoot growth rate was 93% in T76 whereas 45% in G41 at 150 mM NaCl. At 450 mM, the shoot growth rate decreased to 29% in T76 but to 9% in G41 (Fig. 1).

Table 2. Effects of 200 mM NaCl on the vegetative growth of Gwandongpi 41 (G41) and Tunisia 76 (T76). Seedlings at the three-leaf-stage were treated with 200 mM NaCl for five days.

		Length (cm)		Fresh weight (g)		Dry weight (g)	
		G41	T76	G41	T76	G41	T76
Shoot	Control	29.06 ± 2.04[b]	37.47 ± 0.32[a]	5.00 ± 0.83[a]	5.82 ± 0.76[a]	0.48 ± 0.09[a]	0.51 ± 0.07[a]
	NaCl	24.42 ± 0.54[a]	32.90 ± 0.75[b]	4.15 ± 0.57[b]	4.58 ± 0.47[a]	0.47 ± 0.06[a]	0.45 ± 0.05[a]
	P-value	0.068	0.003	0.032	0.131	0.510	0.458
	LSD (p=0.05)	5.478	1.127	0.673	2.161	0.087	0.274
Root	Control	15.61 ± 0.79[a]	21.06 ± 0.45[a]	0.97 ± 0.21[a]	0.81 ± 0.06[a]	0.08 ± 0.02[a]	0.07 ± 0.02[a]
	NaCl	13.04 ± 0.43[b]	17.37 ± 0.73[b]	1.03 ± 0.27[a]	0.70 ± 0.16[a]	0.10 ± 0.02[a]	0.07 ± 0.01[a]
	P-value	0.040	0.002	0.261	0.432	0.110	0.713
	LSD (p=0.05)	2.270	0.717	0.161	0.497	0.020	0.065

Means followed by the same letter in each column are not significantly different (LSD, $p<0.05$).

Seedling growth: Seedlings at the three-leaf stage were subjected to 200 mM NaCl treatment for up to five days and their growth responses were analyzed. In the control condition, shoot length, shoot fresh weight and shoot dry weight were by 22%, 14%, and 6% higher in T76 compared to G41. Root length was by 26% higher but root fresh weight and root dry weight by 19% and 14% lower in T76 than in G41. All the shoot characteristics were decreased by 3% to 21% under the NaCl treatment in the two varieties. Though the root fresh weight and dry weight were increased by 6% and 25%, respectively, in G41, those were decreased by 14% or unchanged, respectively, in T76 (Table 2).

Mineral ion content: Sodium ion and K^+ contents in the three-leaf stage seedlings were analyzed for up to 5 days after 200 mM NaCl treatment. Under the normal condition, Na^+ and K^+ contents in the shoot and root were by 10 to 50% higher in T76 than in G41 except K^+ in the root which was by 10% higher in G41 than in T76. According to expectation, Na^+ content of the shoot and root drastically and gradually increased up to 5 days of NaCl treatment in the two cultivars. In the NaCl-treated shoots and roots of G41, Na^+ content increased by 5- to 72-fold and by 12- to 42-fold, respectively, relative to that in control plants. However, in the NaCl-treated shoots and roots of T76, Na^+ content increased by 3- to 14-fold and by 2- to 5-fold, respectively, relative to that in control plants (Fig. 2 A and B). Potassium ion content in the NaCl-treated shoots and roots of G41 decreased by 13% to 31% and by 69% to 98% compared to that in the control plants, respectively. Potassium ion content in the NaCl-treated shoots and roots of T76 decreased by 8% to 13% and by 77% to 98% compared to that in the control plants, respectively (Fig. 2 C and D). The K^+/Na^+ ratio in the roots and shoots of the NaCl-treated G41 plants decreased by 83% to 99% and by 97% to 99% compared to that in the control plants, respectively. The K^+/Na^+ ratio in the roots and shoots of the NaCl-treated T76 plants decreased by 74% to 94% and by 96% to 99% compared to those in the control plants, respectively (Fig. 3 A and B).

