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 leaf folder [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$_2$O$_2$ 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.

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 aleurome 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
stimulates the expression of several defense proteins that was mediated by JA-dependent pathways (Gatehouse 2002). Hydrogen peroxide (H$_2$O$_2$), 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$_2$O$_2$ 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 and Ryan 1999). Moreover, H$_2$O$_2$ and nitric oxide (NO) interact and NADPH oxidase activity generated by membrane-bound NADPH oxidase and often 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 leaf folder 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 leaf folders [Cnaphalocrosis medinalis (Guènèe)] larvae were reared on maize seedlings, and adults were fed with 10% sucrose solutions (Bentur and Kalode 1990). For feeding treatment, rice leaf folder 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 leaf folder 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$_2$O$_2$ signaling, 100 μM of diphenyleneiodonium chloride (DPI [Sigma-Aldrich, UK], an H$_2$O$_2$ 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® PrimeScript™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 OsJAbmyb (accession no. AY026332) was 5′-TCGGTGGCTGAACCATCTCT-3′. The reverse primer for OsJAbmyb was 5′-CATGGCATCTTGAACTCCTC-3′ (Nahar et al. 2011). The primers for the response to the ABA 16A gene (OsRab16A, accession no. AK121952) were 5′-ACCACAGCACAAGGCCGGAAGC-3′ and 5′-CCGGGT TTCCGCTACTGTC-3′. The primers for detecting OsPAL (accession no. AK068993) mRNA were 5′-TCGGTGGCTGAACCATCTCT-3′ and 5′-GCCCTCACACACACTCTGTGA-3′. Gene expression levels were normalized to the internal control (Ubi).

Hydrogen peroxide (H$_2$O$_2$) 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$_2$O$_2$ 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 H₂O₂ 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% H₂SO₄), 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 larval 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 larval 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
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 (fluridine) pre-treated 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.
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 leafler-enhanced OsSUT4 expression was only slightly influenced by PAC.

**H2O2 participates in the regulatory mechanisms of rice leafler-induced OsSUT4 gene expression**

The 3,3′-diaminobenzidine (DAB) staining results indicated that H2O2 accumulated in the wounded leaves of rice leafler-infested seedlings (Figure 7A). Furthermore, quantitative analysis showed that the H2O2 content in the wounded leaves of rice leafler-infested seedlings was higher than that in noninfested seedlings (Figure 7B). To identify the roles of H2O2 in the regulatory mechanisms of rice leafler-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 leafler 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 leafler-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 leafler 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...
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 leaf folder 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 whitely (Bemisia tabaci) (Kempea et al. 2007). However, both aphid and silverleaf whitely 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 leaf folder larvae caused serious chemical 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 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 leaf folder 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 leaf folder larvae infestation induced changes in JA and ABA contents (Figures 4 and 5). In addition, the leaf folder-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 as 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 leaf folder 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 leaf folder infestation.

H$_2$O$_2$, 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$_2$O$_2$ 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$_2$O$_2$ accumulation was induced in the injured leaves of leaf folder-infested plants (Figure 7A and B). DPI specifically inhibits the activity of NADPH oxidase, which is a key enzyme of H$_2$O$_2$ biosynthesis (Orozco-Cárdenas and Ryan 1999). As shown in Figure 7C, DPI suppressed leaf folder-induced OsSUT4 gene expression. Thus, it was suggested that H$_2$O$_2$ participate in the signaling pathway of leaf folder-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 leaf folder-infested rice plants, and it was suggested that OsSUT4 functions as 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$_2$O$_2$. Our finding provided the basis for addressing the network regulations of herbivory-induced sucrose transport in future studies.
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References
Aoki N, Hirose T, Scofield GN, Whitfield PR, Furbank RT. 2003. The sucrose transporter gene family in rice. Plant Cell Physiol. 44:223–232.
Arnold T, Appel H, Patel V, Stocum E, Kavalier A, Schultz J. 2004. Carbohydrate translocation determines the phenolic content of Populus foliage: a test of the sink-source model of plant defense. New Phytol. 164:157–164.
Bentur JS, Kalode MB. 1990. A feeding test to identify rice varieties resistant to the leaf folder, Cnaphalocrocis medinalis (Guenee). Proc Indian Acad Sci (Anim. Sci.). 99:483–491.
Chen JT, Liu SL, Siao W, Wang SJ. 2010. Hormone and sugar effects on rice sucrose transporter OsSUT1 expression in germinating embryos. Acta Physiol Plant. 32:749–756.
Chen HJ, Wang SJ. 2008. Molecular regulation of sink-source transition in rice leaf sheaths during the heading period. Acta Physiol Plant. 30:639–649.
Chen HJ, Wang SJ. 2012. Abscisic acid enhances starch degradation and sugar transport in rice upper leaf sheaths at the post-heading stage. Acta Physiol Plant. 34:1493–1500.
Chu C, Lee TM. 1989. The relationship between ethylene biosynthesis and chilling tolerance in seedlings of rice (Oryza sativa L.). Bull Bot Acad Sinica. 30:263–273.
Chung P, Hsiao HH, Chen HJ, Chang CW, Wang SJ. 2014. Influence of temperature on the expression of the rice sucrose transporter 4 gene, OsSUT4, in germinating embryos and maturing pollen. Acta Physiol Plant. 36:217–229.
Consales F, Schweizer F, Erb M, Gouhier-Darimont C, Bodenhausen N, Brunessow F, Sobhy I, Reymond P. 2012. Insect oral secretions suppress wound-induced responses in Arabidopsis. J Exp Bot. 63:727–737.
Cui F, Brosché M, Sipari N, Tang S, Overmyer K. 2013. Regulation of ABA dependent wound induced spreading cell death by MYB108. New Phytol. 200:634–640.
Dong CJ, Li L, Shang QM, Liu XY, Zhang ZG. 2014. Endogenous salicylic acid accumulation is required for chilling tolerance in cucumber (Cucumis sativus L.) seedlings. Planta. 240:687–700.

