.J. ISSAAS Vol. 15 No. 1: 32-43 (2009)

EFFECTS OF NaCl SALINITY ON THE SUGAR METABOLISM OF COMMON BEAN (PHASEOLUS VULGARIS L.) CV. 'TSURUNASHI MARUSAYA KUROSANDO' FRUIT GROWN IN SOLUTION CULTURE

Akio Tazuke[1], Teruo Wada[2] and Takuya Kanazawa[1]

[1] College of Agriculture, Ibaraki University, 3-21-1,
Chuo, Ami, Ibaraki, 300-0393, Japan
[2] Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-1,
Gakuen-cho, Naka-ku, Sakai, Osaka, 599-8531, Japan

(Received: August 28, 2008; Accepted: February 13, 2009)

ABSTRACT

Common bean is an important source of amino acid in Southeast Asia, where salinity as well as other abiotic stresses is an important growth limiting factor. In order to examine the effect of salinity on the edible part of common bean, effects of 60 mM NaCl addition to Hoagland No. 2 nutrient solution on the sugar metabolism of developing pericarps and seeds of common bean (*Phaseolus vulgaris* L.) cv. 'Tsurunashi Marusaya Kurosando' were studied. In pericarps, the hexose (glucose and fructose) concentration was slightly affected, but that of sucrose and starch was increased markedly by salinity. The activity of acid invertase (EC 3.2.1.26) was suppressed, and that of sucrose phosphate synthase (EC 2.4.1.14) was enhanced by salinity, suggesting the role of these enzymes in the accumulation of sucrose in the pericarp. The activity of sucrose synthase (EC 2.4.1.13) was not much affected by salinity. Salinity had little effect on the composition and the activities of sucrose metabolizing enzymes of seeds. This experiment showed the severity of the effects of salinity was different among whole plant, pods and seeds. The results show the potential for the production of common bean for seeds in Southeast Asia especially with respect to salinity tolerance.

Key words: invertase, seed, sucrose phosphate synthase, sucrose synthase

INTRODUCTION

In some Southeast Asian countries where cereals are the main dietary supply, deficiency of essential amino acids, e.g. lysine, is a problem (Pellet, 1996). In such countries, beans including common bean are important supplements because they have high lysine content (van der Maesen and Somaatmadja, 1989). Salinity is a serious problem in some places of Southeast Asia, such as Thailand (Dobermann and Fairhurst, 2000; Sinanuwong and Takaya, 1974). In addition, the tsunami in 2004 caused flooding of sea water in farming areas (Slavich et al., 2008).

Common bean is a relatively salt sensitive crop (Greenway and Munns, 1980). So, it is not recommended to intentionally plant common bean in saline soil. However, gradual salinization can occur in common bean farms. The effect of salinity on its growth has been reported (Valdez et al., 2002; Yamauchi et al, 1997). The edible part of common bean is usually the mature seeds, while the whole fruit, consisting of immature pericarp and immature seeds, is sometimes used as vegetable. Even in the case of seed production, however, the metabolism of the pericarp may affect seed growth during development. Thus, the effects of salinity on the growth and composition of common bean fruit should be assessed for pericarp and seeds separately, but there seems to be few reports addressing this problem: most reports are concerned with whole plant growth (Valdez et al., 2002; Yamauchi et al, 1997). In soybean, growth and glucose, sucrose and starch concentrations were studied in seeds and pericarps under varying source condition (Fader and Koller, 1985). In common bean, the translocated sugar is sucrose (Thorne, 1985). Enzymes that metabolize sucrose (acid and neutral invertase (EC

3.2.1.26), sucrose synthase (EC 2.4.1.13) and sucrose phosphate synthase (SPS) (EC 2.4.1.14)) may be involved in the accumulation and usage of the translocated sugar and sink activity in pericarps and seeds.

Here we studied the effects of salinity on the sugar composition and the activities of sucrose metabolizing enzymes of pericarps and seeds of common bean grown in solution culture.

MATERIALS AND METHODS

Cultivation

Common bean (*Phaseolus vulgaris* L.) cv. 'Tsurunashi Marusaya Kurosando' was grown in solution culture in a glasshouse in Osaka, Japan, in the spring of 1991. During the experimental period, the temperature in the glasshouse during the day was about 30^{o}C, night temperature was about 25^{o}C and day length was about 14 h. Seeds were sown on river sand, and after 10 days when the primary leaves have already expanded, four seedlings were transferred to a plastic container (circular holder) holding 14 L Hoagland No. 2 solution. De-ionized water with electric conductivity less than 10^{-8} Sm^{-1} was used to prepare the solution which was aerated at a rate 0.5 L min^{-1}. The pH of the solution was adjusted to 6.0 every two days and replenished every two weeks.

