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Effect of NaCl Stress on Inorganic Ion, L-Proline, Sugar and Starch Content of Soybean Seedlings

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ABSTRACT : This study conducted to elucidate the change of the cation content (Na^+, K^+, and Ca^{2+}), the L-proline content, and the sugar and starch content in the stems, roots, and leaves of three cultivars of the 30 days old seedling soybeans (*Glycine max* L. cv. Danwonkong, Hwangkeumkong, and Kwangankong) after 100 mM NaCl stress containing 1/2 Hoaglands nutrient solution in the sand culture. The reduction of the dry matter weight after 100 mM NaCl treatment among cultivars was higher in the order of Kwangankong, Danwonkong, and Hwangkeumkong. The highest reduction of the dry matter weight was occurred at the roots among three parts of plant. The Na^+ content increased with NaCl treatment in overall and specially greatly increased in roots and stems. The K^+ and Ca^{2+} content decreased with NaCl treatment at the roots and stems. The K^+ content, however, at the leaves increased in all three cultivars with the NaCl treatment. The L-proline content with NaCl stress increased greatly. The increment of the L-proline content at the stems and roots of Hwangkeumkong was lower than that of other two cultivars, Kwangankong and Danwonkong. The sugar content decreased with NaCl treatment at the stems and leaves. The starch content also decreased at the stems and leaves with NaCl treatment.

Keywords *: soybean, NaCl stress, Na^+, K^+, Ca^{2+}, L-proline, Reducing sugar, starch.*

Consideration of interaction between the ion absorption and the soil salinity is very interesting subject. The ions greatly absorbed at the high content of Na^+ and Cl^- media and accumulated continuously in the tissue (Cramer *et al.*, 1995; Lee & Kim, 1995). These ions inhibited absorption of essential elements such as K^+ and Ca^{2+} and derived the element deficiency in tissue by inhibiting the transposition to shoots through xylem and increased Na^+/K^+, Na^+/Ca^{2+} ratio. Lynch and Lauchli (1984) reported that the Na^+/K^+ ratio increased at the shoots of saline stressed barley by absorption of K^+ and inhibiting transposition, and Cramer *et al.* (1987) reported that since K^+ affected the osmoregulation capability and Na^+ content between cells associated with the different osmotic stress the K^+/Na^+ selectivity could be considered to be an important index of the salt tolerant ability. Also, Ca^{2+} was an important element for the cell membrane protection and ion permeability maintenance and a high Ca^{2+} content in a cell expected to have an effect of reduction of the saline stress by inhibiting the Na^+ absorption and reducing the leakage through the membrane. In the case of cotton, there was a report stated that the treatment of the Ca^{2+} in the salinity improved the root growth inhibition. In case of corns, there was a report stated that the amount of Na absorption reduced to around 30% treated with Ca^{2+} 1 mM and up to 60% treated with 5 mM (Shalhevet *et al.*, 1995).

Salt stressed plants, meanwhile, composed or accumulated the various organic mater such as proline, glucose, betaine, sobital, and etc. in the cell membrane for the osmoregulation to continue to growth by protect the enzyme activity by means of the osmoregulation (Cho, 1997; Kim, 1992; Munns and Termeat, 1986). The salt tolerant plant such as tomato showed a high organic mater content (Martinez *et al.*, 1996), and such as rice seedlings, the proline content increased abruptly and impeded the growth or proline content increased by saline stress (Lin & Kao, 1996). There was also a report stated that the sugar content varied as saline stress occurred and salt tolerant plants, however, increased the sugar content that maintain the high growth efficiency.

This research carried out to determine the growth reaction, inorganic ion, proline, and sugar content variation with NaCl stress using the three soybean cultivars; Danwonkong, Hwangkeumkong, and Kwangankong, to clarify the saline stress reaction mechanism, and to provide the data for the upbringing and development of the salt tolerant cultivars.

MATERIALS AND METHODS

This research conducted at the farm attached to Chungnam National University, Daejeon, Korea and used three cultivars; Danwonkong, Hwangkeumkong, and Kwangankong as samples. The NaCl was treated to the samples with the concentration of 100 mM with a 1/2 Hoagland solution. Samples were seeded at June 5 and transplanted to a 1/5,000 a plastic pot with a group of three seedlings. Sand was used

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as media after washed with clean water. The overhead flooding of 200 ml for each pot was done every other day for the first 10 days after transplant and everyday after that. The 200 ml NaCl stress in each pot was done every other day for the first 10 days after transplant and five days period for the rest of the days.

