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Effect of salinity stress on growth and carbohydrate metabolism in three rice (*Oryza sativa* L.) cultivars differing in salinity tolerance

Wattana Pattanagul & Maysaya Thitisaksakul

Applied Taxonomic Research Center, Department of Biology
Faculty of Science, Khon Kaen University, Khon Kaen, 40002 Thailand

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Rice seedlings cv. Khao Dawk Mali 105 (salt-sensitive), Luang Anan (moderately salt-tolerant) and Pokkali (salt-tolerant) were exposed to 0, 50, 100 and 150 m*M* NaCl for 9 d. Salinity stress caused reduction in leaf relative water contents in all cultivars. Shoot length of cv. Pokkali was least affected by salinity stress whereas increased root length in response to salinity stress was apparent in cvs. Khao Dawk Mali 105 and Luang Anan. Increased salinity level also caused reduction in fresh and dry weights in cvs. Khao Dawk Mali 105 and Luang Anan, but had no effect in cv. Pokkali except at 150 mM. Accumulation of total soluble sugars and sucrose in mature leaves were observed in cv. Khao Dawk Mali 105 exposed to high level of salinity whereas their concentrations in cvs. Luang Anan and Pokkali remained the same as control plants. Accumulation of sucrose in cv. Khao Dawk Mali 105 was suggested to be resulted from the alteration of photosynthate partitioning since the activities of sucrose phosphate synthase were not affected by salinity in this cultivar. On the contrary, salinity stress induced an accumulation of starch in cv. Pokkali. It is suggested that partitioning sugars into starch may involve in salinity tolerance by avoiding metabolic alterations.

Keywords: Carbohydrate metabolism, Rice, Salinity stress, Salt tolerant

Salinity is a major environmental stress affecting plant productivity and constitutes a problem concerning many areas, with an emphasis on regions with hot and dry climates. The ability of plants to cope with salinity stress is an important determinant of crop distribution and productivity in many areas, so it is important to understand the mechanisms that confer tolerance to saline environment[1]. Salinity exerts its undesirable effects through osmotic inhibition and ionic toxicity. Osmotic inhibition is the result of the salt presented in the soil solution which reduces the ability of the plant to take up water, and leads to slower growth. Ionic toxicity is caused by an excessive amount of salt entering the transpiration stream which eventually injures cells in the transpiring leaves and may further reduce growth[2]. Reduction in growth and photosynthesis are among the most conspicuous effects of salinity stress. In addition, stomatal closure, in order to reduce transpiration, appears to be the main cause of the decrease in photosynthetic rate[3]. Thus, limited CO_2 availability can alter leaf carbohydrate content and source-to-sink translocation pattern[4].

Telephone: +66-43-342908
Fax: +66-43-364169
E-mail: wattana@biology.kku.ac.th

Plants normally cope with salinity stress in various ways. Among these responses, accumulation of compatible solutes including proline, soluble sugars, sugar alcohols and glycine betaine has received most attention in terms of their functions in osmotic adjustment[5,6]. Osmotic adjustment refers to the net accumulation of solutes in cells in response to a fall in the water potential of their environment. As a consequence, the cell osmotic potential lowers, and turgor pressure tends to be maintained[7]. Increased sugar concentration under salinity stress has been reported in many studies[8-10]. Their role in terms of osmotic adjustment is, however, still under debate. In many species, the absolute osmolyte concentrations are unlikely to mediate osmotic adjustment[11]. Additional benefits of these solutes have been described including buffering cellular redox potential and protecting cellular structure under stress condition. In addition, associated effects on photoassimilate allocation between source and sink tissues may also contribute to the accumulation of these solutes[6]. Although numerous studies have been done in the past few years to develop the rice (*Oryza sativa* L.) cultivars which can tolerate salinity condition, at least to some extent, the mechanisms of salinity tolerance in rice are not yet well understood.

In fact, our understanding in terms of carbohydrate metabolism under salinity condition is still very limited.

