Effects of leaf–sprayed salicylic acid on sucrose to starch conversion in underground tuber of yam (Dioscorea alata L.)

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\textbf{Abstract:} [Objective] Effects of leaf–sprayed salicylic acid (SA) on the conversion of sucrose to starch in yam underground tuber was elucidated in order to provide references on knowing the mechanism of sucrose transformation and enhancing starch formation of yam. [Method] Four treatments of SA with 0.0 (i.e. the control), 0.5, 1.0, and 5.0 mmol/L were respectively sprayed thoroughly on leaves of Dioscorea alata L. cv. Baibian both at early and later of tuber formation stages. Underground tuber was periodically sampled to determine the contents of predominant carbohydrates and the activities of enzymes related to sucrose cleavage and starch biosynthesis. [Result] With the exception of the sharp increase of total soluble sugar (TSS) content at later stage for 5.0 mmol/L SA treatment, the contents of TSS, sucrose, glucose, fructose, starch, amylose (AM), amylpectin (AP), and cellulose among the other treatments had the same profiles of general change during the sampled period. Compared with the control, 0.5 and 5.0 mmol/L SA presented opposed effects on underground tuber development, the former not only significantly increased activities of sucrose synthase (SuSase), soluble acid invertase (SAInv), cell wall–bound acid invertase (CWBAInv), and neutral invertase (NInv), but also significantly enhanced activities of adenosine diphosphate pyrophosphorylase (AGPase), soluble starch synthase (SSS), grain–bound starch synthase (GBSS), and starch branching enzyme (SBE). But 1.0 mmol/L SA treatment had slightly positive effects on these measured enzymes only at middle stage. In all treatments, although sucrose content showed insignificantly positive correlations with activities of both SuSase and related starch–synthesized enzymes, the ratio of sucrose to total soluble sugar was significantly and positively correlated with activities of SuSase, SAInv, CWBAInv, NInv, AGPase, SSS, GBSS, SBE and starch content. The contents of glucose, fructose and their ratios in total soluble sugar significantly and negatively correlated to these measured enzyme activities, respectively. Glucose–fructose ratio had insignificant correlation with the aforementioned enzyme activities, but the ratios of sucrose–glucose and sucrose–fructose with activities of AGPase, SSS, and GBSS were significantly positive correlations. [Conclusion] Leaf–sprayed SA influenced both the transport and the fate of sucrose throughout yam underground tuber development through affecting the activities of enzymes related to both sucrose degradation and starch synthesis. SAs at 1.0 mmol/L or less promoted the transport of sucrose into tuber and the transition of sucrose to starch through increase of enzyme activities, and other contents of SAs had converse effects.

\textbf{Keywords:} yam (Dioscorea alata L.); underground tuber development; carbohydrate; enzyme activities; salicylic acid; effect

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叶喷水杨酸对山药(Dioscorea alata L.)地下块茎
蔗糖—淀粉转化影响

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摘要:[目的]阐明叶喷水杨酸(SA)对山药地下块茎蔗糖—淀粉转化的影响, 为了解山药蔗糖转化机制, 提高其淀粉

