Seasonal and Cultivar Differences in Salt-induced Change in Ascorbic Acid and Dehydroascorbic Acid Contents of Tomato Fruit

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Salt stress has been applied to improve the quality of tomato fruit, but ascorbic acid (ASA) and dehydroascorbic acid (DHA, oxidized form of ASA) contents in salt-stressed fruit have not been clearly understood. We examined the salt-induced changes in ASA and DHA contents of tomato fruit in two cultivars and two cropping seasons. Tomato plants were grown under closed irrigation systems in a greenhouse. Salt stress was applied by adding 50 mM and 100 mM NaCl to the nutrient solution. The results indicated that the ASA and DHA contents are not always increased by salt stress, and the effect depends on cropping seasons and cultivars. In the spring-summer season, the ASA and DHA contents in ‘Mini Carol’ (cherry type cultivar) decreased with an increase in the NaCl concentrations, whereas those in ‘House Momotaro’ (normal-fruiting cultivar) increased. However, in the fall-winter season, the ASA and DHA contents in both the cultivars increased by salt stress. In addition, our data revealed that salt-induced changes in the ASA and DHA contents were not induced by changes in the ASA precursor, and suggest that, according to cultivars and cropping seasons, these changes may relate to the antioxidant systems against salt-induced oxidative stress.

Keywords: ascorbic acid, dehydroascorbic acid, tomato fruit, salt stress

INTRODUCTION

The most important vitamin in vegetables for human nutrition is vitamin C (Lee and Kader, 2000). Ascorbic acid (ASA) is the principal biological active form but dehydroascorbic acid (DHA), an oxidized form of ASA, also exhibits biological activity (Lee and Kader, 2000). In addition, recently, ASA reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer (Witting and Stocker, 2004).

Tomato fruit is an abundant source of vitamin C including ASA and DHA, because tomato is one of the daily consumption vegetables. The ASA content in tomato fruit has been known to fluctuate according to the cropping season, cultivar and several environmental stress conditions (Dumas et al., 2003). We previously showed that the effect of water deficit on the ASA content in tomato fruit was positive or null among five cultivars (Zushi and Matsuzoe, 1998). Furthermore, Santamaria et al. (2004) reported that, with the advance of the growing season, the ASA content increased or decreased in two tomato cultivars.

In tomato production, salt stress has recently been applied to improve the fruit quality. Salt
stress may increase the fruit quality in terms of the concentration of sugar, acid and the percentage of dry matter; however, the fruit size and yield may be reduced (Cuartero and Fernández-Muñoz, 1999). Few studies have addressed the change in the ASA and DHA content in salt-stressed tomato fruit. For example, salt stress (the high electrical conductivity (EC) or addition of NaCl in nutrient solution) in the root zone increased the ASA content on a fresh weight basis but decreased on a net accumulation per dry weight basis (Petersen et al., 1998; Krauss et al., 2006). In contrast, Santamaria et al. (2004) reported that a high EC level did not influence the ASA content in tomato fruit. However, there are no studies detailing the varietal and seasonal differences in salt-induced changes in the ASA and DHA contents. Furthermore, the regulatory mechanisms of ASA content have not been elucidated in these studies.

The regulation of ASA content could either be achieved by altering the turnover of ASA or its synthesis (Conklin, 2001). ASA is synthesized from hexose precursor, especially glucose, which may help in controlling the ASA pool size. For example, a positive relationship between the ASA and carbohydrate contents was observed in wheat leaves (Smirnoff and Pallanca, 1996) and spinach leaves (Toledo et al., 2003). In tomato fruit, many researchers reported that carbohydrate contents were influenced by salt stress, as reviewed by Cuartero and Fernández-Muñoz (1999). Therefore, salt stress-induced changes in the ASA contents may be the result of changes in the carbohydrate such as glucose.

In general, ASA plays an important role as an antioxidant, and protects the plant during oxidative damage by reactive oxygen species (ROS). These ROS enhance during abiotic stresses, include drought stress and desiccation, salt stress, chilling, heat shock, heavy metals, ultraviolet radiation, air pollutants such as ozone and SO2, mechanical stress, nutrient deprivation, pathogen attack and high light stress (Mittler, 2002). Indeed, in the salt-stressed roots and leaves of tomatoes, ASA plays an important role in the ROS detoxification against salt-induced oxidative stress, and then ASA level decreases by oxidizing into DHA (Shalata and Tal, 1998; Shalata et al., 2001). However, the relationship between the ASA and DHA contents and oxidative stress has not yet been elucidated in salt-stressed tomato fruits.

