(Korean J. Crop Sci.) 44(1) : 4~10(1999)

Effect of NaCl on Nitrogen Content of Barley Seedlings

Choong-Soo Kim*, Jin-Woong Cho*, Sok-Young Lee**, and Kwan-Soo Park*

ABSTRACT

This study was conducted to determine the effects of NaCl stress on nitrogen, NH_4^+, and NO_3^- content of 4 barley cultivar seedlings that were cultured for 10 and 30 days with different NaCl levels (0, 50, 100, and 150 mM) containing 1/4 Hoagland solutions.

The sodium ion content in the shoot of barley seedlings sharply increased with an increase of NaCl concentration. After 30 days of NaCl treatment, the sodium content of the shoot at 150 mM NaCl was 27 times higher than in non-saline conditions. The sodium content in the root linearly increased with increasing NaCl concentration. Nitrogen content in the shoot linearly increased with increasing NaCl concentration, but nitrogen content in the root declined above the point where the Na^+ content was 3.0 mM/g Na^+ in the barley seedling. NO_3^- content also decreased with increasing NaCl concentration. NH_4^+ content in the shoot decreased with NaCl condition, but its content in the root increased with NaCl condition. A positive correlation between NO_3^- and NH_4^+ content was found in the shoot, but their relationship was negative in the root.

Key words : NaCl, Na^+, nitrogen, NO_3^-, NH_4^+, barley.

The response of crop to salinity varied among species. Greenway and Munns (1980) reported that barley was considered as a relatively salt-tolerant plant. Thus, it could be cultivated under saline conditions as drainage or reclaimed land area in South Korea. But it is very difficult to obtain good yields because of high salt concentration in the soil.

The major factors of growth inhibition in salinity conditions are nutrient unbalance, low water potential and ion toxicity (Greenway & Munns, 1980). High salt concentration disturbs the ion balance and ion absorption, and interferes with transpiration of essential elements. This will result in plant death.

The effect of salinity on plant varied according to growth stages. Saline responses are the most sensitive at the seedling stage in rice (Akbar & Yabuno, 1974) and barley (Lynch & Laüchli, 1984), the vegatative stage in sorghum (Boursier & Laüchli, 1990), and the germination stage in sugarbeets (Maas & Hoffman, 1977).

Nitrogen is usually the most growth-limiting plant nutrient. The interaction of salinity and nitrogen has been observed in generally deficient nitrogen conditions. In general, addition of nitrogen improved growth and yield of barley, bean, corn, and rice when the degree of salinity was not severe. However, applied nitrogen did not improve the growth and decreased plant salt tolerance under extreme saline conditions (Grattan & Grive, 1994).

The object of this study was to investigate the effects of NaCl concentration on nitrogen, NO_3^- and NH_4^+ contents in barley seedlings.

MATERIALS AND METHODS

Seedlings of four Korean barley cultivars "Neulssalbori", "Chalssalbori", "Bunong" and "Dongbori 1" were grown hydroponically in the growth chamber under approximately 25,000 lux (day/night, circa 12/12 hour and day/night temperatures of 26/21℃) for 10 and 30 days with 4 NaCl treatments (0, 50, 100, and 150 mM). The nutrient solution was modified 1/4 Hoagland's solution (pH 5.8).

Total nitrogen, NO_3^-, and NH_4^+ contents of barley seedlings grown for 10 and 30 days after sowing were measured. All samples were dried at 70℃ for 72 hours in a dryoven and ground with 20 mesh. Total nitrogen content was determined by the modified Kjeldahl method. That was, 1g sub-samples were boiled in a Kjeldahl flask filled with 9 g K_2SO_4, 1 g $CuSO_4$, 25 ml concentrated H_2SO_4 for 2 hours about 550℃. Sample was cooled when it was clear and 200 ml of distilled water was added. After the digested sample was transfered to the Kjeldahl distillation apparatus, filled 60% NaOH of 70~100ml were boiled. They distilled for 7 minutes and then prepared a 250ml Erlenmeyer flask containing 50ml of 4% boric acid and three drops of mixed indicator (0.1% methyl red, 0.5% bromocresol green). There were titrated the solutions of boric acid and mixed indicator containing the distilled off ammonia with the standardized 0.1N HCl.