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0 Introduction

【Research significance】Yam (Dioscorea spp.) possesses plenty of carbohydrates in underground tuber, and so its tuber serves traditionally as food, vegetable, and herbal medicine in the world (Chen et al., 2003; Yang et al., 2003), as well as the promising raw materials of biomass energy in future. The major pathway for carbohydrate formation in such plant non-photosynthetic tissues as underground tuber is sucrose to starch conversion involving in a series of important reactions catalyzed by key enzymes like SuSase (EC 2.4.1.13), invertase (EC 3.2.1.26), AGPase (EC 2.7.7.27), SSS and GBSS (EC 2.4.1.21), and SBE (EC 2.4.1.18) (Fernie et al., 2002; Geigenberger, 2003; Haagensohn et al., 2006). Both carbohydrate contents and aforementioned enzyme activities in higher plants were normally regulated by both endogenous phytohormones and exogenous plant growth regulators (Lopez–Delgado and Scott, 1997). Salicylic acid (SA) is a naturally occurring phenolic compound used as plant growth regulator and plays an important role in the regulation of plant growth, development, ripening, and defense responses (Lopez–Delgado and Scott, 1997; Scott et al., 2004). Therefore, it will be of importance significance to investigate how SA affects the conversion of sucrose to starch of yam tuber to know the mechanism of sucrose transformation and enhancing the starch formation of yam. 【Research progress】SA has few content in plants, but it exerts many and important physiological actions on organisms such as potato (Ervin et al., 2005), garlic (Xian et al., 2007), and Lily (Liu and Wang, 2009). During tuberization, SA can enhance carbon flux from sucrose to starch by its effects on the activities of SuSase, AGPase, SSS, GBSS and SBE in storage organs (Liu et al., 2003; Geigenberger, 2003). The effects of exogenous SA generally vary with SA doses and duration (Ryals et al., 1995). By contrast with SA at other levels, SA at 1.0 mmol/L or less can enhance carbon flux from sucrose to starch by the elevation of the contents of photosynthetic pigments (Rao et al., 1997; Ervin et al., 2005), the protection of photosynthetic apparatus components (Ervin et al., 2005), the promotion of the electron transport in both photosynthesis (Uznova and Popov, 2000) and respiration (Norman et al., 2004), enhancing alternative oxidase activity (Xie and Chen, 1999), increasing antioxidant enzyme activities (Metwally et al., 2003; Ervin et al., 2005) and antioxidant contents (Dat et al., 1998). 【Research breakthrough point】SA may contribute to sucrose synthesis in leaves, and there are some reports on effects of SA on potato underground tuber formation, but it is as yet unclear how the external SA applied to leaves regulates both the bulking and starch accumulation of storage organ such as underground tuber of plant like yam because SA is the signal molecular of non–long–distance transport (Ryals et al., 1995). 【Solving problems】In the present study, 0.0, 0.5, 1.0, and 5.0 mmol/L of SA were designed as foliar application to a tropical Asian species of yam (Dioscorea alata L.) cultivated widely in many countries including China (Chen et al., 2003; Leunufna and Keller, 2003), to elucidate effects of SA on sucrose–to–starch conversion by the analyses of the changes in both major carbohydrate contents and the activities of key enzymes related to the conversion in yam underground tuber.

1 Materials and methods

1.1 Experimental materials

The tested yam (Dioscorea alata L. cv. Baibian) is greenish and cordate leaves, usually single palmate tuber with white interior flesh and brown exterior thin skin for individual plant. All chemical reagents were of analytical reagent grade or above. Major reagents were from Sigma Company.

1.2 Experimental methods

1.2.1 Experimental design Field trials were conducted on the farm of Vegetable Research Institute, Zhejiang University, China (30°16' N, 120°12' E)
from May 4 to November 29 of 2004. After yam seed tuber sets of 100–150 g from healthy and vigorous tubers sprouted and grew to 5–6 fully expanded leaves, the seedlings were transplanted to an open field plot of 2 m x 6 m, in which 6 rows of plants spaced 25 cm and deepened 10 cm were grown according to 100 cm row spacing in west–east direction. At the same time, guard rows of yam around experimental field were also planted against marginal effect on trials. The other cultural practices followed the local standards for yam high yield cultivation.

In this experiment, 0.0, 0.5, 1.0, and 5.0 mmol/L of SA were respectively applied to yam leaves according to the methods of Rao et al. (1997) and Norman et al. (2004). Each of the treatments had three plots as repetitions in a randomized complete block design. After each plot was enclosed with plastic film to prevent interaction of applied SA in plots, each of SA application solution was thoroughly sprayed on the canopy of yam ‘Baibian’ until drips formed in the afternoon of clear and slightly windy day at both early and termination of middle stage of developing tuber (i.e., July 20 and September 23).

1.2.2 Sampling and pretreatment Tuber of individual plant was diagonally sampled from each of 4 middle rows in plot. To maximally reduce the changes of plot environments by the sampling, four underground tubers, in the case of most horizontally extended roots from stolon–tuber corn against harm, were harvested with a shovel from the corn–like stem bases of four uniform- and active–growing yams on the morning (9–12 a.m.) of a fine day. Sampling was made once at intervals of about 30 days besides the one of July 31 during the periods from July 20 to November 26, namely, totally for 6 times at July 20, July 31, August 20, September 23, October 25, and November 26. Sampled tubers were rapidly weighed to calculate the mean fresh weight of individual plant tuber after rinsed soil, sucked dry, and scraped its peel. Then, tubers from the earlier three times sampling were directly chopped into slices and mixed well together on a board at 0–4 °C, whereas those from the latter three times sampling were longitudinally cut into the long slices from the head part to distal end of tuber, of which 500 g was treated the same as the methods of the former times sampling. Half of each composite sample of tubers was weighed immediately before and after freeze–drying to calculate the dry weight, the other, after snap frozen, was ground into a fine powder in liquid N₂ with a mortar and pestle, and stored in air tight plastic bags at −80 °C until assay.