In this study, the salt-induced changes in the ASA and DHA contents of tomato fruit were investigated in two cultivars, which differ in ASA content, and two cropping seasons. In addition, to understand the mechanisms of salt-induced ASA and DHA contents, we examined the relationship between ASA, glucose and the redox ratio of ASA as an indicator of oxidative stress (Lechino et al., 1997).

MATERIALS AND METHODS

Plant materials and growth conditions

Two commercial tomato cultivars (Lycopersicon esculentum Mill.), normal-fruited type ‘House Momotaro’ and cherry type ‘Mini Carol’, which differ in ASA content, were used in this experiment. The ‘House Momotaro’ and ‘Mini Carol’ fruits had the lowest and highest ASA content, respectively, when compared with nine commercial tomato cultivars (unpublished data). The plants were grown in a greenhouse in the spring-summer season from April 2003 to July 2003 and in the fall-winter season from September 2003 to February 2004 in Japan. Daily mean temperatures in the greenhouse varied from 21 to 29°C in the spring-summer season, and from 12 to 22°C in the fall-winter season. In the fall-winter season, the temperature kept at 12°C with heating.

In each cultivar, when the first truss was visible, the plants were transplanted to closed irrigation systems (Kyushu Electric Power Co., Inc., Japan) with a density of four plants per metre. The closed irrigation system consisted of the culture bed filled with pumices (about 5 mm in particle) in a 100 l tank for nutrient solutions, and a timer for irrigation. The plants were fertilized with a complete nutrient solution (half-strength Otsuka-B solution; Otsuka Chemical, Co., Osaka, Japan)
with the EC of 1.6 dS m⁻¹ until the start of treatments. The nutrient solution was supplied through a drip irrigation tube at 2 h-intervals during the daytime (6:00–18:00). Concentrations of N, P, K, Ca, Mg and Na in the nutrient solution were determined using ion chromatography at 4-day (spring-summer season) and 7-day (fall-winter season) intervals and readjusted accordingly. The pH of the nutrient solutions was measured daily and, when necessary, corrected with 1.0 M H₂SO₄ to maintain the pH in 6.5–7.0 interval. Recirculating nutrient solutions were renewed at 4-week intervals. All the plants were de-topped just below the fourth truss, and all lateral shoots were removed periodically.

**Experimental designs and salt treatments**

The experimental design consisted of three rows and two guard rows. Each row had ‘House Momotaro’ at three replications and ‘Mini Carol’ at two replications, 5 plants per a replication. Salt treatments were applied by adding 50 and 100 mM NaCl to the nutrient solution. Nutrient solution without NaCl addition served as the control treatment. The treatments were carried out 2 weeks after the transplantation until the end of the experiment. Each NaCl concentration in the nutrient solutions was readjusted as mentioned above.

**Fruit harvest**

The fruits were harvested when completely red. Fruits of ‘House Momotaro’ were harvested from the distal second or third fruit on the second truss. On all the trusses of ‘Mini Carol’ with more than 20 fruits, fruits from the intermediate position on truss were harvested to improve the uniformity of the fruit size and the growing environment such as solar radiation. For each treatment and cultivar, 15–20 fruits from different plants (1–2 fruit per plant) were harvested as samples of fruit weight and total soluble solid (TSS) contents. From the harvested fruits, at least six fruits were selected with same size and colour (same physiological age). Then, pericarps of the selected fruits were excised from the equator of the fruits using a cork-borer (diameter, 1 cm). The excised pericarps were immediately frozen in liquid nitrogen and stored at −80°C until analysis of sugar, ASA and DHA had been conducted. The TSS contents were measured using a refractometer (PR-101; Atago Co., Tokyo, Japan) by expressing juice from the pericarp.

**Measurements of ASA and DHA contents**

For ASA and DHA extraction, a frozen pericarp (about 0.7 g) was ground in 3 ml of 2% metaphosphoric acid (w/v) using a mortar and pestle on ice. After centrifugation at 10,000 × g for 15 min at 4°C, the supernatant was filtered through a 0.2 μm filter. The ASA and DHA contents in the filtrates were measured using high performance liquid chromatography (HPLC) equipped with a post-column derivatization system. The separation was carried out using a Shim-pack SCR-102H column (300 × 7.9 mm, Shimadzu Co., Kyoto, Japan). The column was maintained at 40°C. The elution was performed using 2 mM perchloric acid at a flow rate of 1.0 ml min⁻¹. The post-column derivatization was carried out using 100 mM sodium borohydride containing 100 mM NaOH at a flow rate of 0.5 ml min⁻¹. The ASA and DHA concentration of the elution was monitored at 300 nm using a UV-VIS detector (SPD-10A; Shimadzu Co., Kyoto, Japan).

