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The addition of gibberellic acid to auxin solutions increases sugar accumulation and sink strength in developing auxin-induced parthenocarpic tomato fruits

K. Kataoka*, Y. Yashiro, T. Habu, K. Sunamoto, A. Kitajima

Graduate School of Agriculture, Kyoto University, Hattyonawate, Takatsuki 569-0096, Japan

*Corresponding author. Tel.: +81-72-685-0134; fax: +81-72-683-1532
E-mail address: kata@kais.kyoto-u.ac.jp (K. Kataoka)
ABSTRACT

The effects of the addition of gibberellic acid (GA) to auxin solutions for tomato fruit setting on sugar content and titratable acidity at maturity were investigated in ‘Louis 60’ tomatoes, and sucrolytic enzyme activities and cell development during the early stages of fruit development were examined to explain the physiological mechanisms. Days to maturity and titratable acidity were not affected by GA addition. The sugar concentration of the pericarp was increased by 50 mg L⁻¹ of GA addition in the summer and the spring, but not in the autumn experiment. The product of sugar concentration and fruit weight, which is thought to reflect the amount of sugar per fruit, was higher in GA-treated fruit in every experiment. Beginning one day after treatment, cell size was larger in GA-treated fruit and resulted in an increase in pericarp thickness at maturity. These results suggest that GA addition at anthesis can promote an increased sink size of individual pericarp cells immediately after treatment. The activities of VAI and neutral invertase (NI) were higher in GA-treated fruit around five days after treatment, and the possibility of VAI and/or NI involvement in sink activity at an early stage of fruit development is suggested.
Key Words: Tomato, Fruit growth, Gibberellin, Sucrolytic enzyme, Sugar concentration, Sink strength

1. Introduction

Tomato (Solanum lycopersicum L.) is one of the most important vegetables and is consumed throughout the year. In Japan, since most tomatoes are used as fresh fruit, the demand for sweetness is high. On the other hand, the year-round demand for fresh fruits forces fruit production even during unfavorable seasons for fruit setting and growth, which presents many difficulties. In the case of the hot season, high temperatures inhibit pollen development and result in failure of fruit setting and growth (Sato et al., 2000, 2002). Synthetic auxins, such as 4-chlorophenoxy acetic acid (4CPA) or 4-chloro-2-(hydroxymethyl) phenoxyacetic acid (HCPA), are well-known plant growth regulators that can substitute for pollination and induce fruit setting and growth, and these are used for stabilizing fruit production in commercial growing. However, they cannot induce a sufficient setting ratio in extreme high temperatures and may also increase the occurrence of puffiness. The use of gibberellic acid (GA$_3$), together with auxin treatment, can reduce the occurrence of puffy fruit (Yamasaki et al., 1961) and raise the setting
ratio under high temperature conditions (Sasaki et al., 2005). However, the effects of GA addition on fruit development and quality are not yet fully understood. Therefore, in this study, we investigated the effects of GA addition to auxin solutions on fruit growth, sugar content, and acidity of tomato fruit.

Fruit development and sugar content depend on sugar accumulation and metabolism within the fruit. Sugar accumulation within a fruit, or translocation of photosynthate, is driven by the sucrose concentration gradient from source to sink. When the amount in the source (leaves) is relatively large compared to the sink, the amount of translocation is dependent on the strength of sucrose cleavage at the unloading site (Islam, 2001). In an early stage of fruit growth, sucrose synthase (SuSy) is responsible for sink strength due to correlations between SuSy activity and both fruit growth (Sun et al., 1992) and sucrose uptake (N’tchobo et al., 1999). Sucrose is unloaded by the symplastic pathway and metabolized into fructose and UDP-glucose by SuSy, leading to a transient accumulation of starch and an increase in the sucrose gradient. In the next stage of development, the sucrose uptake pathway changes from symplastic to apoplastic, where apoplastic acid invertase (CWAI) is responsible for sucrose cleavage into fructose and glucose. During the maturing stage of development, the amount of transient starch is
gradually decreased, and rapid accumulation of hexoses in the vacuole occurs, which is thought to be dependent on the activity of vacuole acid invertase (VAI) (Yelle et al., 1991; Klann et al., 1993).

