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Functional characterization of an invertase inhibitor gene involved in sucrose metabolism in tomato fruit*

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Abstract: In this study, we produced tomato plants overexpressing an invertase inhibitor gene (*Sly-INH*) from tomato, using a simple and efficient transient transformation system. Compared with control plants, the expression of *Sly-INH* was highly upregulated in *Sly-INH* overexpressing plants, as indicated by real-time polymerase chain reaction (PCR). Physiological analysis revealed that *Sly-INH* inhibited the activity of cell wall invertase (CWIN), which increased sugar accumulation in tomato fruit. Furthermore, *Sly-INH* mediated sucrose metabolism by regulating CWIN activity. Our results suggest that invertase activity is potentially regulated by the *Sly-INH* inhibitor at the post-translational level, and they demonstrate that the transient transformation system is an effective method for determining the functions of genes in tomato.

Key words: Invertase inhibitor, Fruit development, Transient transformation system, *Solanum lycopersicum*, Overexpression

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1 Introduction

Sucrose is an important transported sugar in higher plants, which is exported from the source tissues (leaves) via the phloem to various sink tissues (roots, stem, and reproductive organs). Cell wall invertase (CWIN) is an important enzyme in determining the apoplastic sucrose:glucose ratio and regulating related signaling pathways (Hanson and Smeekens, 2009). Importantly, CWINs maintain sucrose concentration gradients, which are considered to be a driving force for sugar transport, partitioning, and storage between the photosynthetic source and sink tissues (Sturm, 1999; Chourey *et al*., 2006). The activity of CWIN is strictly controlled at both the transcriptional and post-transcriptional levels (Huang *et al*., 2007). As a result of glycan decoration, the regulation of apoplasmic and vacuolar invertases may be highly dependent on post-translational mechanisms (Greiner *et al*., 2000; Rausch and Greiner, 2004; Tauzin *et al*., 2014). Recent studies have suggested that invertase activity may be subject to post-translational suppression by its inhibitory protein (Hothorn *et al*., 2004; Rausch and Greiner, 2004).

Biochemical characterization of plant invertase inhibitors was first performed in the 1960s (Schwimmer *et al*., 1961; Pressey, 1966), and the genes encoding these inhibitors were cloned three decades later (Greiner *et al*., 1998). These inhibitors have been identified in a variety of species, such as *Arabidopsis*, potato, tomato, and tobacco (Weil *et al*., 1994; Greiner *et al*., 1999; Bate *et al*., 2004; Link *et al*., 2004; Reca *et al*., 2008; Jin *et al*., 2009; Kusch *et al*., 2009; Brummell *et al*., 2011). The specificity of plant invertase inhibitors was identified *in vitro*

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through the use of recombinant proteins and *in vivo* using transgenic plants (Bate *et al*., 2004; Rausch and Greiner, 2004; Brummell *et al*., 2011). In tomato, silencing the expression of *INH* led to a 40% to 65% increase in apoplastic invertase activity in mature leaves, and the modified levels of invertase activity were specifically targeted to the apoplast (Jin *et al*., 2009). Moreover, modifying invertase activity via specific inhibitors can potentially govern the senescence process in plants to help attain maximum yields and desirable crop quality. However, the realization of this potential will require a more thorough understanding of the involvement of invertase inhibitors in the regulation of growth and development in other plant tissues.

Carbohydrate content and composition are important indicators of tomato fruit quality. Carbohydrates, which represent a major component of soluble solids, greatly contribute to tomato processing quality (Davies and Hobson, 1981; Baxter *et al*., 2005). The tomato is an ideal species for the study of metabolism related to soluble carbohydrate accumulation because of the natural genetic variation in tomato and the well-developed genetic and physiological information about *Solanum lycopersicum* and related species. Fruit quality has increasingly been linked to the activity of invertase inhibitors. The formation of the invertase inhibitor complex may be an important mechanism in the control of invertase activity *in vivo*, subsequently affecting carbon partitioning and fruit development (Fridman *et al*., 2004; Hothorn *et al*., 2010). Therefore, altering the activity of invertase inhibitors may serve as a strategy to increase the sucrose content and quality of fruit.

The biological functionality of invertase inhibitors has largely been identified through *in vitro* assays using recombinant proteins (Greiner *et al*., 1998; Bate *et al*., 2004). In recent years, significant progress has been made in identifying and elucidating the involvement of invertase inhibitors in development and plant responses to various stimuli through the use of transgenic plants. Although some studies have examined the effects of silencing invertase inhibitor genes in various fruit tissues, few studies have examined the role(s) of these inhibitors through overexpressing invertase genes *in vivo* (McLaughlin and Boyer, 2004; Jin *et al*., 2009). Overexpressing endogenous invertase inhibitors in fruits will provide useful information about these proteins, which play important roles in the complex metabolic networks of plants, especially with regard to governing carbon allocation and fruit development. In this study, we selected tomato fruit as a model plant that undergoes fleshy fruit development. To study the role of invertase inhibitors in fruits, an invertase inhibitor complementary DNA (cDNA) from tomato, *Sly-INH*, was introduced into tomato via *Agrobacterium*-mediated transient expression of a construct containing an expression carrier inhibitor. The results help to elucidate the effect of *Sly-INH* expression and invertase activity on tomato fruit development.