Sodium chloride (NaCl) was added to the nutrient solution to reach the NaCl concentration of 0 or 60 mM on 7 days after the start of solution culture in the experiment of the analysis of sugar metabolism, and on 10 days after the start of solution culture in the experiment of plant growth analysis. In preliminary experiments, 100 mM NaCl killed the plants and 80 mM NaCl caused marked plant damage, whereas effects of 20 or 40 mM NaCl on the sugar content of fruits were less marked than those of 60 mM NaCl. Thus, 60 mM, which has marked effect on sugar content of fruits but does not cause severe plant damage, was chosen as the NaCl concentration in this experiment. In the experiment of sugar metabolism, 3 fruits at each NaCl concentration were harvested 10, 12, 14 and 16 days after anthesis (DAA). For the harvest, the most basal fruit on the apical truss of the main stem was used. The seeds and pericarps were separated, weighed and frozen with liquid nitrogen and stored at -80^{o}C until analysis. In the experiment of plant growth analysis, 3 plants in each treatment were harvested 0, 10, 20, 30 and 40 days after the start of solution culture. Each harvested plant was weighed, dried at 70^{o}C for a week and weighed.

Sugar Analysis

Pericarp samples of about 2 g in fresh weight was freeze-dried for 4 days, weighed, powdered with mortar and pestle and extracted with 20 ml 80% ethanol at 80^{o}C for an hour. Portion of seed samples were weighed, oven dried at 70^{o}C for a week and weighed. Frozen seed samples were directly extracted with 20 ml 80% ethanol at 80^{o}C for an hour. After cooling, seed samples were homogenized with mortar and pestle. The extract solution was filtered with a glass filter (Whatman GF/F), and made up to 100 ml with 80% ethanol. The filtrate was used for sugar analysis. The residue (alcohol insoluble solids, AIS) was rinsed with diethyl ether and dried at 35^{o}C, weighed and used for the analysis of starch.

Twenty ml ethanol extract was dried with a rotary evaporator (Model N-1 EYELA, Tokyo Rikakikai Co., Ltd.) at 40^{o}C and re-dissolved with 2 ml de-ionized water. The concentrations of glucose, fructose and sucrose was measured by HPLC (Shimazu Shimpack CLC-NH_2 (M) column) using a re fractometer detector (JASCO RID-300S) with 75% acetonitril solvent system at a flowrate of 1 ml/min for pericarps and enzymatically (Boehringer Mannheim F kit) for seeds. Five ml of 0.5 N NaOH was added to AIS and homogenized to extract starch. After 30 min, 5 ml of 0.5N acetic acid was added to neutralize the solution. Ten ml of amylase solution {120 mg of glucoamylase (Sigma,

11600 Ug^{-1}) dissolved in 100 mM acetate buffer (pH 4.5)} was added to the extract solution, incubated at 37°C for 3 hours to hydrolyze the starch and centrifuged at 3000 g for 15 minutes. The glucose concentration of the supernatant was analyzed enzymatically (Boehringer Mannheim F kit).

Enzyme assay

The methods for the extraction of crude enzyme extract and the assay of enzyme activity for soluble acid invertase, neutral invertase and sucrose synthase were similar to those reported earlier (Tazuke and Wada, 2002). The assay method of insoluble invertase activity followed that of Schaffer et al. (1987). The extraction of SPS followed Hubbard et al. (1989). The frozen sample was ground in a chilled mortar using a 1:5 tissue-to-buffer ratio. The extraction buffer contained 50 mM HEPES (ph 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, 0.5mg ml^{-1} BSA and 0.05% Triton X-100. Homogenates were centrifuged at 20,000g for 3 min with a refrigerated centrifuge (TOMY MRX-150). Supernatants were desalted immediately by centrifugal filtration. 1.7 ml of the supernatant was loaded on a Sephadex G-25 column equilibrated with the extraction buffer minus EDTA and Triton X-100 and centrifuged at 1800 rpm for 2 min. The filtrate was immediately used for the measurement of SPS activity. The reaction mixture for SPS (final volume 0.5 ml) consisted of 50 mM HEPES (pH 7.5), 15 mM $MgCl_2$, 25 mM UDP-glucose, 10 mM fructose-6-phosphate and 25 mM glucose-6-phosphate and 50µl crude extract. The reaction mixture was incubated for 30 min at 30°C. The reaction was stopped by the addition of 0.5 ml 30% KOH. The amount of sucrose generated was measured by anthrone method following the methods of Hubbard et al. (1989)

Experimental Design and Statistical Analysis

In both experiments for plant growth analysis and sugar metabolism, experimental design was completely randomized design with 3 replications. Comparison between treatments was based on SE of means.