The main objective of this study is to determine the effects of salinity stress on growth and carbohydrate metabolism in rice cultivars differing in salinity tolerance. Three rice cultivars were selected including cv. Khao Dawk Mali 105 (salt-sensitive), Luang Anan (moderately salt-tolerant) and Pokkali (salt-tolerant)[2,12,13]. The activities of enzymes involved in sucrose metabolism such as sucrose phosphate synthase (SPS) and invertase in these rice cultivars were determined. This information may provide a background for understanding how salinity stress alters carbohydrate metabolism and the differences that exist in salt-tolerant and salt-sensitive cultivars to salinity stress.

Materials and Methods

Plant materials and treatments—Rice (*Oryza sativa* L.) seeds, cvs. Khao Dawk Mali 105, Luang Anan and Pokkali, were obtained from Rice Research Station, Khon Kaen, Thailand. Seeds were soaked in 5% sodium hypochlorite for 15 min and rinsed thrice with distilled water. Rice seedlings were germinated by placing the seeds on the moisted filter paper for 3 days. Approximately 10 moistened seedlings were then potted in a plastic pot containing soil:sand:peat mix (1:1:2, v/v) and kept in the greenhouse at the Department of Biology, Faculty of Science, Khon Kaen University, Thailand under natural illumination and temperature conditions. The seedlings were watered daily with 100 ml half strength Hoagland solution and allowed to grow for 14 days. The seedlings of each cultivar were then separated into 4 groups as follows: 0 (control), 50, 100, and 150 m*M* NaCl. Control plants were watered daily with 100 ml distilled water whereas salinity-stressed plants were irrigated daily with 100 ml of 50, 100 and 150 m*M* NaCl solution.

Plant growth and relative water content measurements—After exposing to salinity stress for 9 days, the seedlings were randomly sampled from each treatment. Root and shoot lengths of five plants from each treatment were determined. In addition, fresh weights of four plants from each treatment were recorded and then the plants were oven-dried at 80°C for 3 days in order to determine dry weight. Four additional plants from each treatment were randomly selected to determine leaf relative water content[14]. About 0.1 g leaf sample was cut into smaller pieces and weighed to determine fresh weight. The leaf sample was floated in freshly de-ionized water for 12 hr and weighed thereafter to determine fully turgid weight. The leaf sample was oven-dried at 80°C for 3 days and the dry weight was obtained. The relative water content (RWC) was determined using the following formula:

$$RWC = (\text{fresh weight-dry weight})/(\text{turgid weight-dry weight}) \times 100$$

Carbohydrate extraction and analysis—Leaf carbohydrate composition was determined after the rice seedlings were exposed to salinity stress for 9 days. Fully mature leaves were randomly selected from each treatment and frozen on dry ice to transfer to the laboratory. Approximately 0.1 g leaf tissue was used for carbohydrate analysis. Soluble sugars were extracted from the tissues in hot 80% (v/v) ethanol. Total sugar in leaf tissue was determined colorimetrically using phenol, sulfuric acid method[15]. Sucrose was determined according to Robin and Pharr[16]. The leaf residue remaining after ethanolic extraction was hydrolyzed in 1 ml 0.2 *M* KOH and boiled for 30 min. Samples were cooled and adjusted to about *p*H 5.5 by adding 2 ml 1 *M* acetic acid. Starch in the leaf residue was digested with amyloglucosidase overnight and released glucose was quantified enzymically using hexokinase/glucose-6-P dehydrogenase[17].

Enzyme extraction and assay—About 1 g leaf tissue was collected during midday for enzyme extraction and determination of sucrose phosphate synthase and invertase activities. The leaf sample was ground on ice using mortar and pestle in 5 ml grinding buffer containing 50 m*M* HEPES, 10 m*M* $MgCl_2$, 1 m*M* EDTA, 0.25% BSA and 5 m*M* dithiothreitol, *p*H 7.5. The extract was filtered through 4 layers of cheesecloth, then centrifuge for 1 min at 10000 *g*. Crude extract was desalted on 2 ml Sephadex G25 columns equilibrated with the grinding buffer. The amount of protein in the enzyme extract was determined by Bradford method[18].