In the present study, we first investigated the effects of GA addition to auxin solutions at anthesis on sugar content, acidity, fresh weight, and VAI activity at ripening. Next, we compared changes in the activities of VAI, SuSy, CWAI, and neutral invertase (NI, cytoplasmic) during the early stage of fruit development. Finally, we observed pericarp tissue proliferation histologically to determine whether cell division or cell enlargement is enhanced by GA addition.

2. Materials and Methods

2.1. Exp. 1 Effects of GA addition to auxin solutions on the quality of tomato fruits at ripening (summer 2006)

Tomato cultivar ‘Louis 60’ (Takii Seed Co., Japan) seeds were sown on 19 April 2006 and transplanted to rockwool beds in a glasshouse on 13 May 2006. The nutrient solution was adjusted to electrical conductivity (EC) of 2.0 to 2.5 dS m⁻¹
and pH of 5.5 to 6.5 and circulated 5 to 7 times a day. The nutrient solution was maintained by half-strength Ostuka-A nutrient solution for hydroponics (Otsuka Chem. Co. Ltd., Japan) and H₂SO₄. All stigmata were cut off to prevent pollination before anthesis. At the anthesis of the third flower of each cluster, clusters were dipped in a solution of 15 mg L⁻¹ of 4-chlorophenoxy acetic acid (4CPA, Nissan Chem. Ind. Ltd., Japan) that also contained 0, 50, or 100 mg L⁻¹ of gibberellin A₃ (GA, Kyowa Hakko Co., Ltd., Japan). Two or three days after treatment, the first fruit of each cluster was removed, and the number of fruits per cluster was adjusted to 5. Plants were grown vertically with a single stem and detopped at the second upper leaf above the fifth truss. All fruits of the second and fifth clusters were harvested at ripening, and fresh fruit weight, sugar content and titratable acidity of pericarp, as well as VAI activity of the pericarp, were measured. Each treatment consisted of 15 plants.

2.2. Exp. 2 Effects of GA addition to auxin solutions on growth and sucrolytic enzyme activities during the early stages of fruit development (spring 2007).

Seeds of ‘Louis 60’ tomatoes were sown on 15 February 2007. Seedlings
were transplanted to rockwool beds in a glasshouse on 20 March and grown in the same manner as in Exp. 1. When the third flower of each cluster opened, all flowers of the second to fifth clusters, whose stigmata had been cut off before anthesis, were dipped in 15 mg L⁻¹ of 4CPA solution with or without 50 mg L⁻¹ of GA. Fruits were harvested 1, 3, 5, 7, and 10 days after treatment (DAT), and fruit fresh weight and the activity of SuSy, CWAI, VAI, and NI of pericarp tissue were measured. Fresh fruit weight and sugar content of the pericarp were also measured at ripening. Each treatment consisted of 15 plants.

2.3. Exp. 3 Effects of GA addition to auxin solutions on the division and enlargement of pericarp cells (autumn 2007)

Seeds of ‘Louis 60’ tomatoes were sown on 5 September 2007. Seedlings were transplanted to rockwool beds on 26 September in a non-heated glasshouse and grown in the same manner as in Exp. 1. When the third flower of each cluster opened, all flowers of the second cluster, whose stigmata were cut off before anthesis, were dipped in 15 mg L⁻¹ of 4CPA solution with or without 50 mg L⁻¹ of GA. Fruits (except the first flower) were harvested 1, 3, 5, 8, and 13 days after
treatment (DAT) and at ripening. Twelve fruits for 1 DAT and 4 fruits for the other days were used for measurement of fresh fruit weight and pericarp cell size. The number of pericarp cell layers was estimated from the thickness of the pericarp and the cell diameter. The thickness of the pericarp was measured at an equatorial cut surface several times and expressed as a mean value. Cell diameter was estimated from a projection of cell area because the pericarp cell can be thought of as approximately spherical (Cheniclet et al., 2005). The number of pericarp cell layers was estimated by dividing the pericarp thickness by cell diameter. Sugar content of the pericarp was measured at ripening.

