Carbohydrate Metabolism Changes in *Prunus persica* Gummosis Infected with *Lasiodiplodia theobromae*

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ABSTRACT

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Peach gummosis represents a significant global disease of stone fruit trees and a major disease in the south peach production area of the Yangtze River of China. In this study, the carbohydrate composition of peach shoots during infection by *Lasiodiplodia theobromae* was examined. The expression of genes related to metabolic enzymes was also investigated. Control wounded and noninoculated tissue, lesion tissue, and wounded and inoculated surrounding lesion tissue of peach shoots were analyzed. Soluble sugars, glucose, mannose, arabinose, and xylose significantly increased in inoculated tissues of peach shoots compared

with control tissues at different times after inoculation. Accumulation of polysaccharides was also observed by section observation and periodic acid Schiff's reagent staining during infection. Analysis using quantitative reverse-transcription polymerase chain reaction revealed that the abundance of key transcripts on the synthesis pathway of uridine diphosphate (UDP)-D-glucuronate, UDP-D-galactose, and UDP-D-arabinose increased but the synthesis of L-galactose and guanosine diphosphate-L-galactose were inhibited. After inoculation, the transcript levels of sugar transport-related genes (namely, *SUT*, *SOT*, *GMT*, and *UGT*) was induced. These changes in sugar content and gene expression were directly associated with peach gum polysaccharide formation and may be responsible for the symptoms of peach gummosis.

Peach gummosis is one of the most important and damaging diseases of peach (*Prunus persica* L. Batsch) in China, Japan, and the United States (16,28). Peach tree fungal gummosis was first observed in Georgia in 1970 (41). The three species of the genus *Botryosphaeria* (*Lasiodiplodia theobromae*, *Botryosphaeria dothidea*, and *Diplodia seriata*) were the predominant causes of the disease (6,39). All three species infect through pruning wounds and lenticels (6,27,42). Populations of *L. theobromae* increase in summer (7). *L. theobromae* discharges ascospores and conidia from diseased peach bark and dead prunings during or soon after rainfall (27). The common symptoms of fungal gummosis are sunken necrotic lesions, which are characteristically swollen and crusty in appearance, and gum exudates from cracked warts on the trunk and major branches (4). Gum exudates are secreted from diseased peach trunks, branches, or fruit; in more serious cases, fungal gummosis results in reduced vigor, small yellow leaves, earlier defoliation, twig die back, and decreased production (16).

The characteristics of peach gum exudate polysaccharide have been studied. Gum polysaccharides have complex components, including many monosaccharides and glycosidic linkages (33). Peach gum polysaccharides are composed of arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal), and uronic acids at different molar ratios based on a comparison analysis of peach and nectarine (*P. persica* var. *nucipersica* Schneid.) (34,36). The relationship between plant polysaccharides and disease has been

recently studied. The polysaccharide called *Ulva*, isolated from green algae, induces defense responses against powdery mildew in cucumber, common bean, and grapevine. *Ulva* also enhances resistance to powdery mildew in wheat and barley plants by triggering oxidative burst (13,26). In addition, Sun et al. (37) analyzed the polysaccharide composition of intervessel pit membranes and showed that the resistance of grapevine to Pierce's disease depended on the lack of some kinds of polysaccharide. Therefore, polysaccharide formation is closely associated with carbohydrate metabolism and transport.

Carbohydrate components such as soluble sugars, starch, and sucrose display significant variation between inoculated plant tissues and healthy control tissues (21,27,30). These studies suggest that, after pathogen infection, glycometabolism changes to a great extent in plant tissues associated with plant disease. However, the monosaccharide metabolism and the formation mechanisms of peach gum polysaccharides in infected tissues have not been extensively studied. The sucrose transporter (*SUT*) and sorbitol transporter (*SOT*) are localized in the phloem of plants and can be responsible for phloem loading (40,43). The transporters of guanosine diphosphate (GDP)-Man and uridine diphosphate (UDP)-Gal are located in the Golgi apparatus, where polysaccharides are formed. These transporters play a critical role in providing substrates for glycosyltransferases and in the synthesis of glycans (10,22).

