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*Full Length Research Paper*

# Effects of tomato fruit under $Na^+$-salt and $Cl^-$-salt stresses on sucrose metabolism

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**Changes in sucrose metabolism in response to $Na^+$-salt and $Cl^-$-salt stresses were measured in tomato cultivar liaoyuanduoli (*Lycopersicon esculentum* Mill.) and its objective was to provide a new evidence for the ion-specific stress effects of $Na^+$ and $Cl^-$ on sucrose metabolism of tomato fruits. The carbohydrate contents, as well as sucrose metabolizing enzymes activities were determined. The results indicated that hexoses (fructose and glucose) accumulated to higher levels and the content of sucrose and starch were lower in matured fruit under salt stress treatments, which accumulated more hexoses under $Na^+$-salt stress than under $Cl^-$-salt stress. $Na^+$-salt stress and $Cl^-$-salt stress enhanced the acid invertase and decomposition direction of sucrose synthase activities of tomato fruit in a long period of time (30-60 days after anthesis), but little variation of sucrose-phosphate synthase and synthesis direction of sucrose synthase activities were observed. The sucrolytic activities were linked to changes in soluble sugar levels but not with the activities of sucrose biosynthetic. These results point to the importance of sucrolytic activities in sucrose metabolism of tomato fruit under salt stress; the effect of $Na^+$ was more severe than that of $Cl^-$.**

**Key words:** Salt stress, $Na^+$-salt, $Cl^-$-salt, sucrose metabolism.

## INTRODUCTION

Salinity is one of major abiotic stresses and affects almost every aspect of the physiology and biochemistry of plants. This significantly reduces yield. Great effort has been devoted to understanding physiological aspects of response to salinity in plants, as a basis for plant breeders to develop salinity-tolerant genotypes (Cuartero et al., 2006). The crop yield decreased to some extent under salinity, however, at early stage of fruit development, salt stress can increase soluble sugar content and sugar acid ratio in tomato mature fruit (Jiang et al., 2007; Shi et al., 2001; Balibrea et al., 2003).

The yield and quality of tomato appear to be regulated by the net assimilation rate of the crop, the rate of import into individual fruit and sink activity (Yelle et al., 1988). High sink demand can significantly increase the quality of the tomato fruit by high accumulation of soluble solids, an important factor for processing tomatoes. Sugars are the major components of the soluble solids content in tomato mature fruit. The composition of stored sugars in tomato is associated with certain key enzymes responsible for sucrose metabolism. It has been reported that soluble acid invertase is a major sucrolytic activity in cultivated tomato. This activity is considered to be involved in the composition of stored sugars (Klann et al., 1996; Qi et al., 2004). The sucrose synthase activity plays an important function in the control of sucrose import and fruit growth parameters (D'Aoust et al., 1999). NaCl stress can enhance soluble sugar content and sucrolytic activities in tomato fruits (Balibrea et al., 1999, 2000). Moreover, to the authors' knowledge, no information is available about ion-specific stress effects of $Na^+$ and $Cl^-$ on sucrose metabolism of tomato fruit and no clear relationship has been shown between sucrolytic and sucrose biosynthetic under $Na^+$-salt and $Cl^-$-salt stresses in tomato fruit.

The objective of this work was to study the influences of sucrose metabolism in developmental tomato fruit under salt stresses during the fruiting period and to investigate ion-specific stress effects of $Na^+$ and $Cl^-$ on sucrose

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metabolism in tomato sink organs.

## MATERIALS AND METHODS

### Plant material and treatments

The experiments were conducted in solar greenhouse of Science and Research Field of Horticultural Department of Shenyang Agricultural University. The cultivated tomato hybrid (*Lycopersicon esculentum* cv. Liaoyuanduoli) was grown in plug that contained a substrate composed of nutrient soil and vermiculite (2:1, V/V). Seeds were germinated at 28℃ and 90% relative humidity. When the seedlings started to develop the sixth leaf, 30 seedlings of uniform size were transferred to each of the 2 tanks (2m×1.5m×0.1m). The containers, where the plants were to be grown, were filled with 200 L of full-strength Hoagland's solution. The planting pattern was 0.5 m between rows and 0.45 m between plants within rows. Plants with only one stem were cultivated, eliminating all axilary buds. The salt stress treatments were prepared by adding 50 mM $L^{-1}$ NaCl, $NH_4Cl$ and $NaNO_3$ to the tanks (pH 6.5±0.1). The nutrient solution was changed every 7 days and the levels adjusted every 3 days. The flowers were tagged at anthesis in the first trusses and stress treatment started 30 days after anthesis (30 DAA). Fruits were harvested at the start of ripening (40 DAA and 50 DAA) and at the fully ripe stage (60 DAA). After each fruit harvest, a sufficient number of 1 g fresh pericarp tissue samples were frozen with liquid nitrogen and stored at –86℃ until analysis.