2.4. Sugar and starch analysis

Approximately 1 g of fresh pericarp tissue was cut off in a crescent shape as a proportional distribution of pericarp, boiled in 80% methanol using a microwave oven, and homogenized after the addition of raffinose as an internal standard. Homogenates were centrifuged, and supernatants were evaporated to leave an aqueous solution and cleaned with Sep-Pak Plus Accell Plus QMA cartridges (Waters Co., USA) and PTFE filters. Sugars in the supernatant were
analyzed by HPLC (880-PU, JASCO Co., Japan) fitted with an RI-71 refractive index detector (Showa Denko, Japan) and an SC-1011+SC-G column (Showa Denko, Japan). The eluent was water with a 1.0 mL min⁻¹ flow rate at 80 °C. The residues were washed with 80% ethanol three times, followed by drying, and boiled with 5 mL of 0.02 N NaOH for 15 min. After cooling, they were incubated with 60 units of amyloglucosidase in 1.5 M citrate buffer (pH 4.6) at 45 °C overnight. The released glucose amounts were measured by F-kit D-glucose (Roche Diagnostics, Switzerland) and multiplied by 0.9 to calculate the starch contents.

2.5. Titratable acidity

Approximately 1 g of fresh pericarp tissue was homogenized with water to 40 mL. Half of each supernatant from the centrifuged homogenates was combined with a drop of 0.1% phenolephtarane solution and titrated with 0.01 N NaOH. Titratable acidity was described as citrate amount per fresh weight.

2.6. Extraction of enzymes
Approximately 1 g of pericarp tissue was cut and immediately frozen in liquid nitrogen, then kept at -50°C until extraction. Enzyme extraction and desalting were carried out following the methods of Sun et al. (1992) and Xu et al. (1989) with some modifications. Tissue samples were homogenized in 3 mL of extraction buffer (200 mM Hepes [pH 7.0], 3 mM Mg acetate, 1 mM EDTA, 10 mM ascorbate, 5 mM DTT, and glycerol) with 0.1 g of insoluble polyvinylpolylyrrolidone. The extract was filtered with three layers of gauze and centrifuged at 15,000 g for 15 min. The supernatants were desalted in a visking tube with desalting buffer (10 mM Hepes [pH 7.8], 2.5 mM DTT, 1 mM EDTA) at 4°C overnight, and an aliquot of the extract was used as crude enzyme fraction (CF) for SuSy, VAI, and NI activity assays. The pellets were washed with extraction buffer three times, followed by incubation in buffer (0.2 M citrate buffer [pH 5.0], 0.2 M NaCl) at 4°C overnight. The extracts were centrifuged at 15,000g for 15 min, and the supernatants were desalted by a microcentrifuge desalting procedure using ultrafree-MC (Millipore Co., USA) at 3000 g for 20 min at 4°C. An aliquot of the extract was used as CF for CWAI activity assays.

2.7. Enzyme activities
Acid invertase was assayed by incubating a 0.1-mL aliquot of CF at 45°C for 15 min with 0.2 mL of citrate buffer (pH 5.0), 0.1 mL of 0.5 M sucrose, and 0.1 mL distilled water, based on the methods of Islam et al. (1996). The concentration of glucose was determined by F-kit D-glucose. SuSy was assayed by incubating a 30-µL aliquot of CF at 37°C for 30 min with 2 µmol fructose and 1 µmol UDP glucose in 90 µL of 50 mM Hepes buffer (pH 7.8) containing 15 mM MgCl₂. The reaction was stopped by adding 120 µL of 1 N NaOH. After boiling at 100°C for 10 min, sucrose synthesis was measured using an anthron reaction, based on the methods of Wang et al. (1993) and Islam et al. (1996). NI was assayed by incubating a 0.1-mL aliquot of CF at 45°C for 15 min with 0.2 mL of 50 mM Hepes buffer (pH 7.0), 0.1 mL of 0.5 M sucrose, and 0.1 mL of distilled water, based on the methods of Islam et al. (1996) and Vorster and Botha (1998). Liberated glucose was measured by F-kit D-glucose. The amount of soluble protein in the supernatant was determined by the method of Bradford (Bradford, 1976) using bovine serum albumin standards.