Discussion

Germination and seedling growth: It is known that germination and early growth of barley is significantly affected by saline conditions like 250 mM or more of NaCl (Glenn *et al.*, 1998). But there existed a large difference in seed germination and seedling growth rates between T76 and G41 in the saline conditions. NaCl concentrations to inhibit germination by 50% were about 150 mM for G41 and over 450 mM for T76, respectively. NaCl concentrations to inhibit shoot growth of germinating seedlings by 50% were about 150 mM for G41 and 450 mM for T76, respectively (Fig. 1). Significant variations in salt

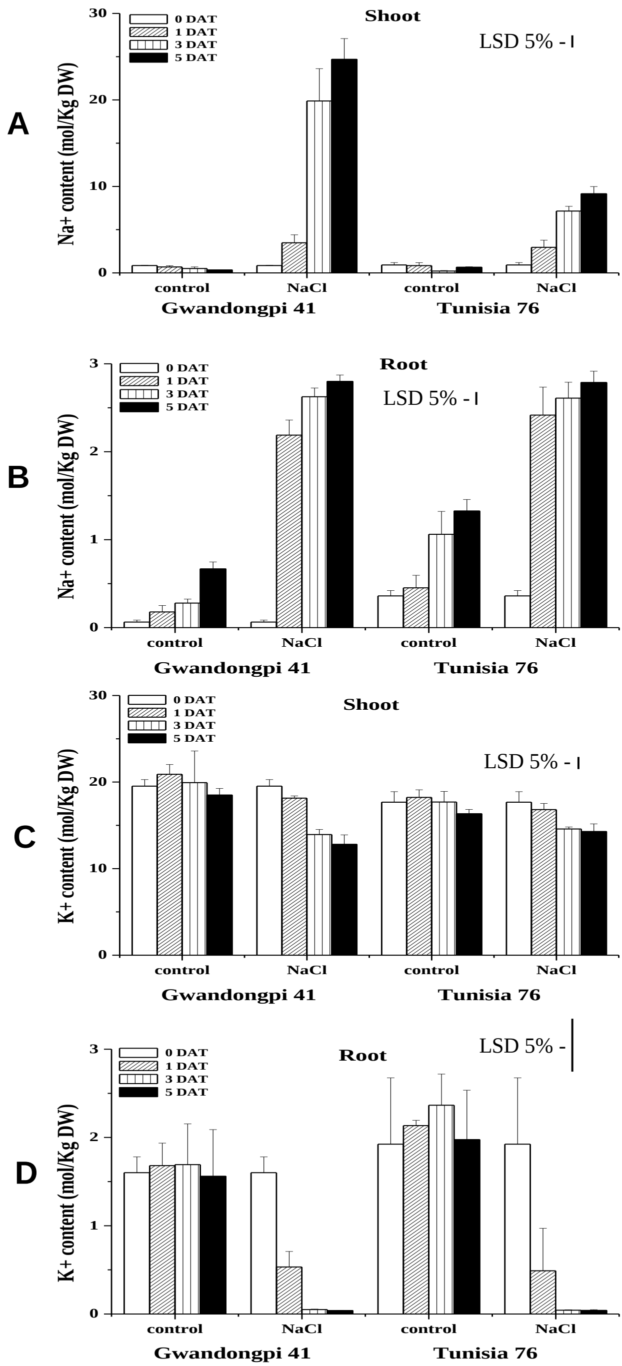


Fig. 2. Na^+ and K^+ contents in the shoot and root of Gwandongpi 41(G41) and Tunisia 76 (T76) plants at the three-leaf-stage. The seedlings were treated at 200 mM NaCl for 0, 1, 3, and 5 days, respectively.