Nitrate content was determined by modified Cataldo et al. (1975) method. For nitrate analysis, the seedling tissue was ground with a mortar and pestle in 2 volumes of deionized water and centrifuged for 15 minutes. The supernatant was used for analysis. The extracted solution of 0.2 ml was mixed with 0.8ml of 5% salicylic acid in sulfuric acid. After 20 minutes, this complex solution was gently mixed with 19.0 ml of 2 N NaOH (above pH 12). Absorbance was read at 410 nm (Shimadzu uv-120-02). Ammonium analysis used 0.2 ml of extracted solution to which was added 1 ml of reagent I (1 L of deionized water containing 50 g phenol, 0.25 g sodium nitroprusside) and 1 ml of reagent II (1 L of deionized water containing 25.0 g sodium hydroxide, 21.0 g sodium hypochlorite). The solution was incubated in a water bath

* College of Agriculture, Chungnam National University., Taejon 305-764, Korea (E-mail : jwcho@hanbat.chungnam.ac.kr).
** National Agricutural Science and Technology Institute, RDA, Suwon 441-707, Korea.
Received 20 Oct. 1998.

at 50~60℃ for 5 minutes. After diluting the solution with 23 ml water, the sample was read by absorbance at 625 nm.

Na^+ was extracted in 1 N HCl at 30℃ for 24 hours and its content was measured by atomic absorption spectrophotometer (Baird Automic Ltd. Model Alpha 4).

RESULTS AND DISCUSSION

Na^+ content in barley seedlings

Na^+ content of 4 barley cultivar seedlings that were grown for 10 and 30 days under four different NaCl treatments are shown in Table 1. In the 10 day old seedlings, in general, the Na^+ content of barley seedlings sharply increased with NaCl treatment. The means of Na^+ content of non-saline condition were 0.34 mM (g dry weight) in the shoot and 0.32 mM in the root. The Na^+ content at 150 mM NaCl treatment was 8.60 mM in the shoot and 8.65 mM in the root approximately 30 times higher than those of non-saline condition.

In the 30 day old seedlings, Na^+ content also sharply increased with NaCl treatments. Sodium contents at 50, 100, and 150 mM NaCl levels in the whole plant (shoot and root) were 7.5, 9.9 and 12.1 times higher than in non-saline condition. In the non-saline condition, Na^+ content was lower in the shoot than in the root, but Na^+ contents with 50, 100 and 150 mM NaCl treatments were higher in the shoot than in the root. The result might indicate that absorbed sodium might be positively translocated to the shoot or it might flow out the roots of 30 day the old barley seedling. Sodium has been shown to be essential for a few species and for improvement in growth and productivity of the chenopodeaceae (Kim, 1992). Rush & Epstein (1981) reported that, under high salt conditions, the wild type tomato showed more sodium accumulation in the shoot than the domestic type, nevertheless, the reduced growth of the wild type tomato was less than that of the domestic type tomato under saline conditions. That is, we think if the crops made be elevation of adaptability to sodium ions or formation of receptive organs of sodium ions in leaf cell according to high translocation of Na^+ to the shoot, they could overcome salt ion toxicity.

Total nitrogen content

Total nitrogen content of barley seedlings with 4 NaCl treatments are shown in Table 2. Nitrogen content in the shoot was higher in NaCl conditions than in non-saline condition. Total nitrogen contents in shoot were 4.31% and 4.27% for 10 and 30 day old seedlings, respectively. They are about 16.8% and 19.9% higher in 150 mM NaCl condition than those found in non-saline conditions. In the root, however, nitrogen content of 10 day old seedlings at 100 mM and 150 mM NaCl condition was less than the control, and nitrogen content at the 50 mM NaCl condition was higher than in non-saline condition. Nitrogen content in root of 30 day old seedlings, compared to 10 day old, was slightly increased, except for the 150 mM NaCl condition. This result may indicate that moderate NaCl concentrations (50 and 100 mM NaCl) increase nitrogen content, but high salt condition (150 mM or more) decreases nitrogen content.