2.8. Measurement of cell size
The fixed pericarp tissue kept in 80% ethanol-formaldehyde-acetic acid (FAA, 90:5:5) was macerated for at least 24 h at room temperature in a mixture of 0.05 M EDTA and 0.4 M mannitol at a pH of 10.3. Thereafter, the incubated tissue was stirred for 15–20 min on a magnetic stirrer. Aliquots of the cell suspension were observed under a differential interference microscope (DP71, Olympus Co., Japan), and the projection area of the cells was measured using an Image-Pro Plus ver. 5.0 (Media Cybernetics, USA).

3. Results

3.1. Exp. 1 Effects of GA addition to auxin solutions on the quality of tomato fruits at ripening (summer 2006)

The average daily maximum and minimum temperatures were 36°C and 23°C, respectively, from the start of anthesis of the second cluster (12 June) to the end of harvest of the fifth cluster (11 August). GA addition had no effect on the days to ripening, while the position of the cluster had a significant effect (Table 1).
GA addition had no effect on fresh fruit weight. Titratable acidity was decreased in the fifth cluster, but was not affected by GA addition. VAI activities were significantly different between the clusters, but not different after GA addition. The concentrations of pericarp sugars, mainly hexoses, were significantly increased by GA addition. Both fructose and glucose concentrations were highest in the fruits treated with GA at 50 mg L⁻¹ in either the second or fifth cluster. The effects of GA addition were remarkably observed at 50 mg L⁻¹ rather than 100 mg L⁻¹.

3.2. Exp. 2 Effects of GA addition to auxin solutions on growth and sucrolytic enzyme activities during early stages of fruit development (spring 2007).

The average daily maximum and minimum temperatures were 33°C and 16°C, respectively, from the start of anthesis of the second cluster (24 April) to the end of harvest of the fifth cluster (27 June). The fresh weight of fruits treated with GA was significantly heavier than those without GA, from 1 to 10 DAT by two-way ANOVA (Fig. 1). At the red-ripe stage, fresh fruit weight and total sugar concentration in the fruits treated with GA were significantly higher than those
without GA, but no treatment differences were detected for any of the sugars (Table 2). Enzyme activities of SuSy and CWAI were not affected by GA treatment, while those of VAI at 5 and 7 DAT and NI from 3 to 7 DAT were higher in fruits treated with GA than those without GA (Fig. 2).

3.3 Exp. 3 Effects of GA addition to auxin solutions on the division and the enlargement of pericarp cells (autumn 2007)

The average daily maximum and minimum temperatures were 36°C and 14°C, respectively, during the two weeks after the start of anthesis (24 October). The fruit development in a later stage was shown to be restricted by low temperature from the middle of November onward. The average daily maximum and minimum temperatures were 31°C and 8°C, respectively, from 7 November (two weeks after the start of anthesis) to the end of harvest (31 December). As a result, 63-68 days were needed for ripening, which was longer than in either Exp. 1 or Exp. 2. While the progress of fruit growth in the early stages seemed to be slower than in Exp. 1, fresh weight of fruit treated with GA was significantly higher than that of fruit without GA treatment from 1 to 13 DAT (Table 3).
Significant differences were also detected by two-way ANOVA between samples with GA and without GA in logarithms of cell area (Table 3). At ripening, both the pericarp thickness and the cell area of fruits treated with GA were larger than those without GA, and, as a result, the number of pericarp cell layers was not different between the treatments (Table 4). At ripening, fresh weight of fruit treated with GA was 102.9 g and significantly higher (by t-test, $p<0.05$) than that of fruit without GA treatment, which was 87.1 g. Total sugar concentration of GA-treated fruit was 40.9 mg g$^{-1}$FW, which was not higher than that of non-GA-treated fruit, which was 40.6 mg g$^{-1}$FW.

