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Analysis of Sugar Content and Expression of Sucrose Transporter Genes (*OsSUTs*) in Rice Tissues in Response to a Chilling Temperature

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Abstract

Changes in sucrose metabolism have been implicated in tissue damage found in rice plants exposed to cold. However, little is known about the effect of low temperature on sucrose transport in cold-sensitive plants such as rice. Here, we investigated the changes induced by a 12°C chilling treatment on the soluble sugar content relative to the expression of sucrose transporter genes (*OsSUT1-5*) in various tissues of young (6-week-old) and mature (booting stage) rice plants. Sucrose levels increased in source tissues but decreased in young panicles during the chilling treatment. Sucrose transporter genes also responded to the chilling treatment: *OsSUT1* in leaf sheaths and *OsSUT2* and *OsSUT4* in panicles were down-regulated. Our results suggested that the sucrose translocation supported by OsSUTs in rice plants decreased during chilling treatment, assuming the involvement of OsSUT1 and OsSUT4 in phloem loading of sucrose. These changes may induce a sugar imbalance in anthers, resulting in impaired pollen development.

Discipline: Plant breeding
Additional key words: cold tolerance, phloem loading

Introduction

Rice is of tropical origin and very sensitive to low temperatures, and chilling injury can occur in rice plants exposed to temperatures below 12°C (Koike & Satake 1987, Satake 1976, Suzuki et al. 2008). Young microspores at the booting stage show the greatest sensitivity to chilling, and male (pollen) sterility induced by chilling temperatures causes serious loss of grain yield in rice (Satake 1976). Changes in starch and sugar metabolism of rice anthers in response to chilling temperatures at the microspore stage have already been reported in the 1970s (Ito 1978, Satake 1976). Ito (1978) reported that non-reducing sugars increased in rice anthers soon after exposure to a temperature of 12°C at the meiotic stage. Since those reports, many researchers have been interested in the relation between sugar metabolism and male sterility induced by chilling. However, the mechanism has not yet been clarified. Low-temperature stress and other environmental stresses can induce essential changes in carbohydrate metabolism in plants (Gupta & Kaur 2005, Guy et al. 2008). The role of sucrose in cold stress in plant tissues has been studied in detail with focus on the responses of related enzymes such as invertase (EC 3.2.1.26) (Richardson 1990), sucrose-phosphate synthase (EC 2.4.1.14) and sucrose synthase (EC 2.4.1.13) (Guy et al. 2008).

Sucrose, an important energy and carbon source in plants, plays a vital role in the control of multiple metabolic pathways by regulating the expression of sugar-sensing genes (Koch 2004, Lalonde et al. 1999). Sucrose also plays important roles as a mediator in carbon partitioning among cells and proximate tissues, and in the long-distance translocation from source to sink tissues in plants by passive diffusion and active transport mechanisms (Eom et al. 2012, Kühn & Grof 2010, Lemoine 2013, Sauer 2007). In plant species including rice, sucrose is predominantly transported from source tissues to sink tissues via phloem; consequently, there is a very high concentration of sucrose in phloem. In rice plants, sucrose transport involves both symplastic phloem loading (or unloading) and apoplastic