2 Materials and methods

2.1 Plant materials

Tomato seeds (Micro-Tom) were surface sterilized with 0.5% (5 g/L) sodium hypochlorite, rinsed with water, and germinated for 3 d in the dark at 25 °C. Uniformly germinated seedlings were transferred to seedling trays containing growth medium comprising 1 part peat:1 part perlite:1 part vermiculite (v/v/v) and grown in a greenhouse under a 12-h day-night period with temperatures of 25 °C during the day and 15 °C at night and with an irradiance of 300 μmol photons/(m^2·s). The relative humidity varied, averaging 80%.

2.2 Plasmid construction and plant transformation

A fruit-specific overexpression construct for *Sly-INH* was produced by cloning the respective full-length cDNA into pCAMBIA1300 downstream of the 2A11 promoter. Full-length *Sly-INH* cDNA was cloned by polymerase chain reaction (PCR) using the following primers: forward 5'-ATGAAAATTTT GATTTTCCC-3' and reverse 5'-TTACAATAAATT TCTTACAA-3'.

Agroinjection was performed as described by Orzaez *et al*. (2006). Tomato fruits (*S. lycopersicum* cv. Micro-Tom) at 22 days after flowering (DAF) were injected with a maximum of 600 μl agroinjection solution into mature green tomatoes. When some drops of infiltration solution appeared in the hydathodes at the tips of sepals, the fruits were determined to be fully infiltrated. Completely infiltrated fruits were used in the experiments.

2.3 Determination of soluble sugar and starch levels

The contents of fruit discs (0.5 cm^2) were extracted by incubating the discs in 1.0 ml 80% ethanol at 80 °C for 60 min and centrifuged at 4 °C for 5 min at 14000 r/min. After they were transferred to new tubes, the cleared supernatants were evaporated to dryness at 40 °C. The dry residue was resolved in 250 μl of ultrapure water and used for soluble sugar analysis. Starch analysis was performed using the pellets derived from the centrifugation step. The materials were homogenized in 0.2 mol/L potassium hydroxide and incubated at 95 °C for 1 h. Then, the pH was adjusted to 5.5 with 1 mol/L acetic acid. Starch and soluble sugar assays were performed as described by Hajirezaei *et al*. (2000).

2.4 Enzyme extraction and activity assays

Fruit material samples (100 mg) were homogenized in 50 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (10 mmol/L magnesium chloride, 2.5 mmol/L dithiothreitol, 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 10 mmol/L ascorbic acid, and 5% polyvinylpyrrolidone, pH 7.5). The homogenates were centrifuged for 20 min at 12000 r/min at 4 °C. An aliquot of the resulting supernatant was desalted through Sephadex G-25 (medium), equilibrated and used for the various assays. Neutral invertase (NI), sucrose synthase (SS), and sucrose phosphate synthase (SPS) activities were determined as described by Zrenner *et al*. (1995). CWIN and cytoplasmic invertase (CIN) activities were enzymatically assayed according to Gibon *et al.* (2004) and Tomlinson *et al*. (2004).

2.5 RNA extraction and quantification

Total RNA (1 g) was extracted from specified fruit tissues using a TIANGEN RNAprep pure plant kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. First-strand cDNA synthesis of 1 μg of RNA in a final volume of 20 μl was performed using Moloney Murine Leukemia Virus Reverse Transcriptase, Point Mutant RNase H Minus (Promega, Beijing, China) following the manufacturer's protocol using oligo$(dT)_{18}$ primer. The expressions of *LIN5*, *LIN6*, *LIN7*, *LIN8*, *TIV1*, and *INH* were examined by real-time quantitative reverse transcription PCR (qRT-PCR) using the fluorescent intercalating dye SYBR Green in an iCycler detection system (Bio-Rad; http://www.bio-rad.com) following the protocol of Schaarschmidt *et al*. (2006). The comparative C_T method was performed for relative quantification of the target gene expression levels. The next primers used were as follows: for analysis of *LIN5* transcript levels (GenBank accession No. AJ272304), forward 5'-AAAGGGATCTCAGCATCACAGG-3', reverse 5'-CGTCTTGGGCATATAGGTCAGC-3'; for *LIN6* (GenBank accession No. AF506006), forward 5'-ATCAAGCCCGATAACAATCCA-3', reverse 5'-CCTCACACTCCCAACCAATACTC-3'; for *LIN7* (GenBank accession No. AF506006), forward 5'-TTTGGTGCTGGTGGAAAGACA-3', reverse 5'-GGCTCCGTTCCGTTGTTAAAC-3'; for *LIN8* (GenBank accession No. AF506007), forward 5'-AAGGATGGGCGGGAATACA-3', reverse 5'-GGCCTGTGCTGGTGTGATT-3'; for *TIV1* (GenBank accession No. AF506007), forward 5'-AGGACTTTAGAGACCCGACTAC-3', reverse 5'-GCAGCACTCCATCCAATAGC-3'; for *Sly-INH* (GenBank accession No. AJ010943), forward 5'-GTATGCCAGAAGCATTAGAAGCA-3', reverse 5'-GCATCACCAGAAGAACCAACC-3'. To normalize gene expression levels (to eliminate differences in the efficiency of cDNA synthesis), the transcript levels of the constitutively expressed *Actin* gene in tomato (XM_004249818) were measured using the following primers: forward 5'-TGTCCCTATTTACGAGGGTTATGC-3'; reverse 5'-AGTTAAATCACGACCAGCAAGAT-3'.