Under salt stress conditions, the uptake of N by the plant generally showed inhibitory (e.g., tomato, cotton) or stimulatory (e.g., rice, bajra) effects (Alam, 1994). Seemann and Sharkey (1986) reported that nitrogen content on the leaves of bean seedlings was reduced by high salinity conditions. Grattan & Maas (1985) reported that accumulated nitrogen with salinity decreased salt stress in plants. In this study, the result was different from previous studies and nitrogen content in the shoot increased

Table 1. Na^+ content of 4 barley cultivar seedlings cultured for 10 and 30 days in different NaCl concentrations containing 1/4 Hoagland solution.

NaCl conc. (mM)	Na^+ content (mM/g D.W.)									
	Neulssalbori		Chalssalbori		Bunong		Dongbori 1		Mean	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
					10 DAY					
0	0.33	0.38	0.41	0.23	0.40	0.40	0.22	0.28	0.34	0.32
50	1.97	1.88	2.17	3.04	2.64	3.25	3.79	2.42	2.64	2.65
100	4.18	3.01	4.45	3.12	4.64	3.83	3.87	3.98	4.28	3.49
150	8.67	8.13	9.00	8.06	9.11	8.81	7.62	9.54	8.60	8.65
LSD(5%)	0.78	0.45	0.42	1.01	0.40	0.42	1.24	0.73	0.24	0.27
					30 DAY					
0	0.31	0.50	0.42	0.90	0.38	1.33	0.44	0.64	0.37	0.85
50	5.59	1.68	5.75	2.24	3.98	3.60	6.12	3.87	6.36	2.85
100	9.48	2.58	8.33	2.35	9.83	4.26	7.51	4.09	8.69	3.35
150	10.59	5.40	10.11	3.67	10.35	4.79	9.71	4.54	10.19	4.60
LSD(5%)	0.71	0.18	1.08	0.33	0.95	0.37	0.80	0.41	0.52	0.21

Table 2. Total nitrogen content of 4 barley cultivar seedlings cultured for 10 and 30 days in different NaCl concentrations containing 1/4 Hoagland solution.

NaCl conc. (mM)	Total nitrogen content (%/g D.W)									
	Neulssalbori		Chalssalbori		Bunong		Dongbori 1		Mean	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
					10 day					
0	3.84	3.06	3.55	3.57	3.68	2.59	3.67	3.94	3.69	3.29
50	3.98	3.14	4.06	3.64	3.82	3.32	3.92	4.21	3.95	3.58
100	4.28	3.54	4.36	3.16	4.26	2.76	4.34	3.56	4.31	3.26
150	3.92	3.07	4.59	2.98	4.38	2.53	4.34	3.44	4.31	3.01
LSD (5%)	0.33	0.19	0.32	0.21	0.39	0.25	0.35	0.21	0.34	0.22
					30 day					
0	3.64	3.12	3.47	3.65	3.65	3.78	3.46	4.02	3.56	3.64
50	3.84	4.02	4.06	4.53	3.86	4.03	3.96	4.65	3.93	4.31
100	3.98	3.26	4.22	4.26	4.06	4.76	4.45	3.28	4.18	3.89
150	3.98	2.97	4.42	3.68	4.21	3.19	4.48	3.26	4.27	3.28
LSD (5%)	0.31	0.28	0.23	0.21	0.19	0.20	0.20	0.28	0.24	0.25

