

# Chapter 1

## Effect of Salt Stress on the Growth and Fruit Quality of Tomato Plants

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**Abstract** During the past several decades, salt injury has arisen as one of the most serious problems in agriculture worldwide, especially in arid and semiarid areas. Generally, excessive exposure of crops to salinity stress leads to yield reduction and loss of quality. However, for tomato crops, moderate salt stress improves the fruit quality, increasing nutritional components but decreasing fruit yield. In the current Japanese market, such fruits are referred to as “fruit tomatoes” and are sold at a higher price compared with normally cultivated tomatoes because of their high Brix (sugar content) and excellent flavor. Previously, the mechanism underlying this phenomenon was referred to as a “concentration effect” because fruit enlargement was suppressed by limited water uptake as a result of salt stress. However, recent studies have suggested that, in addition to the “concentration effect,” certain metabolic and molecular genetic responses to salinity are also involved in the development of fruit tomatoes. Here, we introduce metabolic alterations in major fruit components such as sugars, amino acids, organic acids, and carotenoids in high-Brix fruit, and we describe the physiological changes observed in tomato plants exposed to salt stress. We also discuss possible molecular mechanisms underlying the production of fruit tomatoes.

**Keywords** ADP-glucose pyrophosphorylase • Amino acid • Assimilate transport • Fruit quality • GABA • Invertase • Organic acid • Salt stress • Starch • Tomato

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## 1.1 Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in agriculture, both domestically and worldwide. Because this vegetable can be adapted for cultivation in various environments ranging from tropical to nearly alpine regions, its production area is now expanding worldwide. On the other hand, in the traditional cultivation area, which is concentrated around the Mediterranean Sea and in the southern and western parts of the United States (USA) because of the warm and dry climate that is favorable for tomato cultivation, yield loss from salt injury has arisen as a serious problem in 19.5 % of the irrigated land area and in the irrigation water (Flowers and Yeo 1995; Cuartero and Fernandez-Munoz 1999; Foolad 2004). Additionally, 2 million hectares (ha), equivalent to 1 % of the world's tomato cultivation areas, are lost every year because of environmental factors such as salinity, drought, and soil erosion. In some cases, poor cultivation techniques can also promote the damage caused by environmental stresses (Ashraf and Foolad 2007).

It has been suggested that moderate salinity and drought stress improve the nutrient quality in tomato fruits by increasing sugars, amino acids, carotenoids, and other substances (Adams 1991; Gao et al. 1998; Ho et al. 1987; Krauss et al. 2006; Tal et al. 1979; De Pascale et al. 2001; Saito et al. 2008a). Recently, these cultivation conditions have been introduced in hydroponic cultivation techniques such as the nutrient film technique (NFT), and high-Brix tomatoes are being produced in Japan (Sakamoto et al. 1999; Saito et al. 2006) and in northern European countries (Adams 1991). Such high-Brix fruits are referred to as “fruit tomatoes” and have become popular in the Japanese consumer market because the fruit flavor is much better than that of a tomato cultivated under ordinary conditions. Until recently, the development of “fruit tomatoes” was thought to result from a “concentration effect” caused by the suppression of fruit size caused by salt stress (Ehret and Ho 1986; Ho et al. 1987; Sakamoto et al. 1999). However, evidence uncovered during the past decade indicates that other physiological and molecular alterations might be involved in this phenomenon.

This chapter describes the effects of salt stress on various aspects of tomato growth and on the content of the nutritive components of the fruit, with a specific focus on sugars, and discusses the possible mechanisms that are responsible for the formation of high-Brix fruit.

## 1.2 Effect of Salinity on Plant Growth and Yield

In Mediterranean countries, tomatoes have also been studied as a model crop in salinized or dry land agriculture, and a large amount of data about the effects of salinity on the growth of tomato plants has been produced. Most commercial cultivars are moderately sensitive to salinity at all stages of plant development, including seed germination, vegetative growth, and fruit production (Ashraf and Foolad 2007).

Even at NaCl concentrations as low as 80 mM, salt stress suppresses seed germination (Cuartero and Fernandez-Munoz 1999), vegetative growth, leaf area, and root weight (Papadopoulos and Rendig 1983; Van Ieperen 1996). Predictably, fruit yield is also negatively affected by salt stress because of a decrease in average fruit weight or the number of fruits produced by a single plant (Cuartero and Fernandez-Munoz 1999). At relatively low electrical conductivity (EC), fruit weight rather than fruit number tends affect the fruit yield. In contrast, at an elevated EC, fruit yield is mainly affected by reduction in fruit number (Van Ieperen 1996). In salt-stressed fruit, fruit enlargement is suppressed during the cell expansion phase because water uptake into the fruit, which is the motive force for cellular expansion, is suppressed by the increased salinity (Ehret and Ho 1986). Regarding marketable yield, increased blossom-end rot (BER) hampers tomato production. It has been suggested that BER is a physiological disorder caused by calcium deficiency in certain parts of the fruit (Adams and Ho 1992; Chretien et al. 2000; Franco et al. 1994; Willumsen et al. 1996) because  $\text{Ca}^{2+}$  uptake by the roots and its transport through the xylem to the fruits are both decreased under severe salt stress conditions (Belda et al. 1996; Ho et al. 1993). However, the detailed mechanism underlying BER is still unclear, and an effective method for combating BER has remained elusive.

### 1.3 Effect of Salt Stress on Fruit Qualities

As described in Sect. 1.2, salt stress has unfavorable effects on both plant growth and fruit enlargement in tomatoes. On the other hand, it has been reported that moderate salinity improves fruit quality by affecting the levels of soluble solids, such as sugars and acids, as well as the pH value; these are key factors in quality evaluations of fruit sold in markets, and salt stress generally improves fruit quality by increasing the content of those substances. This phenomenon has been attributed to a “concentration effect” that results from the suppression of fruit enlargement in plants exposed to salt stress. However, during the past decade, increasing evidence has indicated that alterations in assimilatory metabolism and the translocation of assimilates into the fruit are likely to be involved in the increase in soluble solids and other components.