2.6 Statistical analysis

All agroinjection experiments were arranged in a completely randomized split-plot design with three replicates of 30 tomato plants each. Each extract was measured twice, and at least three replications were performed per analysis. For enzyme activity and sugar content assays, each biological replicate was composed of three technical replicates. Each experiment was repeated at least three times, and similar results were obtained. One representative set of data is described here from those that were carried out, and significant differences between the mean values among treatments were determined according to Tukey's Least Significant Difference test ($P<0.05$) using analysis of variance (ANOVA; SPSS 11.0, Chicago, IL, USA). The standard errors (SEs) of the means were also calculated and are presented in the graphs as error bars.

3 Results

3.1 Agroinjection of tomato fruit tissues

To assess the physiological role of *Sly-INH* in tomato fruit, we produced a *Sly-INH* overexpression construct harboring this gene driven by the fruit-specific 2A11 promoter. We introduced this plasmid into tomato via *Agrobacterium tumefaciens*-mediated fruit injection, as described by Orzaez *et al*. (2006), and measured the levels of *Sly-INH* transcript by qRT-PCR (Fig. 1).

As shown in Fig. 1, *Sly-INH* exhibited a tissue-specific expression pattern in p1300-2A11-*INH*-agroinjected tomato fruits, with maximum expression observed in the pericarp, followed by the pectinic placenta and dissepiments, perhaps because these structures form a diffusion barrier in the apoplastic network of the fruit. These results indicate that *Sly-INH* can be effectively expressed in fruit tissues via agroinjection. Notably, the relative expression of *Sly-INH* in sepals was significantly different in tomato fruit agroinjected with EHA105 containing p1300-2A11-*INH* plasmid (OE) plants versus the controls, and a significant reduction in *INH* expression was observed after 5 d, most likely because the infiltration solution in the hydathodes at the tips of sepals elicited *Sly-INH* gene silencing. Further studies are needed to determine whether the expression of *Sly-INH* is regulated via different routes.

3.2 Activity of sugar metabolism-related enzymes

After agroinjection of tomato fruits with EHA105 bacterial solution, CWIN activity in each part of the fruit was altered. The activity of this enzyme was slightly higher than that of uninjected fruit, while CIN, NI, SS, and SPS activities were not significantly altered (Figs. 2 and 3). These results may be because *Agrobacterium* infection triggers a defense response in the plant, and CWIN activity is related to stress. By contrast, CWIN activity was observably reduced in plants transformed with p1300-2A11-*Sly-INH* compared with the control. A remarkable inhibition of CWIN activity was observed in *Sly-INH* OE tomato fruits, especially in sepals and dissepiments, at 3 d after injection, with CWIN activity decreasing by 58.8% and 30.4%, respectively. From 0 to 5 d after injection, CWIN activity decreased by 75.0% and 81.8% in sepals and dissepiments, respectively. In *Sly-INH* OE tomato fruits at 5 d after injection, CIN activity sharply increased in sepals, while CWIN activity decreased. It is worth noting that accurately comparing CWIN and tonoplast invertase (TIN) or CIN activity is difficult since CWIN activity is expressed based on cell-wall proteins whereas CIN and TIN activities are expressed on a soluble protein basis.

Analysis of the activities of other enzymes related to carbohydrate metabolism revealed an increase in the total activity of SS (when assayed in the

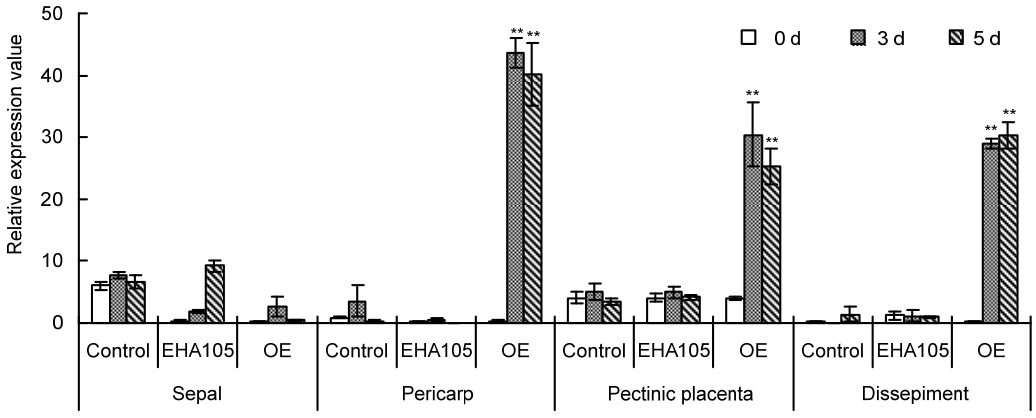

Fig. 1 Relative expression levels of *Sly-INH* in the sepal, pericarp, pectinic placenta, and dissepiment of Micro-Tom tomato fruits without agroinjection treatment (control), agroinjected with EHA105, or agroinjected with EHA105 containing p1300-2A11-*INH* plasmid (OE) at 0, 3, and 5 d, respectively

