Korean J. Crop Sci. 51(2): 137~141(2006)

Early Growth, Carbohydrate and Phytic Acid Contents of Germinating Rice Seeds under NaCl Stress

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ABSTRACT: Germination characteristics and alterations in soluble sugar-starch transition and phytic acid during germination were studied in rice seeds under saline conditions. NaCl significantly reduced the speed of germination. Also, the radicle growth out of seeds was severely inhibited by the exposure to NaCl solution, thus, seeds were almost impossible to grow to seedlings. Soluble sugar was remarkably accumulated, whereas starch was decomposed stepwise during seed germination. The metabolism of soluble sugar and starch in germinating seeds showed a distinct difference. The level of phytic acid in seeds decreased in all NaCl treatments during germination, but the level was affected differently by NaCl concentration in the two varieties. Overall, our results suggest that salt stress retard the radicle growth of rice seeds, and affect the starch-to-sugar conversion and the decomposition of phytic acid differently in two varieties.

Keywords: carbohydrate, germination, phytic acid, rice, salt

Saline condition is a major factor limiting the establishment of plants from seeds. The response to salinity during germination has been reported to be more complex than during plant growth because it depends on the availability of stored compounds (González *et al.*, 1985). Some plants that have been classified as salt sensitive, such as tomato and maize, can germinate under high concentrations of NaCl (Kurt *et al.*, 1986; Maas *et al.*, 1983). Salt stress affects germination percentage, germination rate, and seedling growth in different ways depending on plant species. It has been found that germination percentage and growth of young seedlings were reduced with a high NaCl (Ghoulam & Fares, 2001). Salinity caused a delay in germination and reduced germination percentage in different varieties of melon (Botia *et al.*, 1998), and tomato (Cuartero & Fernández-Muñoz, 1999). A common adaptive mechanism of most plants under salinity stress is to accumulate a number of organic solutes which have little effect on plant metabolism (Bohnert *et al.*, 1995). These metabolites include amino acids, polyamines and carbohydrates. Soluble carbohydrates and their polyol derivatives are the most common osmolytes accumulating in plants in response to low water potentials. Experiments with model systems have demonstrated that sugars can protect the structural integrity of membranes during dehydration by preventing membrane fusion, phase transition and phase separation (Crowe & Crowe, 1992). Phytate is a storage form of phosphorus which is found in plant seeds and in many roots and tubers. Phytic acid has the potential to bind calcium, zinc, iron and other minerals (Oke, 1990). On the other hand, phytate may play an important role as an antioxidant by complexing iron and thereby reducing free radical generation and the peroxidation of membranes (Graf *et al.*, 1987). Decomposition of phytate in germination seeds may provide P, *myo*-inositol (MI) and mineral cations for the growth of seedlings (Raboy, 1991). The growth and development of plants is particularly dependent upon the availability of phosphate, under conditions of phosphate limitation. The demand for phosphorus increases dramatically during periods of rapid cell growth and division, such as seed germination (Hegeman & Grabau, 2001). The aim of the present work was to study the effect of salinity (NaCl) on the germination and early growth of rice seedlings in the two varieties known for different sensitivity to salinity. An additional aim was to determine if changes in storage compounds, such as carbohydrates and phytic acid, can be related to salt stress.

MATERIALS AND METHODS

Plant materials and salt treatment

Rice seeds (*Oryza sativa* L. cvs. Ilpumbyeo and Gancheockbyeo), were surface-sterilized with a 2 % (w/v) solution of sodium hypochlorite. The surface-sterilized seeds were placed on layers of filter paper moistened with NaCl 0, 50 and 100 mM solution in Petri-dishes (20 seeds per dish)

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<Received August 2, 2005>

and the Petri-dishes were transferred into incubators (30°C, 12hr-photocycle) to allow germination.