### Carbohydrate determination

Soluble sugars (fructose, glucose and sucrose) were measured using a water 600E high-performance liquid chromatography (HPLC). Carbohydrate column and 2410 refractive index monitors were used. The mobile phase was 75% acetonitrile and ultra water (75:25). The mobile rate was 1.0 ml $min^{-1}$ and the temperature of the column was 35℃.Water millennium software was used to handle data. The starch content was measured using perchloric acid hydrolyzed method.

### Enzyme extraction and assays

Enzyme extracts were prepared essentially as described by Miron et al. (1991) and Scholes et al. (1996). Sample of fruit material was homogenized in 10 ml of ice-cold homogenizing medium (50 mM Hepes-NaOH, pH 7.5) and centrifuged at 12000 g for 20 min at 4℃. The supernatant were dialyzed for about 20 h against 5 mM Hepes-NaOH (pH 7.5). The supernatant contained soluble sucrose-metabolizing related enzymes. The insoluble pellet was washed 2 times in homogenizing medium and then incubated, with shaking, for 4 h in ice-cold homogenizing medium with 1 M NaCl. Following centrifugation, the supernatant contained apoplasmic invertase activity.

Invertases activities were measured as described by Qi et al. (2005). The soluble and insoluble acid (EC 3.2.1.25) and neutral (EC 3.2.1.26) invertases (6-D-fructofuranoside fructohydrolase) activities were assayed in a final volume of 25 ml, that contained 0.2 ml of dialyzed enzymatic extract and 0.8 ml of reaction solution (pH 4.8 or 7.2, 0.1 M $Na_2HPO_4$-0.1 M sodium citrate, 0.1 M sucrose for acid invertase and neutral invertase, respectively). The activities were measured by the quantity of reducing sugars released in the assay media with dinitrosalicylic acid. The reducing sugars were revealed by incubation at 100℃ for 5 min and read at 520 nm in a Cary 100UV: VIS spectrophotometer (GBC Scientific Equipment Pty Ltd, Heareus, Germany). Decomposition direction of sucrose synthase (EC 2.4.1.13) was measured as described by Balibrea et al. (2000).

Synthesis direction of sucrose synthase (EC 2.4.1.13) was measured by using 0.4 ml reaction solution (0.05 M fructose, 0.82% UDPG, 0.1 M Tris. and 10 mM $MgCl_2$), 0.2 ml enzyme at 37℃ for 30 min and bathing for 1 min at 100 ℃. A volume of 1 ml was added to 0.1 ml 2 M NaOH, placed in boiling water bath for 10 min, cooled in water and 3.5 ml of 30% HCl and 1ml of 0.1% resorcinol were added. Blank controls were obtained by adding the distilled water to the reaction medium containing resorcinol. The reducing sugars were determined by incubation at 80℃ for 10 min and read at 480 nm in a Cary 100UV: VIS spectrophotometer (GBC Scientific Equipment Pty Ltd, Heareus, Germany). Sucrose-phosphate synthase (EC 2.4.1.14) was assayed by measuring sucrose produced from fructose 6-phosphate plus UDP-glucose (Vassey and Sharkey, 1989).

## RESULTS

### Hexose, sucrose and starch contents

As the fruit developed, the hexoses (fructose and glucose), which were the predominant sugars, gradually increased with the highest level observed at the ripe stage (Figure 1). Moderate salinity enhanced the highest hexose concentrations along the growing period of fruits, which accumulated more hexoses under $Na^+$-salt stress than under $Cl^-$-salt stress.

In the early stage of development, sucrose and starch were accumulated but they decreased sharply and remained at low level at the ripe stage. The content of sucrose and starch were lower in tomato matured fruits under both stresses than under control. $Na^+$-salt stress produced similar impact on the concentration of sucrose and starch than $Cl^-$-salt (Figure 1).

### Activities of sucrose-metabolizing related enzymes

In order to determine whether part of the changes in carbohydrate content could be due to an increase in enzymes mobilizing sucrose or hexose, sucrolytic activities and sucrose biosynthetic activities were measured at the start of ripening and at the fully ripe stage under salt stress, respectively.