Data are expressed as mean±SE (n=3). ** P<0.01, compared with the control

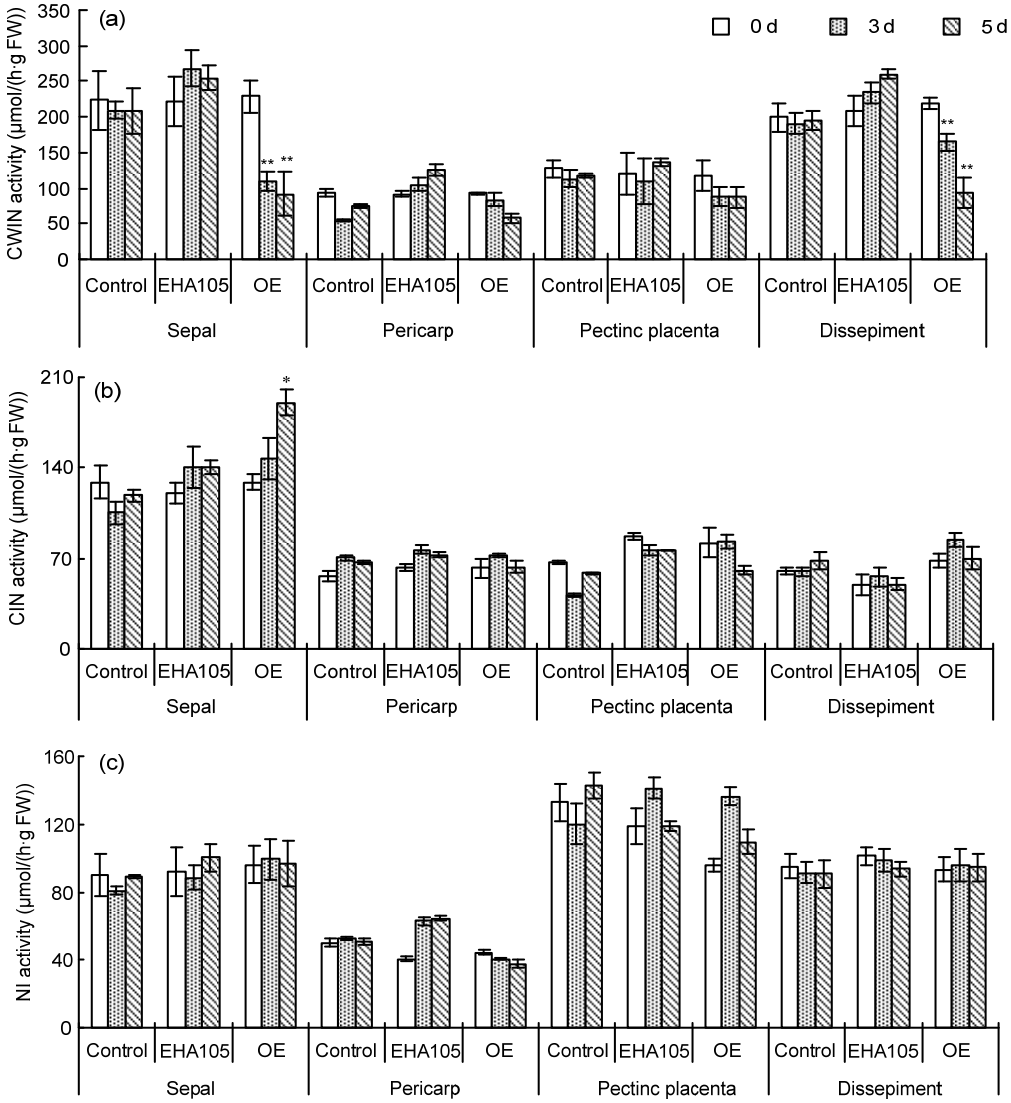

Fig. 2 Activities of invertase in the sepal, pericarp, pectinic placenta, and dissepiment of Micro-Tom tomato fruits without agroinjection treatment (control), agroinjected with EHA105, or agroinjected with EHA105 containing p1300-2A11-*INH* plasmid (OE) at 0, 3, and 5 d, respectively

(a) Cell wall invertase (CWIN) activity; (b) Cytoplasmic invertase (CIN) activity; (c) Neutral invertase (NI) activity. Data are expressed as mean±SE (n=3). * P<0.05, ** P<0.01, compared with the control

direction of sucrose cleavage) and a decrease in the activity of SPS in the young fruits of plants most strongly overexpressing *Sly-INH* in the sepals (Fig. 3). This result suggests that injecting bacteria harboring a *Sly-INH* overexpression vector can reduce the activity of CWIN (Fig. 2). In *Agrobacterium*-mediated injected fruit, the expression of target genes can quickly be detected. A significant increase in the expression of the target gene in tomato fruit was detected at 3 d after injection.

3.3 Relative expression levels and inhibition of sucrose invertase family genes

To demonstrate that the reduction in CWIN expression resulted from a specific reduction in invertase expression, we examined the relative expression levels of CWIN family genes in fruit (Fig. 4). The tomato *CWIN* gene family includes *Lin5*, *Lin6*, *Lin7*, and *Lin8*. In pectinic placenta, the expression level of *Lin5* was high, while there was no significant