#### 1.3.1 Organic Acids

Acids largely affect fruit flavor by interacting with sugars and by creating acidity (Stevens et al. 1977), and organic acids account for approximately 13 % of the fruit dry matter (Davies and Hobson 1981). Malic acid and citric acid are the most abundant organic acids in fruit (4 % malic acid and 9 % citric acid on a dry matter basis); citric acid is more important for the sour flavor of the fruit than malic acid. Moderate salt stress ( $\text{EC } 8.0 \text{ dS m}^{-1}$ ) enhances the accumulation of citric and malic acids by

1.7- and 2.5 fold, respectively, compared to control conditions (EC 2.5 dS m<sup>-1</sup>) at the red-ripe stage (Saito et al. 2008a). Transcriptional analyses showed that the expression of genes involved in organic acid metabolism, such as phosphoenolpyruvate carboxykinase (PEPCK), malate dehydrogenase (MDH), malic enzyme (ME), and pyruvate kinase (PK), is upregulated by both moderate (EC 8.0 dS m<sup>-1</sup>) and severe (EC 15.0 dS m<sup>-1</sup>) salt stress in ripening fruit (Saito et al. 2008a; Yin et al. 2010a). These results suggest that a shunt of the tricarboxylic acid (TCA) cycle (malate–oxaloacetate–PEP–pyruvate–citrate) is involved in organic acid metabolism and is stimulated by salt stress. Davies (1964) suggested that the accumulation of organic acids in the fruit counterbalances excessive cations to maintain the fruit pH. The difference between the cation and anion level tends to be greater in salt-stressed fruits, which leads to a higher concentration of organic acids in these fruits (Cuartero and Fernandez-Munoz 1999).

### 1.3.2 Carotenoids

Tomato fruits contain various carotenoids that are a major source of the antioxidant lycopene in the human diet, although the quantity, distribution, and antioxidant effects vary among cultivars (Minoggio et al. 2003). Fresh tomato fruit and its processed products provide approximately 85 % of the lycopene in the human daily diet (Canene-Adams et al. 2005). Tomatoes are also a notable source of other carotenoids, such as  $\beta$ -carotene, lutein, phytoene, phytofluene, and  $\zeta$ -carotene. Because the carotenoid content is an important trait of tomato, many studies have reported the effects of salinity on the carotenoid content in tomato fruits. Generally, salt stress enhances lycopene and  $\beta$ -carotene accumulation; however, some reports indicated that the increase was observed only on a fresh weight and not a dry weight basis. Thus, these authors concluded that the increase should be attributed to a concentration effect caused by the suppression of fruit expansion (Krauss et al. 2006; Dumas et al. 2003; Shi and Le Maguer 2000). On the other hand, De Pascale et al. (2001) reported that the total carotenoid and lycopene content increased on both a fresh weight and dry weight basis under moderate salt stress (EC 4.0 dS m<sup>-1</sup>) and suggested that metabolic alteration is involved in the increase along with the concentration effect. Our previous study also supports this observation. A key enzyme involved in carotenoid biosynthesis that is produced by the phytoene synthase gene was upregulated by moderate salinity stress (Saito et al. 2008a). Krauss et al. (2006) suggested that reduced leaf area caused by the growth suppression under salt stress and the increased exposure of fruit to sunlight resulted in increased carotenoid accumulation because carotenoid biosynthesis is regulated by light and increased exposure of the fruit to sunlight. Further investigation is required to elucidate the mechanisms underlying the effect of salinity on carotenoid accumulation.

### 1.3.3 Amino Acids

Tomato fruit flavor largely depends on the types and quantity of free amino acids present in the fruit. Several amino acids, such as glutamate,  $\gamma$ -aminobutyric acid (GABA), glutamine, and aspartic acid, account for approximately 80 % of the total free amino acids in the fruit (Kader et al. 1978). Among these amino acids, glutamate is the principal free amino acid in red-ripe fruit in commercial cultivars and it confers the characteristic “umami” flavor to tomato (Sorrequieta et al. 2010). It has been reported that salt stress increases the total amino acid content in tomato fruit, and the effect of the stress is greater in the pericarp than in the columella tissue (Zushi and Matsuzoe 2006; Yin et al. 2010a). Importantly, the proline content is increased markedly in both pericarp and columella tissue. Proline is a well-known indicator that responds to abiotic stresses such as salinity and drought in higher plants and most likely functions as an osmoprotectant in fruit exposed to salt stress (Ashton and Verma 1993; Claussen 2005).