RESULTS

Plant Growth

Sixty mM NaCl markedly suppressed the plant growth. At 40 days after the start of solution culture (i.e. 33 days after the start of treatment), the plant dry weight was about 50% of the control (Fig. 1). The growth reduction was due mainly to the marked reduction in branching which led to the reduction of total leaf area, although expansion of individual leaves was also reduced about 20% at 60 mM NaCl (data not shown).

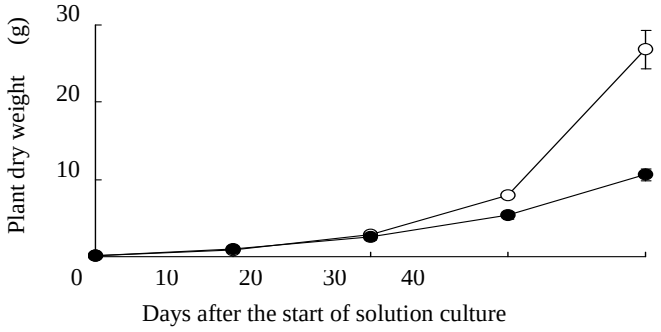

Fig. 1. Effects of NaCl addition to the nutrient solution on the growth in dry weight of common bean plant. NaCl was added 10 days after the start of solution culture. Open circles: 0 mM NaCl, closed circles: 60 mM NaCl. Vertical bars are SE of means.

Fruit Growth

The fresh weight of a pericarp increased linearly in both untreated control and 60 mM NaCl until 14 DAA,after which growth stopped. At 60 mM NaCl, the growth in fresh weight was markedly suppressed (Fig 2a). The percent dry matter of a pericarp was fairly constant during the harvest period. It was about 8% without NaCl and about 10% at 60 mM NaCl. The concentration of AIS of a pericarp was fairly constant during the harvest period and tended to be higher at 60 mM NaCl (data not shown).

A pericarp contained about 6 seeds. At 60 mM NaCl, the growth of as many as 2 seeds stopped growth at an early stage. However, for well-developing seeds, fresh weight of seeds increased linearly during the harvest period, but no difference was observed between the untreated control and at 60 mM NaCl. Thus, when seed weight was expressed as the total fresh weight of seeds per fruit, it tended to be lower at 60 mM NaCl (Fig 2b). The percent dry matter of seeds was fairly constant during the harvest period, being about 15% irrespective of the treatment. The AIS concentration of seeds was fairly constant during the harvest period, being about 8% irrespective of the treatment (data not shown).

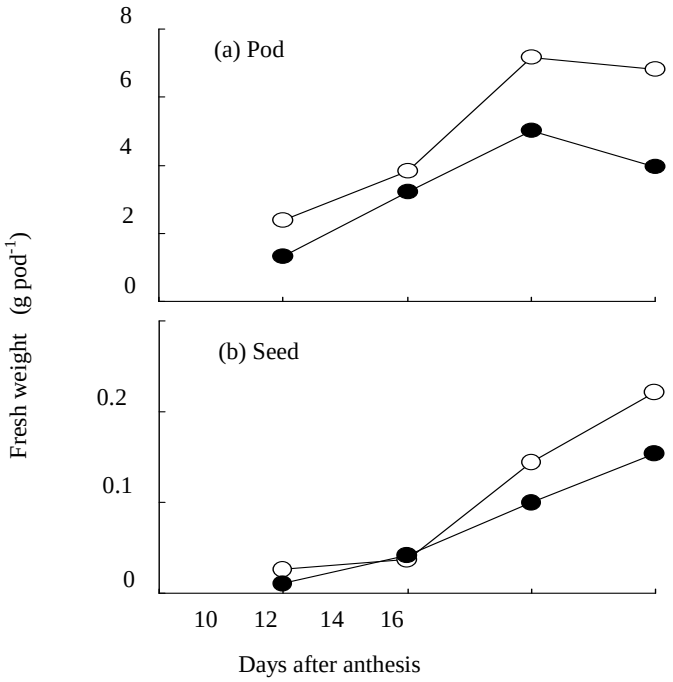

Fig. 2. Effects of NaCl addition to nutrient solution on the growth of pod and seed of common bean. Open circles: 0 mM NaCl, closed circles: 60 mM NaCl.