Germination

Rice seeds were considered as germinated one when the radicle emerged through the seed coat. Germinated seeds were counted daily for 4 days. Four parameters of germination were determined : 1) final germination percentage (FG); 2) number of day to final germination; 3) germination rate (GR, Osboren *et al.*, 1993) is a measure of rapidity of germination with lower values indicating faster germination. It was calculated as follows; GR = $(n_1t_1) + (n_2t_2) +\ldots+ (n_\chi t_\chi)/\chi^n$ where n_1 is the no. of germinants at the first day of germination, t_1 is the day from start to first germination, and χ^n is the total numbers of seeds germinated; 4) mean daily germination (MDG), (Osborne *et al.*, 1993) where MDG = final germination percentage/no. of days to final germination percentage.

Soluble carbohydrate

According to Roe method (1955), soluble sugar (SS) was extracted by heating rice seeds (0.2 g) in 80% ethanol. SS from alcohol extracts was determined with an anthrone procedure. In brief, a mixture of 6 ml of 0.2% anthrone in concentrated H_2SO_4 (w/v) and 3 ml of extract was heated in a boiling water for 7.5 min, and measured at 630 nm using spectrophotometer. The solid fraction was used for starch analysis. Starch was extracted with 9.3 N perchloric acid (PCA). The starch concentration was determined by the anthrone method as described above. Glucose was used as standard for both soluble sugar and starch.

Phytic acid

To determine phytic acid, the method of Garcia-Villanova & Rope (1982) was employed. Dried seed samples (2 g) were extracted with 40 ml of HCl-Na_2SO_4 solution for 90 min at room temperature on a rotary shaker. A mixture of transparent floating liquid, HCl-Na_2SO_4 solution, Fe(III) solution, and 20% sulfosalicylic acid solution (v:v:v:v = 1:1:1:1) was placed in neutral glass tube, and heated in a boiling water for 15 min. After cooling and checking the appearance of ferric phytate precipitate, 20 ml of the supernatant was added in 200 ml of deionized water to increase pH to 2.5 ± 0.5. After heating at 70°C the resultants were titrated with 0.01 M EDTA solution until bright yellow. The percentage of phytic acid was calculated from the following equation:

Phytic acid (%) = 1.32(10-V)/P
V = EDTA solution volume, ml
P = Sample weight (g)

Statistics

The experimental design was a randomized complete design with three replicates. All data were subjected to an analysis of variance and when significance ($P < 0.05$, 0.01, 0.001) occurred for treatment effect, a least significant difference (LSD) was calculated using SAS 8.12.

RESULTS AND DISCUSSION

Under different NaCl concentrations, final germination, GR and MDG of seeds of the two rice varieties were determined (Table 1). The p values of germination characteristics in two varieties had no significant difference (FG, 0.117; GR, 0.903; MDG, 0.924). In contrast with the result observed in two varieties, the treatment of different NaCl concentrations had influence on seed germination. Statistical significance between 0 and 50 mM of NaCl didn't exhibit, whereas 100 mM of NaCl retarded remarkably seed germination, especially for cv. Ilpumbyeo. However although the speed of germination decreased with increasing salinity in cv. Gancheockbyeo, the final % germination was not affected by the level of salinity. The cumulative germination rates with germination time were measured under different NaCl concentrations (Fig. 1). Seed germination at 2 days after

Table 1. Germination characteristics of two rice varieties observed for 4 days.

NaCl (mM)	cv. Ilpumbyeo			cv. Gancheockbyeo		
	FG (%)	GR	MDG	FG (%)	GR	MDG
0	96.7 ± 1.4a†	2.31 ± 0.03b	24.2 ± 0.4a	96.7 ± 2.9a	2.48 ± 0.9b	24.2 ± 0.7a
50	95.0 ± 2.5a	2.51 ± 0.03b	23.8 ± 0.6a	95.0 ± 0.0a	2.74 ± 1.2b	23.8 ± 0.0a
100	78.3 ± 2.9b	3.00 ± 0.09a	19.6 ± 0.7b	91.7 ± 2.9b	3.32 ± 1.9a	22.9 ± 0.7b

†Means within a species that have the same letter are not significantly different ($p < 0.05$, $n = 3$). GR = $(n_1t_1) + (n_2t_2) +\ldots+ (n_\chi t_\chi)/\chi^n$ where n_1 is the no. of germinants at the first day of germination, t_1 is the day from start to first germination, and χ^n is the total numbers of seeds germinated, and MDG indicates final germination percentage/no. of days to final germination percentage.