In this study, current-year peach shoots were inoculated with *L. theobromae* and the content of several soluble carbohydrates was determined from lesion, tissue surrounding lesions, and healthy tissue during the development of peach gummosis. The relative expression of genes related to sugar metabolism was analyzed. This study aimed to gain a better understanding of peach gummosis and provide a basis to control this disease. Hence, the major challenge was to unravel the changes in carbohydrate metabolism and peach gum polysaccharide formation triggered by *L. theobromae* in peach shoots.

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MATERIALS AND METHODS

Plant and pathogen material. Peach plants (*P. persica* L. 'Springsnow') were grown in an experimental field at Huazhong Agricultural University in Wuhan. For all inoculation experiments, current-year shoots were collected from 3-year-old plants of *P. persica* Springsnow from June to August 2011 and 2012. The plants were grafted on wild peach rootstock. *L. theobromae* strain JMB-122 was originally isolated from a diseased peach tree in Hubei province, China and maintained in an incubator with a photoperiod of 12 h of light and 12 h of darkness at 26°C (39).

Inoculation with *L. theobromae* and sample collection. A detached inoculation assay was conducted according to the methods of Britton and Hendrix (6). Strain JMB-122 was cultured on potato dextrose agar (PDA) medium for 3 days prior to inoculation. Current-year shoots were surface sterilized with 70% alcohol for 10 s, rinsed three times with sterile water, and cut into 15-cm-long segments. The shoot segments were wounded with a sterilized needle, and an actively growing isolate of strain JMB-122 on a mycelial agar plug (4 mm in diameter) was placed over the wound. The current-year peach shoots were wounded and inoculated with sterile PDA as controls under the same conditions. All treated shoots were placed in glass bottles containing 100 ml of sterile water, covered with a clear plastic film to maintain humidity, and placed in an incubator. The water in the bottles was changed daily. Unless indicated otherwise, the standard growth conditions were a photoperiod of 12 h of light (20,000 lux) and 12 h of darkness at 26°C and relative humidity of 90%.

Samples were collected as described by Dorey et al. (8). All samples were collected at the same time point. Control wounded and noninoculated tissues, lesion tissues, and wounded and inoculated surrounding lesion tissues were collected at 0 and 12 h and 1, 2, 3, 4, 5, and 6 days postinoculation (dpi). Control and inoculated tissues were collected within a 0.5- to 1.0-cm distance surrounding the wound and lesion, frozen in liquid nitrogen, and stored at -80°C for subsequent use. At each time point, three independent replicates were collected from infected shoots and control shoots, and each replicate consisted of 15 current-year peach shoots for the analysis of carbohydrates and quantitative real-time polymerase chain reaction (qRT-PCR).

Determination of carbohydrates. Sucrose, glucose, fructose, sorbitol, Man, Ara, and Xyl were quantified using a gas chromatography (GC) system (Agilent 6890N, Santa Clara, CA), equipped with an auto-sampler (Agilent 7683), flame ionization detector (FID), HP-5 5% phenyl-methyl polysiloxane column (30 m by 0.25 μ m i.d.), and a data processor (Applied Biosystems, Santa Clara, CA). The flow rates of N₂, H₂, and air to the FID were 25, 30, and 40 ml/min, respectively. The GC conditions were set according to Bartolozzi et al. (3), with slight modifications. The injector and detector temperatures were 270 and 300°C, respectively. The following temperature program was set: from 130 to 152°C at 8°C/min, 152 to 176°C at 12°C/min, 176 to 198°C at 16°C/min, 198 to 238°C at 20°C/min, 239 to 300°C at 24°C/min, and finally 300°C for 4 min. Each compound was quantified using the internal standard method (3).

For the soluble sugar assay, the content was determined following the anthrone method of Spiro (36).