The changes in invertase (soluble and insoluble acid invertase and neutral invertase) activity and decomposition direction of sucrose synthase during the development of the pericarp tissue of tomato fruit are shown in Figure 2. The sucrolytic activities were generally increased toward the end of fruit development with ripening. The acid invertase and decomposition direction of sucrose synthase were the main activity responsible for sucrose cleavage in both control and treatment fruits. The sucrolytic activities of fruits were generally higher under salt stress than under control. Salt stress slightly improved neutral invertase activity and significantly improved the acid invertase activity in ripened fruit. The

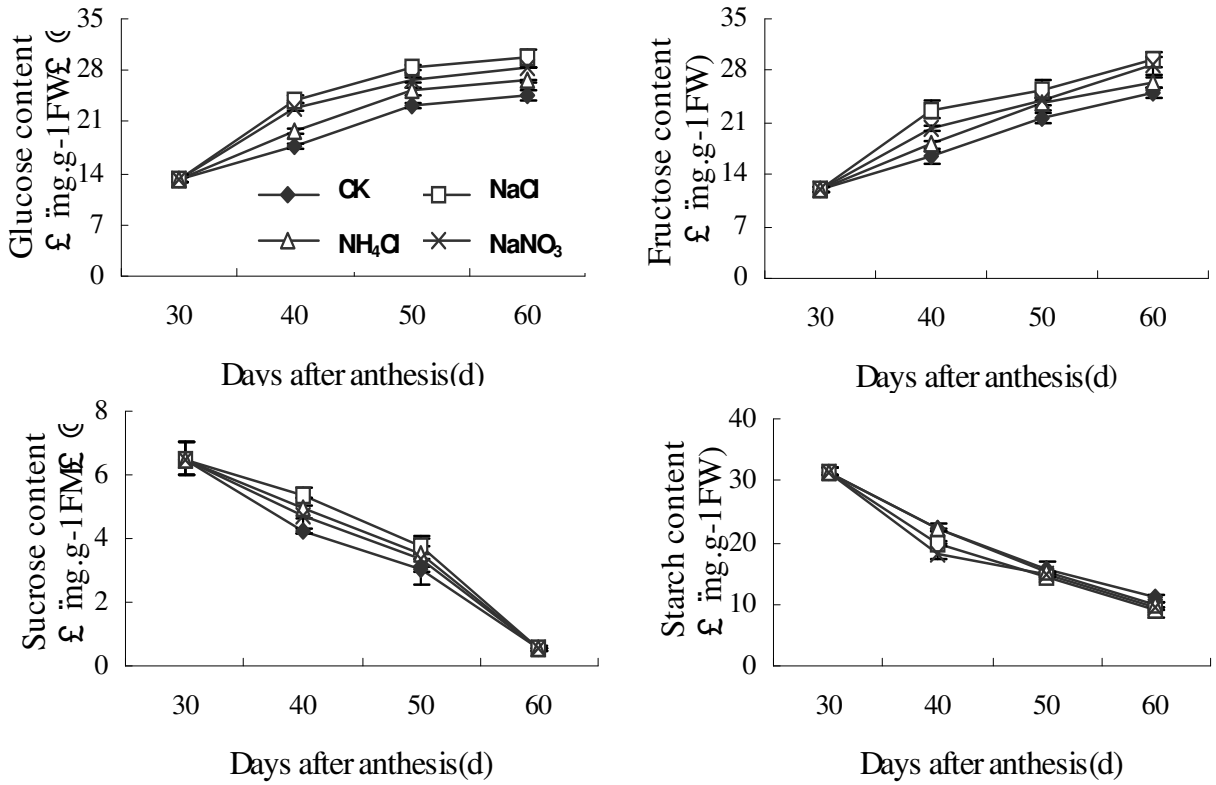


**Figure 1**. Influence of $Na^+$-salt and $Cl^-$ -salt Stresses on tomato fruit carbohydrate content. Glucose, fructose, sucrose and starch were determined in the same samples. Values are the mean ± SD of three replicate samples.