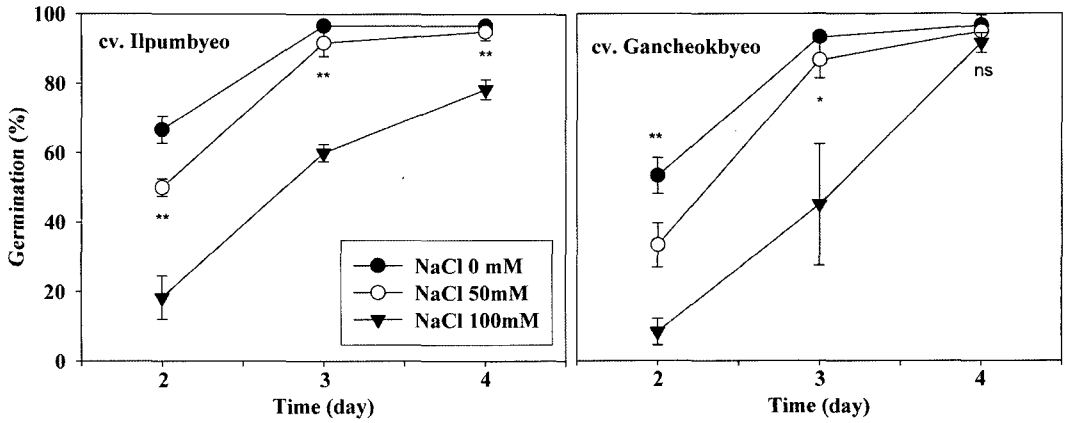

Fig. 1. Effects of NaCl on speed of germination. Rice seeds were exposed to different concentrations of NaCl (0, 50 and 100 mM) for four days. Each value is the mean ± S.D. of three replicates.

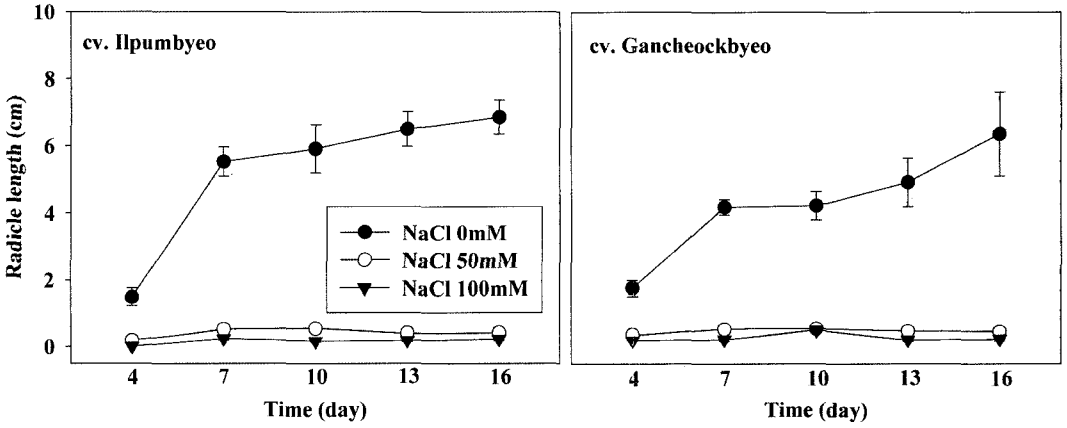

Fig. 2. Effects of NaCl on the radicle growth in germinating seeds. Rice seeds were exposed different concentrations of NaCl (0, 50 and 100 mM) for four days. Each value is the mean ± S.D. of seven replicates.