Sucrose phosphate synthase (SPS) activity was determined by measuring sucrose-6-phosphate produced from the substrates, UDP-glucose and fructose-6-phosphate[16]. Approximately 80 μl desalted enzyme was incubated in a reaction mixture containing 25 m*M* UDP-glucose, 8 m*M* fructose-6-phosphate, 5 m*M* $MgCl_2$ at 25°C for 1 hr and terminated by adding 100 μl of 1 *N* NaOH. Unreacted

fructose-6-phosphate and fructose were destroyed by boiling the tube in a boiling water bath for 10 min. Sucrose-6-phosphate formed during the reaction was determined by reacting with 0.25 ml resorcinol solution and quantified by a spectrophotometer at 520 m*M*.

Invertase activities were determined under acidic (*p*H 4.0) and alkaline (*p*H 7.6) conditions. Approximately 50 µl of desalted enzyme was incubated with 125 mM sucrose (w/v) in the extraction buffer at the appropriate *p*H. Assays were run at 30°C for 30 min and stopped by boiling for 1 min. The glucose content of 10 µl aliquots of the assay mixture was determined spectrophotometrically at 360 nm using hexokinase/glucose-6-P dehydrogenase enzymes[19].

Results

Plant growth and relative water content—Increased salinity level caused reduction in shoot lengths in cvs. Khao Dawk Mali 105 and Luang Anan, but had little effect in cv. Pokkali. Shoot length of cv. Luang Anan increased slightly at 50 m*M* but decreased at 100 and 150 m*M*, while in cv. Pokkali it increased at 50 and 100 m*M*, but decreased at 150 mM (Fig. 1A). Shoot length of cv. Pokkali, however, was consistently higher than those of cvs. Khao Dawk Mali 105 and Luang Anan at all treatments. On the contrary, root lengths of the three cultivars responded differently to salinity stress. Root lengths of cvs. Khao Dawk Mali 105 and Luang Anan increased in response to increased salinity level, while salinity stress caused a reduction in root length in cv. Pokkali (Fig. 1B). Salinity stress also caused reduction in fresh weights in cvs. Khao Dawk Mali 105 and Luang Anan. Fresh weight of cv. Pokkali, however, was not affected by salinity stress except at 150 m*M* (Fig. 1C). Salinity stress also caused reduction in dry weights in cvs. Khao Dawk Mali 105 and Luang Anan, but not in cv. Pokkali (Fig. 1D). In addition, increased salinity level caused reduction in leaf relative water contents in all cultivars. Relative water content of cv. Pokkali was reduced from 90% in the control plants to 77% in the stressed plants. Relative water content of cv. Luang Anan was also reduced from 87% in the control plants to 76% in the stressed plants, while that of cv. Khao Dawk Mali 105 was reduced from 83% in the control plants to 68% in the stressed plant. It is noteworthy that leaf relative water contents in cv. Pokkali, a salt-tolerance cultivar, were higher than those of cvs. Khao Dawk Mali 105 and Luang Anan at all concentrations (Fig. 2).

Carbohydrate contents—Leaf carbohydrate compositions were differently altered by salinity

