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Regulation of rice sucrose transporter 4 gene expression in response to insect herbivore chewing

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ABSTRACT

Sucrose is an important carbon source for plant tissue repairing and also a signal involved in defense responses to against insect herbivores. Allocation of sucrose toward to undamaged tissues is an herbivory tolerance mechanism. Sucrose transporters (SUTs) are key factors to control sucrose flow in plants. In this study, herbivory effects caused by rice leaffolder [*Cnaphalocrocis medinalis* (Guenée)] on *OsSUT4* expressions were investigated in rice plants, and the results showed *OsSUT4* expression was significantly upregulated by larvae infestation and mechanical wounding. The wounding-induced *OsSUT4* expression was not systemically demonstrated in undamaged leaves. The larval chewing-induced *OsSUT4* expression was obviously reduced by blocking jasmonic acid (JA) and abscisic acid (ABA) biosynthesis. In addition to JA and ABA, data showed H_2O_2 generated in wounded tissues also role a signal in the regulatory pathway of herbivore-induced *OsSUT4* expressions. Our finding provide insights for further study in signal network of herbivory-induced sucrose translocation.

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KEYWORDS
Abscisic acid; herbivory; hydrogen peroxide; jasmonic acid; mechanical wounding; sucrose transporter

Introduction

Insect herbivores are one of the serious problems resulting in worldwide crop losses. Plant defense against insect pests is an important trait for crop improvement. Negative effects caused by chewing insects were conducted by both mechanical wounding and stimuli from oral secretions. Wounding causes cell death and water loss at the injured sites, and damaged plants are also easily infected by pathogens through these wounds (Consales et al. 2012; Cui et al. 2013; Savatin et al. 2014). Defense responses and repair mechanisms are important for protecting plants against insect herbivores. In herbivore-attack situation, several secondary metabolites produced in plants have been proved to play an important roles in plant resistance to insect pest (War et al. 2012; Mithöfer and Maffei 2017). Moreover, changes of primary metabolism and carbohydrate re-partition also have been observed in plants (Quilliam et al. 2006; Schwachtje et al. 2006; Schwachtje and Baldwin 2008). Carbohydrates are needed as energy and carbon structure sources to locally repair wounded tissues at injured sites. In addition, sugars also function as signal molecules to activate defense gene expressions (Koch 1996; Rolland et al. 2006). Quilliam et al. (2006) indicated that photosynthetic products accumulated near the injured area of *Arabidopsis*, implying that wounded sites were strong carbohydrate sinks. In contrast, carbohydrate transported away from injured sites and moved to undamaged organs for later growth recovery has been observed in herbivore attacked tobacco (Schwachtje et al. 2006).

Sucrose is the major carbohydrate transported within plants through symplastic or apoplastic pathways (Sauer 2007). Sucrose transporters (SUTs) facilitate the loading of sucrose into the phloem and influence sucrose distribution through apoplastic pathways (Sauer 2007). There are five SUTs (named OsSUT1 to 5) in rice (Aoki et al. 2003). OsSUT1 expression has been observed in several tissues, such as germinating seeds, leaves and panicles (Hirose et al. 1997; Aoki et al. 2003; Scofield et al. 2007). In germinating embryos, *OsSUT1* expression was upregulated by gibberellic acid and repressed by abscisic acid (ABA) (Chen et al. 2010). According to the analysis of *OsSUT2* promoter::*β-glucuronidase* expression in transgenic rice, *OsSUT2* was highly expressed in leaf mesophyll cells, lateral roots, seed coats, fertilized seeds, embryos and aleurone layers (Eom et al. 2011; Siao et al. 2011). Expression of the *OsSUT4* gene in rice leaf tissues was observed in our previous study, and the results showed that *OsSUT4* expression in the leaf sheaths of upper leaves was highly correlated with the sink/source status of tissues during heading periods (Chen and Wang 2008). Moreover, the regulation of *OsSUT4* expression in spikelet tissues was developmental stage dependent (Chung et al. 2014). Expression levels of *OsSUT3* and *OsSUT5* were quite low compared to those of *OsSUT1, 2* and *4* in leaf tissues (Takahashi et al. 2017).