Na^+, K^+, and Ca^{2+} content were injected using 1N HCl with IRRI method (Yoshida *et al.*, 1972). Samples were dried 7 days at 80 8C and pulverized. The samples then were measured with 1 g correctly and extracted for a day, and Na^+, K^+ and Ca^{2+} was measured at 589 nm, 766.5 nm, and 422.7 nm, respectively, using an automic absorption spectrophotometer (Shimazu AA-6800, Japan).

The L-proline content was measured using Faber method (Cho, 1997). Dried sample 1 g was extracted using 5 ml MCW solution (Methanol : Chloroform : Water = 12 : 5 : 3) and boiled for 45 minutes after mixed with 5 ml glacial acid and 5 ml Ninhydrin solution. Then, L-proline content on the toluene layer was measured was using a spectrophotometer (Shimazu uv-120-40s, Japan) at 520 nm after pouring the toluene into the sample. The content of the standard curve was obtained using L-proline.

The sugar and starch content was measured using the IRRI method (Yoshida *et al.*, 1972). The 500 mg of completely dried powder sample was extracted with 80% ethanol. The symbolic liquid and rest of the liquid was used to measure the sugar content and starch content, respectively. These were measured after forming the color using H_2SO_4 with 2% anthorone using a spectrophotometer (Shimazu uv-120-40s, Japen) at 630 nm. The standard curve of content was obtained using glucose.

RESULTS

Fig. 1 showed the results of the dry mater weight and the relative growth rate in percent of control of stems, leaves,

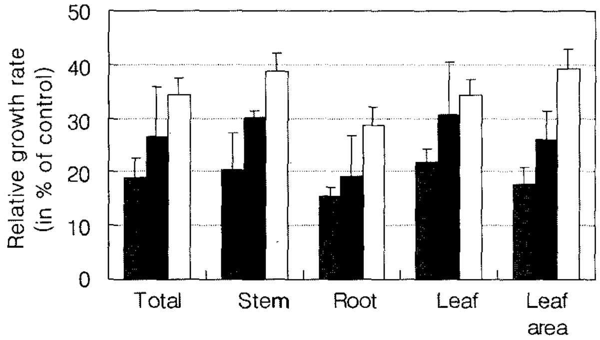

Fig. 1. Relative growth rate (in percent of non-treated control) of 30 day old seedlings in three soybean cultivars to 100 mM NaCl stressed. ■ Kwangankong, ■ Danwonkong and □ Hwangkeumkong. Total is sum of stem, root, and leaf dry weight (g $plant^{-1}$). Vertical bars indicate standard errors.

Table 1. Na^+, K^+ and Ca^{2+} content of three soybean cultivars seedlings cultured for 30 days on 100 mM NaCl concentration containing Hoagland medium in sand soil.

Cultivars	Parts	NaCl conc. (mM)	Na^+ (% $D.W.^{-1}$)	K^+ (% $D.W.^{-1}$)	Ca^{2+} (% $D.W.^{-1}$)
Kwangankong	Stem	0	1.37 ± 0.31	17.27 ± 1.81	5.15 ± 0.51
		100	21.36 ± 1.54	16.24 ± 1.63	5.10 ± 0.63
	Root	0	3.09 ± 0.41	27.98 ± 3.24	5.95 ± 0.63
		100	15.49 ± 2.16	14.58 ± 2.12	4.02 ± 0.45
	Leaf	0	1.11 ± 0.21	16.91 ± 1.46	7.46 ± 0.62
		100	4.34 ± 0.62	22.46 ± 2.54	9.33 ± 0.85
Danwonkong	Stem	0	1.74 ± 0.41	18.13 ± 2.27	6.02 ± 0.45
		100	22.73 ± 2.76	15.46 ± 1.85	6.87 ± 0.67
	Root	0	3.84 ± 0.28	27.23 ± 3.23	4.09 ± 0.33
		100	18.41 ± 1.97	14.95 ± 2.15	4.11 ± 0.24
	Leaf	0	1.08 ± 0.14	15.58 ± 1.93	7.12 ± 0.92
		100	7.51 ± 0.91	23.11 ± 2.57	12.17 ± 1.15
Hwangkeumkong	Stem	0	1.84 ± 0.51	19.53 ± 2.25	4.55 ± 0.33
		100	11.97 ± 0.83	15.72 ± 1.61	5.21 ± 0.56
	Root	0	5.25 ± 0.61	21.54 ± 2.83	4.01 ± 0.38
		100	19.95 ± 1.66	13.13 ± 1.87	4.76 ± 0.23
	Leaf	0	2.28 ± 0.41	19.57 ± 2.04	5.91 ± 0.42
		100	4.18 ± 0.61	27.82 ± 3.32	8.35 ± 0.67