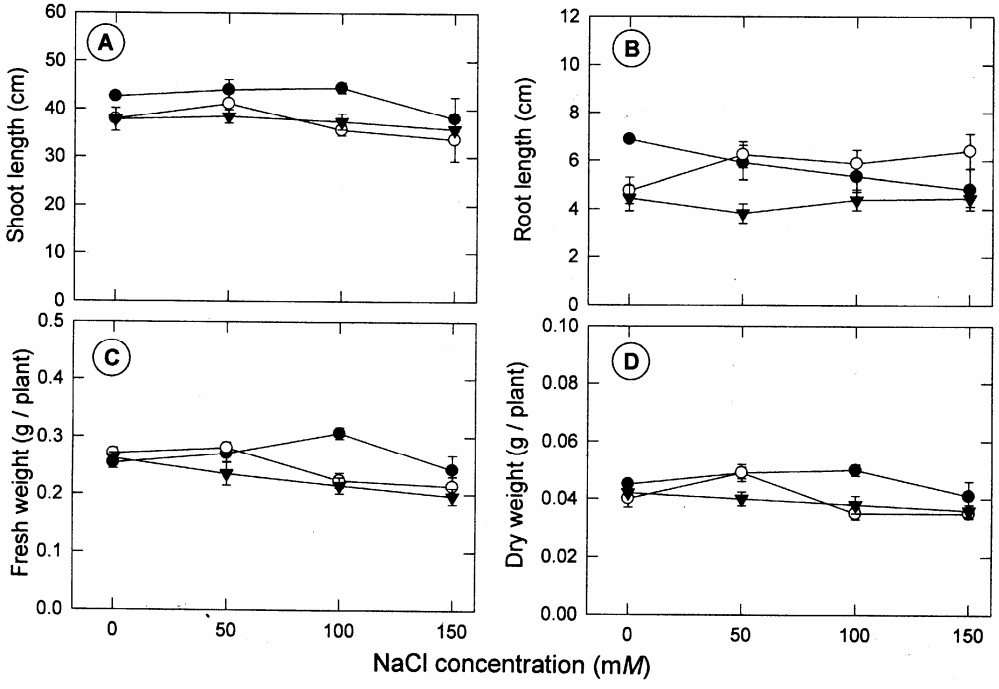

Fig. 1—Effects of increased NaCl concentrations on shoot length (A), root length (B), Fresh weight (C), and dry weight (D) in mature leaves. [Values are mean ± SE of 4 measurements per data point. ▼, cv. Khao Dawk Mali 105; ○, cv. Luang Anan; ●, cv. Pokkali.]

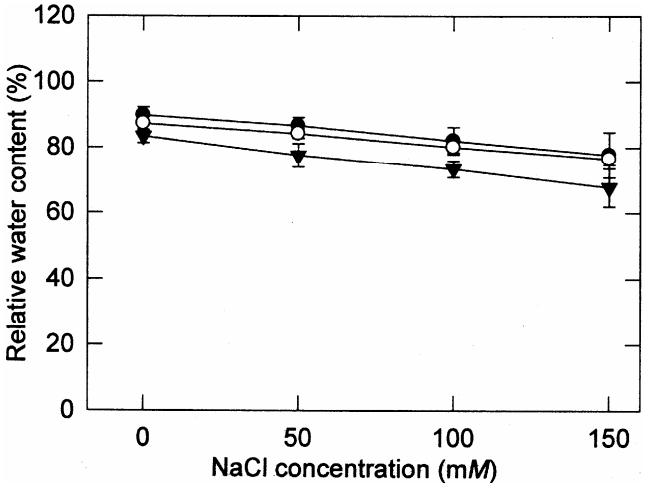

Fig. 2—Effects of increased NaCl concentrations on relative water content. [Values are mean ± SE of 4 measurements per data point. ▼, cv. Khao Dawk Mali 105; ○, cv. Luang Anan; ●, cv. Pokkali.]

stress (Fig. 3). Salinity stress induced total soluble sugar and sucrose accumulation only in cv. Khao Dawk Mali 105, but not in cvs. Luang Anan and Pokkali (Fig. 3A and 3B). In contrast to what was observed for sugar concentration, salinity stress increased starch accumulation in cv. Pokkali, while starch concentrations in cvs. Luang Anan and Khao Dawk Mali 105 were decreased with increasing salinity (Fig. 3D).