The functions of several signaling factors, such as jasmonic acid (JA), salicylic acid (SA), ethylene, ABA, nitric oxide (NO) and reactive oxygen species (ROS), in wounding responses have been discussed for some plant species. JA is one type of oxylipin compound synthesized through the octadecanoid pathway. Increased JA accumulation and JA synthesis-related gene expressions stimulated by mechanical wounding treatments were found in several plant species, such as *Arabidopsis* (Park et al. 2002) and rice (Lee et al. 2004). The enhancement of SA and ABA levels by insect oral secretion stimuli was observed in *Arabidopsis* (Schäfer et al. 2011). In lettuce, the gene expression and activity of phenylalanine ammonia-lyases (PAL), a key enzyme in SA biosynthesis, could be increased by mechanical wounding (López-Gálvez et al. 1996). In potato tubers, ABA biosynthesis could be induced under mechanical wounding stresses (Suttle et al. 2013). In addition, it has been found that ABA

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stimulates the expression of several defense proteins that was mediated by JA-dependent pathways (Gatehouse 2002). Hydrogen peroxidase (H_2O_2), a reactive oxygen species, is generated by membrane-bound NADPH oxidase and often functions as a signal factor to trigger defense responses (Orozco-Cárdenas and Ryan 1999). NADPH oxidase activity and H_2O_2 accumulation were enhanced by mechanical wounding and JA stimuli in the leaves of several plant species, such as pea (Liu et al. 2008) and tomato (Orozco-Cárdenas et al. 2001). Moreover, H_2O_2 and nitric oxide (NO) interact with other secondary signal factors, such as calcium and cGMP, have been reported to be involved in wounding signal transduction pathways (Lin et al. 2011).

Sugar transporter is a key modulator in charge of carbon assimilate partition in plants. Even wounding effects on carbohydrate translocation and *SUT* expression have been shown in some reports (Arnold et al. 2004; Meyer et al. 2004; Tang et al. 2010). However, the related signaling pathways and regulatory mechanisms of *SUT* expressions stimulated by chewing insects is still limited. In this study, an effort was made to reveal the influence of rice leaffolder larvae attacking on changes of sugar accumulation and the signaling involved to the regulatory mechanisms of *OsSUT* expressions induced by larval infestation.

Materials and methods

Plant materials and growth conditions

Rice seeds (*Oryza sativa* L. cv. Tainung 67) were sterilized with 1% (v/v) NaOCl (containing 0.1% Tween-20) and were germinated at 30°C in the dark for 3 d. Germinated seeds were transferred to 30/25°C (day/night) growth chambers and continuously cultured with Kimura B nutrient solutions (Chu and Lee 1989). The leaf number was counted in sequence starting from the first leaf that emerged after the coleoptile. The seedling stage was determined according to the number of leaves. For example, the four-leaf stage means that the collar of the 4th leaf had been presented and was higher than that of the 3rd leaf, but the collar of the 5th leaf still had not emerged. Rice seedlings at the 4- to 5-leaf stages were used in this study.

Leaffolder infestation and mechanical wounding treatments

Rice leaffolders [*Cnaphalocrocis medinalis* (Guenée)] larvae were reared on maize seedlings, and adults were fed with 10% sucrose solutions (Bentur and Kalode 1990). For feeding treatment, rice leaffolder larvae (4th instar) were starved for 4 h before being transferred to the leaves of rice seedlings. Each treatment contained five rice seedlings, and three larvae were released onto each seedling. Leaves or roots were collected for soluble sugar and gene expression analysis. Mechanical wounding was conducted by scissors, and ten cuts were made on each leaf sample.

Chemical treatments

To reveal whether JA is involved in the regulatory pathways of *OsSUT4* expression stimulated by rice leaffolder feeding, aspirin (100 μM, Sigma-Aldrich, UK) dissolved in DMSO was applied to 5-leaf stage rice seedlings for 12 h before leaffolder infestation. Aspirin acts as an inhibitor to suppress JA biosynthesis (Pan et al. 1998). Fluridone is an inhibitor of ABA biosynthesis (Yoshioka et al. 1998). To determine whether ABA is involved in the signaling pathway of leaffolder-stimulated *OsSUT4* expression, fluridone (10 μM, Fluka, Germany) dissolved in ethanol was applied to rice seedlings for 24 h before larvae feeding treatments. To reduce the level of endogenous SA in seedlings, 100 μM of paclobutrazol (PAC [Fluka, Germany], a SA biosynthesis inhibitor [Dong et al. 2014]) was applied to culture solutions for 24 h before leaffolder infestation. To study whether leaffolder-induced *OsSUT4* expression was dependent on H_2O_2 signaling, 100 μM of diphenyleneiodonium chloride (DPI [Sigma-Aldrich, UK], an H_2O_2 synthesis inhibitor [Orozco-Cárdenas and Ryan 1999]) was applied to seedlings for 24 h before rice leaffolder infestation.

Carbohydrate content analysis

Leaf and root tissues of 4-leaf stage seedlings were collected and dried at 80°C and then weighed. Dried samples were ground and extracted twice with 5 mL of ethanol (80%, v/v) at 85°C for 15 min. Samples were centrifuged at 3,000×g for 10 min, and the supernatant was collected for soluble sugar analysis. The total soluble sugar content was determined with anthrone reagent (Yoshida et al. 1976). Sucrose contents were analyzed by enzyme-based detection methods (Nakamura et al. 1989; Spackman and Cobb 2002).