Values represent the means ± standard error.

and roots of the three cultivars of the soybean with 100 mM NaCl stress. The relative growth rate greatly decreased with the NaCl stress. Among three cultivars, Kwangankong showed the highest decrease in dry mater weight and the next was Danwonkong and Hwangkeumkong in the order by the NaCl stress. Regard to the plant parts, the roots showed more to NaCl stress sensitive than the shoots. The leaf area also showed the great decrease due to the NaCl stress. The relative leaf area decreased for Kwangankong, Danwonkong, and Hwangkeumkong against control in percent was 20%, 26%, and 39%, respectively.

The Na^+, K^+, and Ca^{2+} content were as follows. Na^+ content with NaCl stress in stems showed great increase with respect to 1.37% increase in control. Na^+ content in stems with 100 mM NaCl stress showed 21.36%, 22.73%, 11.97% in Kwangankong, Danwonkong, and Hwangkeumkong, respectively (Table 1). In roots, there was 15.49%, 18.41% and 19.95% increase of Na^+ content with 100 mM NaCl stress in Kwangankong, Danwonkong, and Hwangkeumkong, respectively. There was, meanwhile, 3.09-5.25 % increase in control. In leaves, there were relatively low Na^+ content with respect to the stems and roots. For Kwangankong, Danwonkong, and Hwangkeumkong, there was 1.11% and 4.34%, 1.08% and 7.51%, and 2.28% and 4.18% in control and with NaCl stress, respectively.

The change of K^+ content with NaCl stress was small compare with the Na^+ content. The K^+ content in stems with NaCl stress for each cultivar decreased 1.0%, 2.6%, and 3.8% in Kwangankong, Danwonkong, and Hwangkeumkong, respectively. The K^+ content in roots was relatively high with respect to the K^+ content in stems. There was 13.4% point K^+ content reduction from the 27.98% in control to the 14.58% with NaCl stress in Kwangankong. Also, in Danwonkong, and Hwangkeumkong, there was 12.3% point and 8.5% reduction with NaCl stress, respectively. In leaves, however, K^+ content increased with NaCl stress that was differ to the K^+ content variation in stems or roots. From the 16.91% K^+ content increase in control, Kwangankong, Danwonkong, and Hwangkeumkong showed 22.46%, 23.11% and 27.82% increase with NaCl stress, respectively.

The change of Ca^{2+} content showed a little difference that was differed to the Na^+ and K^+ content change. There was almost no Ca^{2+} content in stems and roots for all three cultivars. The Ca^{2+} content in leaves, however, increased with NaCl stress. Kwangankong showed 7.46% Ca^{2+} content in control and 9.33% with NaCl stress. Danwonkong showed the largest increase of 12.17% among the three cultivars. Hwangkeumkong also showed 2.4% point of Ca^{2+} content from the 5.91% in control to 8.35% with NaCl stress.

The ratio, meanwhile, of the Na^+/K^+ and Na^+/Ca^{2+} increased with NaCl stress. In stems, Danwonkong showed the highest ratio of 1.47 and Hwangkeumkong showed the lowest ratio of 0.76 in Na^+/K^+ (Table 2). These ratios showed similar in roots and relatively low Na^+/K^+ ratio in leaves with respect to the stems and roots. The Na^+/K^+ ratio by NaCl stress in leaves for Kwangankong, Danwonkong, and Hwangkeumkong by NaCl stress was 0.19, 0.32, and 0.15, respectively. The Na^+/Ca^{2+} ratio also showed the similar to the Na^+/K^+ ratio such that the Na^+/Ca^{2+} ratio was high in

Table 2. Na^+/K^+ and Na^+/Ca^{2+} ratio of three soybean cultivar seedlings cultured for 30 days with 100 mM NaCl concentration containing Hoagland medium in sand soil.

