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Change in Photosynthesis, Proline Content, and Osmotic Potential of Corn Seedling under High-Saline Condition

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ABSTRACT : To identify salt-tolerance characteristics of corn seedling was treated in solution of 0, 50 and 100 mM NaCl of hydroponic cultivation. In photosynthesis of corn seedling, there was no large difference between 50mM and 0 mM NaCl solution, however, in 100 mM NaCl solution, the tolerance gradually decreased to 76%, 49%, and 31% after one day, four days, and seven days, respectively, in comparison to 0 mM NaCl solution. Osmotic potential of corn in seedling period was significantly decreased with increasing saline level, however, free proline content in the plant on the ground was significantly increased with increasing saline level and with the lapse of time. In terms of correlation among major characteristics, there was a highly significant positive difference between osmotic pressure potential and photosynthesis, However, highly negative correlation was found between osmotic pressure potential and free proline content. In addition, it was expected that young seedling of corn with saline tolerance may be utilized in the transplantation in salt-accumulated land. Based on above-shown result, in terms of saline tolerance of Chalok-2 variety, growth suppression was serious with 100 mM NaCl solution. However, growth was expected that seedling growth would be favorable under 50 mM NaCl solution.

***Keywords*:** seedling corn, saline, NaCl concentration, photosynthetic rate, osmotic potential, free proline content

In general, synthesis of chlorophyll, leaf area, affected photosynthesis and respiration, and enzyme activation was affected under saline condition (Beatriz *et al.*, 2001; Cho & Kim,1998; Lee *et al.*, 1996; Lee & Kim, 1995; Volkmar *et al.*, 1998). This saline stress decreased productivity and quality of farm products. However, it was known that an appropriate increase in saline level results in improvement in quality such as increased sugar contents in tomato Saline damage accelerate the aging of farm products and, especially, photosynthetic capacity was significantly influenced in intracellular ion balance and zoning; Volkmar *et al.* (1998) and Cho *et al.* (2002) reported that optimum levels of Na^+ and K^+ are unbalanced under saline stress; Mohammad (1994) reported that stomatal conductance is decreased due to suppressed water absorption; and Abou-Hadid (1992) reported photosynthesis of plants is affected due to decreased stomatal transpiration. While, there was also a report that affection of water absorption was resulted due to decreased water potential and excessive soil caused by toxic activity of specific ions existing as high levels or salts existing in plant medium (Megel & Kirkby, 1978). Salt gives a large influence on metabolism process of plant. In case of farm products with high salt-tolerant property, accumulation of free amino acid, especially proline, it was apparent under the condition with insufficient water. According to Xiaomu *et al.* (1995), although proline was the amino acid that was shown in small quantity under the favorable plant cultivation condition, it was accumulated in large quantities under the unfavorable physiological condition such as saline damage or drought damage. James *et al.* (1996) reported that proline acted on membrane osmotic pressure between cytoplasm and intercellular space, enzyme defense, and pH of cytoplasm and accumulation of proline is related to tolerance capacity. Corn is C_4 plant with large sizes and excellent carbon dioxide assimilation capacity with light and grows fast with absorption of large quantity of fertilizers with strong fertilizer absorption capacity (Marschner, 1986); Choi *et al.* (1997) reported that the larger photosynthetic capacity and rooting power in species, the higher salt-tolerance. Frensch & Hsiao (1994) reported that corn roots exposed to mannitol or KCl show immediate decrease in water potential and growth rate within 30 minutes; Neumann *et al.* (1994) reported that low growth of root under salts in seedling period of corn wasn't affected influence in the change of osmotic pressure but makes cell walls hard; and Evlagon *et al.* (1992) reported that Ca^{2+} accumulation in the root causes an important role to NaCl reaction. Zidan *et al.* (1990) reported that initial growth of roots in corn seedling period was significantly suppressed by treatment with high-level NaCl. The purpose of these studies were to get the data of salt-tolerance capacity and physiological characteristics of saline reaction for transplantation during seedling period in salt-accumulated cultivation land and reclaimed

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land through determination of photosynthesis, osmotic pressure potential, free prolin in plant under different concentrations of NaCl in water culture of corn seedling.