4. Discussion

In Exp. 1, 50 mg L$^{-1}$ of GA added to the auxin solution for fruit set increased sugar concentration, mainly hexoses, and fruit weight of ‘Louis 60’ tomatoes. It is interesting physiologically that an increase in sugar concentration was induced by GA addition at anthesis. This was not a result of condensation because it occurred without fruit weight reduction. A similar result was also obtained with another cultivar, ‘Mini Carol,’ in a simultaneous experiment (data
not shown). In the cases of Exps. 2 and 3, the increases in sugar concentration were small or nonexistent, but fruit fresh weight was increased markedly, by 13% in Exp. 2 and 18% in Exp. 3, by GA treatment. Here, we tried to calculate the product of pericarp sugar concentration and fruit fresh weight, which was considered to reflect sugar content per fruit, or an approximate value of assimilation to an individual fruit. It was determined that GA addition had a tendency to increase sugar content per fruit by 15-17% in every experiment (Table 5). This indicates that a high GA level just after anthesis can enhance sugar translocation into a fruit, but whether the increased sugar accumulation results in increases in either fruit weight or sugar concentration at maturity may depend on other factors, such as low temperature in later stages, as in Exp. 3.

Tomato fruit development has been divided into four phases. Phase I is generally referred to as fruit set. In phase II, fruit growth is due to cell division, and in phase III, fruit growth continues by cell expansion (Gillaspy, et al. 1993). Sugar accumulation might be highest in phase III and continue 3-5 weeks after phase II, which involves cell division that lasts for 7-14 days after anthesis (Srivastava and Handa, 2005). Because of a time lag between GA application and phase III, it is difficult to imagine the direct effect of GA on accumulation in a later
stage, when the growth rate is highest. Applied GA may induce some physiological changes several days after treatment in phase I or II, resulting in an increase in translocation in phase III. The ability of a sink organ to import assimilate is known as the sink strength (Ho, 1988). Sink strength is a product of sink size and sink activity. Sink size is defined as cell number and cell size of the sink organ, and sink activity is the physiological constraint upon a sink organ’s assimilate import (Ho, 1988, Baldet et al., 2006). Therefore, we examined activities of some sucrose cleavage enzymes and fruit growth from 1 to 10 days after treatment in Exp. 2. The results showed that fruit growth from 1 to 10 DAT was enhanced by GA addition. During the early stages of fruit development, SuSy is the dominant enzyme involved in cleaving sucrose and is responsible for fruit growth (Wang et al., 1993). In our results, treatment with GA did not affect SuSy activity between the treatments. Therefore, it is difficult to say whether SuSy activity accounted for the effects of GA on fruit growth. During the following stage of fruit development, there is a transition in the uptake pathway from symplastic to apoplastic, during which CWAI plays an important role in the hydrolysis of sucrose (Miron et al., 2002). CWAI, or extracellular invertase, has an important function in establishing and maintaining sink metabolism (Godt and Roitsch, 1997). In Exp. 2, CWAI
activities became slightly higher at 7 and 10 DAT in GA-treated fruit, although it may be necessary to examine whether or not the difference becomes even larger from 10 DAT onward. On the other hand, it is interesting to note that VAI and NI activities in the fruit treated with GA were higher than those of untreated fruit at 5 DAT, when the difference in fruit weight was relatively large.