Cultivars	Parts	NaCl conc. (mM)	Na^+/K^+	Na^+/Ca^{2+}
Kwangankong	Stem	0	0.08	0.27
		100	1.31	4.19
	Root	0	0.11	0.52
		100	1.06	3.85
	Leaf	0	0.07	0.15
		100	0.19	0.47
Danwonkong	Stem	0	0.10	0.29
		100	1.47	3.31
	Root	0	0.14	0.94
		100	1.23	4.48
	Leaf	0	0.07	0.15
		100	0.32	0.62
Hwangkeumkong	Stem	0	0.09	0.40
		100	0.76	2.30
	Root	0	0.24	1.31
		100	1.52	4.19
	Leaf	0	0.12	0.39
		100	0.15	0.50

Table 3. L-proline content (% $D.M.^{-1}$) by parts of three soybean cultivar seedlings with 100 mM NaCl concentration containing Hoagland medium in sand soil.

NaCl conc. (mM)	Kwanankong			Danwonkong			Hwangkeumkong		
	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf
0	26.5 ± 7.9	15.0 ± 1.0	154.2 ± 10.0	24.5 ± 7.1	15.6 ± 7.3	150.1 ± 60.3	28.1 ± 2.0	23.9 ± 0.9	104.6 ± 18.6
100	114.1 ± 6.7	62.3 ± 5.0	597.1 ± 31.3	61.9 ± 2.1	41.1 ± 14.1	621.1 ± 2.6	58.4 ± 22.5	25.8 ± 8.2	620.0 ± 02.1

Values represent the means ± standard error.

stems and roots with NaCl stress. The Na^+ content in leaves, however, higher than the Ca^{2+} content.

Table 3 showed the L-proline content with NaCl stress for Kwangankong, Danwonkong, and Hwangkeumkong. The L-proline content with the NaCl stress increased against in control (without NaCl stress). From the results of the change of L-proline content in each part of the plant, in stems, there were no significant differences of L-proline content of 24.5% 28.1% in control among the three cultivars. There were, however, significant differences of the L-proline content after the 100 mM NaCl stress among the three cultivars. Kwangankong, Danwonkong, and Hwangkeumkong showed 114.1% point, 61.9% point, and 58.4% point L-proline content increase, respectively, with NaCl stress and, hence, Danwonkong had the greatest increase. But, in the roots, there was small difference of L-proline content increase between cultivars compare with the stems. These variations were 62.3%, 41.1%, and 25.8% in Kwangankong, Danwonkong, and Hwangkeumkong respectively. Also, the L-proline content in leaves showed no significant difference among cultivars, but there were more L-proline content in stems and roots without NaCl stress and very high L-proline content with NaCl stress.

Table 4 showed the soluble sugar and starch content with NaCl stress of the three cultivars of soybeans. The soluble sugar content in the stems decreased with the NaCl stress. The Kwangankong showed 4.1% of the soluble sugar content, which was 2.4% point decrease with the NaCl stress. The soluble sugar content, meanwhile, in control was 6.5%. In Danwonkong and Hwangkeumkong, the soluble sugar content by the NaCl stress was 3.3% and 3.0%, respectively. Differ to the stems, in roots, the soluble sugar content of the Kwangankong with the NaCl stress was 5%, which was higher than in control. Meanwhile, the soluble sugar content of Danwonkong and Hwangkeumkong was decreased with respect to control. Similar to the stems, the soluble sugar content of the leaves decreased with the NaCl stress. Among the three cultivars, Danwonkong showed the highest decrease of the soluble sugar content from the 23.4% of without treatment to 9.5% with NaCl stress.

The starch content in stems generally decreased with NaCl stress. Danwonkong and Hwangkeumkong showed 3.5% and 1.2%, respectively. Danwonkong, however, showed no difference. In roots, there were no big differences of starch content among cultivars, and Hwangkeumkong showed decrease of the starch content with the NaCl stress. In leaves, Danwonkong and Hwangkeumkong showed the starch content decrease with NaCl stress, meanwhile, Danwonkong showed no difference.

DISSCUSSION

This research conducted to elucidate the change of the Na^+, K^+, and Ca^{2+} content, the L-proline content, and the affect of the sugar and starch content in the stems, roots, and leaves with the NaCl stress using the three representative cultivars of soybeans in Korea.