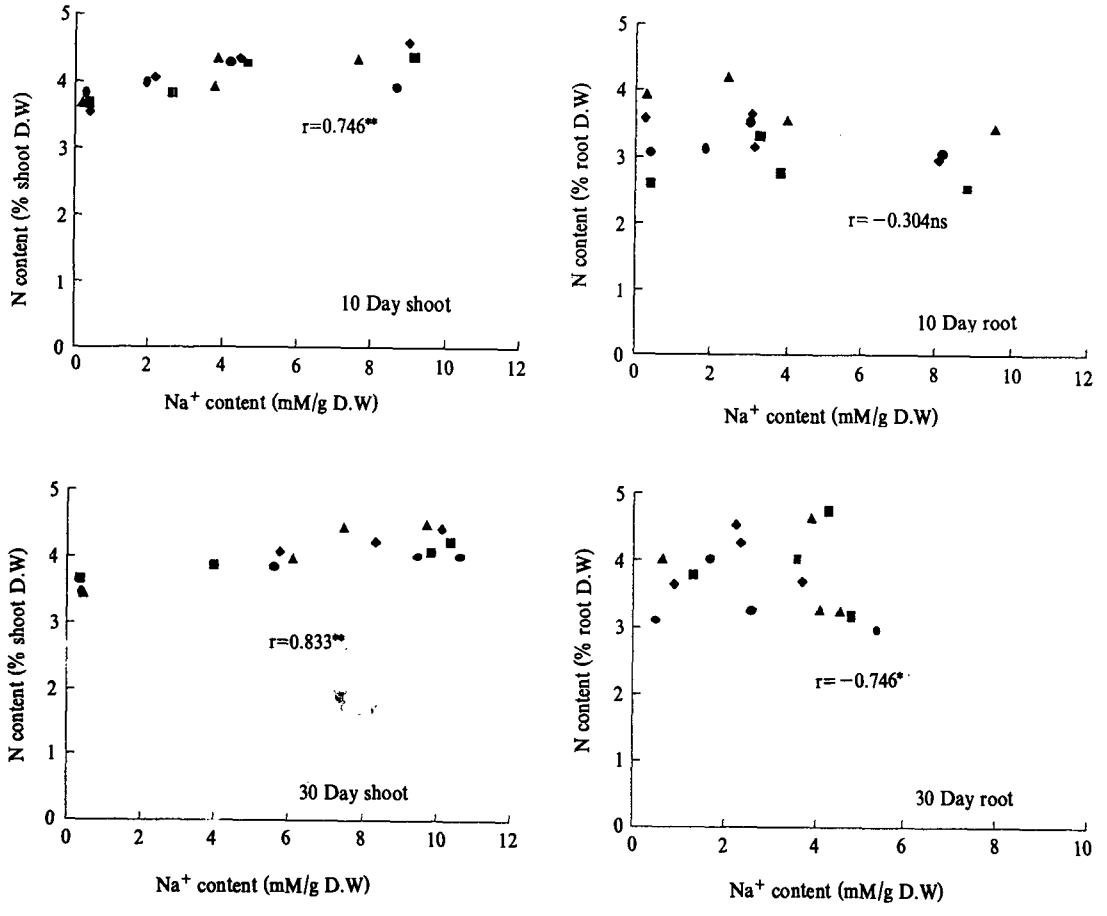

Fig. 1. Relationship between Na^+ content and nitrogen content of 4 barley cultivar seedlings cultured for 10 and 30 days in different NaCl solution. ● ; Neulssalbori, ◆ ; Chalssalbori, ■ ; Bunong, ▲ ; Dongbori 1.

with NaCl treatments. However, in the root, nitrogen content decreased at high NaCl levels (150 mM NaCl) compared to non-saline conditions.

There was a positive correlation between total nitrogen and Na^+ content in the shoot of 10 and 30 day old seedlings (Fig. 1). The result means that a higher content of Na^+ have higher total nitrogen under NaCl stress. However, there was a significantly negative correlation between total nitrogen and Na^+ content in the roots of 30 day old seedlings. The result may indicate that NaCl -stressed barley supressed total nitrogen accumulation in the root of barley seedlings regardless of Na^+ level.

NO_3^- and NH_4^+ content

NO_3^- content of 4 barley cultivar seedlings cultured for 10 and 30 days in different NaCl concentrations are shown in Table 3. NO_3^- content in the seedling slightly decreased with increased NaCl concentration as sharply drop for 30 day old seedling greater than 10 day old seedling. NO_3^- content of the plant decreased and the decreased rate was higher in the root than in the shoot. NO_3^- content in the root at 150 mM NaCl level was 63% of the NO_3^- content of non-slaine conditions, but in the shoot the content was about 1/3 of that of the control for 10 day old seedlings. 30 day old seedlings were more rapidly decreased than that of 10 day old seedlings. In all parts of 10 day old seedlings, NO_3^- content among cultivars were lower in naked barley (Neulssalbori and Chanssalbori) than in hulled barley (Bunong and Dongbori 1) with increased NaCl level. But there was no tendency in roots of 30 day old seedlings of the other three cultivars except for Chalssalbori.