1.2.3 Carbohydrate extraction and analysis The method for carbohydrate extraction of sample was modified from both Gomez et al. (2002) and Chinnasamy and Bal (2003). Exact 1.0000 g of the stored and lyophilized fine powder was placed in a 50 mL centrifuge tube with screw cap, and 20 mL of petroleum ether, 10 mL of methanol, and 10 mL of deionized water were added. After tightly closed, vortexed well, shaken constantly on an oscillator in the dark at 0–4 °C and 200 r/min for 12 h and then centrifuged at 9000 r/min and 0–4 °C for 10 min, pellets were formed at the bottom, the upper liquid phase was petroleum ether and the lower liquid phase was methanol–water. Subsequently, petroleum ether was discarded, methanol–water was carefully transferred into a large beaker with pipette, while the remainder was again extracted several times with half of initial volume for 3 h each as the above–mentioned procedures until no soluble sugar was detected with the anthrone–H₂SO₄ method. The combined methanol–water solution was evaporated to dryness in rotatory evaporator at 50 °C under vacuum, and then completely redissolved in ddH₂O, and transferred into 100 mL volumetric flask and precisely made up to 100 mL with ddH₂O and stored at 0–4 °C for analysis of soluble sugars. The finally–resultant pellet was dried to constant weight at 60 °C after rinsing 2–3 times with ethanol and then ground into fine powder for analysis of starch and cellulose.

Total soluble sugar (TSS), glucose, fructose were determined according to the anthrone–H₂SO₄ method (Chang, 1979), glucose determining Kit (Dahlqvist, 1964), the method of Anderson et al. (1979), respectively. Sucrose was enzymatically hydrolyzed by invertase according to Frei et al. (2003) and Chinnasamy and Bal (2003). These determined values were all expressed as glucose equivalent on basis of dry weight (DW).

Starch was completely hydrolyzed into glucose by amyloligosaccharide based on methods of both Asp et al. (1983) and Frei et al. (2003), the resulting glucose was measured by Dahlqvist (1964) with glucose determining Kit. Cellulose was carried out by both Paul Barratt et al. (2001) and the anthrone–H₂SO₄ method (Chang, 1979). Amylose (AM) was determined by iodine colorimetry based on methods of both Lewis et al. (1994) and Liu et al. (2003) with the mixture of AM and amylopectin (AP) as standard and ddH₂O as blank, and AP was determined by subtracting the content of AM from the amount of total starch. These results were expressed on the basis of DW.

1.2.4 Enzyme extraction and analysis All procedures were carried out at 0–4 °C except specific illustrations. All enzyme analysis were optimized for substrate concentrations and pH and were within the linear phase with respect to incubation time and protein concentration. Because insoluble enzyme activities were not expressed and compared on the basis of soluble protein content, enzyme activities in the present study were expressed on the basis of per g DW.

Crude enzyme extract was prepared according to Jiang et al. (2004). Homogenate was divided into three parts. Of these, 2 mL was pipetted into 2.5 mL
Eppendorf tube and centrifuged at 12000 r/min for 10 min to remove supernatant, while the residual was washed several times with the extraction buffer until no protein was detected in the washing solution and re-suspended in the extraction buffer supplemented with 1.0 mol/L NaCl, vortexed for 5 min, stood for 12 h, centrifuged at 12000 r/min for 10 min, and then the resultant supernatant was assayed for CWBAInv (Ranwala and Miller, 1998). After another part of 2 mL in the 2.5 mL Eppendorf tube was centrifuged (4500 r/min, 20 min) and supernatant was discarded, the sediment was washed several times with the extraction buffer and centrifuged, and finally—remaining pellet was resuspended in the same buffer of 2 mL in ice-cold bath for 5 min and centrifuged at 12000 r/min for 10 min to obtain the supernatant for analysis of GBSS and SBE (Schaffer and Petreikov, 1997; Jiang et al., 2003). After the rest of the homogenate was transferred to 10 mL Eppendorf tube and centrifuged at 12000 r/min for 10 min, the resulting supernatant was analyzed for SuSase, SAInv, NInv, AGPase, SSS (Jiang et al., 2004). Before analysis of these enzyme activities, an aliquot of crude enzyme extract was desalted by a microcentrifuged desalting procedure using Sephadex G–25 column as described by Helmerhorst and Stokes (1980) with 50 mmol/L HEPES–NaOH buffer (pH 7.5). During analysis, the background value of crude enzyme extract was determined by adding the same volume of denatured enzyme extract in boiling water to correct the possible substrate in the crude enzyme extract.