Sugar Concentration

Hexose (glucose + fructose) concentration of the pericarp was fairly constant (about 2.5%) during the harvest period (Fig 3a). Sucrose concentration in the pericarp was about 1/10 of hexose concentration. It tended to increase as the fruit grew, and was markedly higher at 60 mM NaCl (Fig 3b). Starch concentration of a pericarp began to increase from 10 DAA. It continued to increase until 16 DAA at 0 mM NaCl. At 60 mM NaCl, starch increased more rapidly than at 0 mM NaCl until 14 DAA and then stopped to accumulate (Fig 3c).

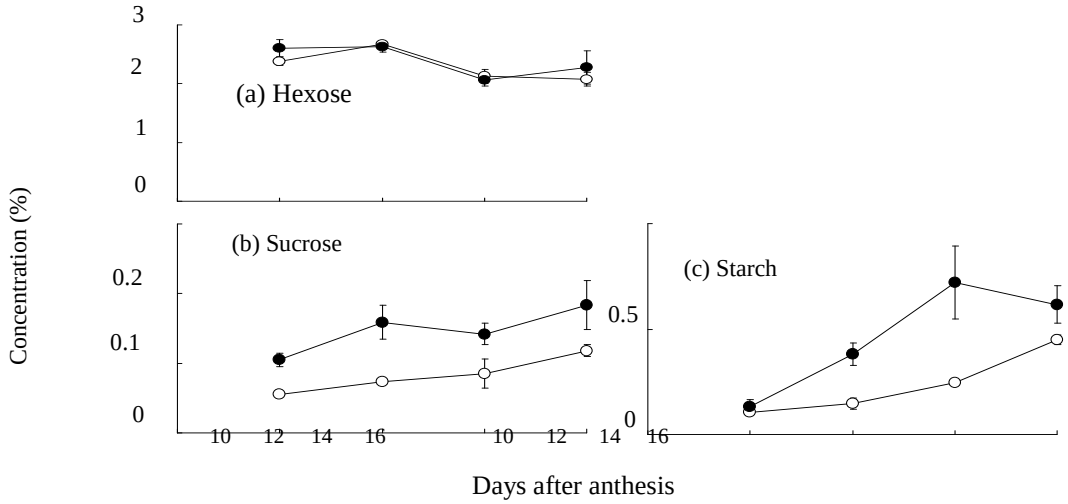

Fig. 3. Effects of NaCl addition to the nutrient solution on the concentration of hexose (glucose + fructose), sucrose and starch of common bean pericarp. Open circles: 0 mM NaCl, closed circles: 60 mM NaCl. Vertical bars are SE of means. The concentrations are expressed as percentages on the basis of fresh weight.

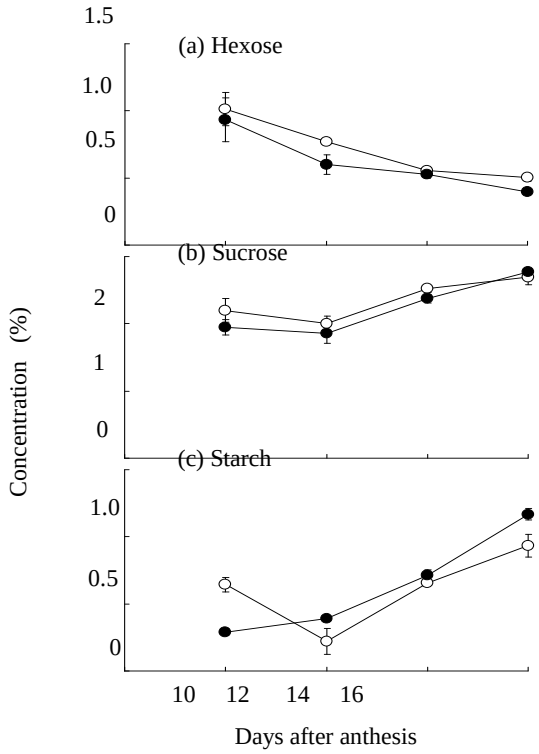

Fig. 4. Effects of NaCl addition to the nutrient solution on the concentration of hexose (glucose + fructose), sucrose and starch of common bean seed. Open circles: 0 mM NaCl, closed circles: 60 mM NaCl. Vertical bars are SE of means. The concentrations are expressed as percentages on the basis of fresh weight.