Glutamate and GABA are the most abundant amino acids in tomato fruits (Inaba et al. 1980; Rolin et al. 2000). Tomato accumulates higher amounts of GABA in its edible parts than most other vegetable crops (Matsumoto et al. 1997). GABA is a four-carbon, nonprotein amino acid commonly found in diverse organisms, and it serves as a major inhibitory neurotransmitter in vertebrates (Zhang and Jackson 1993). It has been known to play a role in reducing blood pressure in the human body (Inoue et al. 2003). GABA is synthesized from glutamate by glutamate decarboxylase (GAD), which is activated by  $\text{Ca}^{2+}/\text{CaM}$  (Aurisano et al. 1995; Snedden et al. 1995; Turano and Fang 1998) and acidic pH (Johnson et al. 1997; Snedden et al. 1996). Salt stress also enhances GABA accumulation in the fruit at the red stage (Saito et al. 2008a, b). However, although salinity enhances fruit acidity and stimulates cellular  $\text{Ca}^{2+}$  in plant cells (Sanders et al. 1999), we cannot determine whether these factors directly promote GABA accumulation in tomato fruit because our previous data showed that glutamic acid decarboxylase (GAD) activity was unchanged in salt-stressed fruit; in fact, our data indicated that only the protein content increased (Yin et al. 2010a). Consequently, substrate availability and an increased concentration of GAD would promote GABA accumulation in salt-stressed tomato fruit. In fact, the GABA metabolic pathway is TCA cycle shunt (Bouchè and Fromm 2004). GABA accumulation reaches a maximum in mature green fruit and is rapidly degraded during fruit ripening (Rolin et al. 2000; Akihiro et al. 2008). Tracer analyses utilizing [ $^{14}\text{C}$ ]-labeled GABA demonstrated that GABA in mature green fruit is converted to malate, citrate, *cis*-aconitate, isocitrate, and 2-oxoglutarate in red-ripe fruit; furthermore, [ $^{14}\text{C}$ ] was also detected in the  $\text{CO}_2$  gas fraction during ripening (Yin et al. 2010a). Those results indicate that dissimilated GABA flows back into the TCA cycle, is metabolized to organic acids, and is utilized as a substrate for respiration during climacteric ripening of fruit.

It was also reported that GABA accumulation in the fruit was enhanced 1.6- to 1.9 fold during the postharvest storage of red-ripe fruit under anaerobic (low  $\text{O}_2$ ) conditions compared with normal air conditions, regardless of the  $\text{CO}_2$  concentration (Mae et al. 2012). This increase was caused by increased GAD activity and decreased GABA transaminase (GABA-T) activity.

### 1.3.4 Ascorbic Acid

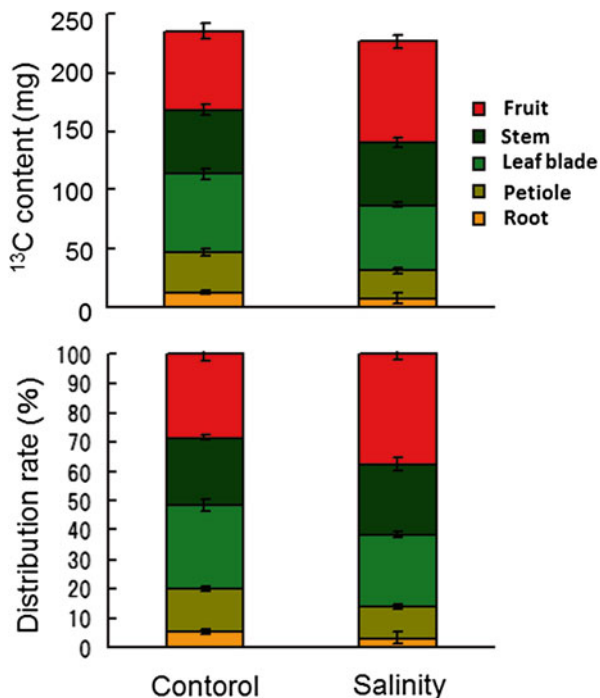
Ascorbic acid (ASA), which is present in tomato fruit, has an important role in the maintenance of human health by serving as an antioxidant that scavenges reactive oxygen species (ROS). In addition, ASA-related antioxidant systems, including the ascorbate–glutathione cycle (in which ascorbate peroxidase is involved), prevent the accumulation of toxic ROS levels under environmental stress conditions (Zhang 2013). Fruit ASA content is affected by cropping season, cultivar, and environmental stress (Dumas et al. 2003). However, the effect of salinity on fruit ASA content is actually not as clear as its effects on other metabolites such as sugars and amino acids. Several studies have shown positive results (Krauss et al. 2006), negative results (Zushi and Matsuzoe 2009), or no change (Fanasca et al. 2007) in the ASA content, and an interaction with other factors such as cultivars and growth conditions (e.g., temperature and light intensity) was suggested. Zushi et al. (2014) examined the combined effect of salt stress and light intensity on ASA content and the activity of ASA-related antioxidant enzymes in tomato fruits using tissue culture experiments. The results revealed that changes in the ASA content and the activity of antioxidant enzymes under salt stress conditions could not be explained only by salinity, because light intensity could also be involved in the regulation of antioxidant enzyme activity.

## 1.4 Effect of Salinity on Carbohydrate Allocation to Fruits

Salinity stress inhibits water uptake by the roots and water influx into the fruits as a result of high osmotic pressure around the root system, resulting in a decrease in fruit size and an increase in quality constituents. As described in Sect. 1.3, enhanced metabolite accumulation in salinity-stressed fruit has been attributed to a “concentration effect.” However, our previous studies revealed that the ratio of sucrose to the total sugar content in salt-stressed fruits was increased compared with that of the control (Saito et al. 2006), and the effect of salt stress on the sugar concentration was apparently more severe than its suppressive effect on fruit weight (Yin et al. 2010b). Gao et al. (1998) reported that salinity enhanced the transport of assimilates from leaves to adjacent fruits. These results indicate that the increase in sugar content from salinity is not simply a result associated with the suppression of fruit growth; rather, it is also caused by alterations in sugar metabolism or sugar translocation into the fruit.