During the assays of SAInv, CWBAInv, NInv, and SuSase in cleavage direction, the reaction mixtures were incubated as described by Ranwala and Miller (1998), and then the concentration of reducing sugars liberated in mixture were determined by the Nelson’s method (Chinnasamy and Bal, 2003). Enzyme activity was defined as the amount of enzyme catalyzing the formation of μg reducing sugar with glucose as standard per min at 30℃ on basis of per g DW. Activities of AGPase, SSS, GBSS, and SBE were determined according to the methods of Nakamura et al. (1989) and Jiang et al. (2003). One unit of AGPase or SBE activity was defined as the amount causing an increase in absorbance of one unit at 540 nm in 1 min on basis of per g DW. For absorbance of SSS or GBSS was determined at 340 nm.

### 1.3 Statistical Analysis of data

All experimental treatments were repeated three times in open field and each sample from each plot was separately analyzed three times. Means and standard errors were calculated from pooled data of three repeated experiments. The results were analyzed for variance and linear regression using SAS 8.2 statistical analysis package (version 6.12; SAS Institute, Cary, NC). Means were compared by Duncan’s multiple comparison test at P=0.05 or 0.01. Linear regression analysis was used to evaluate the relationships among enzymatic activities, among carbohydrate contents,

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**Fig.1** Changes in contents of TSS (A), sucrose (B), glucose (C) and fructose (D) in yam tubers during development after tuber initiation (○, ▲, ● and ■) denoted the applications of 0, 0.5, 1.0, 5.0 mmol/L SA treatment, respectively. Data are the means of three replicates (n=3) with standard error (±SE) shown by vertical bars on the sampled date points, error bars smaller than symbol size are not shown, the same was applied in the following figures.
and between enzymatic activities and carbohydrate contents in the tuber.

2 Results and analysis

2.1 Changes in TSS component contents

Over the sampled ranges, TSS (Fig.1-A) in yam tubers of all treatments were mainly composed of sucrose (Fig.1-B), glucose (Fig.1-C), fructose (Fig.1-D), accounting for 39.0%-81.0%, 4.0%-24.0%, 7.0%-31.0% of TSS, respectively, of which sucrose content in every treatment was the highest at the same periods and glucose the lowest. Not only the contents of both glucose and fructose in the same treatment had the similar change trends throughout the periods of tuber expansion, but also all treatments presented the similar fluctuating trend in contents of each of TSS, sucrose, glucose, and fructose before October 25. Of these, the overall changes in concentrations of TSS, glucose, fructose declined with the advance of tuber development during the periods from the first application of SA at July 20 to the second at September 23, while sucrose content declined sharply to the lowest at September 23 after initially increasing for a time, and followed by more or less steady increases to October 25 for these soluble sugars. Thereafter, these soluble sugar contents of 5.0 mmoll/L SA drastically increased until the harvesting time unlike those of the other treatments with smaller fluctuations.

Compared with the other treatments at every sampled time, 0.5 mmoll/L SA presented the highest in TSS, fructose and sucrose contents, and had only an insignificant difference in glucose content of the control at August 20. Although the contents of both sucrose at every phase and TSS at the other phases excluding November 26 were less for 1.0 mmoll/L SA than those of 0.0 mmoll/L, while the contents of both glucose and fructose of 0 mmoll/L SA treatment were higher at other times except July 31 and August 20. The 5.0 mmoll/L SA was superior in TSS and sucrose contents at August 20 and November 26, and glucose and fructose contents at October 25 and November 26 to the control. These results might revealed that 0.5 mmoll/L SA remarkably contributed to the synthesis, transformation, and conversion of photo-assimilating sucrose in yam in contrast to 1.0 mmoll/L SA with indistinct effect, and 5.0 mmoll/L SA with severely negative action.