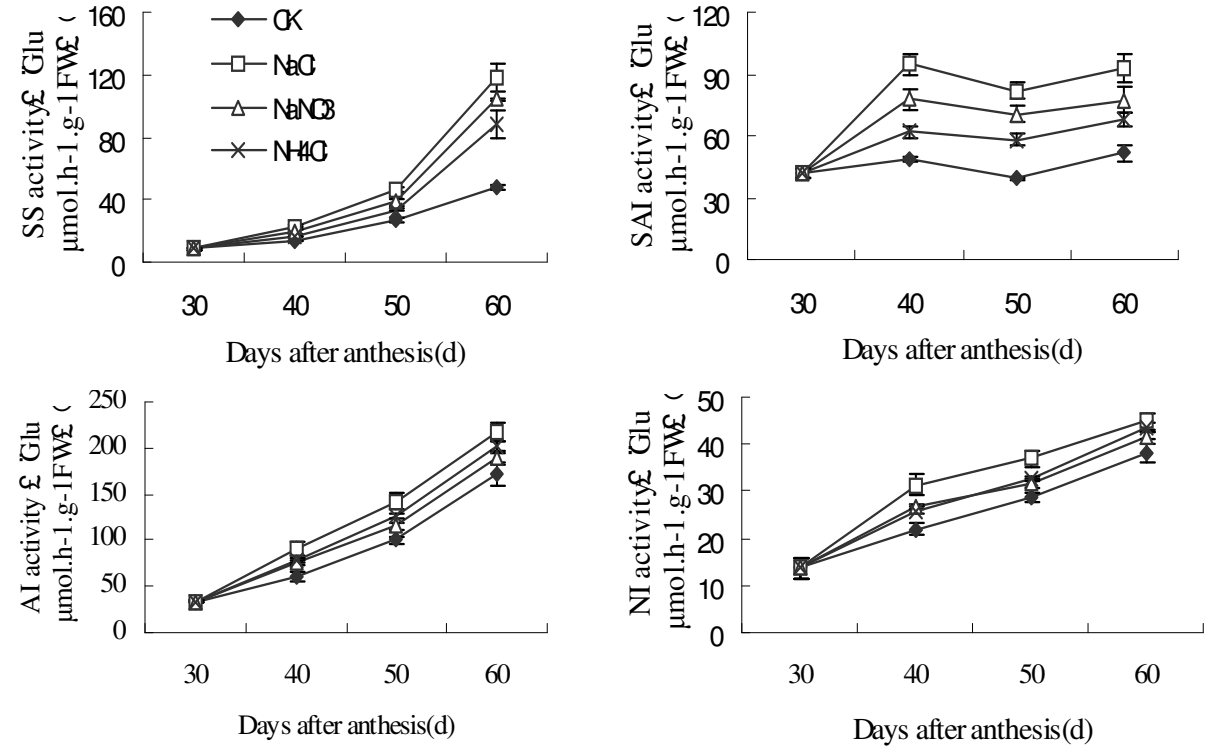


**Figure 2.** Effect of $Na^+$-salt and $Cl^-$-salt stresses on sucrolytic activities in tomato fruit. The enzymes were determined in crude extracts in the same samples. Values are the mean ± SD of three replicate samples.

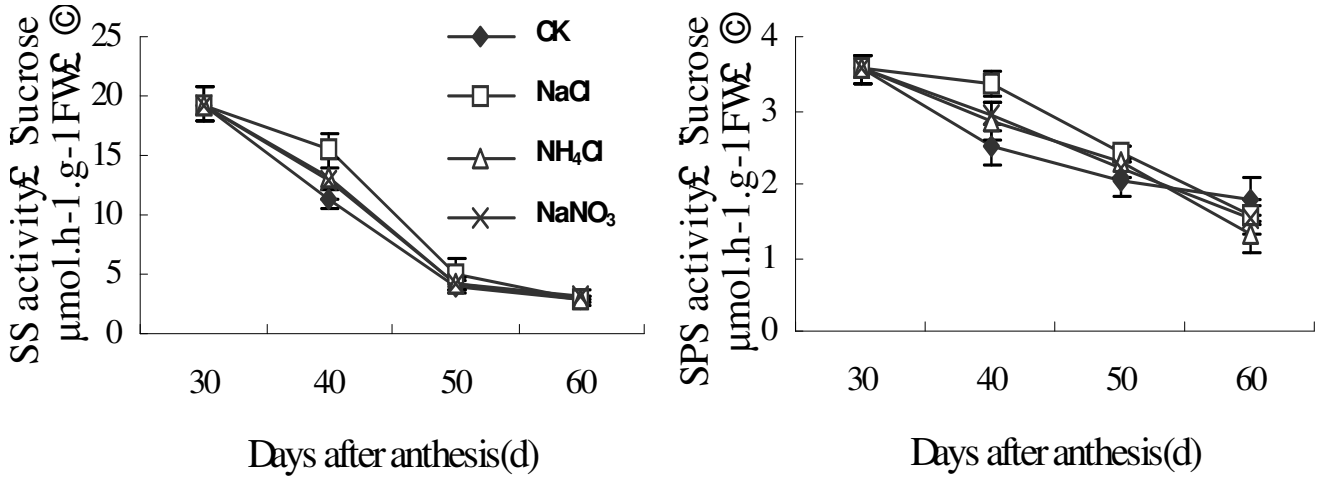


**Figure 3.** Effect of $Na^+$-salt and $Cl^-$ -salt stresses on activities of sucrose biosynthetic in tomato fruit. The enzymes were determined in crude extracts in the same samples. Values are the mean ± SD of three replicate samples.