To examine alterations in carbohydrate partitioning in plants exposed to salt stress, Saito et al. (2009) investigated the distribution of carbohydrates under moderate salt stress conditions ( $EC\ 8.0\ dS\ m^{-1}$ ) in tomato plants by feeding  $[^{13}C]$ -labeled  $CO_2$  to source leaves at 16 days after anthesis, which is the time when a tomato plant shows the greatest transport activity of photoassimilates from the source leaves to the fruits (Ho 1986). Although both photosynthetic and transpiration ratios were reduced by approximately 60 % under salt stress, the content of  $[^{13}C]$ -labeled assimilate to

**Fig. 1.1** Effect of salinity treatment on  $^{13}\text{C}$  content and distribution in tomato plants 48 h after feeding  $^{13}\text{CO}_2$ . Bars indicate SE ( $n=3$ )



fruits was 28 % higher compared with that observed in the control (Fig. 1.1). The distribution ratio of [ $^{13}\text{C}$ ]-labeled assimilate in fruits was 32 % higher under saline conditions than under control conditions (Fig. 1.1). A similar result was also observed in the [ $^{13}\text{C}$ ] tracer analyses performed under different salt stress conditions ( $\text{EC } 15.0 \text{ dS m}^{-1}$ ), in which the carbohydrate influx into the fruit was strongly enhanced by salinity in immature and mature green fruits, even though the stress severely suppressed plant growth (Yin et al. 2010b). These results indicate that salt stress alters the allocation of photoassimilate in the entire plant and enhances the sink strength of fruits.

## 1.5 Salinity Stress Enhances Starch and Sugar Accumulation in Fruit

### 1.5.1 Sugar Metabolism

In addition to the enhancement of photoassimilate translocation and the sink strength of fruit, salt stress increases the sugar content in fruit. This sugar mainly consists of hexoses produced by the cleavage of sucrose. Interestingly, sucrolytic activity is

also thought to be an index of sink strength (Yamaki 2010). There are two notable enzymes involved in the cleavage of sucrose to monosaccharides: sucrose synthase and invertase. Sucrose synthase (Susy) converts sucrose into fructose and UDP-glucose. By contrast, invertase irreversibly catalyzes the hydrolytic cleavage of sucrose into glucose and fructose. In tomato, Susy was considered to be a major factor determining the fruit sink strength because a strong correlation among Susy activity, ADP-glucose pyrophosphorylase activity, and starch accumulation in early developing fruit was found (Robinson et al. 1988; Yelle et al. 1988). Moreover, antisense transgenic plants in which Susy activity was suppressed displayed reduced fruit setting and sucrose import capacity in young fruit (D'Aoust et al. 1999). However, the suppression of the Susy-encoding gene in tomato did not lead to remarkable alterations in starch and sugar accumulation in the fruit (Chengappa et al. 1999). There is minimal evidence that sucrose synthase is directly involved in the control of fruit sugar content and composition in tomato.

On the other hand, during the past two decades, increasing evidence has indicated that invertase is an essential factor involved in the regulation of sugar content in tomato fruit. In plants, invertases are classified into three isozyme types according to their solubility, subcellular localization, isoelectric point (pI), and optimal pH (Sturm 1999): cell wall invertase (CWIN), vacuolar invertase (VIN), and cytoplasmic invertase (CIN). Among these isozyme types, CWIN and VIN are characterized as acid invertases because of their acidic optimal pH, whereas CIN is characterized as a neutral invertase because of its neutral optimal pH. Several studies have revealed the diverse roles of invertases in the plant life cycle, including their participation in various responses to abiotic and biotic stresses such as drought, hypoxia, high temperature, wounding, and pathogen infection (Roitsch and González 2004).

The relationship between fruit sugar content and abiotic stress suggests that the most important invertase is CWIN. During the past decade, CWIN has attracted attention as an essential enzyme for determination of the total soluble solids level in tomato (Fridman et al. 2002, 2004; Zantor et al. 2009). Among the genes encoding CWIN (*LINs*), *LIN6* responds to various biotic and abiotic stimuli, including wounding, pathogen infection, and sugars (Godt and Roitsch 1997; Ohshima et al. 1998; Sinha et al. 2002). Additionally, *LIN7* was suggested to be involved in heat stress tolerance because its expression was specifically promoted by heat stress in heat-tolerant varieties (Li et al. 2012). Li et al. (2012) also suggested that the increased ability of young fruits to import sucrose contributes to the heat tolerance of the variety and is likely to be governed by *LIN7* expression. However, reports that fruit VIN and CWIN activities were not affected by salt stress (Carvajal et al. 2000; Saito et al. 2009) suggest that these two isozymes are most likely not involved in the phenomenon. In contrast, there was a positive correlation between CIN activity and hexose levels in the fruit of plants exposed to salinity stress (Balibrea et al. 1996, 2006). However, minimal information is available on CIN in tomato, and its physiological function has yet to be elucidated. These results suggest that CIN functions under specific environmental conditions such as salinity stress.