Ward et al. (1986) reported that NO_3^- uptake rates and growth of barley seedlings were reduced under saline conditions. Aslam et al. (1984) reported that Cl^- inhibited NO_3^- uptake, and Gorham et al. (1985) observed that despite drastic reductions in leaf NO_3^- contents in response to salinity, other nitrogen-containing fractions either increased (e.g., proline, glycine, and total soluble protein) or were not greatly reduced (e.g., total amino acid content). In contrast to the effect of NaCl on NO_3^- uptake, reported data indicate that increased NO_3^- in the substrate decreased Cl^- uptake and accumulation (Bernstein et al., 1974).

The relationships between Na^+ and NO_3^- showed highly negative correlation for 10 and 30 day old seedlings in the shoot and only 10 day old seedling in the root (Fig. 2). These results indicate that Na^+ accumulation in the plant decreased NO_3^- content in plant.

NH_4^+ content of NaCl stressed barley is shown in Table 4. NH_4^+ content in barley seedlings with different NaCl treatments tend to be decreased for 10 day old seedling. In roots, NH_4^+ content at 50 mM NaCl level showed the lowest as 9.97 mM, but increasing the NaCl level to 150 mM NaCl condition. NH_4^+ content at the 150 mM NaCl condition was about 48 % higher than at the non-NaCl treatment. NH_4^+ content of the shoots was the highest as 7.79 mM at the 50 mM NaCl but NH_4^+ content of high NaCl level tended to decrease slightly in seedlings. Therefore, NH_4^+ contents were 5.94 mM and 4. 80 mM for 100 mM and 150 mM NaCl treatment, respectively. In the roots, NH_4^+ content was lower with NaCl condition than that of non-NaCl treatment but NH_4^+ content under high NaCl level was slightly increased. Moreover, in 30 day old seedlings, NH_4^+ content with NaCl treatment was abundant that it was increased at the high NaCl level such as 11.98 mM and 18.96 mM at 100 mM and 150 mM NaCl conditions, respectively. Leidi et al. (1991) suggested that NO_3^- was a better N source than NH_4^+ for wheat grown in salt-affected areas and its grain weight negatively correlated with

Table 3. NO_3^- content of 4 barley cultivar seedlings cultured for 10 and 30 days in different NaCl concentrations containing 1/4 Hoagland solution.

NaCl conc. (mM)	NO_3^- content (mM /g F.W.)									
	Neulssalbori		Chalssalbori		Bunong		Dongbori 1		Mean	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
					10 day					
0	81.92	88.71	105.82	59.88	71.33	64.49	66.61	70.28	81.42	70.84
50	62.36	53.20	79.15	59.34	69.18	69.55	65.04	64.31	68.93	61.60
100	52.62	32.96	61.63	44.70	51.75	49.40	75.98	55.91	60.50	45.74
150	43.38	23.34	55.04	18.77	51.13	26.45	50.03	34.94	49.90	26.00
LSD (5%)	2.06	2.30	1.39	1.34	1.92	0.75	0.98	1.10	1.45	1.54
					30 day					
0	105.31	106.70	146.50	78.86	77.54	67.11	145.78	81.12	118.78	83.45
50	75.58	60.42	105.41	45.24	91.02	88.26	118.41	108.32	97.61	75.56
100	69.50	101.81	63.64	30.43	36.71	19.40	97.15	92.96	66.75	61.14
150	50.16	25.46	56.66	11.85	45.26	10.55	63.06	22.74	53.79	17.68
LSD (5%)	1.33	0.88	1.01	1.20	1.53	1.04	2.42	2.20	1.35	1.40