**Measurement of glucose content**

Glucose was extracted by placing tubes containing lyophilized pericarp with 10 ml of 80% (v/v) ethanol in a hot water bath (80°C) for 10 min; the extraction was repeated three times. The pooled extracts were evaporated to dryness, and then re-dissolved in 7 ml of distilled water and filtered through a 0.2 μm filter. The filtrates were analyzed by HPLC according to Zushi and Matsuzoe (2006).

**Statistical analyses**

Data were analyzed by the analysis of variance (ANOVA) to test the significance of the observed differences. When the ANOVA was significant at P < 0.05, mean differences were statistically assessed at P < 0.05 by Tukey-Kramer test.
RESULTS AND DISCUSSION

Many studies have investigated the effect of salt stress on the quality of tomato fruit (Cuartero and Fernández-Muñoz, 1999). There is a general agreement that salt stress may increase the quality of the tomato fruit because of the increase of TSS, sugar and organic acid contents (Balibrea et al., 1997; Petersen et al., 1998). In this study, although the fruit weight was reduced in 50 mM and 100 mM NaCl in comparison with the control, TSS contents increased with the increase in the NaCl concentrations (Table 1). However, the effects of salt stress on ASA and DHA contents differ from that on TSS as discussed below.

The ASA and DHA contents on a fresh weight basis are shown in Figs. 1 and 2. In this study, salt-induced changes in ASA and DHA contents depended on cultivars and cropping seasons. For example, in the spring-summer season, the ASA content of ‘House Momotaro’ fruit in 100 mM

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>NaCl (mM)</th>
<th>Spring-summer season</th>
<th>Fall-winter season</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Weight (g fruit⁻¹)</td>
<td>TSS (%)</td>
<td>Weight (g fruit⁻¹)</td>
</tr>
<tr>
<td>House</td>
<td>0</td>
<td>166.9 c'</td>
<td>4.2 a</td>
</tr>
<tr>
<td>Momotaro</td>
<td>50</td>
<td>109.7 b</td>
<td>5.6 b</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>72.6 a</td>
<td>7.5 c</td>
</tr>
<tr>
<td>Mini Carol</td>
<td>0</td>
<td>22.4 c</td>
<td>4.9 a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>16.6 b</td>
<td>6.6 b</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>11.0 a</td>
<td>7.5 c</td>
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</table>

' Within each cultivar and cropping season, means with different letters indicate significant difference by Tukey-Kramer test at $P < 0.05$ (n=20–25).

**Table 1** Effect of NaCl stress on fruit weight and total soluble solid (TSS) contents in fruit of two tomato cultivars grown under spring-summer and fall-winter seasons.

![Fig. 1](image1.png) **Fig. 1** Effect of NaCl stress on ascorbic acid (A) and dehydroascorbic acid (B) contents in tomato fruit grown under spring-summer season. Values indicate means ± SE (n=6). Different letters indicate significant difference by Tukey-Kramer test at $P < 0.05$.

![Fig. 2](image2.png) **Fig. 2** Effect of NaCl stress on ascorbic acid (A) and dehydroascorbic acid (B) contents in tomato fruit grown under fall-winter season. Values indicate means ± SE (n=6). Different letters indicate significant difference by Tukey-Kramer test at $P < 0.05$. 

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NaCl was higher than that in the control but that of ‘Mini Carol’ fruit in 100 mM NaCl was lower than that in the control (Fig. 1A). The DHA content tended to decrease in salt-stressed fruit of both the cultivars (Fig. 1B). In contrast, in the fall-winter season, the ASA and DHA contents tended to increase in 50 mM and 100 mM NaCl in both the cultivars (Fig. 2A and B). These results clearly show that the ASA and DHA contents were not always increased by salt stress.

In salt-stressed tomato fruit, the enhancement of chemical compositions is a concentration effect by the reduction of water from the fruit (Petersen et al., 1998). However, the net accumulations in chemical compositions such as sugars were found in salt-stressed tomato fruit (Balibrea et al., 1997). In this study, to elucidate whether the salt-induced changes in ASA and DHA contents occurred by the concentration effect, we calculated the ASA and DHA contents on a dry weight basis. In both the cultivars, the ASA and DHA contents were not affected by salt stress in the fall-winter season, while those in the spring-summer season decreased in 50 mM and 100 mM NaCl (data not shown). These results indicate that the salt-induced changes in the ASA and DHA contents in the fall-winter season were induced by the concentration effect; however, in the spring-summer season they were induced by other factors.