Hexose concentration of seeds declined during the harvest period. It was slightly lower at 60 mM NaCl than in untreated controls (Fig 4a). Sucrose concentration of seeds was apparently higher than that of pericarps, and increased slightly during the harvest period. There was no significant difference between the treatments (Fig 4b). Starch concentration of seeds tended to increase as fruits grew, and it was slightly higher at 60 mM NaCl at 12 DAA and onwards (Fig 4c).

Enzyme Activity

In pericarps, soluble acid invertase activity decreased as fruits grew, and it was lower at 60 mM NaCl as compared with the control. The activities of neutral invertase and insoluble invertase were negligible in both treatments (Fig 5a). In seeds, both soluble acid invertase and neutral invertase activities decreased as fruits grew, but there was not much difference between the treatments. The activity of insoluble invertase was fairly high and constant, but, again, there was no difference between treatments (Fig 5b).

Sucrose synthase activity was fairly constant in pericarps but decreased as fruits grew in seeds. In both pericarps and seeds, no difference in sucrose synthase activity between the treatments had been observed (Fig 6). In pericarps, sucrose phosphate synthase activity was fairly constant and tended to be higher at 60 mM NaCl (Fig 7a). In seeds, sucrose phosphate synthase activity decreased as fruits grew, but there was no difference between the treatments (Fig 7b).

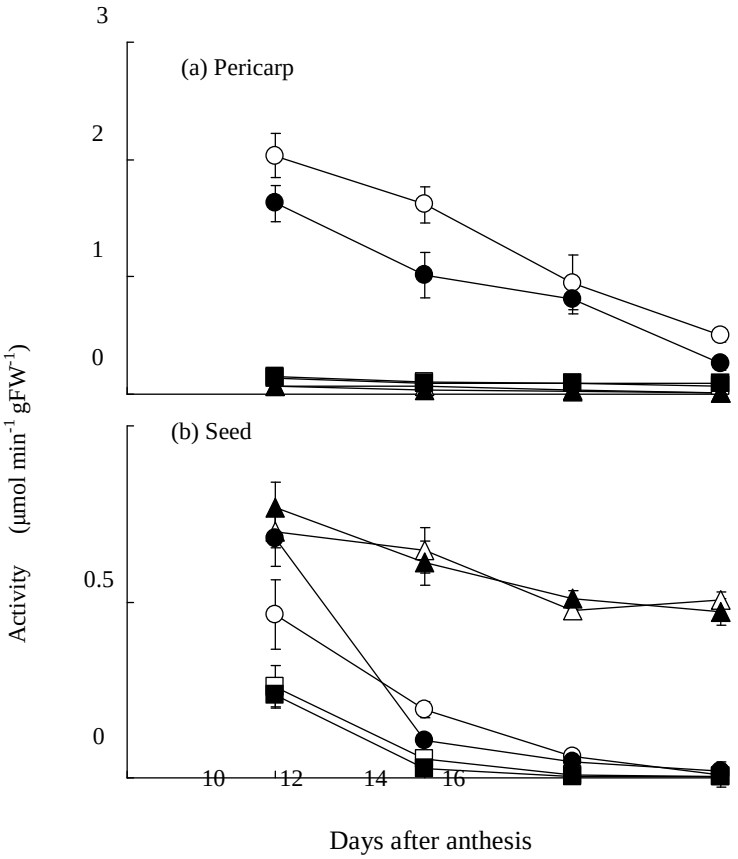

Fig. 5. Effects of NaCl addition to the nutrient solution on the activities of soluble acid invertase (circles), neutral invertase (squares) and insoluble invertase (triangles). Open symbols: 0 mM NaCl, closed symbols: 60 mM NaCl. Vertical bars are SE of means.