2.2 Changes in the contents of starch and cellulose

In all the treatments, starch (Fig.2-A) and cellulose (Fig.2-B) accounted respectively for 43.3%-62.4% and 3.0%-12.2% of tuber dry weight over the sampling period, starch increased from start to finish, and the latter straightly declined for the other treatments except 5.0 mmoll/L SA with larger increase on November 26. AM (Fig.2-C) with 12.2%-50.2% of starch always increased in the range of 52-310 mg/gDW, while AP (Fig.2-D) with 49.9%-87.8% of starch generally decreased between 275 and 406 mg/gDW with the exception of slight increase at both earlier-

![Fig.2 Changes in contents of starch (A), cellulose (B), amylose (AM, C), and amylpectin (AP, D) in yam tubers during development after tuber initiation (∗, △, ○ and ■ was 0, 0.5, 1.0, 5.0 mmoll/L SA treatment, respectively)](image-url)
and later-stage of tuber formation. The AM–AP ratio ranging from 0.14 to 1.0 all along increased in treatments of 0.5 and 1.0 mmol/L SA. At the same time, all SA treatments had lower in AM content and higher in cellulose content than the control within 10 days after the first application of SA.

SA at 0.5 mmol/L showed significantly higher in AM content than the control, while 1.0 mmol/L SA insignificantly. Cellulose content of all SA treatments was higher than the control at both earlier and later stages in contrast to middle stage, and there was significant difference in cellulose contents between 5.0 mmol/L SA and the control at the harvesting.

As seen from the results of Fig.2–A, 0.5 mmol/L SA presented the highest in starch content which was significantly higher than the control during the sampled periods, and followed by 1.0 mmol/L SA at other sampled times except July 31, while insignificant differences in starch content between 5.0 mmol/L and the control were observed. Although having insignificant differences in AP content at every stage, all SA treatment presented significantly higher in AP content than the control before October 25. The AP content of 1.0 and 5.0 mmol/L SA treatments were lower than that of the control which was nearly equal to that of 0.5 mmol/L SA treatments (Fig.2–D).

2.3 Changes in sucrose–cleaved enzyme activities

In underground tuber of all treatments, the activity of SuSase in the cleavage direction (Fig.3–A) increased straightly from the initial stage and reached the highest at October 25, and then decreased to harvesting time, while the activities of CWBAInv (Fig.3–B), NlInv (Fig.3–C), and SAInv (Fig.3–D) all increased to the peak at September 23 or October 25 after rapidly decreasing at the initiation of early stage, then followed by slowly decreasing to harvesting time.

During the tuber expanding stage, SAInv and SuSase were respectively 1.5– to 4.5–times higher in total enzyme activities than CWBAInv and NlInv, and showed the highest activities in 0.5 mmol/L SA treatment at every stage. SAInv and SuSase were significantly higher in enzyme activities for 0.5 mmol/L SA than that of the other treatments at middle and later stages.

Meanwhile, SAInv activity in 1.0 mmol/L SA treatment were always higher than that of the control, while SuSase activity in 1.0 mmol/L SA treatment being over that of the control at September 23 and November 26 was always higher than that of 5.0 mmol/L SA treatment. Additionally, in contrast to 5.0 mmol/L SA, 0.5 mmol/L SA was higher in CWBAInv activity than the control all along, and 1.0 mmol/L SA presented higher CWBAInv activity than the control except September 23. Furthermore, both 0.5 and 1.0 mmol/L SA treatments presented higher NlInv activity than that of the control only at August 20 and September 23, while 5.0 mmol/L SA treatment presented lower NlInv activity than the control at all stages.

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**Fig.3** Changes in activities of such sucrose-cleaved enzymes as SuSase (A), CWBAInv (B), NlInv (C) and SAInv in cleavage direction (D) in yam tubers during development after tuber initiation (◇, ▲, ● and ■ was 0.5, 1.0, 5.0 mmol/L SA)}
except August 20 and November 26.

2.4 Changes in starch–synthesized enzymes activities

The activities of AGPase (Fig.4–A), SSS (Fig.4–B), and GBSS (Fig.4–C) for all treatments in underground tuber at sampled periods, being in the ranges of 3.0–16.6, 0.32–0.89, 0.19–1.1 U/gDW·min, respectively, increased from the lowest at the beginning to the highest at middle stage and then drastically decreased to harvesting. Meantime, the activity ratio of GBSS to SSS generally increased from <1 to >1, which paralleled with the changes in content ratio of amylase to amyllopectin (0.14–1.0). Nevertheless, SBE activity of all treatments presented two peaks at both July 31 and September 23 and a lower value at August 20, while SBE activity with 46.8–119.6 U/gDW·min reduced slowly from September 23 till harvesting, but slightly increased for 0.5 and 1.0 mmol/L SA treatments after October 25.