Enzyme activities—Salinity stress had no or little effect on SPS activities in cv. Pokkali. SPS activities in cv. Khao Dawk Mali 105, however, was reduced in cv. Luang Anan was induced. It is noteworthy that SPS activities of cv. Pokkali were higher than those of cvs. Khao Dawk Mali 105 and Luang Anan at all NaCl concentrations (Fig. 4A). Salinity stress also caused reduction in acidic invertase activities in cv. Khao Dawk Mali 105 while its activity in cv. Luang Anan was reduced at 50 m*M* and increased at 100 and 150 m*M* (Fig. 4B). Activities of acidic invertase in cv. Pokkali were induced by salinity at 50 and 150 m*M*. Activities of alkaline invertase were induced by salinity stress in all cultivars (Fig. 4 C).

Discussion

Growth reduction is generally observed in plants exposed to salinity stress. This may be partly due to lower water potential in the cells which, in turn, causes stomatal closure and limits CO_2 assimilation. In the present study, the salt-sensitive and moderately salt-tolerant cultivars showed growth reduction when

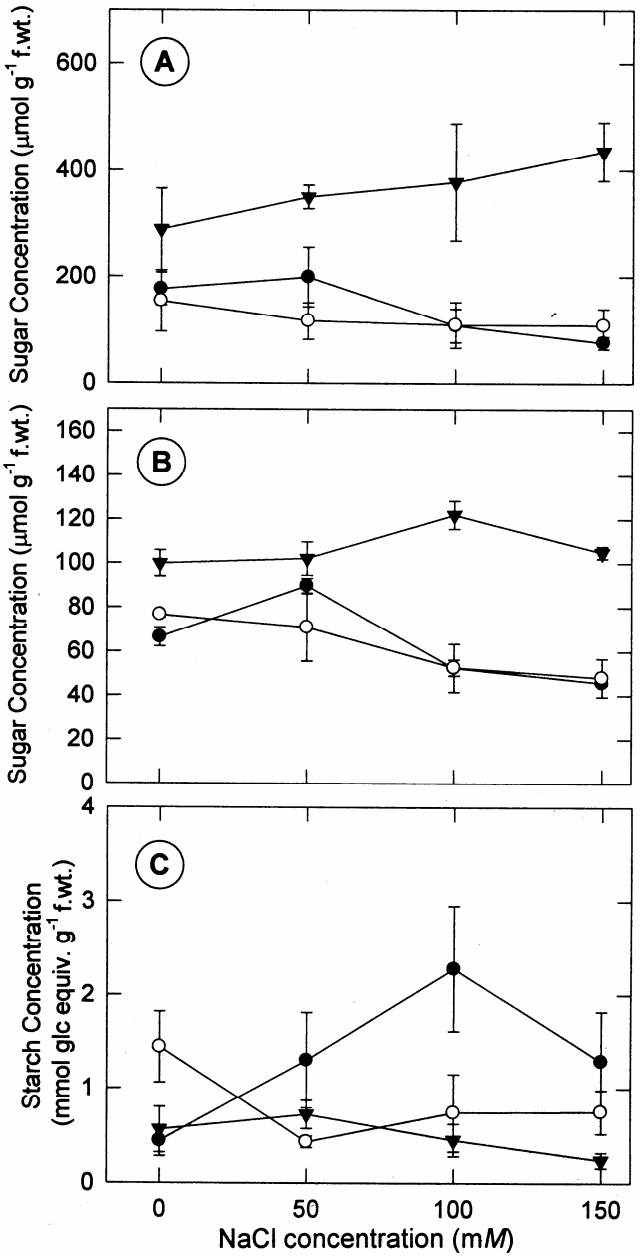

Fig. 3—Effects of increased NaCl concentrations on total soluble sugars (A), sucrose (B), and starch (C) contents in mature leaves. [Values are mean ± SE of 4 measurements per data point. ▼, cv. Khao Dawk Mali 105; ○, cv. Luang Anan; ●, cv. Pokkali.]