Dubre N, Idris A, Verma AK, Chandrashekhar K, Pandey KD. 2013. Expression pattern of sucrose transporters in Arabidopsis thaliana during aphid (Myzus persicae) infestation. Am J Plant Sci. 4:47–51.
Eom JS, Cho JI, Reinders A, Lee SW, Yoo Y, Tuan PQ, Choi SB, Bang G, Park YI, Cho MH, et al. 2011. Impaired function of the tonoplast-localized sucrose transporter in rice, OsSUT2, limits the transport of vacuolar reserve sucrose and affects plant growth. Plant Physiol. 157:109–119.
Gatehouse JA. 2002. Plant resistance towards insect herbivores: a dynamic interaction. New Phytol. 156:145–159.
Hirose T, Inaizumi N, Scofield GN, Furbank RT, Oh sugi R. 1997. cDNA cloning and tissue specific expression of a gene for sucrose transporter from rice (Oryza sativa L.). Plant Cell Physiol. 38:1389–1396.
Ibraheem O, Botha CE, Bradley G. 2010. In silico analysis of cis-acting regulatory elements in 5’ regulatory regions of sucrose transporter gene families in rice (Oryza sativa japonica) and Arabidopsis thaliana. Comput Biol Chem. 34:268–283.
Kempema LA, Cui X, Holzer FM, Walling LL. 2007. Arabidopsis transcriptome changes in response to phloem-feeding silverleaf whitley nymphs. Similarities and distinctions in responses to aphids. Plant Physiol. 143:849–865.
Kim SR, Costa MA, An GH. 1991. Sugar response element enhances wound response of potato proteinase inhibitor II promoter in transgenic tobacco. Plant Mol Biol. 17:973–983.
Koch KE. 1996. Carbohydrate-modulated gene expression in plants. Annu Rev Plant Physiol Plant Mol Biol. 47:509–540.
Lee A, Cho K, Jang S, Rakwal R, Iwashashi H, Agrawal GK, Shim J, Han O. 2004. Inverse correlation between jasmonic acid and salicylic acid during early wound response in rice. Biochem Biophys Res Commun. 318:734–738.
Lin CC, Jih PJ, Lin HH, Lin JS, Chang LL, Shen YH, Jeng ST. 2011. Nitric oxide activate superoxide dismutase and ascorbate peroxidase to repress the cell death induced by wounding. Plant Mol Biol. 77:235–249.
Liu CH, Chao YY, Kao CH. 2012. Abscisic acid is an inducer of hydrogen peroxide production in leaves of rice seedlings grown under potassium deficiency. Bot Studies. 53:229–237.
Liu Y, Pan QH, Yang HR, Liu YY, Huang WD. 2008. Relationship between H2O2 and jasmonic acid in pea leaf wounding response. Russ J Plant Physiol. 55:765–775.
López-Galvèz G, Salvièt M, Cantwell M. 1996. Wound-induced phenylalanine ammonia lyase activity: factors affecting its induction and correlation with the quality of minimally processed lettuces. Postharvest Biol Tec. 9:233–239.
Machado RAR, Baldwin IT, Erb M. 2017. Herbivory-induced jasmonates constrain plant sugar accumulation and growth by antagonizing gibberellin signaling and not by promoting secondary metabolite production. New Phytol. 215:803–812.
Meyer S, Lauterbach C, Niedermeier M, Barth J, Sjölund RD, Sauer N. 2004. Wounding enhances expression of AtSUC3, a sucrose transporter from Arabidopsis sieve elements and sink tissues. Plant Physiol. 134:684–693.
Mithöfer A, Mafiei ME. 2017. General mechanisms of plant defense and plant toxins. In: Gopalakrishnakone P, Carlini C, Ligabue-Braun R, editors. Plant toxins. Toxinology. Dordrecht: Springer; p. 3–24.
Nahar K, Kynidl T, De Vleeschauwer D, Höf t M, Gheyssen G. 2011. The jasmonate pathway is a key player in systematically induced defense against root knot nematodes in rice. Plant Physiol. 157:305–316.
Nakamura Y, Yuki K, Park SY, Ohy a T. 1989. Carbohydrate metabolism in the developing endosperm of rice grains. Plant Cell Physiol. 30:833–839.
Orozco-Cárdenas ML, Narváez-Vásquez J, Ryan CA. 2001. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. Plant Cell. 13:179–191.