### 1.5.2 Starch Biosynthesis

Several studies have reported that salt stress enhances starch accumulation in early-developing tomato fruit (Balibrea et al. 1996; Gao et al. 1998; Yin et al. 2010b). In fact, this phenomenon involves an increase in ADP-glucose pyrophosphorylase (AGPase) activity. AGPase plays a role in the regulation of starch accumulation in early-developing fruit (Schaffer and Petreikov 1997; Schaffer et al. 2000). This enzyme catalyzes the synthesis of ADP-glucose from glucose-1-phosphate and ATP, which is the first regulatory step in starch biosynthesis in plants (Tsai and Neleson 1966; Lin et al. 1988; Stark et al. 1992). Plant AGPase is a heterotetrameric enzyme composed of two small and two large subunits (Morell et al. 1987). In tomato, four AGPase-encoding genes were isolated: one encodes the small subunit (*AgpSI*) and the other three genes encode the large subunit (*AgpLI*, *L2*, and *L3*) (Chen et al. 1998; Park and Chung 1998). Among these genes, *AgpLI* and *AgpSI* are predominantly expressed in fruit, and both genes show the highest expression during early developmental stages (Petreikov et al. 2006; Yin et al. 2010b). It has been reported that plant AGPase-encoding genes are regulated at the transcriptional level by phosphates, nitrates, and sugars (Müller-Röber et al. 1990; Scheible et al. 1997; Nielsen et al. 1998; Sokolov et al. 1998; Li et al. 2002). Additionally, our previous work showed that *AgpSI* and *AgpLI* expression was specifically enhanced by salt stress in early developing fruits in an ABA- and osmotic stress-independent manner (Yin et al. 2010b). Detailed expression analyses utilizing detached fruits revealed that the response of *AgpLI* expression to salt stress is a sugar-mediated response. This observation was indirectly supported by the [<sup>13</sup>C] tracer analyses, in which the carbohydrate influx into the fruit was notably enhanced under salinity stress conditions in immature green fruits (Yin et al. 2010b). The observation that starch biosynthesis in fruit is dependent on the sugar supply is consistent with the results of N'tchobo et al. (1999). It has been reported that total soluble sugar contents and sucrose phosphate synthase (SPS) activity were increased (Carvajal et al. 2000) and the expression of the sucrose transporter gene *LeSUT1* was enhanced (Yin et al. 2010b) by salinity stress in leaves. Considering the results of the tracer analyses, which showed that the allocation of photosynthetic [<sup>14</sup>C]/[<sup>13</sup>C] to fruits and roots was increased under saline conditions (Gao et al. 1998; Saito et al. 2009), those responses are most likely a result of a systemic response to salt stress that promotes assimilate accumulation in sink organs. Salt stress expands carbohydrate availability in the developing fruits and promotes AGPase gene expression and consequent starch biosynthesis, followed by starch breakdown during ripening, which results in a high sugar content in red-ripe fruit. Similar phenomena were observed in different germplasms, such an *Solanum pennellii*-derived introgression line possessing the *Brix9-2-5* allele, in which enhanced starch accumulation in young fruit results in a higher content of total soluble solids in red-ripe fruit compared with normal tomato cultivars (Robinson et al. 1988; Baxter et al. 2005). These observations support the view that salt stress enhances sugar accumulation in red-ripe fruit through activation of starch biosynthesis in immature green fruit.

## 1.6 Conclusion

In this chapter, we introduced the concept of active alteration of fruit metabolism in response to salinity stress and discussed the proposed molecular mechanisms underlying the development of the “fruit tomato.” As described in Sect. 1.5 and elsewhere, increased sugar levels cannot be explained only by a “concentration effect” caused by the suppression of fruit enlargement. This insight indicates that some metabolic pathways, at least those related to sugar and starch biosynthesis, have functions that are independent from that of the suppression of fruit enlargement. Generally, moderate salt stress simultaneously causes a reduction in fruit yield with a concomitant improvement in fruit quality. However, utilizing current biotechnological techniques, we are interested in producing a high-Brix fruit without yield reduction; this could be accomplished, for example, by manipulating the expression of starch biosynthesis genes. Another possibility entails the use of a reverse genetic approach to obtain a mutant in which a metabolic pathway is modified by knockout of a protein that degrades a particular metabolite (for example, GABA-T) utilizing a targeted mutant screen, such as the TILLING (targeting-induced local lesions in genomes) technique. Additionally, starch accumulation under salt stress would be a useful marker for identifying candidates that can be used in breeding varieties with high-Brix fruit. We hope that the information described in this chapter will be useful for researchers studying salt stress responses and those who are interested in the development of novel, high-value-added fruit crop varieties.

## References

- Adams P (1991) Effects of increasing the salinity of nutrient solution with major nutrients or sodium chloride on the yield, quality and composition of tomatoes grown in rockwool. *J Hort Sci* 66:201–207
- Adams P, Ho LC (1992) The susceptibility of modern tomato cultivars to blossom-end rot in relation to salinity. *J Hort Sci* 67:827–839
- Akihiro T, Koike S, Tani R, Tominaga T, Watanabe S, Iijima Y, Aoki K, Shibata D, Ashihara H, Matsukura C, Akama K, Fujimura T, Ezura H (2008) Biochemical mechanism on GABA accumulation during fruit development in tomato. *Plant Cell Physiol* 49:1378–1389
- Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* 59:206–216
- Ashton JD, Verma DPS (1993) Proline biosynthesis and osmoregulation in plants. *Plant J* 4:215–223
- Aurisano N, Bertani A, Reggiani R (1995) Involvement of calcium and calmodulin in protein and amino acid metabolism in rice roots under anoxia. *Plant Cell Physiol* 36:1525–1529
- Balibrea ME, Santa Cruz AM, Bolarin MC, Perez-Alfocea F (1996) Sucrolytic activities in relation to sink strength and carbohydrate composition in tomato fruit growing under salinity. *Plant Sci* 118:47–55
- Balibrea ME, Martínez-Andújar C, Cuartero J, Bolarín M, Pérez-Alfocea F (2006) The high fruit soluble sugar content in wild *Lycopersicon* species and their hybrids with cultivars depends on sucrose import during ripening rather than on sucrose metabolism. *Funct Plant Biol* 33: 279–288