Materials and Methods

Chalok-2 corn (*Zea mays* L.) was cultivated in a growing seedling box for 25 days and, after transplantation in a water culture box with salt concentration of 0 mM, 50 mM and 100 mM NaCl. this process was repeated three times after one, four, and seven days. Leaf photosynthetic rates (LPS) were measured with a photosynthesis measuring apparatus LAC-4 (Analytical Development Company, Ltd. UK). Stomatal conductance (Gs) and intercellular CO_2 concentrations (Ci) were also concurrently measured. Those leaves were ground; 0.8 μl was taken into a chamber and stabilized for 25 minutes; and then, the chamber was connected with water potential measuring apparatus (HR-33T Dew point Microvoltmeter, INC. USA) to measure osmotic potential of leaves. Photometric method was used in measurement of free proline according to the method of Troll & Lindsley (1955). 1.0 g of living body of above ground plant was ground in centrifuge tube with liquid nitrogen. 10 ml of MCW solution (methanol : chloroform : water = 12 : 5 : 1) was added to the ground solution and centrifuged at 5,000 rpm with 4 °C for 10 minutes. 2 ml of upper layer in centrifuged tube was taken after centrifuge and, 3 ml of glacial acid and 3 ml of ninhydrine reagent (ninhydrine : glacial acid : 6M-phosphoric acid = 6.25 g : 150 ml : 100 ml) was added and boiled in water bath for 1 hour; after cooling of the solution at normal temperature, 5 ml of toluene was added and shaked vertially for overnight; absorption of spectrophotometer was measured at 520 nm. L-proline was used to get standard curve of proline.

RESULTS AND DISCUSSION

Table 1 showed the differences of photosynthetic rate, stomatal conductance, and intercellular CO_2 concentration by saline level in water culture of corn seedling. Photosynthetic rate and stomatal conductance decreased significantly as the level of saline increased and, although no apparent difference was shown between 0 mM and 50 mM NaCl in which photosynthetic rate in 50 mM treatment was 86 - 90% in comparison to 0 mM with the lapse of time. But, in case of 100 mM NaCl solution, photosynthetic rate was 76% of 0 mM at one day after saline treatment and decreased significantly to 31% in comparison to 0 mM at seven days after saline treatment. In case of 100 mM, stomatal conductance was 75% of 0 mM at one day after treatment and decreased significantly with 33% in photosynthetic rate in comparison to 0 mM at seven days after saline treatment. Photosynthetic rate and stomatal conductance were closely related and showed positive correlation. Stomatal conductance is used in diagnosis of opening of stoma on the leaves. Stomata were not only the pathway of CO_2 dispersion but also the pathway of stoma transpiration. Decrease in stomatal conductance with increase of saline was a protective mechanism to suppress water loss caused by stomatal transpiration, so stomatal conductance is related to photosynthesis. In this study, in case of 50 mM which was low-saline, plant did not receive large saline damage. However, in case of 100 mM which was high-saline treatment, photosynthetic rate is significantly decreased due to closing of stoma. Xu *et al.* (1994) reported that decreased photosynthesis under high saline level is the cause of the decrease of stomatal conductance. The results of this study were consistent with result of Lee *et al.* (1998) in which photosynthesis of tobacco decreased significantly with elevated saline level under

Table 1. Changes in leaf photosynthetic rate of corn seedling grown under different NaCl treatment

Days after treatment	NaCl concentration (mM)	A^1 (μmol $m^{-2} s^{-1}$)	Gs (μmol $m^{-2} s^{-1}$)	Ci (ppm)
1	0	21.55 ± 0.55(100)*	0.16 ± 0.01	148.2 ± 14.3
	50	19.35 ± 0.70(90)	0.12 ± 0.01	110.7 ± 17.4
	100	16.45 ± 0.66(76)	0.12 ± 0.01	140.6 ± 17.6
4	0	19.00 ± 1.08(100)	0.16 ± 0.02	114.2 ± 9.4
	50	16.29 ± 0.14(86)	0.12 ± 0.00	99.6 ± 6.6
	100	9.38 ± 0.78(49)	0.07 ± 0.00	90.2 ± 1.7
7	0	15.81 ± 0.66(100)	0.12 ± 0.01	105.7 ± 10.7
	50	14.16 ± 0.24(90)	0.10 ± 0.01	109.3 ± 13.7
	100	4.95 ± 0.44(31)	0.04 ± 0.00	120.1 ± 12.7

A^1, Leaf photosynthetic rate, Gs, Stomatal conductance
Ci, Intercellular CO_2 concentration
*, Figures in the parentheses are percentage ratio to the control

saline stress condition. Fig. 2 showed the changes of osmotic pressure potential of leaves juice by saline level in water culture of corn seedling Osmotic pressure potential of leaves juice of 50 mM decreased to 20% of 0 mM at one day after treatment and it decreased to 58% in case of 100 mM NaCl solution. Luttus *et al.* (1995) reported that significant decrease in water potential of leaves with saline stress was caused by intracellular accumulation of organic or inorganic solute. Katsuhiro *et al.* (1996) reported that when water potential of plant decreased to -2.0 or lower MPa, there were suppression of cell division, suppression of cell wall and

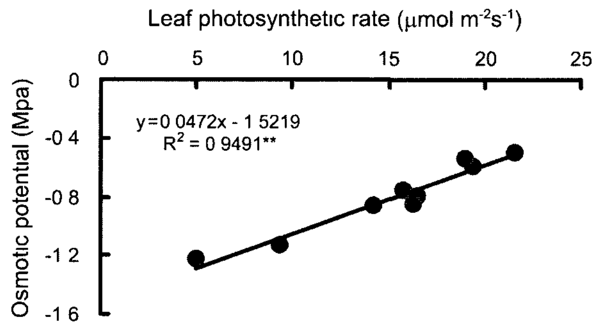

Fig. 1. Relationship between leaf osmotic potential and leaf photosynthetic rate of corn seedling under different NaCl treatment.