Orozco-Cárdenas M, Ryan CA. 1999. Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. Proc Natl Acad Sci USA. 96:6553–6557.
Pan Z, Camara B, Gardner HW, Backhaus RA. 1998. Aspirin inhibition and acetylation of the plant cytochrome P450, allene oxide synthase, resembles that of animal prostaglandin endoperoxide H synthase. J Biol Chem. 273:18139–18145.
Park JH, Halitschke R, Kim HB, Baldwin IT, Feldmann KA, Feyerisen R. 2002. A knockout mutation in allene oxide synthase results in
male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. Plant J. 31:1–12.
Quilliam RS, Swarbrick PJ, Scholes JD, Rolfe SA. 2006. Imaging photosynthesis in wounded leaves of *Arabidopsis thaliana*. J Exp Bot. 57:55–69.
Rolland F, Baena-Gonzalez E, Sheen J. 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol. 57:675–709.
Rolland F, Moore B, Sheen J. 2002. Sugar sensing and signaling in plants. Plant Cell. 14 (suppl 1):S185–S205.
Sauer N. 2007. Molecular physiology of higher plant sucrose transporters. FEBS Lett. 581:2309–2317.
Savatin DV, Gramegna G, Modesti V, Cervone F. 2014. Wounding in the plant tissue: the defense of a dangerous passage. Front Plant Sci. 5:470.
Schäfer M, Fischer C, Baldwin IT, Meldau S. 2011. Grasshopper oral secretions increase salicylic acid and abscisic acid levels in wounded leaves of *Arabidopsis thaliana*. Plant Signal Behav. 6:1256–1258.
Schwachtje J, Baldwin IT. 2008. Why does herbivore attack reconfigure primary metabolism? Plant Physiol. 146:845–851.
Schwachtje J, Minchin PEH, Jahnke S, van Dongen JT, Schittko U, Baldwin IT. 2006. SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. Proc Natl Acad Sci USA. 103:12935–12940.
Scofield GN, Hirose T, Aoki N, Furbank RT. 2007. Involvement of the sucrose transporter, OsSUT1, in the long-distance pathway for assimilate transport in rice. J Exp Bot. 58:3155–3169.
Siao W, Chen JY, Hsiao HH, Chung P, Wang SJ. 2011. Characterization of OsSUT2 expression and regulation in germinating embryos of rice seeds. Rice. 4:39–49.
Spackman VMT, Cobb AH. 2002. An enzyme-based method for the rapid determination of sucrose, glucose and fructose in sugar beet roots and the effects of impact damage and postharvest storage in clamps. J Sci Food Agric. 82:80–86.
Suttle JC, Lulai EC, Huckle LL, Neubauer JD. 2013. Wounding of potato tubers induces increases in ABA biosynthesis and catabolism and alters expression of aba metabolic genes. J Plant Physiol. 170:560–566.
Takahashi S, Meguro-Maoka A, Yoshida M. 2017. Analysis of sugar content and expression of sucrose transporter genes (OsSUTs) in rice tissues in response to a chilling temperature. JARQ-JPN AGR RES Q. 51:137–146.
Tang C, Huang D, Yang J, Liu S, Sakr S, Li H, Zhou Y, Qin Y. 2010. The sucrose transporter HbSUT3 plays an active role in sucrose loading to laticifer and rubber productivity in exploited trees of *Hevea brasiliensis* (para rubber tree). Plant Cell Environ. 33:1708–1720.
Wang SJ, Ho CH, Chen HW. 2011. Rice develop wavy seminal roots in response to light stimulus. Plant Cell Rep. 30:1747–1758.
War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, Sharma HC. 2012. Mechanisms of plant defense against insect herbivores. Plant Signal Behav. 7:1306–1320.
Yoshida S, Forno DA, Cock JH, Gomez KA. 1976. Laboratory manual for physiological studies of rice, 3rd ed. Manila: The International Rice Research Institute.
Yoshioka T, Endo T, Satoh S. 1998. Restoration of seed germination at supraoptimal temperatures by fluridone, an inhibitor of abscisic acid biosynthesis. Plant Cell Physiol. 39:307–312.
Zhou S, Lou YR, Tzin V, Jander G. 2015. Alteration of plant primary metabolism in response to insect herbivory. Plant Physiol. 169:1488–1498.