- Baxter CJ, Carrari F, Bauke A, Overy S, Hill SA, Quick PW, Fernie AR, Sweetlove LJ (2005) Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. *Plant Cell Physiol* 46:425–437
- Belda RM, Fenlon JS, Ho LC (1996) Salinity effects on the xylem vessels in tomato fruit among cultivars with different susceptibilities to blossom-end rot. *J Hortic Sci* 71:173–179
- Bouchè N, Fromm H (2004) GABA in plants: just a metabolite? *Trends Plant Sci* 9:110–115
- Canene-Adams K, Campbell JK, Zaripheh S, Jeffery EH, Erdman JW (2005) The tomato as a functional food. Symposium: relative bioactivity of functional foods and related dietary supplements. *J Nutr* 135:1226–1230
- Carvajal M, Cerda A, Martinez V (2000) Modification of the response of saline stressed tomato plants by the correction of cation disorders. *Plant Growth Regul* 30:37–47
- Chen BY, Janes HW, Gianfagna T (1998) PCR cloning and characterization of multiple ADP-glucose pyrophosphorylase cDNA from tomato. *Plant Sci* 6:59–67
- Chengappa S, Guilleroux M, Wendy P, Shields R (1999) Transgenic tomato plants with decreased sucrose synthase are unaltered in starch and sugar accumulation in the fruit. *Plant Mol Biol* 40:213–221
- Chretien S, Gosselin A, Dorais M (2000) High electrical conductivity and radiation-based water management improve fruit quality of greenhouse tomatoes grown in rockwool. *HortScience* 35:627–631
- Claussen W (2005) Proline as a measure of stress in tomato plants. *Plant Sci* 168:241–248
- Cuartero J, Fernandez-Munoz R (1999) Tomato and salinity. *Sci Hortic* 78:83–125
- D'Aoust MA, Yelle S, Nguyen-Quoc B (1999) Antisense inhibition of tomato fruit sucrose synthase decrease fruit setting and the sucrose unloading capacity of young fruit. *Plant Cell* 11:2407–2418
- Davies JN (1964) Effects of nitrogen, phosphorus and potassium fertilizers on the non-volatile organic acids of tomato fruit. *J Sci Food Agric* 15:665–673
- Davies JN, Hobson GE (1981) The constituents of tomato fruit: the influence of environment, nutrition, and genotype. *CRC Crit Rev Food Sci Nutr* 15:205–280
- De Pascale S, Maggio A, Fogliano V, Ambrosino P, Ritieni A (2001) Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. *J Hortic Sci Biotechnol* 76:447–453
- Dumas Y, Dadomo M, Di Lucca G, Grolier P (2003) Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *J Sci Food Agric* 83:369–382
- Ehret DL, Ho LC (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
- Fanasca S, Martino A, Heuvelink E, Stanghellini C (2007) Effect of electrical conductivity, fruit pruning, and truss position on quality in greenhouse tomato fruit. *J Hortic Sci Biotechnol* 82:488–494
- Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants: where next? *Aust J Plant Physiol* 22:875–884
- Foolad MR (2004) Recent advances in genetics of salt tolerance in tomato. *Plant Cell Tissue Organ Cult* 76:101–119
- Franco JA, Banon S, Madrid R (1994) Effects of protein hydrolysate applied by fertigation on the effectiveness of calcium as a corrector of blossom-end rot in tomato cultivated under saline condition. *Sci Hortic* 57:283–292
- Fridman E, Liu YS, Carmel-Goren L, Shoresh AGM, Pleban T, Eshed Y, Zamir D (2002) Two tightly linked QTLs modify tomato sugar content via different physiological pathways. *Mol Gen Genet* 266:821–826
- Fridman E, Carrari F, Liu YS, Fernie AR ZD (2004) Zooming in on a quantitative trait for tomato yield using interspecific introgressions. *Science* 305:1786–1789
- Gao Z, Sagi M, Lips SH (1998) Carbohydrate metabolism in leaves and assimilate partitioning in fruits of tomato (*Lycopersicon esculentum* Mill.) as affected by salinity. *Plant Sci* 135:149–159