Periodic acid Schiff's reagent staining. To identify polysaccharides, the samples were cut into 5-by-5-mm pieces. The tangential sections of the peach shoots were cut from control and inoculated tissues (within and near the lesion) at different positions, respectively. Periodic acid Schiff's reagent was used to visualize the polysaccharides of the sectioned materials (1). Sections were incubated in 1% (wt/vol) periodic acid (HIO₄) for 30 min, rinsed in running water for 5 min, then immersed in periodic acid Schiff's reagent for 30 min in the dark. After staining, the slides were washed three times for 3 min with 2%

(wt/vol) sodium metabisulfite solution and in running water for 5 min. The slides were dried, observed, and photographed using a microscope.

RNA isolation and qRT-PCR. Total RNA from different samples was extracted from 0.5 g of frozen tissue of peach current-year shoots using an EASYspin Plus RNA kit (Aidlab, Beijing). DNase I (TaKaRa, Dalian, China) was added to remove genomic DNA. RNA yield and purity were analyzed with NANODROP 2000 (Thermo, Waltham, USA), whereas RNA integrity was detected by electrophoresis in 1.5% agarose gel. First-strand cDNA was synthesized with total RNA using oligo (dT) primers following the manufacturer's instructions on a PrimeScript RT reagent kit (TaKaRa).

Relative expression was determined by performing qRT-PCR in a LightCycler 480 Sequence Detection System (Roche, Basel, Switzerland) using the intercalation dye SYBR Green as a fluorescent reporter. Reactions were carried out in a final volume of 15 μ l containing 30 ng of cDNA, 10 μ mol of each primer, and 7.5 μ l of 2 $\times$ SYBR Green PCR master mixes (TaKaRa) according to the manufacturer's instructions. PCR primers were designed based on peach cDNA sequences published in the GenBank database and described in Supplemental Table 1. The thermal cycling conditions were 10 min of denaturation at 95°C followed by 40 cycles of amplification (95°C for 15 s, 60°C for 15 s, and 72°C for 1 min). Relative expression levels were analyzed with LightCycler 480 Sequence Detection System software and normalized with the results of *actin* as a reference. The qRT-PCR results are shown as the mean values of three independent biological replicates $\pm$ standard deviation.

Statistical analysis. The one-way analysis of variance (ANOVA) of SAS program package (version 8.1; SAS Institute, Cary, NC) was used for statistical analysis between inoculated peach shoots and control shoots. Statistically significant differences are reported in the text and shown in the figures.

Accession numbers. Sequence data for the genes in this study can be found in the EMBL/GenBank data libraries under the following accession numbers: L-arabinose kinase (*ARA*) (KC345021), fructokinase (*FRK*) (KC339524), D-galactokinase (*AGK*) (KC345020), GDP-Man transporter (*GMT*) (KC339528), *PGI* (KC345022), phosphomannomutase (*PMM*) (KC339527), *SUT* (KC345023), UDP-D-glucose dehydrogenase (*UGD*) (KC339532), UDP-glucose 4-epimerase (*UGE*) (KC339533), *UGP* (KC339525), UDP-Gal transporter (*UGT*) (KC339529), UDP-D-Xyl 4-epimerase (*UXE*) (KC339531), and UDP-D-Xyl synthase (*UXS*) (KC339530).

RESULTS

Symptoms. Gum exudates from diseased trunk and branches are a typical symptom of peach gummosis (Fig. 1A and B). The most serious symptom observed was that the trunk and most branches were covered by brown gum exudates (Fig. 1C). The colony morphology of *L. theobromae* strain JMB-122 was cultured on PDA medium for 3 days (Fig. 1D). Under laboratory conditions, a similar symptom was observed in current-year shoot phenotypes at 3 dpi with field symptoms (Fig. 1F), and 129.8 mg of gum exudates was obtained, on average, for every lesion (Supplemental Figure 1). No symptom was observed in control shoots (Fig. 1E). Interestingly, numerous gum exudates were observed from the tangential section surrounding the inoculation point of peach current-year shoots (Fig. 1H), and no symptom was observed in the control shoots (Fig. 1G).