The damage on plant by various stresses during a growing season showed diversely in each growth stage. Generally, the disaster occurred vigorously at seedling, reproductive growth, and germination stage and, hence, the reaction of the saline stress at seedlings is very important. Dry mater of each part decrease with the NaCl stress was similar for the three parts; stems, roots, and leaves, and, specifically, the decrease in roots was higher than those of in leaves and stems. These results were opposite to the results, the decrease in the shoots was higher than the roots with the saline stress, reported by Munns & Termeat (1986) and Cho (1997). But, this result was the same as the result from Grattan & Maas (1985), which reported that the dry mater decrease in shoots and roots of soybeans with the saline stress was similar or roots was higher than the shoots. From the result of the difference of the dry mater decrease with NaCl stress among cultivars, Hwangkeumkong showed relatively small decrease compare with other two cultivars. This was considered due to the bigger seed size of the

Table 4. Soluble sugar and starch content (% $D.M.^{-1}$) by parts of three soybean cultivar seedlings with 100 mM NaCl concentration containing Hoagland medium in sand soil.

Cultivars	NaCl conc. (mM)	Soluble sugar			Starch		
		Stem	Root	Leaf	Stem	Root	Leaf
Kwangankong	0	6.5 ± 0.1	4.3 ± 0.6	16.5 ± 1.2	10.5 ± 1.3	6.3 ± 0.7	10.3 ± 1.1
	100	4.1 ± 0.4	5.0 ± 0.4	13.6 ± 1.6	7.0 ± 0.1	7.0 ± 0.1	11.2 ± 2.0
Danwonkong	0	8.3 ± 1.2	5.2 ± 0.9	23.4 ± 0.1	5.2 ± 2.3	5.2 ± 2.3	14.2 ± 0.7
	100	3.3 ± 0.4	3.7 ± 0.2	9.5 ± 0.3	5.2 ± 1.7	5.2 ± 1.7	10.8 ± 0.6
Hwangkeumkong	0	9.9 ± 0.8	6.1 ± 2.3	21.9 ± 1.0	6.1 ± 0.4	6.1 ± 0.4	12.0 ± 0.8
	100	3.0 ± 1.1	4.6 ± 0.1	10.8 ± 0.6	4.9 ± 0.1	4.9 ± 0.1	9.6 ± 0.6

Values represent the meansstandard error.

Hwangkeumkong than the other two cultivars. As Amthor (1983) and Grieve & Frncois (1992) reported that there was difference of salt tolerance depended on the seed size, and large seed had stronger salt tolerance than small seed in the wheat and corn.

The plants grown in high NaCl content soil excessively absorbed ions such as Na^+ or Cl^- and relatively inhibited absorption or transfer of the essential elements such as K^+ or Ca^{2+}; as a result, this interfered the metabolism or induced the nutritional unbalance and, finally, lead to withering to death by the nutritional starvation. Results from the 100 mM NaCl stress, the Na^+ content increased abruptly and K^+ content decreased. The Ca^{2+} content, however, did not decrease with NaCl stress; conversely, the Ca^{2+} content increased in leaves and showed different results from other researches, and it needed to be more study in the future.

Plants product the protective materials for the plant tissue or for the metabolism when the plant damaged by the salt or different stress control the osmoregulation with the this material. The L-proline was known as a representative osmoregulation material (Almansour *et al.* 1999, Cho, 1997; Greenway & Munns, 1980; Kim, 1992; Lin & Kao, 1996; Martinez *et al.*, 1996; Moftah & Michel, 1987). Greenway & Munns (1980) reported that the L-proline production had more close relation on the survival than the growth, but it was not clear that more production of the L-proline in salt tolerant plants than others. Results from this research, the L-proline content sharply increased with the NaCl stress, and the increase of the L-proline content in the stems was relatively higher than in the roots. Also, salt damaged plants should inhibited the growth by the L-proline accumulation, and, furthermore, regard to the facts that the less salt tolerant rice cultivars had more L-proline content and it was hard to use it as the salt tolerance index although the salt sensitive cultivars had more L-proline content (Lin & Kao, 1996), the results from this research, the difference of the L-proline content among cultivars considered being due to the genetic characteristics of the plants rather than the difference of the salt tolerance among cultivars.

From sited reference, the salt damaged barley should increase the carbohydrate content and it was due to the soluble sugar content increase (Haung & Redman, 1996). The result from this research, the content of the soluble sugar and starch decreased with the NaCl stress, and it was considered being due to sensitivity to the salt stress in soybeans growth characteristics such as decrease of the metabolism and productivity by the salt stress.

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