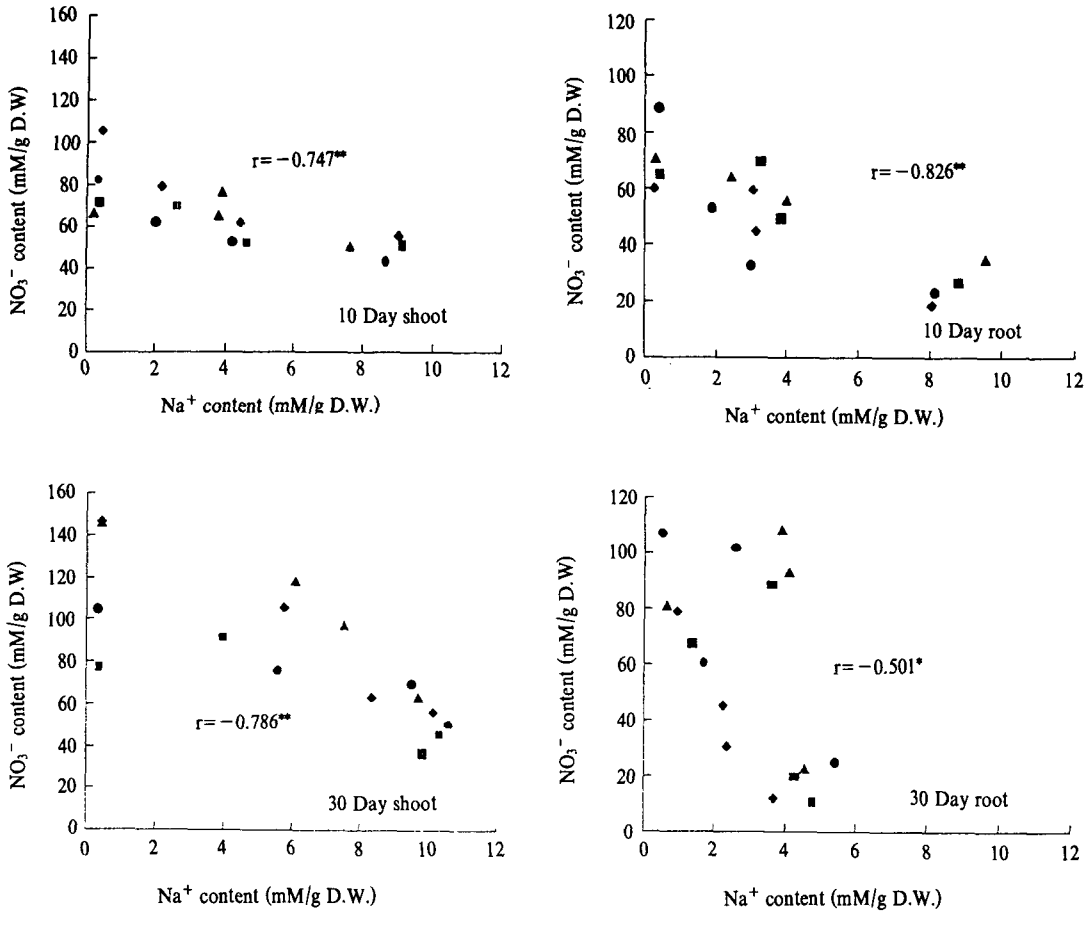

Fig. 2. Relationship between Na^+ content and NO_3^- content of 4 barley cultivar seedlings cultured for 10 and 30 days in different NaCl solution. ● ; Neulssalbori, ◆ ; Chalssalbori, ■ ; Bunong, ▲ ; Dongbori 1.

Table 4. NH_4^+ content of 4 barley cultivar seedlings cultured for 10 and 30 days in different NaCl concentrations containing 1/4 Hoagland solution.