Determination of carbohydrate during the disease. Soluble sugar sucrose, glucose, fructose, sorbitol, Man, Xyl, and Ara production was measured during the infection process of *L. theobromae* in peach current-year shoots and healthy shoots (Figs. 2 and 3). In comparison with the control tissue, the production of sucrose, glucose, and fructose from the lesion decreased after

inoculation with *L. theobromae* (Fig. 2A). In tissue surrounding lesion, the soluble sugar level significantly increased at 4 and 5 dpi, except in the gum exudates (Fig. 2A). In addition to the increase in soluble sugar content, glucose significantly increased at 1 and 2 dpi, reaching the highest value at 1 dpi compared with the control shoots (Fig. 2C). The sucrose and fructose contents of the tissue surrounding the lesion were significantly lower than those of the controls, although the sucrose content increased at 2 dpi (Fig. 2B and D).

The other sugar distribution patterns (sorbitol, Man, Xyl, and Ara) differed between the controls and inoculated samples throughout the process (Fig. 3A to D, respectively). The sorbitol content of the inoculated surrounding tissues was significantly lower during infection (Fig. 3A). The Man content was higher in the inoculated samples, with significant differences at all inoculation time points (Fig. 3B). Differences in Xyl content between inoculated and control shoots were concentrated at 1, 3, and 4 dpi, with significant increments at 3 and 4 dpi compared with the control shoots (Fig. 3C). The Ara levels were higher in inoculated shoots than uninoculated shoots, with significant differences at 4 and 5 dpi (Fig. 3D).

qRT-PCR of metabolic genes involved in sugar metabolism during the infection process of peach shoots. A simplified metabolic scheme was established, and the expression of genes involved in sugar metabolism in peach shoots was analyzed by qRT-PCR (Fig. 4).

Sucrose, glucose, fructose, and sorbitol metabolism. Neutral invertase (NI) and acid invertase (AI) are involved in sucrose degradation. The relative expression of putative NI and AI was significantly higher in inoculated peach shoots than control shoots at different times after inoculation but their expression patterns were opposite (i.e., NI increased and AI decreased gradually). Regarding the enzymes involved in sucrose synthesis, the expression of sucrose synthase (SS) was significantly higher than

that in the control shoots. Sucrose phosphate synthase (PSS) expression only significantly increased at 1 dpi, and then no significant decrease was observed in inoculated shoots compared with the control shoots (Supplemental Figure 2A).

The transcript levels of NAD-dependent sorbitol dehydrogenase (*SDH*) significantly increased throughout the course of infection, which was responsible for the degradation of sorbitol. Nevertheless, the expression abundance of sorbitol-6-phosphate dehydrogenase (*S6PDH*) was inhibited during the infection process compared with the controls. Together with the contents of sucrose and sorbitol, they may be used as fuel during infection.

Man and Gal metabolism. D-Man can be synthesized as a free monosaccharide through the fructose pathway (31). Three genes (*FRK*, phosphoglucose isomerase [*PGI*], and *PMM*) involved in the metabolism of Man were assayed in the present research. A significant increase in the expression of *FRK* was detected at 1 dpi compared with the control shoots. However, the expression level of *PGI* appeared to be higher at 5 dpi. The transcript levels of *PMM* also significantly increased at 1 and 5 dpi in inoculated shoots compared with the control shoots. Therefore, the changes in transcript levels had similar patterns with the changes in Man contents.

D-Man serves as the substrate for sugar interconversion enzymes producing GDP-L-Gal or L-Gal. The expression abundance of genes (such as GDP-D-mannose pyrophosphorylase [*GMP*], GDP-D-Man 3,5-epimerase [*GME*], L-galactose-1-phosphate phosphatase (*GPP*), and GDP-L-Gal guanylyltransferase [*GGGT*]) involved in the L-Gal pathway were inhibited during the infection of peach shoots (Fig. 4). However, compared with the control shoots, the transcript abundance of *UGE* and *AGK* involved in UGP-D-Gal significantly increased in inoculated shoots.