By comparison with the control, 0.5 mmol/L SA treatment presented higher in the activities of AGPase and SSS during the periods of pre–harvesting, and that of GBSS, SBE were higher than the control in the sampled ranges. But as to 5.0 mmol/L SA treatments, the activities of AGPase and GBSS at every stage, SSS during the periods after July 31, and SBE at September 23 and October 25 were lower than that of the control. For 1.0 mmol/L SA treatment, it showed the larger fluctuation in these enzyme activities and was higher in the activities of AGPase, SSS, GBSS and lower in SBE activity than the control at both September 23 and October 25. These results indicated that, as opposed to 5.0 mmol/L SA, 0.5 mmol/L SA treatment markedly enhanced the activities of starch–synthesized enzymes as a whole.

2.5 Correlations of both carbohydrate contents and their ratios with enzyme activities

During yam underground tuber expending of all treatments, correlations of carbohydrate contents and their ratios with the activities of enzymes involved in sucrose to starch conversion were analyzed (Tab.1). The results showed that invertase activities were positively correlated with the contents of TSS, glucose, and fructose, of which Nnv activity had a significant or very significant correlation coefficients ($R^2$=0.462, 0.783, 0.742, respectively, $P<0.05$ or 0.01). Nevertheless, the activities of SuSase, AGPase, SSS, GBSS, and SBE were negatively correlated to the contents of TSS, glucose, and fructose, and only the correlation of TSS content with SBE activity was insignificant ($R^2$=−0.386). Although sucrose content was negatively correlated to invertase activity and had significantly negative correlation with Nnv activity ($R^2$=−0.514, $P<0.05$), it was insignificantly and positively correlated with the activities of SuSase, AGPase, SSS, GBSS, SBE. With the exception of insignificant correlations of the activities of SAInv and CWBAInv with the ratios of Suc/TSS, Suc/Glc, Suc/Frc, Glc/TSS, and Frc/TSS, SuSase activity was significantly or very significantly and positively correlated with the ratios of Suc/TSS, Suc/Glc, Suc/Frc ($R^2$=0.718, 0.515, 0.597) and markedly significantly and negatively correlated with the ratios of Glc/TSS, Frc/
Tab. 1 Relationships of both carbohydrate contents and their ratios with enzymes activities involved in sucrose to starch metabolism during yam undergound tuber

<table>
<thead>
<tr>
<th>Item</th>
<th>SAInv</th>
<th>CWBAInv</th>
<th>Nniv</th>
<th>SuSase</th>
<th>AGPase</th>
<th>SSS</th>
<th>GBSS</th>
<th>SBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>0.067</td>
<td>0.194</td>
<td>0.462*</td>
<td>-0.608**</td>
<td>-0.655**</td>
<td>-0.596**</td>
<td>-0.582**</td>
<td>-0.386</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-0.224</td>
<td>-0.020</td>
<td>-0.514*</td>
<td>0.272</td>
<td>0.310</td>
<td>0.091</td>
<td>0.281</td>
<td>0.159</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.123</td>
<td>0.244</td>
<td>0.785**</td>
<td>-0.725**</td>
<td>-0.809**</td>
<td>-0.630**</td>
<td>-0.701**</td>
<td>-0.490*</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.104</td>
<td>0.257</td>
<td>0.742**</td>
<td>-0.742**</td>
<td>-0.826**</td>
<td>-0.646**</td>
<td>-0.703**</td>
<td>-0.479*</td>
</tr>
<tr>
<td>Sucrose/TSS</td>
<td>-0.138</td>
<td>-0.148</td>
<td>-0.789**</td>
<td>0.718**</td>
<td>0.802**</td>
<td>0.596**</td>
<td>0.726**</td>
<td>0.416*</td>
</tr>
<tr>
<td>Sucrose/Glucose</td>
<td>-0.053</td>
<td>0.083</td>
<td>-0.592**</td>
<td>0.515*</td>
<td>0.567**</td>
<td>0.432**</td>
<td>0.573**</td>
<td>0.206</td>
</tr>
<tr>
<td>Sucrose/Fructose</td>
<td>-0.045</td>
<td>0.015</td>
<td>-0.549**</td>
<td>0.597**</td>
<td>0.657**</td>
<td>0.522**</td>
<td>0.619**</td>
<td>0.198</td>
</tr>
<tr>
<td>Glucose/TSS</td>
<td>0.184</td>
<td>0.195</td>
<td>0.818**</td>
<td>-0.685**</td>
<td>-0.777**</td>
<td>-0.579**</td>
<td>-0.685**</td>
<td>-0.446*</td>
</tr>
<tr>
<td>Fructose/TSS</td>
<td>0.133</td>
<td>0.217</td>
<td>0.751**</td>
<td>-0.736**</td>
<td>-0.823**</td>
<td>-0.623**</td>
<td>-0.701**</td>
<td>-0.442*</td>
</tr>
<tr>
<td>Glucose/Fructose</td>
<td>0.200</td>
<td>-0.107</td>
<td>0.297</td>
<td>0.233</td>
<td>0.218</td>
<td>0.220</td>
<td>0.082</td>
<td>-0.034</td>
</tr>
<tr>
<td>Starch</td>
<td>0.089</td>
<td>0.208</td>
<td>-0.621**</td>
<td>0.725**</td>
<td>0.714**</td>
<td>0.517**</td>
<td>0.824**</td>
<td>0.220</td>
</tr>
<tr>
<td>AM</td>
<td>0.133</td>
<td>0.317</td>
<td>-0.505*</td>
<td>0.761**</td>
<td>0.750**</td>
<td>0.586**</td>
<td>0.905**</td>
<td>0.040</td>
</tr>
<tr>
<td>AP</td>
<td>-0.174</td>
<td>-0.432*</td>
<td>0.267</td>
<td>-0.705**</td>
<td>-0.693**</td>
<td>-0.594**</td>
<td>-0.891**</td>
<td>0.219</td>
</tr>
</tbody>
</table>