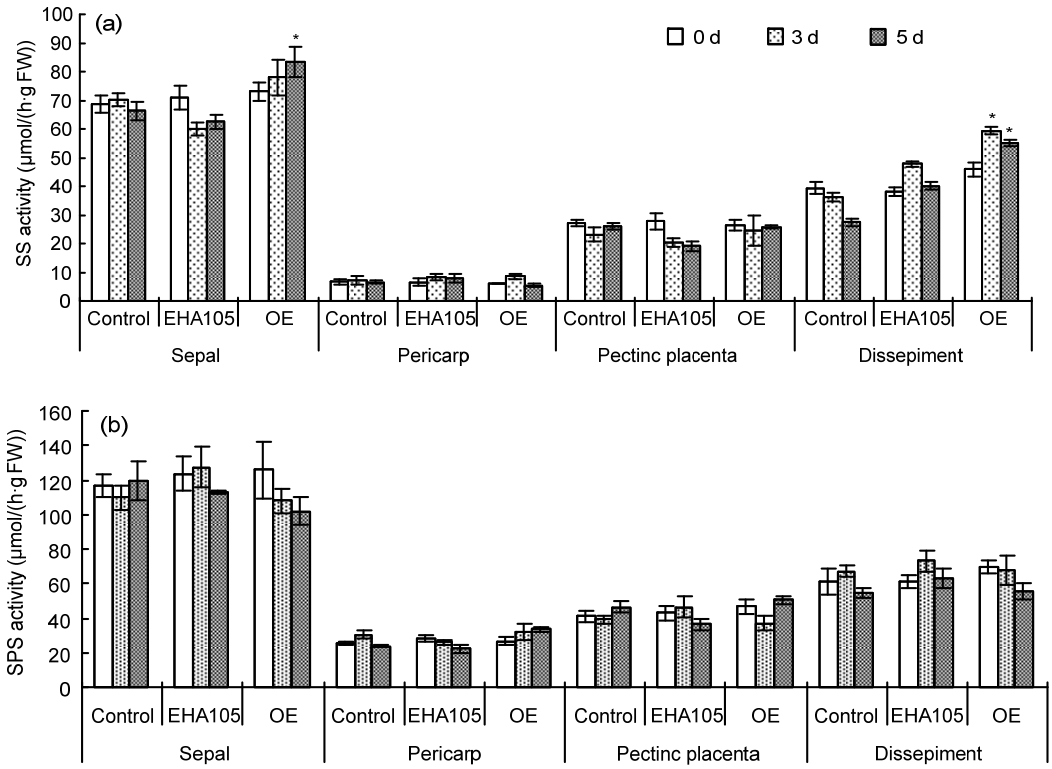

Fig. 3 Activities of sucrose synthase (SS) and sucrose phosphate synthase (SPS) in the sepal, pericarp, pectinic placenta, and dissepiment of Micro-Tom tomato fruits without agroinjection treatment (control), agroinjected with EHA105, or agroinjected with EHA105 containing p1300-2A11-*INH* plasmid (OE) at 0, 3, and 5 d, respectively

(a) SS activity; (b) SPS activity. Data are expressed as mean±SE (n=3). * P<0.05, compared with the control

expression in other parts of the fruit. *Lin6* was mainly expressed in sepals, while *Lin7* and *Lin8* were not significantly expressed in any part of the tomato fruit examined. At 3 and 5 d after injection with bacteria harboring the *Sly-INH* overexpression vector, the expression of *CWIN* genes was not obviously affected. Interestingly, previous qRT-PCR analysis revealed no changes in the expression of *Lin7* or the closest homolog *Lin5* in transgenic tomato fruits subjected to agroinjection (Godt and Roitsch, 1997; Fridman and Zamir, 2003). Moreover, the expression of vacuolar invertase gene *TIV1* in the *Sly-INH* overexpression lines was not obviously altered compared with the control (Fig. 5).

3.4 Carbohydrate composition and content

To investigate the consequences of reduced fruit CWIN activity on carbohydrate metabolism, we measured the levels of soluble sugars and starch in fruits in the three treatment groups. Tomato fruits primarily contain glucose, fructose, and sucrose, which largely affect the flavor and quality of the fruit. Starch is an important storage material in plants, as starch that accumulates during early fruit development is stored and serves as a carbon source for subsequent sugar synthesis. Developing fruits represent strong sinks for sugars during fruit development. After agroinjection of tomato fruits, reduced CWIN activity caused an approximately 50% decrease in starch contents compared with the control fruits, especially in sepals, pectinic placentas, and dissepiment (Fig. 6). While the amounts of glucose and fructose decreased slightly in injected fruits, sucrose levels increased approximately two-fold in injected fruits compared with controls. By contrast, there were no significant differences in carbohydrate contents between control fruits and fruits agroinjected with EHA105 (Fig. 6).