RNA extraction and quantitative real-time RT–PCR analysis

Total RNA from rice tissues was extracted using Azol® RNA Isolation Reagent (Arrowtec, Taiwan), and RNA samples were treated with DNase with a TURBO DNA-*free*™ kit (Ambion, USA) to prevent DNA contamination. RNA was used as a template for quantitative RT–PCR with a One Step SYBR® PrimeScriptTM RT–PCR Kit II (Takara, USA). The RT–PCR reactions were processed on a Multiplex 3000P Real-Time PCR System (Stratagene, USA). The sequences of primers used for analysis of *ubiquitin* (*Ubi*), *OsSUT1*, *OsSUT2*, *OsSUT4* and *allene oxide synthase* (*OsAOS1*, *OsAOS2*) were described previously (Aoki et al. 2003; Chen and Wang 2008; Chen et al. 2010; Nahar et al. 2011; Wang et al. 2011). The forward primer for *OsJAmyb* (accession no. AY026332) was 5′-TCCGGTGGCTGAACTATCTC-3′. The reverse primer for *OsJAmyb* was 5′-CATGGCATCCTTGAACCTCT-3′ (Nahar et al. 2011). The primers for the response to the ABA 16A gene (*OsRab16A*, accession no. AK121952) were 5′-ACCACAGCAAGAGCTAAGTGAG-3′ and 5′-CCGGGTTGCCGTACTCGTC-3′. The primers for detecting *OsPAL* (accession no. AK068993) mRNA were 5′-TCGTATCCGCTCTACCGGTT-3′ and 5′-GCCTCCACACTCCACTGTTA-3′. Gene expression levels were normalized to the internal control (*Ubi*).

Hydrogen peroxide (H_2O_2) detection and quantification

The 3rd leaf of 5-leaf stage rice seedlings was treated with rice leaffolder larvae for 1 h and collected after removal of the larvae. H_2O_2 accumulation on wounded leaves was detected by histochemical staining. The detached leaves were soaked in

0.5 mg mL^{-1} of 3,3′-diaminobenzidine (DAB, Sigma-Aldrich, USA) solution at 37°C for 12 h. The brownish area was observed after chlorophyll was removed with 95% ethanol (Orozco-Cárdenas and Ryan 1999). For H_2O_2 quantification, leaffolder-infested leaves were homogenized with 50 mM sodium phosphate buffer (pH 6.8) and 1 mM hydroxylamine. After centrifugation, the supernatant was mixed with 0.1% titanium chloride (in 20% H_2SO_4), and then absorbance was detected at 410 nm (Liu et al. 2012).

Statistical analysis

The results showed the means of three independent experiments, and significant differences between experimental and control samples were analyzed by Student's t-test.

Results

Effects of rice leaffolder infestation on changes in sugar contents

To determine the effects of rice leaffolder larvae infestation on soluble sugar content in leaf and root tissues, leaffolder larvae were released on the 3rd leaves of rice seedlings for 1 h. The soluble sugar content of wounded leaves (3rd leaves), the unwounded youngest leaves (5th leaves, yet to become fully elongated) and roots were determined at 0, 24 and 48 h after larvae were removed. The results showed that the total soluble sugar and sucrose contents of the 3rd leaf of wounded seedlings were decreased after larvae were removed for 24 h compared to that of non-treated seedlings, although the difference was not statistically significant (Figure 1). In unwounded youngest leaves (5th leaves), increase of total soluble sugar and sucrose were performed in larvae-treated seedlings and the significant difference was observed after larvae were removed for 24 h (Figure 1). On the other hand, the soluble sugars of roots were not significantly influenced in leaffolder-stimulated rice seedlings within 24 h after larvae were removed (Figure 1). After larvae were removed for 48 h, the total soluble sugar content of roots was higher than that in non-treated seedlings (Figure 1).