Ara and Xyl metabolism. UDP-D-glucuronate is a major branch-point in the biosynthesis of UDP sugars, including UDP-D-Gal,

Fig. 1. Typical symptoms of peach gummosis on peach tree. Typical symptoms of peach gummosis on **A**, 2-year-old shoots and **B**, trunk and **C**, 7-year-old tree; **D**, colony morphology of *Lasiodiplodia theobromae* strain JMB-122 (3 days, potato dextrose agar [PDA]); peach current-year shoot inoculated with **E**, PDA and **F**, mycelium plug of *L. theobromae* at 3 days postinoculation; **G**, tangential section of peach current-year shoot inoculation with PDA plug after 3 days; **H**, tangential section of peach current-year shoot inoculation with *L. theobromae* after 3 days. Scale bars: D = 1 cm and E to H = 5 mm.

Fig. 2. Production of **A**, total soluble sugars; **B**, sucrose; **C**, glucose; and **D**, fructose (milligram per gram fresh weight [FW]) in peach shoots from healthy control tissue (W), lesion tissue (W+Lt), and lesion surrounding tissue (W+LtA). Values represent the mean of three independent determinations $\pm$ standard deviation at different times after inoculation. Asterisks indicate a significant difference (*, **, and *** indicate $P < 0.05$, 0.01 , and 0.001 , respectively).

Fig. 3. Levels of **A**, total sorbitol (milligram per gram fresh weight [FW]); **B**, D-mannose; **C**, D-xylose; and **D**, L-arabinose (microgram per gram FW) in peach shoots from healthy control tissue (W) and lesion surrounding tissue (W+LtA) at different times after inoculation. Values represent the mean of three independent determinations $\pm$ standard deviation at different times after inoculation. Asterisks indicate a significant difference (*, **, and *** indicate $P < 0.05$, 0.01 , and 0.001 , respectively).

Fig. 4. Simplified metabolic scheme and expression analysis of transcripts of genes involved in sugar metabolism in peach shoots analyzed by quantitative real-time polymerase chain reaction from control tissue (W) and tissue surrounding lesion (W+LtA) an inoculated with *Lasiodiplodia theobromae* at 1, 3, and 5 days postinoculation (dpi). The relative expression of transcripts of metabolism genes that are increased during infection are indicated in bold with a gray background, whereas the transcripts of metabolism genes that decreased are indicated in normal font with a white background compared with control tissue. The mRNA levels of the genes were normalized to the transcript of actin in the same sample (fold change 1 = time 0 dpi). Means of the results obtained using three independent RNAs as templates are shown. Standard deviations are shown. Asterisks indicate a significant difference (*, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively).

UDP-Ara, and UDP-Xyl (29). In this biosynthesis pathway, the transcript level of *UGD* increased by ≈ 2.6 -fold in inoculated shoots at 1 dpi compared with the control shoots. The relative expression of *UXS* increased with significant differences throughout the infection course.

UDP-L-Ara is synthesized from UDP-D-Xyl by reversible *UXE*. Accordingly, the relative expression of *UXE* increased to a greater extent in inoculated shoots than in the control shoots at 1 and 3 dpi. On the other hand, the expression level of *ARA* increased throughout the infection course, with 2.5-, 3.3-, and 4.1-fold changes at 1, 3, and 5 dpi, respectively, compared with the control shoots.

Accumulation and transport of sugars. The current-year shoots infected with *L. theobromae* began to produce gum at 3 dpi under laboratory conditions. Interestingly, excluding the gum exudates, a significant increase in the soluble sugar level was observed at 4 and 5 dpi in inoculated shoots compared with uninoculated shoots (Fig. 2A). We observed that the tangential section of inoculated shoots contained more gum from the parenchyma cells of phloem than the control shoots (Fig. 1D and E). In addition, the parenchyma cells of the phloem showed more prunosus in inoculated shoots than the control shoots through periodic acid Schiff (PAS) staining. This finding indicated that more polysaccharides accumulated in the parenchyma cell of peach phloem in inoculated shoots (Fig. 5). These data suggested that the pronounced increase in soluble sugars and parts of monosaccharide levels were associated with polysaccharide formation during the infection of *L. theobromae* in the peach current-year shoots.