The superscripts *, ** of R² values represented statistical insignificance, and significance at 0.05 and 0.01 levels, respectively

TSS (R²= -0.685, -0.736). At the same time, the activities of AGPase, SSS, GBSS, and SBE were positively correlated with the ratios of Suc/TSS, Suc/Glcsuc/Fructose, and significantly or markedly significantly and negatively correlated with the ratios of Glc/TSS and Fruc/TSS, of which SBE activity was insignificant correlation with the ratios of both Suc/Glc and Suc/Fructose, while there were insignificant correlation of Glc/Fructose ratio to the activities of enzymes. Although being highly significantly and negatively correlated to Nniv activity (R²= -0.621), starch content had not only positive correlation with the activities of SAInv, CWBAInv, and SBE, but also highly significantly and positively correlated with the activities of SuSase, AGPase, SSS, GBSS (R²=0.725, 0.714, 0.464, 0.815). In addition, the contents of AM and AP were correspondingly significantly positive and negative correlations with the activities of AGPase, SSS, and GBSS, while SBE activity was insignificantly and positively correlated with the contents of starch, AM, and AP.

3 Discussion

Sucrose as one of main assimilates is the major carbon flux from source tissues to sink in yam similar to that of the most plants (Koch, 1996). Sucrose concentration difference between source leaves and developmental sinks is the major determinant of the rate, amount, routes, and direction of sucrose translocation (N’tchobo et al., 1999), and is generally determined by both the capacity of sucrose synthesis in leaves and the ability of sucrose cleavage by SuSase and invertase for both symplastic and apoplastic unloading of sucrose in sink tissues (Fernie et al., 2002; Yang et al., 2004). In the present study, invertase activities at the initiation of tuberization (i.e. the period of stolon-to-tuber transition) were higher than that of SuSase in tuber of all treatments, while during the periods of plentiful starch accumulation of tuber, SuSase activity sharply increased and dominated in the case of invertase activities with more or less increases. The result indicated that sucrose was transferred into yam tuber via a switch from main apoplastic to predominant symplastic phloem unloading, and was consistent with that of potato (Fernie et al., 2002). SA as a phytohormone-like simple phenol, is often used as plant growth regulator, which both involves in response to morphological and physiological levels (Xian et al., 2007; Liu and Wang, 2009; Kang et al., 2012). Compared with the control, on the one hand, 0.5 mmol/L SA treatment not only had higher activities of SuSase, SAInv, and CWBAInv in tuber at every stage, but also had higher activity of Nniv at middle stage, showing that higher sucrose-cleaved enzyme activities in tubers of 0.5 mmol/L SA treatment promoted sucrose cleavage and increased sucrose concentration gradient from the source leaves to the tubers as well as led to the higher ability to attract sucrose into tubers. On the other hand, the higher content of sucrose also existed in tuber of 0.5 mmol/L SA throughout the development period, which, in conjunction with correlation analysis among the activities of SAInv, CWBAInv, Nniv, and SuSase with contents of TSS and sucrose, illustrated that there was a plentiful of sucrose synthesized in leaves and transported into tubers. Consequently, leaf-sprayed 0.5 mmol/L SA could facilitate sucrose into tubers through sucrose concentration gradient formed between leaves and tubers in terms of the levels of both sucrose synthesis in leaves and its degradation in tubers. SA at 5.0 mmol/L treatment showed generally lower sucrose-cleaved enzyme activities and sucrose content in tubers than the control, but 1.0 mmol/L SA had higher in overall sucrose-cleaved enzyme activities in tubers than the control, and lower in sucrose content during development stage. The result was mainly in the comparatively relative smaller amount of sucrose synthesized in leaves of 1.0 mmol/L SA treatment. The aforementioned results supported the notion that smaller than 1.0 mmol/L SA treatment is beneficial to protect and improve photosynthetic organs, and vice versa (Rao et al., 1997; Uzunova and Popov, 2000; Ervin et al., 2005). Therefore, in combination with the assays of soluble sugar content in
both tubers and leaves as well as stolons, SA controlled sucrose synthesis through affecting firstly and directly the assembly of components of photosynthetic apparatus, and then modulated the transport of sucrose to tuber.