Effects of rice leaffolder infestation on OsSUT gene expression

Effects of rice leaffolder infestation on *OsSUT1*, *2* and *4* expression in the damaged leaves were observed after rice leaffolder feeding for 1 and 2 h. *OsSUT1* and *OsSUT2* mRNA levels were not significantly changed after larvae infestation treatment. However, the expression of *OsSUT4* was significantly upregulated by stimuli caused by larval chewing (Figure 2). The expression of *OsSUT4* increased 4.34-fold and 2.95-fold after 1 and 2 h of rice leaffolder treatment, respectively (Figure 2). Moreover, mechanical wounding effects on leaf and root tissues were also observed. One of

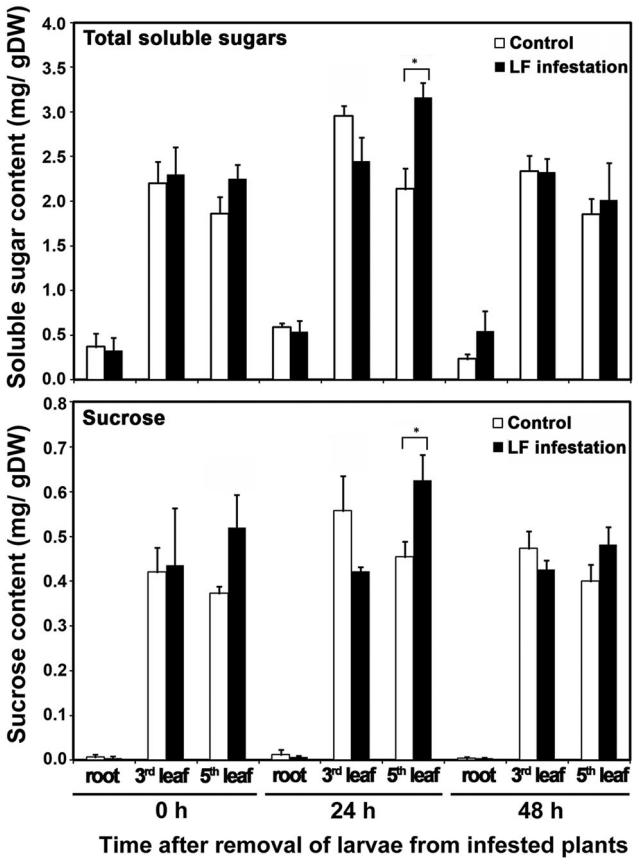

Figure 1. Effects of leaffolder infestation on sugar contents in different tissues of rice plants. 4th-instar leaffolders were placed on the 3rd leaf of 4-leaf stage rice seedlings for 1 h and then removed. The amounts of total soluble sugars and sucrose in the root, 3rd- and 5th-leaf of rice plants were measured at 0, 24 and 48 h after larvae removal. White bars indicate non-treated plants (control), and black bars indicate the plants exposed to rice leaffolder larvae. LF: rice leaffolder. Asterisks indicate significant differences (*$P < 0.05$).

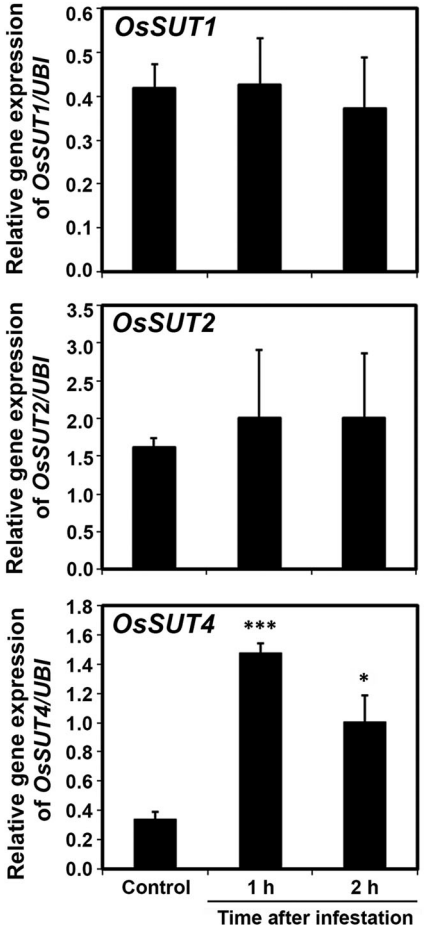

Figure 2. Effects of rice leaffolder infestation on the expression of OsSUT genes. The 3rd leaves of rice seedlings were exposed to rice leaffolders, and then the wounded 3rd leaves were collected for OsSUT gene expression analysis after larvae infestation for 1 and 2 h. Asterisks indicate significant differences (*$P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

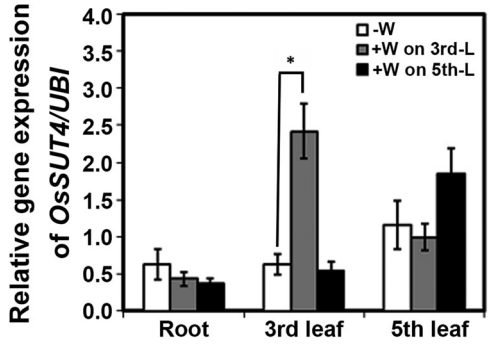

Figure 3. Influence of mechanical wounding on OsSUT4 gene expression in different tissues of rice plants. Rice plants were wounded by scissors either on the 3rd-leaf (+W on 3rd-L) or the 5th-leaf (+W on 5th-L) for 1 h. The roots, 3rd leaves and 5th leaves were collected to analyze *OsSUT4* gene expressions. Asterisks indicate significant differences (*$P < 0.05$).