Four genes involved in sugar transport were analyzed in peach shoots during the disease process by qRT-PCR (Fig. 6). The levels of *SUT* increased by nearly 1.4- and 1.8-fold at 1 and 5 dpi, respectively, whereas no significant difference was observed at 3 dpi (Fig. 6A). Almost 1.5- and 2.6-fold increases in the transcript level of *SOT* were found at 3 and 5 dpi, respectively, compared with the control shoots (Fig. 6B). The transcript level of *GMT* significantly increased 3.3-, 1.3-, and 1.7-fold at 1, 3, and 5 dpi, respectively, in inoculated peach shoots compared with the controls (Fig. 6C). The transcript levels of *UGT* only significantly increased 1.5-fold at 3 dpi (Fig. 6D).

DISCUSSION

Peach gummosis caused by *Botryosphaeria* spp. infection results in very distinctive gum exudates in peach trunks and shoots. In this work, we confirmed that inoculated detached peach shoots had the same symptoms as peach shoots under field conditions (Fig. 1). After infection with *Botryosphaeria* spp., necrosis and death of infected tissues were triggered by unknown factors and processes (39). Previous reports have suggested that programmed cell death is induced as a restriction for pathogen infection (17). The surrounding cells of the hypersensitive response lesion were stimulated at the same time, leading to metabolic changes, decreased mRNA levels, and defensive signals. Different zones far from the lesion displayed different spatial and temporal expression of defense responses (8). Thus, the peach shoot lesion and the tissue surrounding the lesion were separately collected to evaluate the sugar distribution patterns.

Partial changes in sugar metabolites were analyzed during the infection process of *L. theobromae* (Fig. 2). Although the sucrose, glucose, and fructose levels in lesions and surrounding tissues decreased, the amounts of soluble sugars increased after 3 dpi. These changes can be associated with the disintegration of dead cells and the degradation of cell wall polysaccharides (32). Nevertheless, sorbitol, Man, Ara, and Xyl in lesions were not detected, possibly because their amounts were very low in the samples (Fig. 3).

In the tissues surrounding the lesion, the concentrations of soluble sugars, Man, Xyl, and Ara significantly increased from 3 to 5 dpi, whereas the glucose amounts only exhibited a notable increase at 1 and 2 dpi. The high content of soluble sugars, glucose, and sucrose in cocoa have been observed after infection with *Crinipellis perniciosus* (30). Our results support previous ones that the soluble sugar content of inoculated tissues significantly increases in comparison with noninoculated tissues. Gum exudates were first observed in the current-year shoots at 3 dpi (Fig. 1). The Man and Ara contents were at a lower level in inoculated tissues at 3 dpi compared with the control tissues. By contrast, the concentrations of soluble sugars and sucrose showed a lower level at 2 dpi. This finding can be attributed to the fact that sucrose cleavage is vital to multicellular plants for the

Fig. 5. Tangential section of peach current-year shoots stained with periodic acid Schiff (PAS) staining for polysaccharides. Black arrow indicates that the parenchyma cells of peach phloem are stained prunosus; it is very positive to this staining in inoculated peach shoots. Empty arrow indicates the fungal hyphae of *Lasiodiplodia theobromae*. W = control wounded and noninfected tissue and W+Lt = wounded and infected tissue. Scale bars = 30 μ m.

allocation of crucial carbon resources and for the initiation of hexose-based sugar signals (15). The pathways for the synthesis of Xyl and Ara are catalyzed by sucrose synthase, which converts UDP and sucrose into UDP-D-glucose and fructose, whereas the pathway for the synthesis of Man involves the conversion pathway of fructose (29). The relative expression levels of *SS* and *UGP* showed a similar result and significantly increased after inoculation (Fig. 4). In summary, the increased amount of several monosaccharides (Ara, Man, and Xyl) in inoculated peach shoots was consistent with the composition of gum exudate polysaccharides of peach trees.