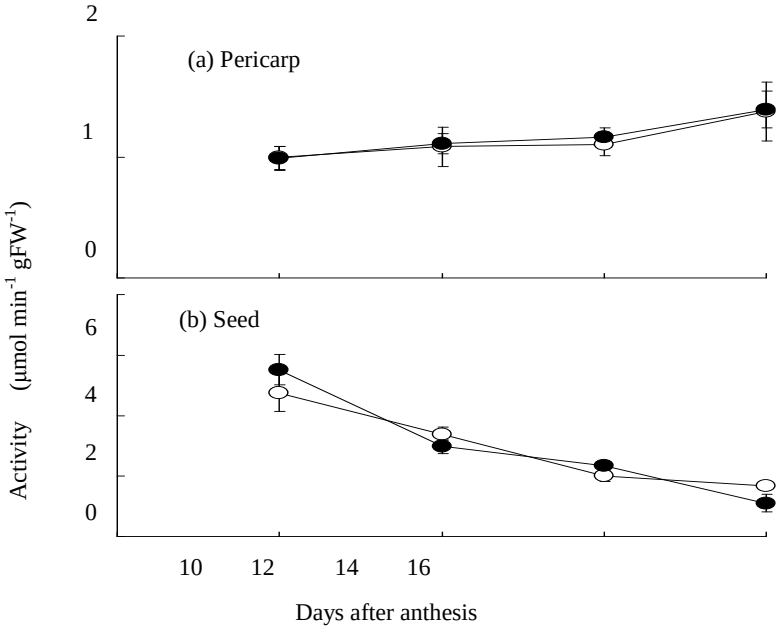

Fig. 6. Effects of NaCl addition to the nutrient solution on the activity of sucrose synthase of (a) pericarp and (b) seed. Open circles: 0 mM NaCl, closed circles: 60 mM NaCl. Vertical bars are SE of means.

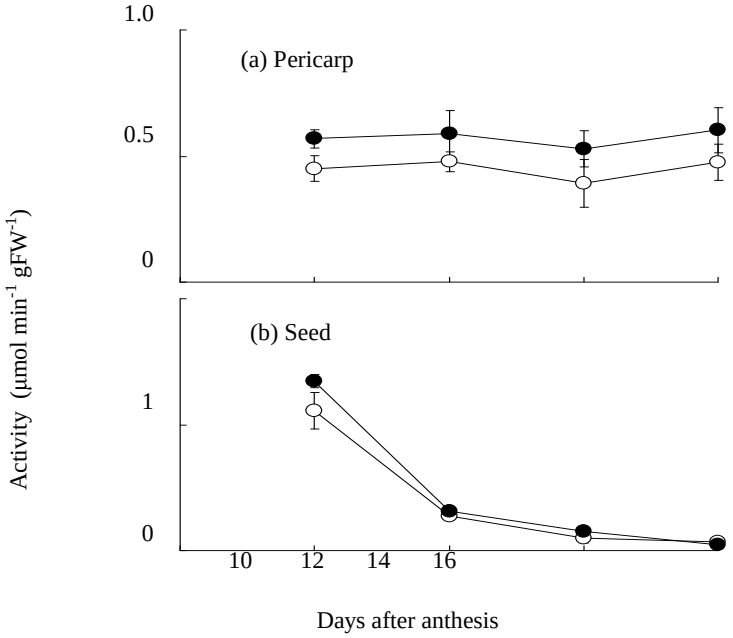

Fig. 7. Effects of NaCl addition to the nutrient solution on the activity of sucrose phosphate synthase of (a) pericarp and (b) seed. Open circles: 0 mM NaCl, closed circles: 60 mM NaCl. Vertical bars are SE of means.

DISCUSSION

The extent of growth suppression under salinity was different among whole plant, pericarps and seeds. It is interesting that pericarps and seeds showed different response to salinity. The reduction of pericarp growth is similar to the reduction of fruit growth in tomato (Adams, 1991). At 60 mM NaCl, some seeds in a pericarp stopped to grow, but other seeds grew at the same rate as those at 0 mM NaCl. In pea, soybean and lupin (Munier-Jolain et al., 1998), seed abortion was affected by treatments that affect source-sink relationship, i.e., depodding, defoliation, shading or changes in air CO_2 concentration. However, these treatments did not affect the growth rate of filling seeds. In soybean, water deficit causes the decrease in seed size, but this is due to the shortened growth period, and seed growth rate is unaffected (Westgate et al., 1989). These results suggest that in rapidly growing seeds, high sink activity is maintained. Our results conformed with this view and also showed the homeostasis of seeds at the sugar metabolism level.