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phloem loading (Fig. 1). A part of the sucrose translocation is mediated by sucrose transporters, cellular membrane-localized H^+-symporting carrier proteins (Kühn & Grof 2010, Marger & Saier 1993, Sauer 2007). A number of sucrose transporter genes (*SUTs* or *SUCs*) have been isolated from various species and are now classified into five subfamilies based on their molecular characteristics (Eom et al. 2011). However, the actual *in planta* functions and localizations of most sucrose transporter genes remain unclear. In rice, five sucrose transporter genes (*OsSUTs*) have thus far been cloned or annotated (Aoki et al. 2003). These five genes were shown to be expressed in most tissues of the rice plant but with different expression patterns (Aoki et al. 2003). The transport activities of OsSUT1, OsSUT2, OsSUT3 and OsSUT5 have been confirmed by complementation methods using yeast (Aoki et al. 2003, Eom et al. 2011) or by a system using *Xenopus laevis* oocytes (Sun et al. 2010). The function and localization of OsSUT1 have been investigated in most detail (Hirose et al. 1997, Matsukura et al. 2000, Scofield et al. 2007a, 2007b). OsSUT1 is found in the sieve elements and companion cells of phloem (SE/CC complex; see Fig. 1), and may play a role in phloem loading of sucrose (Matsukura et al. 2000, Scofield et al. 2007a, 2007b). Scofield et al. (2007b) analyzed the expression of *OsSUT1* in relation to the long-distance transport of assimilates from the flag leaf to the base of the filling grain via the uppermost internode in rice plants and proposed that the primary role of OsSUT1 is the retrieval of leaked sucrose from the apoplasm to the phloem along the transport pathway to maintain the supply of assimilate to the filling grain. Recently, it was also suggested that OsSUT4 might be responsible for sucrose loading into the phloem of upper source leaf sheaths during heading (Chen et al. 2008), although the exact protein function of OsSUT4 remains unclear. On the other hand, OsSUT2, which is localized in the tonoplast, has been proposed as being involved in sucrose transport from the vacuole to cytosol (Eom et al. 2011).

Recent studies have shown that the expression of OsSUT genes is clearly related to plant growth, the development of reproductive organs, and grain filling.