Our results showed that the synthesis of L-Gal and GDP-L-Gal was inhibited after inoculation with *L. theobromae*. However, the relative expression levels of critical genes involved in the synthesis of UDP-D-Gal notably increased (Fig. 4). This phenomenon can be explained by the fact that UDP-D-Gal was ultimately needed in the Golgi apparatus for the synthesis of cell wall polysaccharides and chloroplast galactolipids (14). However, GDP-L-Gal has been confirmed to contribute to the pathway to L-ascorbate biosynthesis (12). Alternatively, GDP-L-Gal may not be uniformly distributed throughout the Golgi stacks with other monosaccharides (29). Thus, the high molecular weight polysaccharides of the cell wall played a role in the outcome of host-pathogen interactions (38). Therefore, UDP-D-Gal was more important for peach gum polysaccharides than GDP-L-Gal.

The expression of four genes involved in sugar transport was analyzed, and results confirmed that sugar transport was enhanced after shoots were inoculated with *L. theobromae* (Fig. 6). Moreover, the accumulation of polysaccharides in inoculated tissues as observed by section observation and PAS staining supported this conclusion (Figs. 1E and 5). These data suggested that the changes in and pathways of glycometabolism were regulated in peach shoots during infection with *L. theobromae* (Supplemental Figure 3). The accumulation of monosaccharides may be due to the change in and transformation of the glycometabolism pathway. Polysaccharides were synthesized by certain types of mono-

saccharides in the Golgi apparatus and transported by the endomembrane system.

Sugars can contribute to immune responses and function as priming molecules (20). A phenomenon termed “high-sugar resistance” involves high levels of sugars for enhancing defense against pathogens and decreasing susceptibility (11). Previous reports have shown that pretreatment of rice plants with sucrose enhances resistance to *Magnaporthe oryzae* infection (9). Some polysaccharides also induce a defense response in tomato plants for wilt and enhanced resistance in wheat against powdery mildew (19,23). Therefore, biodegradable sugar-like extracts may be used to counteract peach gummosis from high-sugar-content plants (such as sugarcane and beet) and some polysaccharides (such as peach gum), which may be cheaper and safer than chemical pesticides. Most importantly, peach trees avoid waste of sugars against pathogen infection.

In summary, various polysaccharide elicitors were characterized from pathogens and host plants (25). Some polysaccharides can act as signal molecules to elicit defensive responses and protect plants against phytopathogenic fungi (2,24). Furthermore, sugars such as sucrose, glucose, and fructose are involved in many metabolic and signal pathways that interact with hormonal pathways. They also contribute to immune responses against pathogens (5,20). Gum exudates mainly comprise polysaccharides containing different monosaccharides at different molar ratios (33,35). Plant gum exudates appeared to act as protective agents in response to microbial invasion (18). However, this phenomenon cannot be sufficient to explain why, in peach gummosis, a large amount of gum exudates is continually produced by peach trees without a regulation mechanism. The changes in carbohydrate content and expression of genes related to carbohydrates may indicate the formation of peach gum polysaccharide in response to *L. theobromae* infection.

The relationship between peach gum polysaccharide and glycometabolism is important. Therefore, to confirm polysaccharide formation and gain further insight into peach-*L. theobromae*

Fig. 6. Relative expression analysis of transcripts of sugars transporter in peach shoots infected with *Lasiodiplodia theobromae* and healthy control shoots with quantitative real-time polymerase chain reaction. **A**, SUT = sucrose transporter; **B**, SOT = Sorbitol transporter; **C**, GMT = GDP-mannose transporter; and **D**, UGT = UDP-galactose transporter. mRNA levels of the genes were normalized to the transcript of actin in the same sample (fold change 1 = time 0 days postinoculation). Relative values are the means of three biological replicates $\pm$ standard deviation. Asterisks indicate a significant difference (*, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively).

interaction, additional biochemical studies on enzyme activity involved in glycometabolism and detailed analysis on histopathology are required. The pathogenicity factors and fungal metabolism of *L. theobromae* are also critical in understanding polysaccharide formation during this process.

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