NaCl conc. (mM)	NH_4^+ content (mM/g F.W.)									
	Neulssalbori		Chalssalbori		Bunong		Dongbori 1		Mean	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
					10 day					
0	4.76	8.80	5.71	19.60	2.83	17.11	3.61	11.57	4.23	14.27
50	4.40	7.08	4.59	14.01	3.00	13.31	3.19	5.46	3.80	9.97
100	3.95	7.84	4.23	19.51	4.84	10.25	3.19	4.67	4.05	10.32
150	3.59	7.94	3.37	16.89	3.37	9.74	2.49	12.43	3.21	11.75
LSD (5%)	0.31	0.77	0.26	0.44	0.30	0.55	0.18	0.45	0.29	0.37
					30 day					
0	5.37	11.83	4.84	10.78	6.24	11.23	5.71	8.08	5.54	10.48
50	7.90	7.20	7.03	15.15	7.81	8.93	8.42	7.40	7.79	9.67
100	6.85	7.64	4.32	14.26	4.15	12.00	8.43	14.00	5.94	11.98
150	4.93	12.53	4.32	22.13	5.01	19.01	4.93	22.16	4.80	18.96
LSD (5%)	0.15	0.46	0.39	0.83	0.21	0.63	0.23	0.76	0.23	0.58

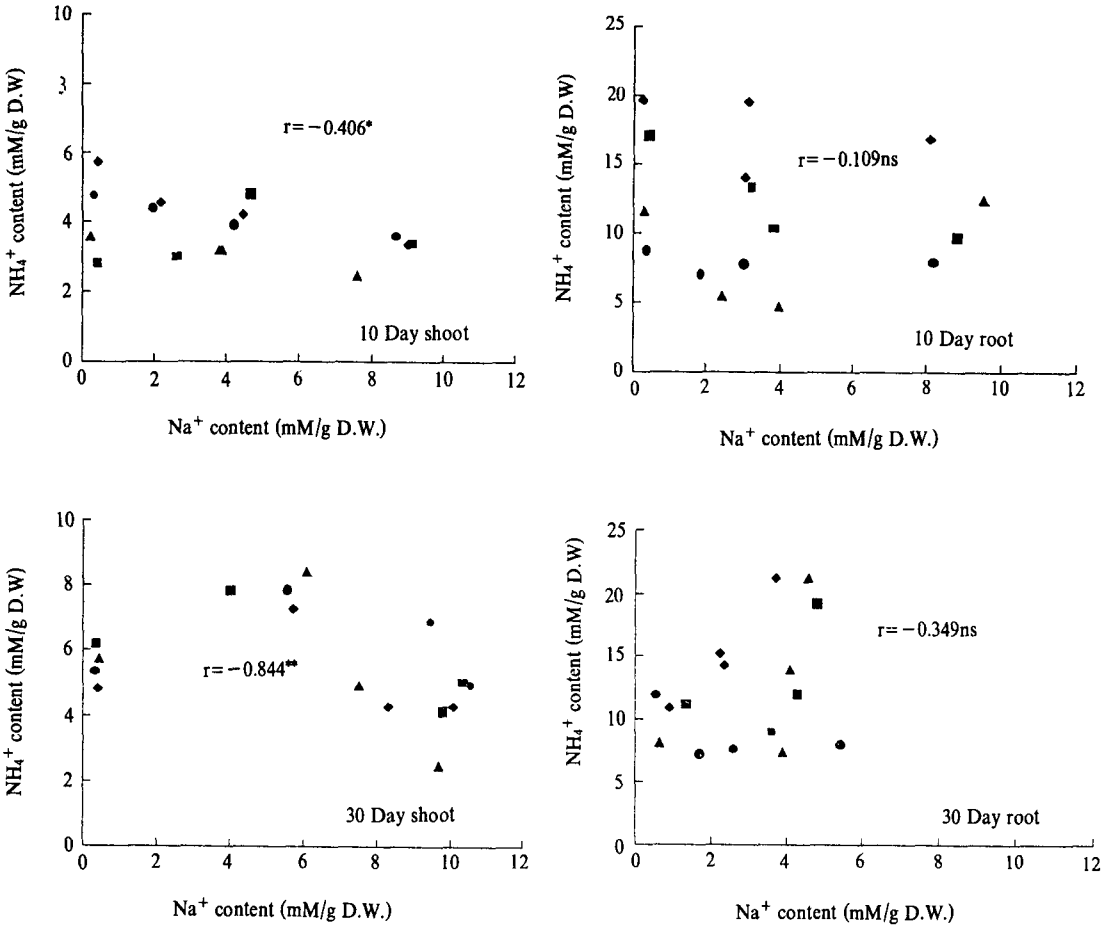

Fig. 3. Relationship between Na^+ content and NH_4^+ content of 4 barley cultivar seedlings cultured for 10 and 30 days in different NaCl solution. ● ; Neulssalbori, ◆ ; Chalssalbori, ■ ; Bunong, ▲ ; Dongbori 1.