VAI is thought to play a major role in the regulation of hexose levels in mature fruits (Klann et al., 1993, Ohyama et al., 1995), and its activity increases as the fruit matures (Yelle et al., 1991); nevertheless, the VAI activity in mature fruits was not affected by GA addition in Exp. 1. However, the expression of an antisense TIV1, which corresponds to soluble acid invertase, resulted in higher sucrose concentrations and smaller size in tomato fruit without an effect on the amount of sugar accumulated per fruit (Klann et al., 1996). This suggests that the water influx that drives fruit expansion is closely related to the concentration of osmotically active soluble sugars, which might be controlled by VAI. It may be necessary to examine the role of VAI at an early stage of fruit development.

The function of NI is not very well understood, in part because of generally low enzyme activity (Roitsh and González, 2004). However, in relation to the regulatory mechanism necessary for maintaining sink capacity under salinity
stress, the induction of cytoplasmic NI activity probably also maintains the continuation of sugar accumulation during ripening (Balibrea et al., 2003, 2006).

In peach fruit development, there is a relationship between the dynamics of fruit growth, sugar metabolism and the expression of a gene encoding an NI, suggesting a regulatory role in plant development (Nonis et al., 2007). As for the relationship with GA, the high expression and activity of NI and VAI were coordinated with active growth regulated by gibberellin in the petiole growth of sugar beet plants (González and Cejudo, 2007), and increased sink demand induced by applied GA was shown to be closely related to the activation of NI in fresh tissue during rapid fruit growth in pear fruit (Zhang et al., 2007). In the pod growth of *Pisum sativum*, NI activities are associated with elongation of both pods induced by GA and pollination (Estruch and Beltran, 1991). These results are consistent with our own findings and suggest that NI activity may play an important role in the regulation of fruit set and developmental growth coordinated by a GA-regulatory mechanism. Therefore, further investigations may be warranted.

In *pat-2*-induced parthenocarpy in tomatoes, the endogenous GA synthesis activity is high (Fos et al., 2000). In non-parthenocarpic cultivars, this GA-mediated response may be suspended until release by an auxin-response
reaction (Vivian-Smith et al., 2001). These results, taken together, suggest the existence of a growth potency of pericarp tissue by nature, which may depend on sink establishment through sucrolytic activities regulated by GA. Our results suggest that the applied GA could slightly strengthen the sink activity of fruit at an early stage through a rise in sucrolytic activities.

The sink strength of a developing fruit depends on both sink activity and sink size, which, in turn, depend on both the number and the size of the fruit cells (Baldet et al., 2006). If GA addition at anthesis can increase sink size at an early stage, the sink strength in subsequent stages will be enhanced and result in a sugar assimilation increase. In Exp. 3, we examined the changes in pericarp cell size and cell number to determine whether these were affected by GA treatment. The results showed that cell enlargement and fruit growth were slightly accelerated by GA treatment from 1 to 13 DAT. The pericarp was thicker in GA-treated fruit, but the number of cell layers of pericarp was not affected at maturity. These results indicate that the addition of GA at anthesis enhanced cell enlargement at an early stage of fruit development, resulting in an increase in final fruit weight through an increase in the translocation of photosynthate into the individual fruit cell. This is most likely related to the fact that GA-induced
parthenocarpic fruit has larger and fewer cells than auxin-induced fruit (Bünger-Kibler and Bangerth, 1982, Serrani et al., 2007). To explain the role of GA in cell expansion, points to be considered are the turgor pressure with osmotically active sugars, sink strength through the maintenance of a sugar gradient controlled by sucrolytic enzymes, cell wall loosening, and the effects on other enzymes. The enlargement of fruit cells at an early stage induced by GA treatment may determine the future sink capacity and carbon accumulation, which will be distributed between the increase in sugar concentration and/or in fruit weight at maturity, forced by environmental or physiological conditions from phase III onward. In Exp. 3, low temperature conditions throughout the later period of fruit growth were thought to have greatly influenced this distribution.