the treatment groups was wounded mechanically on the 3rd leaves, and the results showed that *OsSUT4* expression was highly upregulated 3.87-fold compared to that of unwounded seedlings (Figure 3), but *OsSUT4* mRNA levels were not significantly affected in the 5th leaves (Figure 3). The other treatment group was treated with mechanical wounding on the 5th leaves, and the gene expression data indicated that the increase of *OsSUT4* transcripts only occurred in the wounded 5th leaves but not in the unwounded 3rd leaves (Figure 3). In root tissues of both the 3rd leaf wounded and 5th leaf wounded seedlings, there were no significant difference in *OsSUT4* expressions (Figure 3).

JA biosynthetic inhibitor represses rice leaffolder-induced OsSUT4 *gene expression*

Expressions of genes encoded allene oxide synthase (AOS), a key enzyme in JA biosynthesis, were detected in rice leaffolder-infested leaf tissues. Data in Figure 4A and B showed the gene expression levels of *OsAOS1* and *OsAOS2* were enhanced 8.16- and 3.6-fold, respectively, after rice leaffolder infestation for 1 h. Moreover, the expression of *OsJAmyb*, as an indicator gene of JA levels, was also significantly upregulated by rice leaffolder stimulation (Figure 4C). To reveal whether the rice leaffolder-induced *OsSUT4* expression was regulated through JA signaling, aspirin was applied to repress JA biosynthesis. The results showed that rice leaffolder-induced *OsSUT4* expression was repressed in aspirin pretreated seedlings (Figure 4D).

ABA participates in the regulation of leaffolder-induced OsSUT4 *gene expression*

Expression levels of the ABA responsive gene *OsRab16A* were analyzed after rice leaffolder treatment for 1 h. The mRNA levels of *OsRab16A* were increased 3.06-fold after rice leaffolder larvae infestation (Figure 5A). It was suggested that rice leaffolder infestation could promote ABA biosynthesis. Moreover, rice leaffolder-induced *OsSUT4* gene expression was repressed in ABA inhibitor (fluridone) pretreated seedlings (Figure 5B). It was suggested that ABA was involved in the regulatory mechanisms of rice leaffolder-induced *OsSUT4* gene expressions.

Effects of SA on rice leaffolder-induced OsSUT4 *expression*

Gene expression of the key enzyme of SA biosynthesis, PAL, was determined in rice leaffolder-infested leaf tissues. *OsPAL* expression was highly enhanced in infested leaves (Figure 6A). Furthermore, PAC was applied to culture solution to repress SA biosynthesis and then the *OsSUT4* expressions were determined after larvae-infestation for 1 h.