- Godt D, Roitsch T (1997) Regulation and tissue-specific distribution of mRNAs for three extracellular invertase isoenzymes of tomato suggests an important function in establishing and maintaining sink metabolism. *Plant Physiol* 115:273–282
- Ho LC (1986) Metabolism and compartmentation of translocates in sink organs. In: Cronshaw J, Lucas WJ, Giaquinta RT (eds) *Phloem transport*. Liss, New York, pp 317–324
- Ho LC, Grange RI, Picken AJ (1987) An analysis of the accumulation of water and dry matter in tomato fruit. *Plant Cell Environ* 10:157–162
- Ho LC, Belda R, Brown M, Andrews J, Adams P (1993) Uptake and transport of calcium and the possible causes of blossom-end rot in tomato. *J Hortic Sci* 44:509–518
- Inaba A, Yamamoto T, Ito T, Nakamura R (1980) Changes in the concentrations of free amino acids and soluble nucleotide in attached and detached tomato fruits during ripening. *J Jpn Soc Hortic Sci* 49:435–441
- Inoue K, Shirai T, Ochiai H, Kasao M, Hayakawa K, Kimura M, Sansawa H (2003) Blood-pressure-lowering effect of a novel fermented milk containing gamma-aminobutyric acid (GABA) in mild hypertensives. *Eur J Clin Nutr* 57:490–495
- Johnson BS, Singh NK, Cherry JH, Locy RD (1997) Purification and characterization of glutamate decarboxylase from cowpea. *Phytochemistry* 46:39–44
- Kader AA, Stevens MA, Albright M, Morris LL (1978) Amino acid composition and flavor of fresh market tomatoes as influenced by fruit ripeness when harvested. *J Am Soc Hortic Sci* 103:541–544
- Krauss S, Schnitzler WH, Grassmann J, Woitke M (2006) The influence of different electrical conductivity values in a simplified recalculating soilless system on inner and outer fruit quality characteristics of tomato. *J Agric Food Chem* 54:441–448
- Li XY, Xing JP, Thomas JG, Harry JW (2002) Sucrose regulation of ADP-glucose pyrophosphorylase subunit genes transcript levels in leaves and fruits. *Plant Sci* 162:239–244
- Li Z, Palmer WM, Martin AP, Wang R, Rainsford F, Jin Y, Patrick JW, Yang Y, Ruan YL (2012) High invertase activity in tomato reproductive organs correlates with enhanced sucrose import into, and heat tolerance of, young fruit. *J Exp Bot* 63:1155–1166
- Lin TP, Caspar T, Somerville C, Preiss J (1988) A starch-deficient mutant of *Arabidopsis thaliana* with low ADP-glucose pyrophosphorylase activity lacks one of the two subunits of the enzyme. *Plant Physiol* 88:1175–1181
- Mae M, Makino Y, Oshita S, Kawagoe Y, Tanaka A, Aoki K, Kurabayashi A, Akihiro T, Akama K, Koike S, Takayama M, Matsukura C, Ezura H (2012) Accumulation mechanism of  $\gamma$ -aminobutyric acid in tomatoes (*Solanum lycopersicum* L.) under low O<sub>2</sub> with and without CO<sub>2</sub>. *J Agric Food Chem* 60:1013–1019
- Matsumoto Y, Ohno K, Hiraoka Y (1997) Studies on the utilization of functional food materials containing high levels of gamma-aminobutyric acid (part1). *Ehime Kougi Kenkyu Houkoku* 35:97–100 (in Japanese)
- Minoggio M, Bramati L, Simonetti P, Gardana C, Lemoli L, Santangelo E, Mauri PL, Spigno P, Soressi GP, Pietta PG (2003) Polyphenol pattern and antioxidant activity of different tomato lines and cultivars. *Ann Nutr Metab* 47:64–69
- Morell MK, Bloom M, Knowles V, Preiss J (1987) Subunit structure of spinach leaf ADP glucose pyrophosphorylase. *Plant Physiol* 85:182–187
- Müller-Röber B, Kossamann J, Hannah LC, Willmitzer L, Sonnewald U (1990) One of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. *Mol Gen Genet* 224:136–146
- N'tchobo H, Dali N, Nguyen-Quoc B, Foyer CH, Yelle S (1999) Starch synthesis in tomato remains constant throughout fruit development and is dependent on sucrose supply and sucrose synthase activity. *J Exp Bot* 50:1457–1463
- Nielsen TH, Krapp A, Röper-Schwarz U, Stitt M (1998) The sugar-mediated regulation of genes encoding the small subunit of Rubisco and the regulatory subunit of ADP-glucose pyrophosphorylase is modified by nitrogen and phosphate. *Plant Cell Environ* 21:443–455
- Ohyama A, Nishimura S, Hirai M (1998) Cloning of cDNA for a cell wall-bound acid invertase from tomato (*Lycopersicon esculentum*) and expression of soluble and cell wall-bound invertases in plants and wounded leaves of *L. esculentum* and *L. peruvianum*. *Genes Genet Syst* 73:149–157

- Papadopoulos I, Rendig VV (1983) Tomato plant response to salinity. *Agron J* 75:696–700
- Park SW, Chung WI (1998) Molecular cloning and organ-specific expression of three isoforms of tomato ADP-glucose pyrophosphorylase gene. *Gene (Amst)* 206:215–221
- Petreikov M, Shen S, Yeselson Y, Levin I, Bar M, Schaffer AA (2006) Temporally extended gene expression of the ADP-Glc pyrophosphorylase large subunit (*AgpL1*) leads to increased enzyme activity in developing tomato fruit. *Planta (Berl)* 224:1465–1479
- Robinson NL, Hewitt JD, Bennett AB (1988) Sink metabolism in tomato fruit. I. Developmental changes in carbohydrate metabolizing enzymes. *Plant Physiol* 87:727–730
- Roitsch T, González MC (2004) Function and regulation of plant invertases: sweet sensations. *Trends Plant Sci* 9:606–613
- Rolin D, Baldet P, Just D, Chevalier C, Biran M, Raymond P (2000) NMR study of low subcellular pH during the development of cherry tomato fruit. *Aust J Plant Physiol* 27:61–69
- Saito T, Fukuda N, Nishimura S (2006) Effects of salinity treatment duration and planting density on size and sugar content of hydroponically grown tomato fruits. *J Jpn Soc Hortic Sci* 75:392–398
- Saito T, Matsukura C, Ban Y, Shoji K, Sugiyama M, Fukuda N, Nishimura S (2008a) Salinity stress affects assimilate metabolism at the gene-expression level during fruit development and improves fruit quality in tomato (*Solanum lycopersicum* L.). *J Jpn Soc Hortic Sci* 77:61–68
- Saito T, Matsukura C, Sugiyama M, Watahiki A, Ohshima I, Iijima Y, Konishi C, Fujii T, Inai S, Nishimura S, Ezura H (2008b) Screening for  $\gamma$ -aminobutyric acid (GABA)-rich tomato varieties. *J Jpn Soc Hortic Sci* 77:242–250
- Saito T, Fukuda N, Matsukura C, Nishimura S (2009) Effects of salinity on distribution of photosynthates and carbohydrate metabolism in tomato grown using nutrient film technique. *J Jpn Soc Hortic Sci* 78:90–96
- Sakamoto Y, Watanabe S, Nakashima T, Okano K (1999) Effects of salinity at two ripening stages on the fruit quality of single-truss tomato grown in hydroponics. *J Hortic Sci Biotechnol* 74:690–693
- Sanders D, Brownlee C, Harper JF (1999) Communicating with calcium. *Plant Cell* 11:691–706
- Schaffer AA, Petreikov M (1997) Sucrose-to-starch metabolism in tomato fruit undergoing transient starch accumulation. *Plant Physiol* 113:739–746
- Schaffer AA, Levin I, Ogus I, Petreikov M, Cincarevsky F, Yeselson E, Shen S, Gilboa N, Bar M (2000) ADP-glucose pyrophosphorylase activity and starch accumulation in immature tomato fruit: the effect of a *Lycopersicon hirsutum*-derived introgression encoding for the large subunit. *Plant Sci* 152:135–144
- Scheible WR, González-Fontes A, Lauerer M, Müller-Röber B, Caboche M, Stitt M (1997) Nitrate acts as a signal to induce organic acid metabolism and repress starch metabolism in tobacco. *Plant Cell* 9:783–798
- Shi J, Le Maguer M (2000) Lycopene in tomatoes: chemical and physical properties affected by food processing. *Crit Rev Food Sci Nutr* 40:1–42
- Sinha AK, Hofmann MG, Römer U, Köckenberger W, Elling L, Roitsch T (2002) Metabolizable and non-metabolizable sugars activate different signal transduction pathways in tomato. *Plant Physiol* 128:1480–1489
- Snedden WA, Arazi T, Fromm H, Shelp BJ (1995) Calcium/calmodulin activation of soybean glutamate decarboxylase. *Plant Physiol* 108:543–549
- Snedden WA, Koutsia N, Baum G, Fromm H (1996) Activation of a petunia glutamate decarboxylase by calcium/calmodulin or by a monoclonal antibody which recognizes the calmodulin binding domain. *J Biol Chem* 271:4148–4153
- Sokolov LN, Dejardin A, Kleczkowski LA (1998) Sugars and light/dark exposure trigger differential regulation of ADP-glucose pyrophosphorylase genes in *Arabidopsis thaliana* (thale cress). *Biochem J* 336:681–687
- Sorrequieta A, Ferraro G, Boggio SB, Valle EM (2010) Free amino acid production during tomato fruit ripening: a focus on L-glutamate. *Amino Acids* 38:1523–1532
- Stark DM, Timmerman KP, Barry GF, Preiss J, Kishore GM (1992) Regulation of the amount of starch in plant tissues by ADP-glucose pyrophosphorylase. *Science* 258:287–292
- Stevens MA, Kader AA, Albright-Holton M, Algazi M (1977) Genotypic variation for flavour and composition in fresh market tomatoes. *J Am Soc Hortic Sci* 102:680–689