To understand the mechanism of salt-induced changes in the ASA and DHA contents, we presented the relationship between ASA and glucose contents (Fig. 3), and the redox ratio of ASA (Fig. 4). In the spring-summer season, negative relationships between the ASA and glucose contents were observed in ‘Mini Carol’ fruit (Fig. 3A). Therefore, these results indicate that the salt-induced changes in the ASA content were not induced by changes in the ASA precursor. In addition, the redox ratio of ASA was maintained at a constant level in ‘Mini Carol’ fruit, but in-

\[ y = ax + b \]

\[ R^2 = 0.999 \]

\[ y = ax + b \]

\[ R^2 = 0.998 \]

\[ y = ax + b \]

\[ R^2 = 0.981 \]

**Fig. 3** Relationship between ascorbic acid and glucose contents in tomato fruit grown under salt stress and control conditions during spring-summer (A) and fall-winter (B) seasons. Values indicate means ± SE (n = 6).

**Fig. 4** Effect of NaCl stress on redox ratio (%) of ascorbic acid (ASA/(ASA + DHA) × 100) in tomato fruit during spring-summer (A) and fall-winter (B) seasons. Values indicate means ± SE (n = 6). Different letters indicate significant difference by Tukey-Kramer test at P<0.05.
increased in 50 mM and 100 mM NaCl in ‘House Momotaro’ fruit (Fig. 4A). In general, plant under oxidative stress induce the decrease of the redox ratio of ASA (Conklin, 2001). In salt-stressed tomato leaves and roots, salt stress induced oxidative stress and the decrease of redox ratio of ASA (Shalata and Tal, 1998; Shalata et al., 2001). Therefore, in spring-summer season, changes in ASA and DHA contents may relate to the antioxidant systems against salt-induced oxidative stress. However, our results disagreed with other researches (Shalata and Tal, 1998; Shalata et al., 2001). These disagreements may have resulted from the tissue differences, such as leaves (Shalata and Tal, 1998), roots (Shalata et al., 2001) and fruits (in this study).

In contrast, in the fall-winter season, positive relationships between ASA and glucose contents were observed in both the cultivars (Fig. 3B), and both the contents per dry weight basis were not affected by salt stress (data not shown). Furthermore, the redox state of ASA was not changed in 50 mM and 100 mM NaCl (Fig. 4B). Thus, these findings support that the increases in the ASA and DHA contents in salt-stressed fruit result from the concentration effect.

Cultivar and genotypic difference in ASA content and antioxidant system have been observed in numerous plant species. For example, in wheat leaves, the capacities of ASA synthesis and the antioxidant system were different between two cultivars which differ in ASA content (Bartoli et al., 2005). They suggested that, the cultivar with higher total ASA content is more competent at ASA regeneration than cultivar with lower ASA content under water stress. In the spring-summer season, ‘Mini Carol’ fruit had twice as much ASA and DHA as those of ‘House Momotaro’ fruit under the control condition (Fig. 1). However, under 100 mM NaCl, both cultivars had a similar values of ASA and DHA content. These suggest that, the different response between two cultivars in ASA content may be resulted from the varietal difference of ASA synthesis and the antioxidant system such as ASA regeneration under salt stress. The significance of varietal differences in salt-induced changes in ASA and DHA remains an important open question at the present stage.

In the antioxidant system of plants, a strong correlation have been observed between the growth light intensity and the antioxidant content such as ASA (Logan, 2005). Furthermore, activities of antioxidant enzymes is higher in high light intensity condition than in low light intensity (Logan, 2005). In this study, the light conditions during the experiment different between the spring-summer season (high light intensity) and the fall-winter season (low light intensity). Therefore, seasonal differences in salt-induced changes in ASA and DHA may resulted from the difference of light-dependent antioxidant systems in two seasons.

In conclusion, this study revealed that ASA and DHA contents in tomato fruit were not always increased by salt stress, and these changes depend on the cultivars and cropping seasons. In agricultural practice, these conclusions have important implications for tomato growers that the optimum cultivar and cropping season must be selected to the increase of ASA and DHA contents by salt stress.

REFERENCES


ASA AND DHA OF SALT-STRESSED TOMATO