treatment was observed, and germination rate was different with NaCl concentrations. However, seeds exposed to 50 mM of NaCl recovered germination ability after 3 days, and the cumulative germination rate was similar to the control, whereas it was as inhibited as ever. Consequently, it was presumed that the inhibition of germination was caused by ion imbalance and hyperosmotic stresses. Based on the results of germination experiment, in order to know whether seed could be developed to seedling, the radicle length under different NaCl concentrations was measured (Fig. 2). The radicle growth of the seedlings was entirely sensitive to NaCl. The growth of seed radicle under 0 mM (distilled H_2O) showed a steady increase until 7^{th} day, and then it became gradually blunt, however, NaCl-treated seeds were affected severely for their radicle growth. Extension of the NaCl treatment period resulted in more rapid reduction in subsequent radicle elongation. This is coincident with the earlier finding of high concentration of salt tends to slow down or stop root elongation (Kramer, 1983), and causes reduction in root production (Garg & Gupta, 1997). As a result of this experiment, even though seeds germinated under saline, it was almost impossible for them to be grown to seedlings. We also examined the levels of soluble sugar and starch in germinating rice seeds under salt stress (Fig. 3). Soluble carbohydrates and their polyol derivatives are the most common osmolytes accumulating in plants in response to low water potentials. The conversion of starch to soluble sugar in endosperm was rapidly proceeded to maintain osmotic potential and produce a number of organic solutes during germination. Namely, the soluble sugar was markedly accumulated during germination period, whereas starch was gradually decomposed. The sugar content in cv. Ilpumbyeo lowered 2-fold at a concentration of NaCl treatment compared with the control, whereas in cv. Gancheockbyeo increased 5- and 2-fold at 50 mM and 100 mM of NaCl, respectively. Plants under salinity stress accumulate organic solutes which have little effect on plant metabolism (Bohnert *et al.*, 1995; Gilbert *et al.*, 1998). Lacerda *et al.*

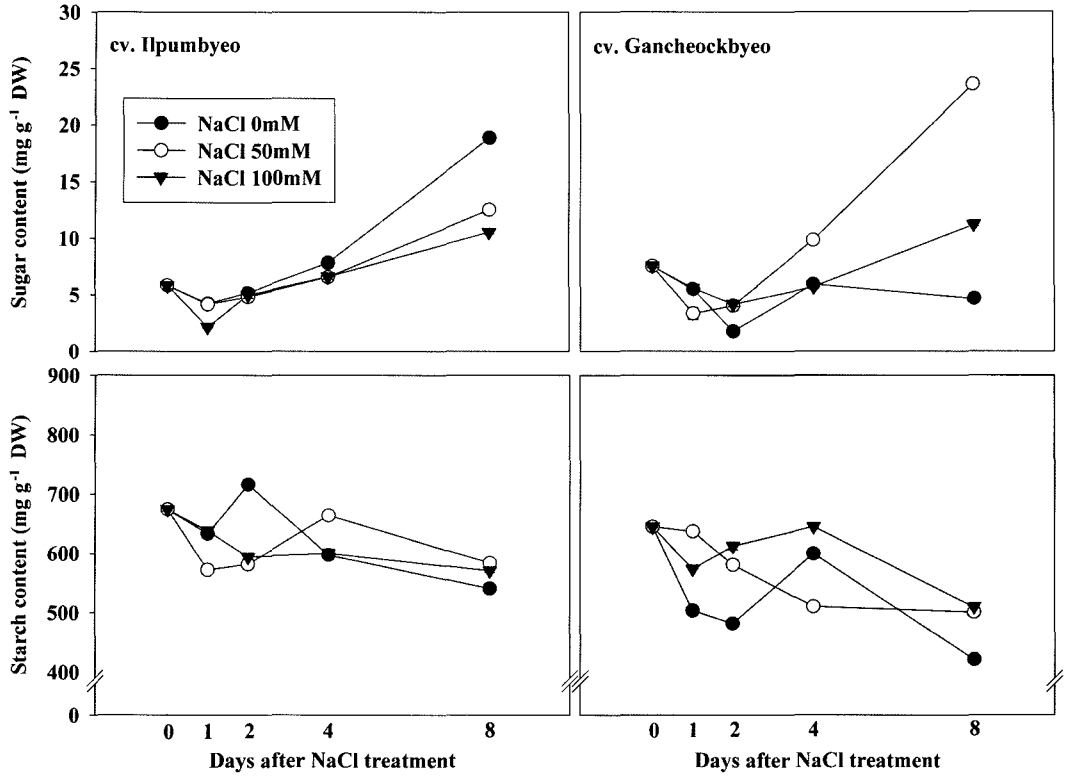

Fig. 3. Salt stress-induced changes in soluble sugar and starch in germinating seeds. Rice seeds were exposed to different concentrations of NaCl (0, 50 and 100 mM) for eight days. Each value is the mean ± S.D. of three replicates, and the *p* value for each treatment is below 0.001.