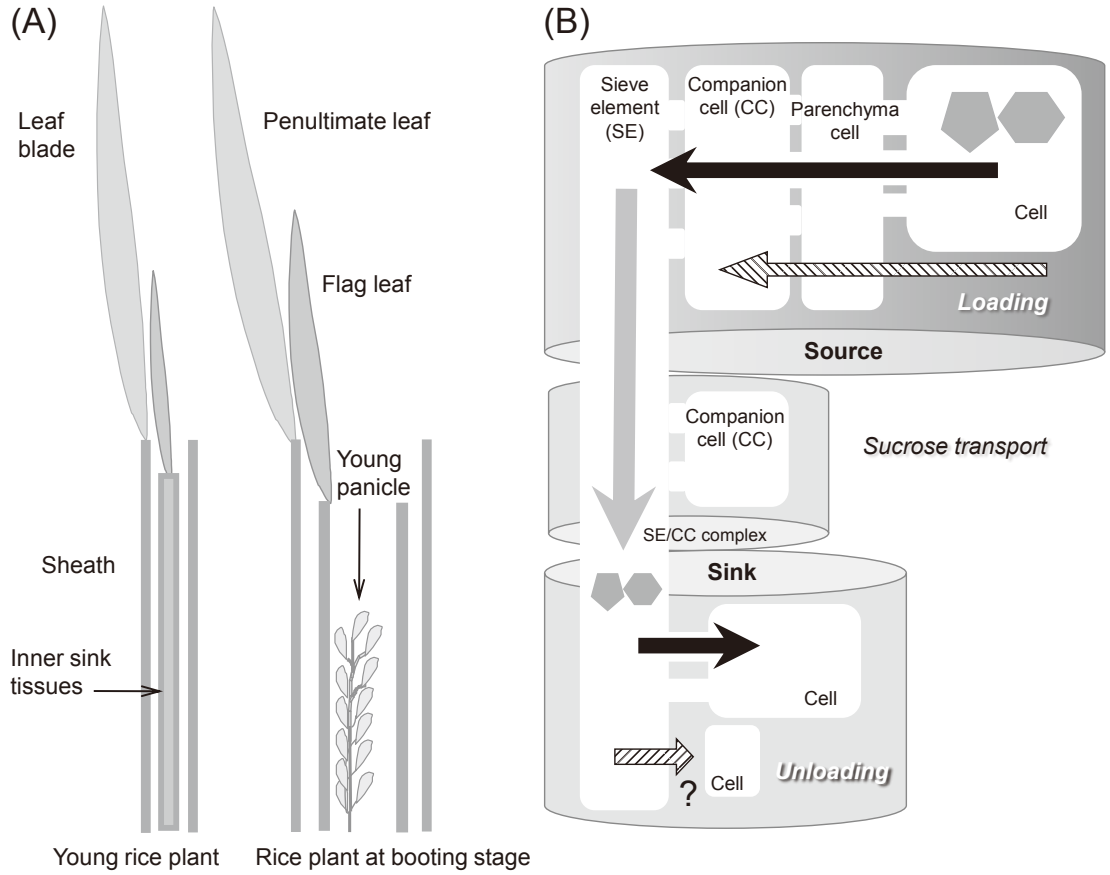

Fig. 1. Schematic illustration of the anatomy of rice plants showing the tissues sampled in the present study (A) and the sucrose transport system from source to sink tissues (B).
Black arrows and hatched arrows in panel (B) indicate symplastic loading (unloading) and apoplastic loading (unloading), respectively.

However, the role of OsSUTs in chilling stress is still unclear. In the present study, we investigated the changes in sucrose content and the expression of *OsSUTs* in various tissues of rice plants exposed to a chilling temperature, in order to determine the response of the sucrose transport system to chilling stress.

Materials and methods

1. Plant growth and chilling treatment conditions

Japonica rice cv. Yukihikari and Oborozuki (*Oryza sativa* L.), which are varieties for northern Japan, were grown in a phytotron, mainly with sunlight illumination, from spring to summer. However, additional lighting was used when necessary to maintain a minimum photoperiod of 15 h. Temperatures were artificially controlled at 25°C/19°C (day/night). In the experiment using young plants, Yukihikari was grown for about 40 days until eruption of the 7th leaf, and then subjected to a chilling temperature (12°C, day and night) for three days in the phytotron. Cold-stressed rice plants were again subjected to temperatures of 25°C/19°C (day/night) for one day. The following tissues were removed from three independent plants (typically around 1 p.m.) for analyses: the blade and sheath of the 6th leaf, and the sheath including inner sink tissues below the 7th leaf blade. Plants were sampled on day 0 (25°C/19°C), on days 1, 2 and 3 of the chilling treatment (12°C), and at one day (day +1) after the return to the non-chilling temperatures (25°C/19°C). In the experiment for another stage, Yukihikari and Oborozuki were grown until the booting stage, and then subjected to chilling treatment (12°C) for four days at around the young microspore stage. The penultimate leaf blade and its sheath, the sheath of the flag leaf, and young panicles were sampled from mature rice plants at 1 p.m. on day 0 (25°C/19°C), on days 2 and 4 of the chilling treatment (12°C), and on day +1 (25°C/19°C). The experiments were repeated at various times under natural sunlight conditions, with essentially identical results. Figure 1 shows the tissues selected for sampling.

2. Measurement of water-soluble carbohydrate content

Soluble carbohydrates were extracted and measured by using the methods described by Yoshida et al.[35]. Each sample was stored at -80°C until the extraction of carbohydrates. Water-soluble carbohydrates were extracted from tissues (approximate sample F.W.: blade and sheath of the 6th leaf, 0.1-0.3 g; sheath and inner sink tissues below the 7th leaf blade, 0.2-0.4 g; penultimate leaf blade, 0.3-0.6 g; penultimate or flag leaf sheath, 0.4-0.7 g; panicle, 0.1-0.4 g) that had been finely chopped and subjected to boiling in deionized water for 1 h. Total carbohydrates were analyzed by HPLC with a combination of Shodex KS-802 and KS-803 columns (Shodex, Tokyo, Japan) using a refractive index detector.