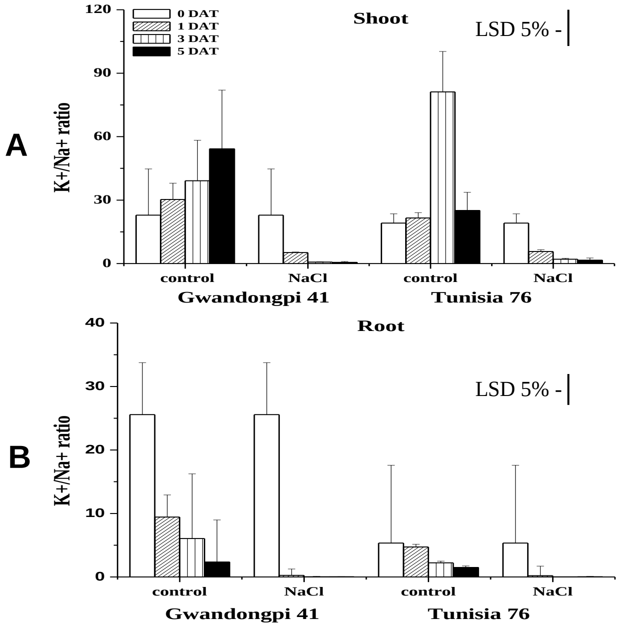


Fig. 3. K^+/Na^+ ratio in the shoot and root of Gwandongpi 41(G41) and Tunisia 76 (T76) plants at the three-leaf-stage. The seedlings were treated at 200 mM NaCl for 0, 1, 3, and 5 days, respectively.

tolerance of barley at germination stage were also reported among Korean cultivars (Lee *et al*., 1997), Australian and Chinese cultivars (Tajbakhsh *et al*., 2006), cultivars of world barley collection (Mano *et al*., 1996) and wild barley species (Mano & Takeda, 1998).

Contrary to the drastic difference in germination between T76 and G41 under saline conditions, difference in growth retardation of young seedlings at the three-leaf stage under 200 mM NaCl was relatively small (Table 2). Interestingly, the degree of retardation of seedling growth, especially that of root growth under saline stress was relatively greater in the salt-tolerant T76, indicating that higher salt tolerance of T76 at germination stage was not maintained at the seedling stage. Similarly, salt tolerance at germination was not significantly related to the rate of decrease in seedling growth in Australian and Chinese barley cultivars (Tajbakhsh *et al*., 2006). Likewise, no relation was found between salt tolerance at the germination stage and that at the seedling stage in a comprehensive study with over 6,000 world collection of barley cultivars (Mano *et al*., 1996). Conversely, growth parameters of young seedlings may not effectively represent salt tolerance levels. Seedling growth traits were valued little as selection indices for salt tolerance in barley (Tajbakhsh *et al*., 2006).

Na^+ concentration in the shoot: Sodium ion is the primary cause of ion-specific damage for most graminaceous crops under saline conditions. Thus, the higher Na^+ concentration, the more severe is the damage caused by the ion. Under the saline condition, Na^+ concentration increased about 5-fold more rapidly and the final content was 2.7-fold higher in the shoot of G41 than in that of T76. Na^+ concentration in the root also increased about 2-fold more rapidly in G41 than in T76. Under the saline condition, K^+

content in the shoot and root decreased more significantly in G41 than in T76. Thus, the K^+/Na^+ ratio in the shoot of salt-treated plants was maintained significantly higher in T76 than in G41. These results indicate that the ability to restrict entry of the potentially toxic Na^+ into the shoot is greater in the salt-tolerant T76 than in salt-susceptible G41. The ability to restrict entry of the potentially toxic Na^+ into the shoot, often termed ion exclusion, is known to be the most useful trait for salt tolerance in barley and wheat (Colmer *et al*., 2005). Sodium ion in the xylem can be removed by the exclusion systems operating in the upper part of the root, the stem, petiole or leaf sheath (Munns, 2002; Tester & Davenport, 2003).

Metabolic toxicity of Na^+ is largely caused by competition with K^+ for binding sites of protein components essential for cellular processes where Na^+ cannot substitute the role of K^+. Thus, maintaining optimal levels of K^+ under saline conditions can alleviate toxic effect of Na^+. Hence, concurrent maintenance of low levels of Na^+ and high levels of K^+ can mitigate toxicity of Na^+ (Munns *et al*., 2000b; Flowers & Hajibagheri, 2001). In this regard, T76 is favored for salt tolerance by retaining more K^+ under high NaCl conditions.

T76 was selected for salt-tolerance based on whole plant performance for an entire life cycle in the saline field. Thus, multiple processes operating both in the specific cells and a whole plant may contribute to salt tolerance of T76. As salt tolerance is a quantitative trait which is affected by many genes, other traits such as osmotic adjustment, tissue tolerance of high Na^+ (Yeo & Flowers, 1983), accumulation of Na^+ in older leaves (Wolf *et al*., 1991), and enhanced vigor (Colmer *et al.*, 2005) also could contribute to tolerance of T76 in the saline conditions.

Taken together, the results from this study indicate that the greater ability to prevent Na^+ from accumulation in leaves is an important trait for salt tolerance of T76.

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