exposed to increased salinity level, whereas the salt-tolerant cultivar was able to tolerate to a greater extent. A marked growth reduction was reported earlier in sensitive varieties of japonica and indica rice seedlings exposed to salinity stress[20]. In addition, salinity caused pronounced effect on fresh and dry weights in salt-sensitive and moderately salt-tolerant cultivars, while salt-tolerant cultivar was able to maintain its fresh and dry weights. Root length,

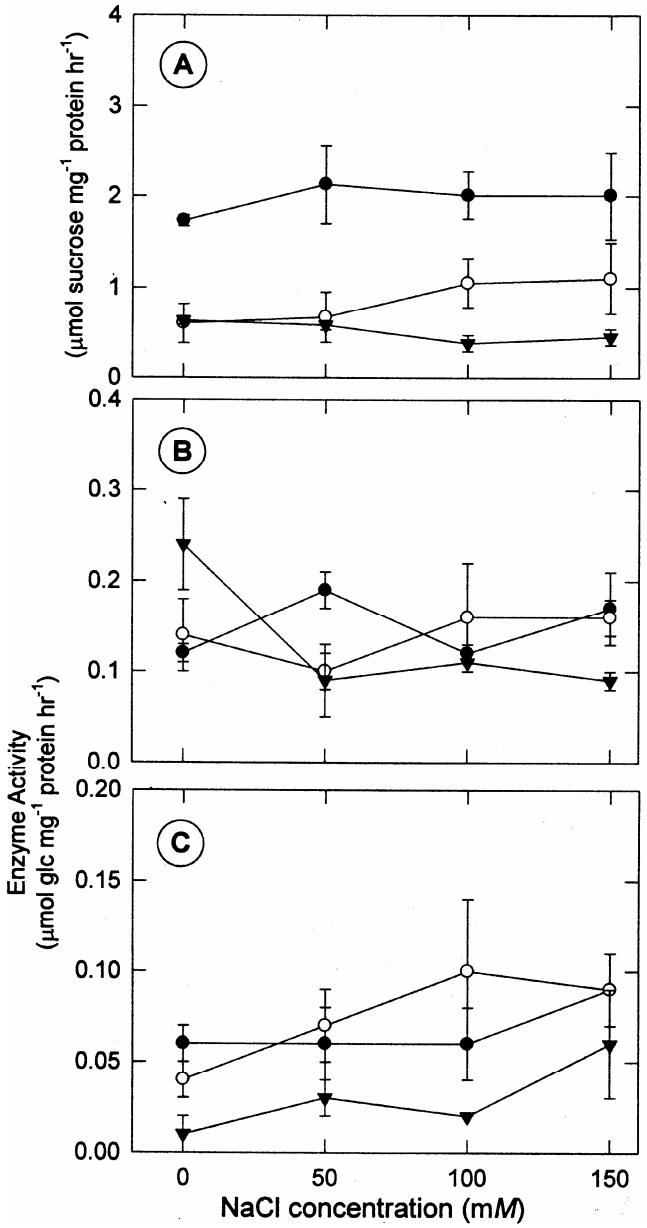

Fig. 4—Effects of increased NaCl concentrations on activities of sucrose phosphate synthase (A), acid invertase (B), and alkaline invertase (C) in mature leaves. [Values are mean ± SE of 4 measurements per data point. ▼, cv. Khao Dawk Mali 105; ○, cv. Luang Anan; ●, cv. Pokkali.]

however, showed a marked difference in response to salinity stress. Salt-sensitive and moderately salt-tolerant cultivars showed an increase in root length in response to salinity stress. This probably reflects the maintenance or even induction of the root elongation at low water potential, which can be considered as an adaptive response to drought and salinity[21]. Increased root length may possibly be resulted from reallocation of photosynthates into the root, in stead of the shoot, thus causing a reduction in shoot growth. On the other hand, root length of salt-tolerant cultivar was reduced by salinity stress. While an increase in root growth in order to increase water influx is usually documented as a general response to salinity, experimental evidence indicates that reduced root and increased shoot growth may improve salinity tolerance by restricting the flux of toxic ions to the shoot and consequently by delaying the onset of the tolerance threshold[22-24]. This factor may possibly contribute to salinity tolerance in the salt-tolerant rice cultivar.