In conclusion, the addition of 50 mg L⁻¹ of GA to auxin solutions for fruit setting at anthesis increased the amount of sugar content per fruit at maturity in tomato cultivar ‘Louis 60.’ At an early stage of fruit development, cell enlargement was accelerated by GA treatment, resulting in higher sink size and sugar accumulation. Simultaneously, the activities of NI and VAI were higher in GA-treated fruit. Further study regarding the relationship of NI and VAI activities to cell enlargement and the establishment of the sink is required.
Acknowledgments

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Table 1. Effects of fruit cluster position and gibberellic acid addition to auxin solution on days to ripening, fresh weight, sugar concentration, titratable acidity, and VAI activity of red-ripe fruit of ‘Louis 60’ tomatoes (summer 2006).

<table>
<thead>
<tr>
<th>Fruit cluster</th>
<th>Gibberellic acid (mg·L⁻¹)</th>
<th>Days to ripening</th>
<th>Fresh fruit weight (g)</th>
<th>Sugar concentration (mg·g⁻¹FW)</th>
<th>Titratable acidity (citric acid mg·g⁻¹FW)</th>
<th>VAI activity (µmol glu·min⁻¹·mg⁻¹proteín)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sucrose</td>
<td>Fructose</td>
<td>Glucose</td>
</tr>
<tr>
<td>2nd</td>
<td>0</td>
<td>33.3</td>
<td>52.3</td>
<td>1.0</td>
<td>17.6</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>33.5</td>
<td>55.6</td>
<td>1.1</td>
<td>22.9</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>35.0</td>
<td>56.3</td>
<td>1.0</td>
<td>20.8</td>
<td>18.7</td>
</tr>
<tr>
<td>5th</td>
<td>0</td>
<td>36.4</td>
<td>52.1</td>
<td>1.5</td>
<td>22.0</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>35.6</td>
<td>55.1</td>
<td>1.8</td>
<td>23.5</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>36.6</td>
<td>51.8</td>
<td>1.9</td>
<td>20.5</td>
<td>18.2</td>
</tr>
</tbody>
</table>

Significance:
- NS and *, ** denote non-significant and significant differences at \( p = 0.05 \) or \( p = 0.01 \), respectively, as determined using two-way ANOVA.
Table 2. Effect of 50 mg L\(^{-1}\) of gibberellic acid added to auxin solution on fruit weight and sugar concentration of red-ripe fruit of ‘Louis 60’ tomatoes (spring 2007).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh fruit weight (g)</th>
<th>Sugar concentration of pericarp (mg·g(^{-1})FW)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sucrose</td>
<td>Fructose</td>
<td>Glucose</td>
<td>Total</td>
</tr>
<tr>
<td>without GA</td>
<td>69.5 ± 1.5</td>
<td>1.1 ± 0.2</td>
<td>21.1 ± 0.5</td>
<td>19.3 ± 0.4</td>
<td>41.6 ± 0.6</td>
</tr>
<tr>
<td>with GA</td>
<td>78.5 ± 1.5</td>
<td>1.3 ± 0.1</td>
<td>22.7 ± 0.8</td>
<td>20.2 ± 0.5</td>
<td>44.3 ± 1.1</td>
</tr>
<tr>
<td>Significance(^\ast)</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

Values represent the mean of 18 to 20 samples ± SE
\(^\ast\)NS and * denote non-significant or significant differences at \(p < 0.05\) as determined using t-test.
Table 3. Changes of fresh fruit weight and cell area in the pericarp of ‘Louis 60’ tomatoes from 1 day to 13 days after setting treatment with or without 50 mg L\(^{-1}\) gibberellic acid at anthesis (autumn 2007).