In pericarps, concentrations of sucrose and starch markedly increased at 60 mM NaCl (Fig 3b, c). The increase of sucrose concentration of fruit under salinity is also reported for tomato (Saito et al., 2008) and cucumber (Tazuke, 2001). The decrease in the activity of soluble acid invertase (Fig 5a) and the increase in the activity of sucrose phosphate synthase (Fig 7a) in pericarps are consistent with the metabolic control of sucrose concentration as suggested for melon fruit (Hubbard et al., 1989). However, it is also possible that the increase in sucrose concentration is the result of negative correlation between sink activity and sucrose concentration in the sink (Walker and Ho, 1977). In tomato, starch concentration of a fruit increases temporally before maturation (Ehret and Ho, 1986; Gao, et al., 1998; Robinson et al., 1988), and it is suggested that the increase in hexose concentration of mature fruit is due to the increase in the temporal accumulation of starch (Gao et al., 1998). The temporal starch accumulation in tomato fruit is enhanced by salinity (Ehret and Ho, 1986; Gao et al., 1998). Hexose concentration was not much affected by salinity. Hexose concentration was not much affected (Saito et al., 2008) or rather reduced (Ehret and Ho, 1986) in immature tomato fruit under salinity. The mechanism of the increase of sugar concentration under salinity has been studied by many workers (Adams, 1991; Ehret and Ho, 1986; Gao et al., 1998; Saito et al., 2008), but it is still not elucidated. One simple interpretation may be the condensation effect: the decline in fruit growth under salinity condenses sugars. However, in our experiment, hexose concentration did not increase under salinity, which contradicts the simple condensation interpretation.

In seeds, neither sugar concentration (Fig 4) nor the activities of sucrose metabolizing enzymes (Fig 5b, Fig 6b, Fig 7b) was much affected by salinity. This indicates that the maintenance of sink activity of seeds under salinity also accompanies the homeostasis at the level of sugar metabolism. In legume seeds, the seeds have no symplastic connection to the maternal tissue, and seeds are nourished by the exudate from the seed coat (maternal tissue symplastically connected to pericarp) (Thorne, 1985). It is possible that the change in the sucrose metabolism in pericarp, the immediate source to the developing seeds, is involved in the homeostasis of sugar metabolism in seeds by compensating the perturbation caused by salinity. In cowpea, recovery from the water stress increases the water potential of pericarp, but the turgor pressure of the cells of seed coat is relatively low and essentially unchanged (Shackel and Turner, 2000). This suggests that the homeostasis is working at the level of seed coat. Geromel et al. (2006) measured the activities of acid invertase, sucrose phosphate synthase and sucrose synthase in pericarp, persperm and endosperm of coffee fruit, and found the activities are different between perisperm and endosperm. In our experiment, seed coat (maternal tissue) and embryo (offspring tissue) were not separated. Such a separation can yield further useful information about the sink control of the embryo.

Because common bean is a relatively salt sensitive crop (Greenway and Munns, 1980), it is not realistic to intentionally plant it to saline soil. However, gradual salinization can occur in coastal area and tsunamis (Slavich et al, 2008) can cause sea water flooding. The result of this experiment

suggests that common bean might be cultivated for seed production, not pod production, in these salinized soil.

CONCLUSION

The growth suppression by salinity was seen in whole plant and pericarps, but not in seeds. Also, the concentration of sugars and the activity of sucrose catalyzing enzymes were affected in pericarps, but not in seeds. The cause of these differences among plant parts in the response to salinity needs further study.

REFERENCES

Adams, P. 1991. Effects of increasing the salinity of the nutrient solution with major nutrients or sodium chloride on the yield, quality and composition of tomatoes grown in rockwool. J. Hortic. Sci. 66: 201–207.

Dobermann A. and T. Fairhurst. 2000. Rice. Nutrient disorders & nutrient management. Handbook series. Potash & Phosphate Institute (PPI), Potash & Phosphate Institute of Canada (PPIC) and International Rice Research Institute. 191 p.

Ehret, D.L. and L.C. Ho. 1986. The effects of salinity on dry matter partitioning and fruit growth in tomatoes grown in nutrient film culture. J. Hortic. Sci. 61: 361–367.

Fader, G.M. and H.R. Koller. 1985. Seed growth rate and carbohydrate pool sizes of the soybean fruit. Plant Physiol. 79: 663-666.