sucrolytic activities were higher under $Na^+$-salt stress than $Cl^-$-salt stress; this tendency was coincidental with the content of hexose.

The synthesis direction of sucrose synthase (SS) showed low activity at later stages of fruit development (Figure 3). At 50 DAA, the activity remained approximately the same in the pericarp tissue. SS activity was also greater both under $Na^+$-salt stress and $Cl^-$-salt stress; the activity remained approximately the same under salt stress and control in tomato mature fruits. Sucrose phosphate synthase was in the same range as the SS and it also showed low activity at all stages with a small decrease toward the end of fruit development that increased with ripening. A slight increase in activity was observed under salt stress than under control in pericarp tissue of tomato fruit at 40 DAA. The activity remained approximately the same in the placental tissue under salt stress and control at ripe fruit.

## DISCUSSION

A survey was conducted on key sucrose metabolizing enzymes activities on development in tomato fruit under 4 varieties condition. The results showed that under normal and stress conditions, there were differences in the activities of sucrose metabolism enzymes. It is interesting to note that the invertase activity increased dramatically in the final stages of fruit development. This coincides with a reduction in the sucrose content (Konno et al. 1993). These results suggest that sucrose hydrolysis by invertase is important. Hexose (fructose and glucose) levels were relatively high in tomato fruit, as was measured in this study. It has been shown that under *in vitro* conditions SS is inhibited by high concentrations of fructose and this is suggested to also be the case *in vivo* (Schaffer and Peterikov, 1997). Based on the inverse relationship between sucrose levels and the import rate, it has been suggested that diffusion along a sucrose concentration gradient is probably the driving force for unloading and translocation in the fruit. This sucrose gradient may be maintained by the metabolic conversion of sucrose to starch for storage in the plastid or to hexose for storage in the vacuole. This is because hydrolysis of sucrose in the sink is the initial step of sucrose metabolism; invertase and decomposition direction of sucrose synthase, catalyzing the breakdown of sucrose, are expected to play a major role (Guan and Harry, 1991).

Under $Na^+$-salt and $Cl^-$-salt stresses, osmotic adjustment is usually achieved by the uptake of $Na^+$ and $Cl^-$ from the soil solution. Demming and Winter (1986) suggested that a great deal of harmless and compatible solutes were synthesized and accumulated in plant leaves, thus maintaining the osmotic balance. Osmotic adjustment by inorganic ions accumulation is less energy and carbon-demanding than adjustment by organic solutes (Yeo, 1983). The growth capacity of tomato plants under salinity have been related to the increase in sink activity of young leaves and roots by the induction of vacuolar acid invertase and sucrose synthase activities (Balibrea et al. 2000; Qi et al. 2003). It has been suggested that invertase activity may play a major role in regulating the rate of carbon translocation in tomato fruit. In tomato, most of the invertase activity is attributable to soluble acid invertase. The soluble neutral invertase and insoluble acid invertase are negligible (Husain et al., 2001). The major function of the high and constant invertase activity in red tomato fruit is to maintain the cellular hexose concentrations. This study found that acid invertase and neutral invertase activity was higher under salinity than in control, while the SS and SPS activity was slightly difference when compared with salinity in the later developmental stage of fruit. This may be appropriately used to speculate that salt stress improved the fruit flavor

of tomato and this was due to invertase playing a major role. In another research, the effects on sucrose metabolism were more severe under $Na^+$-salt stress than under $Cl^-$ -salt stress in tomato fruit. These results point to the fact that $Na^+$ is the main inorganic ions which influence sucrose metabolism under NaCl stress.

Although, the results show that increase in fruit flavor and nutritional quality by salinity in tomato fruits was related to the sucrolytic activities, the identification of different isoforms, location and regulatory mechanisms by endogenous factors such as hormones or sugars could be important in order to determine the role of these enzymes in maintaining sink capacity under salinity. They should also be considered in the scope of the tomato sucrose metabolism in salinity.

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