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>Fresh fruit weight (g)</th>
<th>Mean cell area (μm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with GA</td>
<td>without GA</td>
</tr>
<tr>
<td>1</td>
<td>0.009</td>
<td>0.007</td>
</tr>
<tr>
<td>3</td>
<td>0.028</td>
<td>0.018</td>
</tr>
<tr>
<td>5</td>
<td>0.113</td>
<td>0.066</td>
</tr>
<tr>
<td>8</td>
<td>0.907</td>
<td>0.672</td>
</tr>
<tr>
<td>13</td>
<td>4.805</td>
<td>3.273</td>
</tr>
</tbody>
</table>

Significance

GA addition

<table>
<thead>
<tr>
<th></th>
<th>with GA</th>
<th>without GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days after treatment</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Determined by two-way ANOVA in logarithms of cell area and fruit weight.
Table 4. Effect of 50 mg L$^{-1}$ of gibberellic acid added to auxin solution on cell area, thickness, and number of cell layers of pericarp of red-ripe fruit of ‘Louis 60’ tomatoes (autumn 2007).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell area (x10$^{5}$μm$^2$)</th>
<th>Pericarp thickness (mm)</th>
<th>Number of cell layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>without GA</td>
<td>1.96 ± 0.05</td>
<td>7.28 ± 0.09</td>
<td>14.6 ± 0.2</td>
</tr>
<tr>
<td>with GA</td>
<td>2.33 ± 0.06</td>
<td>7.72 ± 0.14</td>
<td>14.2 ± 0.2</td>
</tr>
</tbody>
</table>

Significance$^*$

$^*$NS and * denote non-significant or significant differences at $p<0.05$, respectively, as determined using t-test.
Table 5. Effects of 50 mg L\(^{-1}\) of gibberellic acid added to auxin solution on the product of sugar concentration and fruit fresh weight of red-ripe fruit of ‘Louis 60’ tomatoes in Experiments 1-3.

<table>
<thead>
<tr>
<th></th>
<th>Exp. 1 (summer 2006)</th>
<th>Exp. 2 (spring 2007)</th>
<th>Exp. 3 (autumn 2007)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd cluster</td>
<td>5th cluster</td>
<td></td>
</tr>
<tr>
<td>without GA</td>
<td>1837 ± 81.7</td>
<td>2138 ± 136.0</td>
<td>3014.0 ± 115.0</td>
</tr>
<tr>
<td>with GA</td>
<td>2408 ± 100.9</td>
<td>2508 ± 148.0</td>
<td>3480.0 ± 142.0</td>
</tr>
<tr>
<td>Significance(^f)</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

\(^f\) NS and * denote non-significant or non-significant differences at \(p<0.05\)
Fig. 1. Changes of fresh weight per fruit of ‘Louis 60’ tomatoes from 1 day to 10 days after setting treatment with or without 50 mg L\(^{-1}\) gibberellic acid (spring 2007). Solid lines represent the value of the fruits treated by 4CPA with GA; broken lines represent the value of the fruit treated by 4CPA without GA. Values represent the mean of 8 or 12 samples ± SE.

Fig. 2. Changes of specific activities of SuSy, VAI, NI, and CWAI from fruit of ‘Louis 60’ tomatoes from 1 day to 10 days after setting treatment with or without 50 mg L\(^{-1}\) gibberellic acid (spring 2007). Solid lines represent activities of the fruits treated by 4CPA with GA; broken lines represent activities of the fruit treated by 4CPA without GA. Values represent the mean of 4 or 5 samples ± SE.
Fig. 1. Changes of fresh weight per fruit of ‘Louis 60’ tomatoes from 1 day to 10 days after setting treatment with or without 50 mg·L⁻¹ gibberellic acid (spring 2007). Solid lines represent the value of the fruits treated by 4CPA with GA; broken lines represent the value of the fruit treated by 4CPA without GA. Values represent the mean of 8 or 12 samples ± SE.
Fig. 2. Changes of specific activities of SuSy, VAI, NI, and CWAI from fruit of ‘Louis 60’ tomatoes from 1 day to 10 days after
setting treatment with or without 50 mg L\textsuperscript{–1} gibberellic acid (spring 2007). Solid lines represent activities of the fruits treated by 4CPA with GA; broken lines represent activities of the fruit treated by 4CPA without GA. Values represent the mean of 4 or 5 samples ± SE.