- Sturm A (1999) Invertases. primary structures, functions, and roles in plant development and sucrose partitioning. *Plant Physiol* 121:1–7
- Tal M, Katz A, Heikin H, Dehan K (1979) Salt tolerance in the wild relatives of the cultivated tomato: proline accumulation in *Lycopersicon esculentum* Mill., *L. peruvianum* Mill. and *Solanum pennellii* Cor. treated with NaCl and polyethyleneglycol. *New Phytol* 82:349–355
- Tsai CY, Neleson OE (1966) Starch-deficient maize mutant lacking adenosine diphosphate glucose pyrophosphorylase activity. *Science* 151:341–343
- Turano FJ, Fang TK (1998) Characterization of two glutamate decarboxylase cDNA clones from *Arabidopsis*. *Plant Physiol* 117:1411–1421
- Van Ieperen W (1996) Effects of different day and night salinity levels on vegetative growth, yield and quality of tomato. *J Hort Sci* 71:99–111
- Willumsen J, Petersen KK, Kaack K (1996) Yield and blossom-end rot of tomato affected by salinity and cation activity ratios in the root zone. *J Hort Sci* 71:81–98
- Yamaki S (2010) Metabolism and accumulation of sugars translocated to fruit and their regulation. *J Jpn Soc Hort Sci* 79:1–15
- Yelle S, Hewitt JD, Nieder M, Robinson NL, Damon S, Bennett AB (1988) Sink metabolism in tomato fruit. III. Analysis of carbohydrate assimilation in a wild species. *Plant Physiol* 87:731–736
- Yin YG, Tominaga T, Iijima Y, Aoki K, Shibata D, Ashihara H, Nishimura S, Ezura H, Matsukura C (2010a) Metabolic alterations in organic acids and  $\gamma$ -amino butyric acid in developing tomato (*Solanum lycopersicum* L.) fruits. *Plant Cell Physiol* 51:1300–1314
- Yin YG, Kobayashi Y, Sanuki A, Kondo S, Fukuda N, Ezura H, Sugaya S, Matsukura C (2010b) Salinity induces carbohydrate accumulation and sugar-regulated starch biosynthetic genes in tomato (*Solanum lycopersicum* L. cv. 'Micro-Tom') fruits in an ABA- and osmotic stress-independent manner. *J Exp Bot* 61:563–574
- Zanor MI, Osorio S, Nunes-Nesi A, Carrari F, Lohse M, Usadel B, Kühn C, Bleiss W, Giavalisco P, Willmitzer L, Sulpice R, Zhou YH, Fernie AR (2009) RNA interference of LIN5 in tomato confirms its role in controlling Brix content, uncovers the influence of sugars on the levels of fruit hormones, and demonstrates the importance of sucrose cleavage for normal fruit development and fertility. *Plant Physiol* 150:1204–1218
- Zhang Y (2013) Regulation of ascorbate synthesis in plants. In: Zhang Y (ed) *Ascorbic acid in plants. Biosynthesis, regulation and enhancement*. Springer, New York, pp 87–99
- Zhang SJ, Jackson MB (1993) GABA-activated chloride channels in secretory nerve endings. *Science* 259:531–534
- Zushi K, Matsuzoe N (2006) Free amino acid contents of tomato fruit grown under water and salinity stresses. *Acta Hort* 724:91–96
- Zushi K, Matsuzoe N (2009) Seasonal and cultivar differences in salt-induced changes in antioxidant system in tomato. *Sci Hort* 120:181–187
- Zushi K, Ono M, Matsuzoe N (2014) Light intensity modulates antioxidant systems in salt-stressed tomato (*Solanum lycopersicum* L. cv. Micro-Tom) fruits. *Sci Hort* 165:384–391