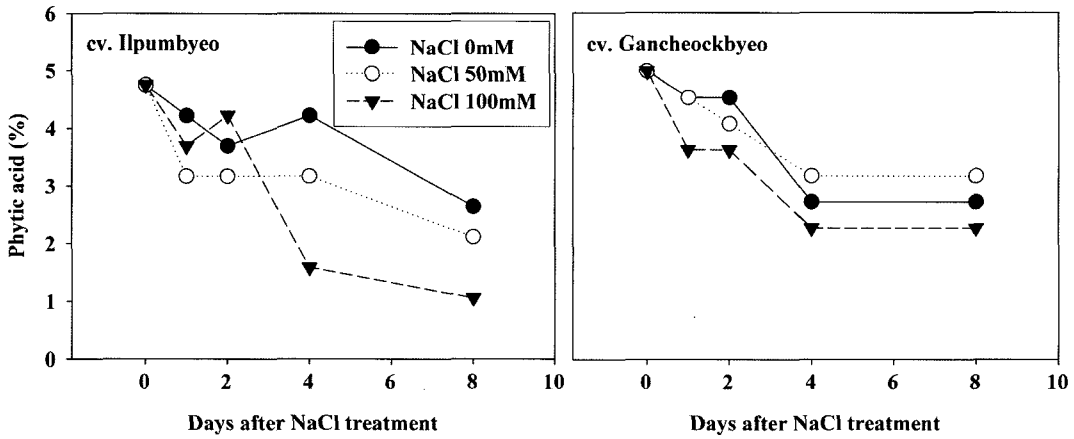

Fig. 4. The phytic acid content (%) of rice seeds as affected by germination time and variety under different NaCl concentrations. Rice seeds were exposed to different concentrations of NaCl (0, 50 and 100 mM) for eight days. Each value is the mean ± S.D. of three replicates, and the *p* value for each treatment is below 0.001.

(2003b) reported that if carbohydrate accumulation was due to a reduced utilization during salt stress period, the restriction to its utilization as a source of energy has not been eliminated. Our results indicate differences in the responses of sugar-starch metabolism to salinity in two varieties. The storage starch in seed of both varieties decreased slowly due to the hydrolysis by hydrolytic enzymes. The starch content of cvs. Ilpumbyeo and Gancheockbyeo decreased up to 15% and 23%, respectively, compared with that of non-germinated seeds. Phosphorus is stored in plant seeds as phytic acid during seed development. Thus, phosphorus, which is decomposed from phytic acid during germination, not only plays a vital role in energy transfer and metabolic regulation but is also an important macromolecular constituent of mol-

ecules such as phospholipids, proteins and nucleic acids. The phytic acid content in seeds of both varieties was presented in Fig. 4. The phytic acid for cv. Ilpumbyeo and cv. Gancheockbyeo before NaCl treatment was detected in 4.85% and 5.81%, respectively. For Ilpumbyeo, the phytic acid content at a concentration of 100 mM NaCl decreased significantly within the first 2 days of germination, however, it under 0 and 50 mM of NaCl dropped after 4 days of germination. Unlike Ilpumbyeo, the phytic acid content of Gancheockbyeo had a similar pattern of decrease without respect of NaCl concentrations. It decreased steeply within 4 days of germination, and then almost unchanged until the end of experiment. The decrease in the level of phytic acid during soaking may be attributed to leaching out inot soaking water under the concentration gradient (Abdullah *et al.*, 1984). Other researchers have reported decrease in the level of phytic acid during germination due to phytase activity in the germinating grain (Borade *et al.*, 1984; Mandal *et al.*, 1972). However, it was no report that the decrease of phytic acid content during germination was closely correlated with NaCl treatment. Considering our results, it was assumed that more rapid reduction of phytic acid under higher concentration of NaCl during germination was one way to increase inorganic and HCl-extractable divalent minerals, and to escape the detrimental effects caused by an ion imbalance.

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