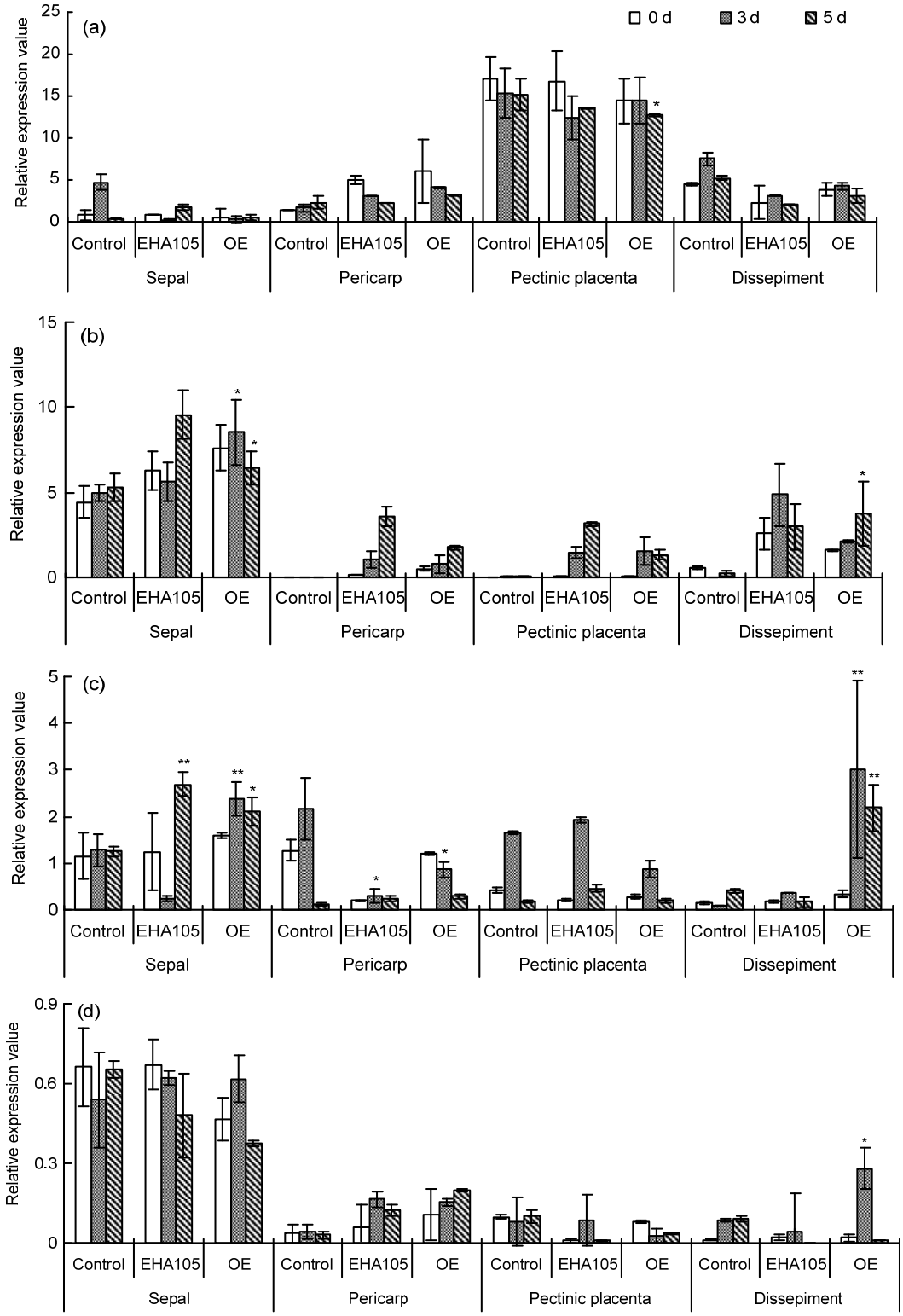

Fig. 4 Relative expression levels of cell wall invertase (*CWIN*) gene families in the sepal, pericarp, pectinic placenta, and dissepiment of Micro-Tom tomato fruits without agroinjection treatment (control), agroinjected with EHA105, or agroinjected with EHA105 containing p1300-2A11-*INH* plasmid (OE) at 0, 3, and 5 d, respectively

(a) Relative expression value of *LIN5*; (b) Relative expression value of *LIN6*; (c) Relative expression value of *LIN7*; (d) Relative expression value of *LIN8*. Data are expressed as mean±SE (n=3). * P<0.05, ** P<0.01, compared with the control

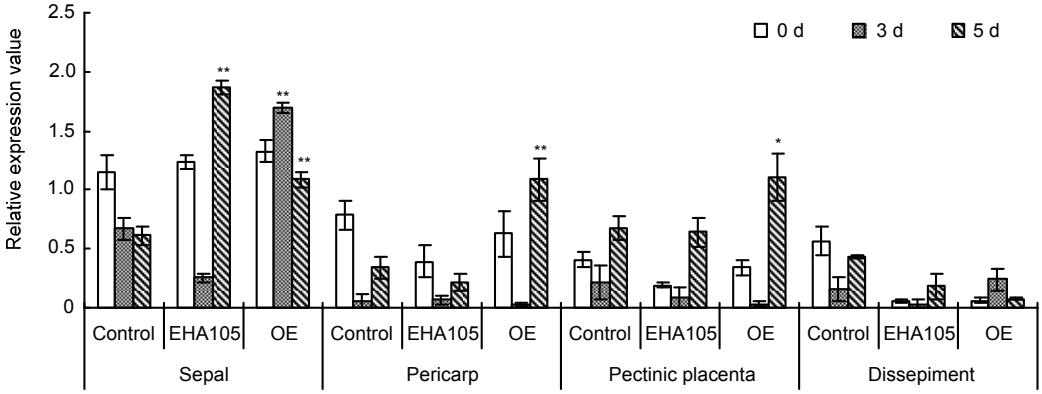

Fig. 5 Relative expression levels of vacuolar invertase *TIV1* in the sepal, pericarp, pectinic placenta, and dissepiment of Micro-Tom tomato fruits without agroinjection treatment (control), agroinjected with EHA105, or agroinjected with EHA105 containing p1300-2A11-*INH* plasmid (OE) at 0, 3, and 5 d, respectively

Data are expressed as mean±SE (n=3). * P<0.05, ** P<0.01, compared with the control