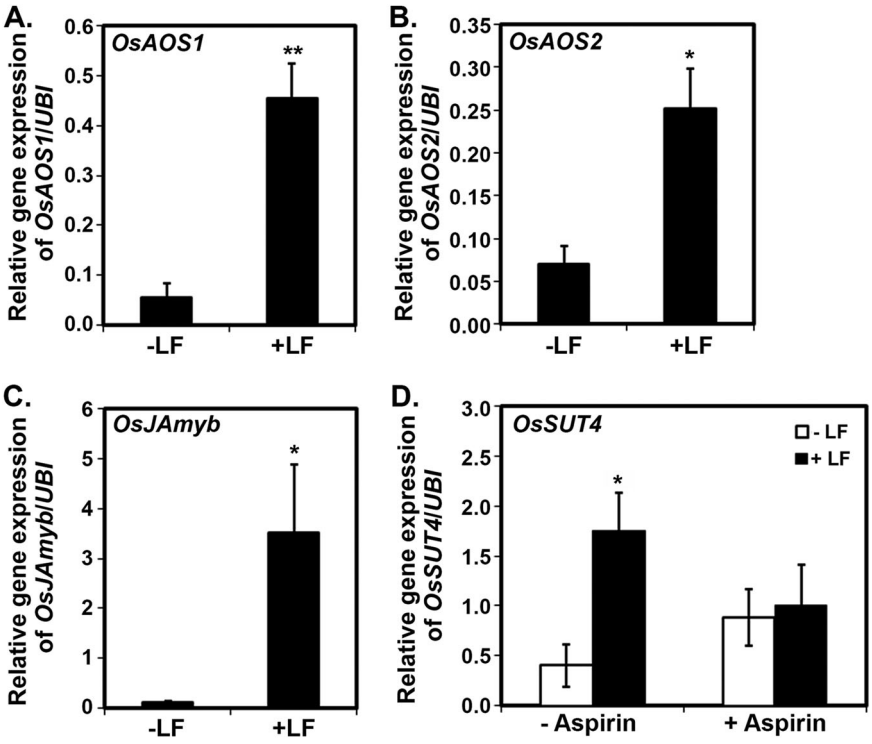

Figure 4. Effects of JA on leaffolder-induced OsSUT4 gene expression. (A-C) Transcript levels of JA biosynthesis and response genes were analyzed after leaffolder treatment for 1 h. (D) *OsSUT4* expression was analyzed in seedlings pretreated with 100 μM aspirin for 12 h before leaffolder infestation. –LF: plants without leaffolder treatment. +LF: samples treated with leaffolder for 1 h. Asterisks indicate significant differences (*$P < 0.05$, ** $P < 0.01$).

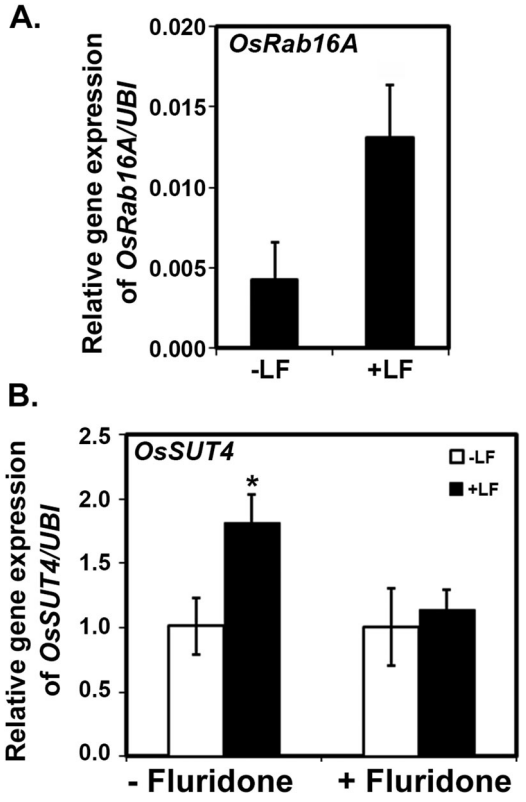

Figure 5. Effects of ABA on leaffolder-induced OsSUT4 gene expression. (A) Rice seedling leaves were treated with leaffolders for 1 h and then collected for *OsRab16A* gene expression analyses. (B) Rice seedlings were pretreated with 10 μM ABA inhibitor (fluridone) for 24 h before leaffolder infestation treatment for 1 h, and then leaves were collected for OsSUT4 gene expression analyses. The control group (-Fluridone) was treated with 0.01% ethanol. −LF: plants without leaffolder treatment. +LF: samples treated with leaffolder for 1 h. Asterisks indicate significant difference (*$P < 0.05$).

The data showed the herbivore-induced *OsPAL* expression was significantly repressed in PAC-treated seedlings (Figure 6A). However, the data in Figure 6B shows that rice leaffolder-enhanced *OsSUT4* expression was only slightly influenced by PAC.

H_2O_2 participates in the regulatory mechanisms of rice leaffolder-induced OsSUT4 *gene expression*

The 3,3′-diaminobenzidine (DAB) staining results indicated that H_2O_2 accumulated in the wounded leaves of rice leaffolder-infested seedlings (Figure 7A). Furthermore, quantitative analysis showed that the H_2O_2 content in the wounded leaves of rice leaffolder-infested seedlings was higher than that in noninfested seedlings (Figure 7B). To identify the roles of H_2O_2 in the regulatory mechanisms of rice leaffolder-induced *OsSUT4* gene expression, *OsSUT4* transcript levels were analyzed in the plants pretreated with diphenyleneiodonium chloride (DPI, an inhibitor of NADPH oxidase synthesis) before larvae infestation treatment. The data showed that DPI treatment significantly reduced the effects of rice leaffolder infestation on *OsSUT4* gene expressions (Figure 7C).