Increased accumulation of sugars was reported in many species exposed to salinity[25]. In rice, concentrations of reducing sugars and non-reducing sugars as well as activities of sucrose phosphate synthase were induced by salinity stress[9]. Accumulation of sugars has been associated with drought- and salinity-tolerant mechanisms in many species. It is generally accepted that the increase in cellular osmolarity which results from the accumulation of compatible solutes is accompanied by the influx of water into, or at least a reduced efflux from, cell, thus providing the turgor necessary for cell expansion[6]. In the present study, however, increased concentrations of sugars in response to salinity stress were found only in the salt-sensitive cultivar, but not in moderately-tolerant and tolerant cultivars. Dubey and Singh[9] also reported that the sugar contents increased more in the sensitive than in the tolerant rice cultivars. In tomato, salt-sensitive cultivar was able to accumulate hexoses and sucrose under salinity stress while their concentrations remained unchanged or decreased in salt-tolerant cultivar[26]. Therefore, present results agreed with the previous reports on the differences in sugar accumulation in cultivars differing in salt tolerance which may explain tolerance differences between these cultivars and may provide details about the sugar components possibly involved in salt tolerance.

Salinity-induced accumulation of sucrose in salt-sensitive cultivar was not correlated with the activity of sucrose phosphate synthase, a key enzyme in sucrose biosynthetic pathway, as also reported in other studies[27]. Interestingly, moderately salt-tolerant and salt-tolerant rice cultivars showed no sucrose accumulation under saline stress. Activity of acid invertase in the salt-sensitive cultivar was reduced which may suggest the possible reduction in source-to-sink translocation. Accordingly, it is possible that utilization of carbohydrate could be a limiting factor of growth under salinity and the accumulation of

sugar in the salt-sensitive cultivar is probably due to reduced utilization in the actively growing tissue[28]. Present results agreed with Liu and Staden[29] which demonstrated that salt tolerant soybean is characterized by a decreased sucrose accumulation under saline conditions. Higher concentration of sucrose in cytoplasm could exert a feed-back inhibition on carbon metabolism which results in lower CO_2 assimilation[30,31]. The expression of Rubisco could be repressed by excess amount of sugars in cytoplasm[32,33]. In addition, the effect of carbohydrate accumulation on photosynthesis is significant in the source leaves, but not in the young sink leaves as pointed out by Araya *et al*[34]. Therefore, reduction of photosynthesis and metabolic alterations by sugar accumulation could contribute to salt sensitivity, which limits growth of the salt-sensitive cultivar under salt stress conditions. On the contrary, starch accumulation was noticed in salt-tolerant cultivar exposed to stress. Starch accumulation perhaps resulted from the increased activity of alkaline invertase activity which hydrolyzes sucrose and converts into simpler sugars. Starch may be synthesized from such sugars. High starch accumulation in mature leaves of the salt-tolerant cultivar of tomato was reported earlier[26]. Although starch may not play a crucial role in salt-tolerance mechanism, it was suggested that the ability of plants to partition sugars into starch may help to avoid metabolic alterations by lowering feedback inhibition caused by excess amount of sucrose in cytoplasm[30].

In summary, salinity stress alters carbohydrate metabolism differently in salt-tolerant and salt-sensitive rice cultivars. Salinity stress induced accumulation of total soluble sugars and sucrose in the leaves of salt-sensitive cultivars without a concomitant increase in the activity of sucrose phosphate synthase. Therefore, it is suggested that accumulation of these sugars may be the result of reduction of sink demand due to growth limitation. On the other hand, starch concentration increased markedly in salt-tolerant cultivar when grown under salinity stress. Although it is unclear whether starch accumulation may play a role in salt-tolerant cultivar, it is possible that adjusted carbon partitioning and allocation could have an important implication on the overall plant growth under salinity.

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