4 Discussion

A. tumefaciens-mediated transient transformation is a simple, quick method that avoids the tedious process of tissue culture (Janssen and Gardner, 1990; Kapila *et al*., 1997). Most transferred DNA (T-DNA) that enters plant cells is not integrated into the plant genome but is instead temporarily stored in the nucleus, with a high copy number. Position effects and gene silencing have no effect on transient gene expression (Kapila *et al*., l997; Wroblewski *et al*., 2005). In addition, traditional transformation systems require the use of antibiotics or other marker genes for initial screening; even so, false-positive plants are often selected, complicating analysis of test results. The agroinjection transformation system does not require the use of marker genes, avoiding the safety factor of antibiotics and transgenes and enabling the use of smaller vectors to transform larger fragments or more genes. Although agroinjection has many advantages, this method has certain limitations that should be considered when designing experiments. For example, its application is limited by the requirement to use only *A. tumefaciens* and similar species. Moreover, overexpression may affect the function and orientation of the gene product, and the stability is poor. In the current study, overexpression of *Sly-INH* has an inhibitory effect on apoplasmic invertase activity in tomato. The results suggest that at 3 to 5 d after *Agrobacterium* infection, high levels of expression of the transgenic product could be detected. Ripe fruits of commercial tomato variants are a good source of glucose and fructose (at equimolar concentrations), but they contain little sucrose (Klee and Giovannoni, 2011) owing to the effects of cell wall-bound and vacuolar invertases on fruit sink strength (Yelle *et al*., 1991; Klann *et al*., 1996; Jin *et al*., 2009; Zanor *et al*., 2009). In the current study, in Micro-Tom tomato injected with blank bacterial solution (EHA105), invertase activity in all parts of the fruit increased slightly, perhaps because the defense response triggered by *Agrobacterium* infection increased CWIN activity. This result is consistent with previous findings (Roitsch *et al*., 2003). After pathogen injection, the hexose content rapidly increases in plants, as hexose functions in the host defense response against pathogens (Herbers *et al*., 1996; Biemelt and Sonnewald, 2006). Scharte *et al*. (2005) found that during the early defense process in tobacco leaves injected with tobacco mosaic virus, CWIN is rapidly induced. The induction of CWIN is assumed to favor sink development, enabling the pathogen to withdraw carbohydrates for its nutrition. In addition, the levels of hexoses formed via CWIN activity increase to support defense responses. In the current study, the reduced invertase activity in *Sly-INH*-overexpressing tomato tissues resulted in reduced hexose and starch levels and increased sucrose levels (Fig. 6). These findings demonstrate that the activation of metabolic carbohydrate fluxes maintains the balance between starch and sucrose metabolism.

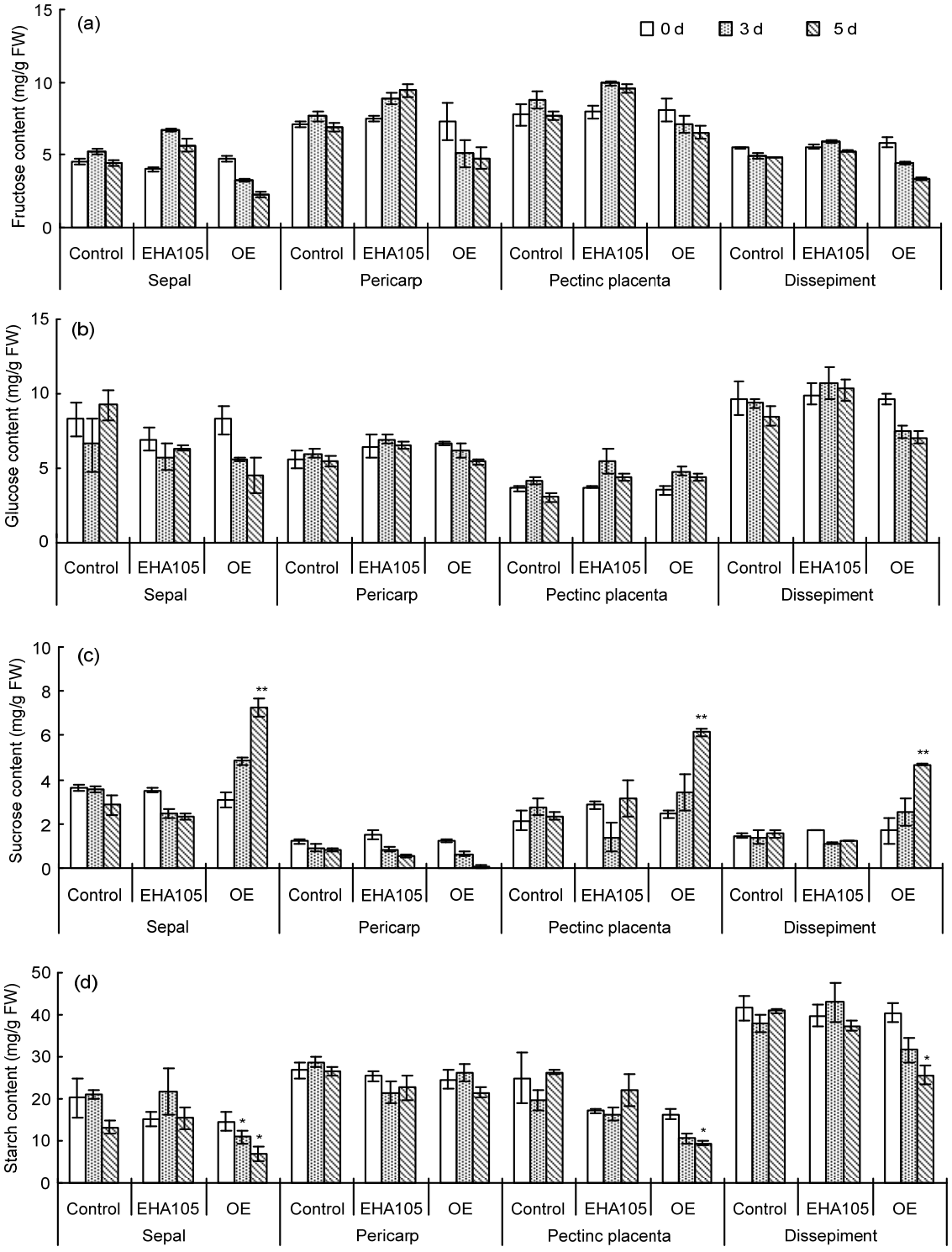

Fig. 6 Carbohydrate contents in different parts of Micro-Tom tomato fruits without agroinjection treatment (control), agroinjected with EHA105, or agroinjected with EHA105 containing p1300-2A11-*INH* plasmid (OE) at 0, 3, and 5 d, respectively