Discussion

Herbivore-attacking or mechanical-wounding stimuli often conduce the changes in sink and source status of various organs in plants (Quilliam et al. 2006). Primary metabolites such as carbohydrates could be allocated in injured sites as resources for defense and repair. However, it could also be removed from local injured tissues to prevent continuous nutrient uptake by herbivorous insects, and carbon assimilates might also be transported to un-damaged tissues for growth recovery (Schwachtje and Baldwin 2008; Zhou et al. 2015). Several studies have indicated that sucrose functions as a regulator to modulate the expression of defense genes (e.g. proteinase inhibitors and pathogenesis-related genes) (reviewed by Rolland et al. 2002). It has also been observed that sucrose could enhance wound-stimulated responses (Kim et al. 1991). Our data showed that total soluble sugar and sucrose contents were increased in undamaged young leaves and decreased in larvae-infested leaves (Figure 1). Sugar content decreasing in rice leaffolder-damaged leaf tissues could be the results of modulation of photosynthesis or carbohydrate metabolism efficiency, and it may also contribute to defense responses. On the other hand, sucrose content of the undamaged of developing sink leaf tissues in leaffolder larvae-treated plants was increased. It was also considered that carbohydrate reallocation from injury site toward to undamaged young tissues were occurred and provided carbon source for maintaining young non-infested leaf growth.

Sucrose transport and repartition relies on SUT. In rice plants, SUTs are encoded by five gene family members that

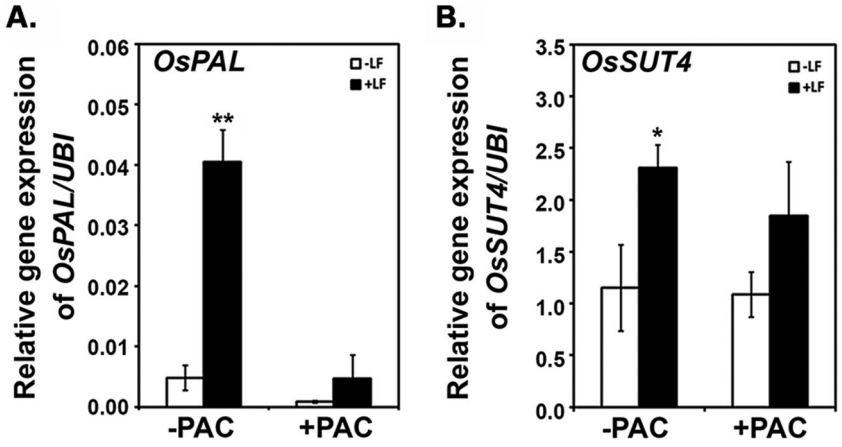

Figure 6. Effects of SA on leaffolder-induced OsSUT4 gene expression. Rice plants were pretreated with 100 μM PAC (+PAC) for 24 h before exposure to leaffolders. OsPAL (A) and OsSUT4 (B) gene expressions were determined in leaf tissues of leaffolder-infested plants (+LF) and non-infested plants (−LF). The leaffolder-infested leaves were collected after larvae infestation for 1 h. Asterisks indicate significant differences (*$P < 0.05$ and ** $P < 0.01$).

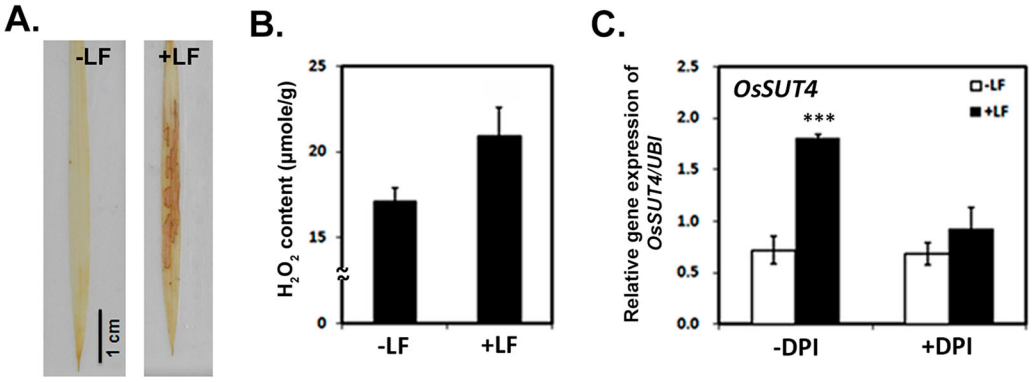

Figure 7. Hydrogen peroxide (H_2O_2) mediates leaffolder-induced OsSUT4 gene expression. (A) Accumulation of H_2O_2 detected with DAB staining after leaffolder infestation. (B) Quantitative analysis of H_2O_2 in leaffolder-infested rice leaves. (C) Changes in larvae infestation-induced *OsSUT4* expressions in DPI-treated seedlings. Rice plants were pretreated with H_2O_2 inhibitor (DPI) for 24 h, and then the 3rd-leaves were exposed to leaffolder larvae. The wounded leaves were collected to analyze *OsSUT4* gene expression after rice leaffolder infestation for 1 h. −LF: plants without leaffolder treatment. +LF: samples treated with leaffolder for 1 h. Asterisks indicate significant differences (*** $P < 0.001$).