the NO_3^-/NH_4^+ ratio. Therefore, over accumulated NH_4^+ content in plant under salinity may induce ammonia toxicity that inhibits growth and eventually the stressed-plant will die (Marschner 1995).

There was a significant negative correlation between Na^+ content and NH_4^+ content in the shoot, but not in the root (Fig. 3).

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for Scientific Research from the Research Foundation, Chungnam National University.

REFERENCES

Alam, S. M. 1994. Nutrient uptake by plants under stress conditions. in. M. Pessarakli. Handbook of plant and crop stress. pp. 227-246. Harcel. Dekker, Inc. New York.

Akbar, M. and T. Yabuno. 1974. Greeding for saline-resistant varieties of rice. II. Jap. J. Breed. 24: 176-181.

Aslam, M., R. C. Huffaker, and D. W. Rains. 1984. Early effects of salinity on nitrate assimilation in barley seedlings. Plant Physiol. 76: 321-335

Bernstein, L., L. E. Francois, and R. A. Clark. 1974. Interactive effects of salinity and fertility on yields of grains and vegetable. Agron. J. 66: 412-421.

Boursier, P. and A. Laüchli. 1990. Growth response and mineral nutrient relations of salt-stressed sorghum. Crop Sci. 30: 1226-1233.

Cataldo, D. A., M. Haroon, L. E. Schrader, and V. L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue. Commun. Soil Science and Plant Analysis. 6: 71-80.

Gorham, J., R. G. Wyn Jones, and E. McDonnell. 1985. Some mechanisms of salt tolerance in crop plants. Plant Soil. 89: 15-40.

Grattan, S. R. and C. M. Grieve. 1994. Mineral nutrient acquisition and response by plants grown in saline environments. in. M. Pessarakli. Handbook of plant and crop stress. pp. 203-226. Harcel. Dekker, Inc. New York.

_____ and E. V. Maas. 1985. Root control of leaf

phosphorus and chloride accumulation in soybean under salinity stress. Agron. J. 77: 890-895.

Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol. 31: 149-190.

Kim, C. S. 1992. Physiological Mechanisms of Halophytes. R.D.A. Symposium 17: 100-123.

Leidi, E. O., M. Silberbush, and S. H. Lips. 1991. Wheat growth as affected by nitrogen type, pH and salinity. I. Biomass production and mineral composition. J. Plant Nutr. 14: 235-246.

Lynch, J. and A. Laüchli. 1984. Potassium transport in salt-stressed barley roots. Planta. 161: 295-301.

Maas, E. V. and G. J. Hoffman. 1977. Crop salt tolerance-current assessment. J. Irrig. Drin. Div. Am. Soc. Civ. Eng. 103: 115-134.

Marschner, H. 1995. Mineral nutrition of higher plants. Second edition. Academic press. London. pp. 239-242, 657-680.

Rush, D. W. and E. Epstein. 1981. Camparative studies on the sodium, potassium, and chloride relation of a wild halophyteic and a domestic salt-sensitive tomato species. Plant Physiol. 68: 1308-1313.

Seemann, J. R. and T. D. Sharkey. 1986. Salinity and nitrogen on photosynthesis ribulose-1,5-bisphosphate carboxylase and metabolitie pool sizes in *Phaseolus vulgaris* L. Plant Physiol. 82: 555-560.

Ward, M. R., M. Aslam, and R. C. Huffaker. 1986. Enhancement of nitrate uptake and growth of barley seedling by Ca^{2+} under saline condition. Plant Physiol. 80: 520-524.