(a) Fructose content; (b) Glucose content; (c) Sucrose content; (d) Starch content. Data are expressed as mean±SE (n=3). * P<0.05, ** P<0.01, compared with the control

Recently, a number of related studies have shown that *INH* inhibits invertase activity *in vitro* (Greiner *et al*., 1998). However, these studies, which used heterologous expression systems, did not elucidate the function of the endogenous *INH* gene. Jin *et al*. (2009) found that inhibiting the expression of *INH* in tomato increased the activity of CWIN 40%–65% in mature leaves. In mature leaves, fruits, and seeds, inhibiting the expression of *Sly-INH* has an effect on the contents of *INV* mRNA. In the current study, CWIN activity decreased in sepals, pericarp, and pectinic placenta in tomato fruit after injection with *Sly-INH*, especially in sepals and dissepiment, whereas the activities of other sugar metabolism-related enzymes remained unchanged. These findings indicate that overexpression of *Sly-INH* inhibits the activity of apoplasmic invertase in developing tomato fruit, which increases fruit sucrose levels.

Emerging evidence indicates that CWIN activity is tightly controlled at both the transcriptional and post-transcriptional levels (Huang *et al*., 2007). Invertase activity may be subject to post-translational suppression by its inhibitory protein (Hothorn *et al*., 2004; Rausch and Greiner, 2004; Jin *et al*., 2009). Our results suggest that *Agrobacterium*-mediated fruit injection greatly affected the expression of the target gene *Sly-INH* but had no effect on the transcription levels of genes in the invertase gene family (Fig. 4). However, physiological analysis indicated that CWIN activity was inhibited in these fruits. Taken together, these results suggest that the inhibitor *Sly-INH* regulates the activity of CWIN at the post-translational level. This finding is in accordance with previous invertase structural analysis (Jin *et al*., 2009; Hothorn *et al*., 2010).

Notably, the relative expression of *Sly-INH* was lower in sepals after injection compared with the control (Fig. 1), while reduced CWIN activity was evident in the sepals of OE plants (Fig. 2). These results raise the possibility that the reduced CWIN activity in 5-d sepals might have been due to different routes in the regulation of the inhibitory effect of *Sly-INH* against CWIN. For example, a recent site-directed mutagenesis study showed that the presence of defective CWIN increased the inhibitory effect of *INHs* on CWIN activity (le Roy *et al*., 2013; Palmer *et al*., 2015).

5 Conclusions

This study demonstrates that CWIN and CIN exist in glycosylated form and are relatively stable, suggesting that the activities of these two invertases largely depend on post-translational regulation. The results suggest that the expression of *Sly-INH* increased significantly at 3 d after injection, while CWIN activity decreased significantly. These results demonstrate that the invertase inhibitor *Sly-INH* has the potential to regulate CWIN activity at the post-translational level.

Compliance with ethics guidelines

Ning ZHANG, Jing JIANG, Yan-li YANG, and Zhi-he WANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：转化酶抑制子调控番茄果实糖代谢的功能分析

目　的：研究转化酶抑制子基因对番茄果实糖转运与积累的影响，并初步探讨其调控机制。

创新点：首次在番茄果实瞬时表达系统中证明转化酶抑制子可明显抑制细胞壁转化酶活性，并且此抑制作用是通过翻译后水平发挥作用。

方　法：将 Micro-Tom 番茄分为对照组（果实未注射农杆菌及注射不含载体的空白农杆菌）和实验组（果实注射含有过表达转化酶抑制子基因的农杆菌），番茄果实分为萼片、中果肉、胶质胎座和心室隔壁四个部位，用实时定量聚合酶链式反应（qRT-PCR）技术检测转化酶抑制子和转化酶基因家族的表达变化，利用比色法分别检测了蔗糖代谢关键酶活性的变化，用高效液相色谱检测果实各部位果糖、葡萄糖和蔗糖的含量，利用高氯酸水解法测定淀粉的含量。

结　论：利用农杆菌注射进行果实瞬时表达后果实各部位的细胞壁转化酶活性受到明显抑制，但转化酶基因家族的转录水平表达变化不大，果糖和葡萄糖含量下降，蔗糖含量有所升高。这一结果表明 *Sly-INH* 基因主要是通过在翻译后水平对番茄细胞壁转化酶进行调控，进而影响番茄果实糖的组成与含量。本研究从分子水平对转化酶抑制子及其调控的转化酶基因家族进行了系统性研究，这为利用栽培手段调控植物体内转化酶的表达和活性，调节库组织的蔗糖的输入速率以及同化产物的分配以改善品质提供依据与技术支持。

关键词：转化酶抑制子；果实发育；瞬时表达体系；过表达；番茄