are different in amino acid sequences and tissue-specific expression patterns, implying that they are responsible for different physiological functions (Aoki et al. 2003). In this study, changes in the expression of *OsSUT1*, *2* and *4* were analyzed by real-time RT–PCR. The results show that only *OsSUT4* is significantly upregulated by rice leaffolder larvae stimuli (Figure 2). The effects of aphid (*Myzus persicae*) infestation on nine *Arabidopsis* sucrose transporter genes (*AtSUCs*) have been investigated, and the expression levels of several AtSUC genes were enhanced in aphid-stimulated plants (Dubey et al. 2013). However, the effects of aphid infestation on *AtSUC* gene expression declined after a longer time of infestation, and it was suggested that the expression of AtSUCs was repressed to prevent further sugar loss (Dubey et al. 2013). AtSUC1 gene expression could also be enhanced by infestation by silverleaf whitefly (*Bemisia tabaci*) (Kempema et al. 2007). However, both aphid and silverleaf whitefly are phloem-sucking insects. Whether AtSUC gene expression is responsive to the damage caused by chewing insects remains unclear. Enhanced expression of *Arabidopsis* AtSUC3 by mechanical wounding was reported by Meyer et al. (2004). The chewing behavior of rice leaffolder larvae caused serious mechanical damage. The effects of mechanical wounding on *OsSUT4* expressions were investigated in this study (Figure 3), and the results showed mechanical wound-induced expression of *OsSUT4* was not a systemic response in shoots. Furthermore, the data presented here showed that the effects of wounding on *OsSUT4* expression in mature leaves (i.e. the 3rd leaf in Figure 3) was more obvious than that in young developing leaves (i.e. the 5th leaf in Figure 3), and it was suggested the strength of wounding effects on the regulation of *OsSUT4* expression was dependent on the sink and source status of leaf tissues.

To clarify the signal transduction and regulatory mechanisms of *OsSUT4* under rice leaffolder infestation, the effects of different hormones on insect-induced expression of *OsSUT4* were examined. Ibraheem et al. (2010) reported that the *OsSUT4* gene promoter contains some *cis*-acting elements involved in the regulation of JA and ABA. Based on the expression of various hormone biosynthesis genes and hormone-induced genes, it was suggested that rice leaffolder larvae infestation induced changes in JA and ABA contents (Figures 4 and 5). In addition, the leaffolder-induced *OsSUT4* expressions were repressed by both JA and ABA inhibitors (Figure 4D, Figure 5B). It has been observed that sugar accumulation and plant growth were suppressed by herbivore-induced phytohormone such as JA (Machado et al. 2017). In this study, it was revealed that JA functions a regulator to enhance the expression of *OsSUT4* in leaf tissues damaged by insect larvae chewing. It was suggested that JA also play a role to promote herbivore-induced sucrose translocation. In our previous study, *OsSUT4* expression and carbohydrate metabolism were upregulated by ABA in rice leaf sheaths during the heading period (Chen and Wang 2012). These results show that JA and ABA are important signals involved in the regulation of *OsSUT4* expression in leaves under stress caused by rice leaffolder chewing. However, applying PAC to repress SA biosynthesis slightly reduced the effect of larvae infestation on *OsSUT4* expressions (Figure 6B), suggesting that SA might function as a factor to modulate *OsSUT4* expression levels induced by rice leaffolder infestation.

H_2O_2, a non-hormonal signal factor, accumulated around injured areas in corn, potato and cotton (Orozco-Cárdenas and Ryan 1999), and it was suggested that H_2O_2 could function as a secondary message to regulate the expression of mechanical wounding- and JA-induced defense genes (Orozco-Cárdenas et al. 2001). In our study, H_2O_2 accumulation was induced in the injured leaves of leaffolder-infested plants (Figure 7A and B). DPI specifically inhibits the activity of NADPH oxidase, which is a key enzyme of H_2O_2 biosynthesis (Orozco-Cárdenas and Ryan 1999). As shown in Figure 7C, DPI suppressed leaffolder-induced *OsSUT4* gene expression. Thus, it was suggested that H_2O_2 participate in the signaling pathway of leaffolder-induced *OsSUT4* gene expression.

In conclusion, the data we presented here showed the total soluble and sucrose content would be increased in non-damaged young leaves but decreased in injury leaf tissues in rice leaffolder-infested rice plants, and it was suggested that OsSUT4 functions the major regulator to control sucrose transport and modulate sucrose partition under insect herbivores stresses. Furthermore, the regulation of herbivore chewing stimulated *OsSUT4* expressions were mediated the stress signaling such as JA, ABA and H_2O_2. Our finding provided the basis for addressing the network regulations of herbivory-induced sucrose transport in future studies.

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Disclosure statement

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