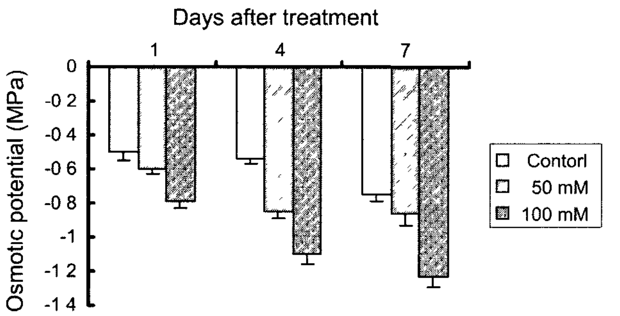

Fig. 2. Changes of osmotic potential of leaf of corn seedling under different NaCl treatment.

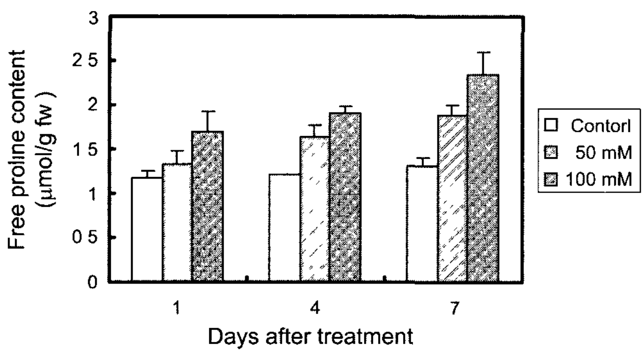

Fig. 3. Changes of free proline content of corn seedling under different NaCl treatment

protein synthesis, accumulation of solute, colsing of stoma, and suppression of photosynthesis were resulted with withering to die finally. In this study, photosynthesis in low-saline as much as 50 mM showed slight decrease comparison to 0 mM due to salt tolerance of corn but high-saline as much as 100 mM large decrease which exceeded the limit of salt tolerance of corn. Fig. 3 showed changes of the content of free proline of above ground corn seedling by saline level in water culture. The content of free proline was increased 1.14 time of 0 mM in case of 50 mM at one day after saline treatment and to 1.45 time in case of 100 mM. The content of free proline increased to 1.45 time of 0 mM in case of 50 mM at seven days after saline treatment add to 1.80 time in case of 100 mM. The content of free proline increased with higher level of saline and increased by the lapse of time also. This result was consistent with the report of Lee *et al.* (1992) in which proline content increased significantly with elevated NaCl level in rice plant, and consistent with the report of Heuer & Nadler (1998) in which proline was accumulated and adjusted osmic pressure in the plant under saline damage in potatoes. Hanson *et al.* (1979) reported that carboxy group of glutamic acid which the precusor of proline, was reoxidated to aldehyde group to synthesize proline under water insufficient situation, and the synthesized proline was translocation to other site. Huber *et al.* (1974) reported that NaCl promoted the activation of Δ-pyrroline-5-carboxylase that induce proline accumulation, and Choi *et al.* (1997) reported that increase of free amino acid under drought damage or saline damage was caused by complicated factors of metabolism process with resolution of protein, suppression of protein synthesis in amino acid, and suppression of photosynthesis. He also reported that in terms of metabolism of free amino acid, it might be considered as adaption to inappropriate environment. In this study also, it was found that content of proline increased with the elevated saline level of corn by the lapse of time. Osmotic pressure potential and photosynthetic rate had highly positive correlation (Fig. 1). That means that the higher osmic pressure potential was, the more photosynthetic rate increased; the lower the osmotic pressure potential was, the less photosynthetic rate decreased. This phenomonon may considered as the result of suppressed photosynthetic rate due to insufficient water condition (Katsuhiro *et al.*, 1996). Stomatal conductance has highly positive correlation with osmic pressure potential. Bethke *et al.* (1992) reported that low water potential closes stoma. Stomatal conductance is presumed to be changed by cellular turgor pressure caused by osmotic pressure potential. Osmotic pressure potential and free proline content shows highly positive correlation; this was consistent with the report by James *et al.* (1996) in which osmotic pressure adjust is made in the plant body through accumulation of chloride and proline under saline damage on potatos, and with consistent the report by McNulty (1985) that proline was synthesized by supplement cellular water stress caused by salt and it took an important role in osmotic pressure adjustment.

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