Gao, Z., M. Sagi and S.H. Lips. 1998. Carbohydrate metabolism in leaves and assimilate partitioning in fruits of tomato (*Lycopersicon esculentum* L.) as affected by salinity. Plant Sci. 135: 149-159.

Geromel, C., L.P. Ferreira, S.M.C. Guerreiro , A.A. Cavalari, D. Pot, L.F.P. Pereira, T. Leroy, L.G.E. Vieira, P. Mazzafera and P. Marraccini. 2006. Biochemical and genomic analysis of sucrose metabolism during coffee (*Coffea arabica*) fruit development. J. Exp. Bot. 57: 3243-3258.

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

Hubbard, N.L., S.C. Huber and D.M. Pharr. 1989. Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. Plant Physiol. 91: 1527-1534.

Munier-Jolain, N.G., N.M. Munier-Jolain, R. Roche, B. Ney and C. Duthion.. 1998. Seed growth rate in grain legumes. I. Effect of photoassimilate availability on seed growth rate. J. Exp. Bot. 49: 1963-1969.

Pellet, P.L. 1996. World essential amino acid supply with special attention to South-East Asia. United Nations University Press Food and Nutrient Bulletin 17: 204-234.

Robinson, N.L., J.D. Hewitt and A.B. Bennett. 1988. Sink metabolism in tomato fruit. I. Developmental changes in carbohydrate metabolizing enzymes. Plant Physiol. 87: 727-730.

Saito, T., C. Matsukura, Y. Ban, K. Shoji, M. Sugiyama, N. Fukuda and S. Nishimura. 2008. Salinity stress affects assimilate metabolism at the gene-expression level during fruit development and improves fruit quality in tomato (*Solanum lycopersicum* L.). J. Japan. Soc. Hort. Sci. 77: 61-68.

Schaffer, A.A., B. Aloni and E. Fogelman. 1987. Sucrose metabolism and accumulation in developing fruit of *Cucumis*. Phytochem. 26: 1883-1887.

Shackel, K.A. and N.C. Turner. 2000. Seed coat tugor in chickpea is independent of changes in plant and pod water potential. J. Exp. Bot. 51: 895-900.

Sinanuwong, S. and Y. Takaya. 1974. Distribution of saline soils in the Khorat basin of Thailand. Southeast Asia Studies 12: 365-382.

Slavich, P., M. McLeod, N. Moor, G. Tinning, R. Lines-Kelly, T. Iskandar, A. Rachman, F. Agus and P. Yufdy. 2008. Tsunami impacts on farming in Aceh and Nias, Indonesia. 2nd International Salinity Forum. 4 p.

Tazuke, A. 2001. Sugar concentration of cucumber fruits as affected by NaCl addition to nutrient solution. (Japanese text with English abstract). Environ. Control in Biol. 39: 281-288.

Tazuke, A. and T. Wada. 2002. Activities of sucrose catalyzing enzymes of cucumber fruits as affected by NaCl addition to nutrient solution. (Japanese text with English abstract). Environ. Control in Biol. 40: 321-325.

Thorne, J.H. 1985. Phloem unloading of C and N assimilates in developing seeds. Ann. Rev. Plant Physiol. 36: 317-343.

Valdez, M.T., T. Ito, Y. Shinohara and T. Maruo. 2002. Effects of nutrient solution levels on the growth, yield and mineral contents in hydroponically-grown bush snap bean. Environ. Control in Biol. 40: 167-176.

van der Maesen, L.J.G. and S. Somaatmadja. 1989. PROSEA 1: Pulses. Universal Book Serv, Bogor. 108 p.

Walker, A.J. and L.C. Ho. 1977. Carbon translocation in the tomato: Effects of fruit temperature on carbon metabolism and the rate of translocation. Ann. Bot. 41: 825-832.

Westgate, M.E., J.R. Schussler, D.C. Reicosky and M.L. Brenner. 1989. Effect of water deficits on seed development in soybean. II. Conservation of seed growth rate. Plant Physiol. 91: 980-985.

Yamauchi, M., S. Tanaka and H. Fujiyama. 1997. The cultivarietal difference in salt-tolerance and the effect of NaCl on the absorption and translocation of K, Ca and Mg ions in *Phaseolus vulgaris* L. (Japanese text with English abstract). J. Japan. Soc. Hort. Sci. 65: 737-745.