SA is a non-long-distance signal molecular (Rylas et al., 1995), and so the regulation of sucrose to starch conversion by leaf-sprayed SA was carried out via effects of other endogenous factors. In present study, leaf-sprayed SA directly affected the synthesis of sucrose in leaves and subsequently indirectly modulated the metabolisms including sucrose–starch conversion in tuber.

Up till now, although it was not known completely how assimilates synthesized in leaves after leaf–sprayed SA influenced tuber metabolisms, results in this study showed that there were significant differences in both sucrose and its cleavage products such as glucose and fructose among the treatments in the sampled ranges, and 0.5 mmol/L SA treatment always had the highest sucrose content in tubers at every stage. Previous studies showed that the increased contents of hexoses enhanced the metabolic activities like respiration and the increased sucrose content promoted starch accumulation (Hajirezaei et al., 2003), suggesting that the two kinds of soluble sugars could serve as signal molecular to regulate plant growth and development (Ho et al., 2001; Rook et al., 2001; Hajirezaei et al., 2003; Hendriks et al., 2003). Then we supposed both sucrose and hexoses in tuber might also be endogenous factors regulating sucrose to starch conversion in response to leaf–sprayed SA. Meantime, the correlation analysis results indicated that, under conditions of the final fact that sucrose content was strongly and significantly positive correlation with starch content. Sucrose content was insignificantly correlated with the activities of SuSase and related starch–synthesized enzymes, but Suc/TSS ratio had at least significant and positive correlation coefficients with SuSase and related starch–synthesized enzymes, and also the ratios of both Suc/Glc and Suc/Frc significantly and positively correlated with SuSase and related starch–synthesized enzymes but SBE. On the contrary, both the contents of Glc and Frc, and the ratios of Glc/TSS and Frc/TSS significantly and negatively correlated with SuSase and related starch–synthesized enzymes, and Glc/Frc ratios insignificantly correlated with SuSase and related starch–synthesized enzymes. In combination with sucrose firstly delivered into tubers, the aforesaid results revealed that sucrose was the initial and endogenous signal molecular, and leaf–sprayed SA regulated sucrose to starch conversion through sucrose in yam underground tuber, and played a role via the ratios of sucrose to soluble sugar as endogenous signal.

4 Conclusion

Leaf–sprayed SA regulated the transportation of sucrose throughout yam underground tuber development through affecting the activities of enzymes related to both sucrose degradation and starch synthesis. SA at 1.0 mmol/L or less promoted the transportation of sucrose into tuber and the transition of sucrose to starch through increase of enzyme activities, and other concentrations of SA had converse effects. Leaf–sprayed SA affected the formation of yam underground tuber by regulating the partition of sucrose as photosynthetic assimilates product in yam leaves into yam underground tubers. Sucrose acts as endogenous signal molecule in the conversion of sucrose–starch and the ratio of sucrose in total soluble sugar as internal signaling.

Reference:
Gomez L, Rubio E, Auge M. 2002. A new procedure for extraction and measurement of soluble sugars in ligneous
Starch synthesis in tomato remains constant throughout fruit development and is dependent on sucrose supply and sucrose synthase activity[1]. Journal of Experimental Botany, 50(338): 1457–1463.