3. Gene expression analysis by quantitative real-time RT-PCR

Total RNA was extracted from each sample using an RNeasy plant mini kit (Qiagen USA, Valencia, CA) as per the manufacturer's instructions. After treatment with DNase I (Invitrogen, amplification grade), cDNA was synthesized from 1 μg of total RNA using SuperScript®III (Invitrogen) with an oligo-dT_{20} primer. Using cDNA corresponding to 5 ng RNA, the transcript levels of the sucrose transporter genes (*OsSUT1-5*) were quantified by real-time RT-PCR using the 7300 Real Time PCR System (Applied Biosystems) and SYBR Premix Ex Taq II (Perfect Real Time) (Takara Bio) as per the manufacturer's instructions. Table 1 lists the gene accession and LOC numbers and the primer pairs used in the real-time PCR. A poly-ubiquitin gene was used as an internal reference gene (Moritoh et al. 2005). Three determinations were made for each sample. The transcript level of each gene was obtained by first calibrating against a plasmid control possessing the same amplified target sequence and then calculating the relative value for the expression level of each *OsSUT* gene against the level of the internal reference gene.

4. Statistical analysis

Multiple comparisons of the sugar content and mRNA levels of gene expression were made by using Dunnett's method. Significant differences compared to values on day 0 (before treatment) were determined (*, $P < 0.05$; **, $P < 0.01$).

Results

1. Changes in soluble sugar contents in young rice plants exposed to chilling

Figure 2 shows representative changes in soluble sugar content in the tissues of rice plants subjected to chilling (12°C for three days) and then returned to normal growth temperatures (25°C/19°C, day/night) for one day at the active grow stage (40 days of growth, cv. Yukihikari). The plants looked normal after the treatment without any visible injury. Sucrose was the major soluble sugar detected in the rice tissues, and changes in the content of sucrose roughly reflected changes in the content of total soluble sugars. The sucrose content in the tissues increased during the chilling treatment and decreased one day after chilling to a level approximating that before chilling. However, the sucrose content in the leaf blades (source tissues) was double and almost triple that before the treatment, on the first and third days of treatment, respectively. In the sheaths, the sucrose content on the third day was about five time higher than

Table 1. Gene-specific primer pairs for amplification of sucrose transporter (SUT) and ubiquitin cDNAs by real-time PCR.

Primer	Position		Sequence
	from	to	5'→3'
OsUbq-F	335	373	AACCAGCTGAGGCCCAAGA
OsUbq-R	431	408	ACGATTGATTTAACCAGTCCATGA
OsSUT1-F	1876	1897	CCACCTCGGTAGAAGAGAATAA
OsSUT1-R	1985	1961	CCATTCATTACACACTAATTACCAA
OsSUT2-F	1561	1581	AGGAGGAGAGGTCACCGATAA
OsSUT2-R	1800	1778	CCAACATCCAATGTACAACAGCA
OsSUT3-F	1564	1583	AAGGTCTCCGTCCGCTCCGT
OsSUT3-R	1698	1679	CCTGCTATAGTACCCGCTCT
OsSUT4-F	1819	1838	TTTGGCTGAGCAGAACACCA
OsSUT4-R	2067	2048	ATGTCATTCGGGCAGAGCTT
OsSUT5-F	1672	1690	CCATGAATTGGGAGTGCAT
OsSUT5-R	1741	1722	ACACAGCGAACGAATCAACA

OsSUT1, *Oryza sativa* sucrose transporter 1 (AF280050, Os03g07480); *OsSUT2*, *O. sativa* sucrose transporter 2 (AB091672, Os12g44380); *OsSUT3*, *O. sativa* sucrose transporter 3 (AB071809, Os10g26470); *OsSUT4*, *O. sativa* sucrose transporter 4 (AB091673, Os02g0827200); *OsSUT5*, *O. sativa* sucrose transporter 5 (AB091674, Os02g36700). The sequences of the primer pair for ubiquitin were adopted as an internal standard from the method reported by Moritoh et al. (2005).

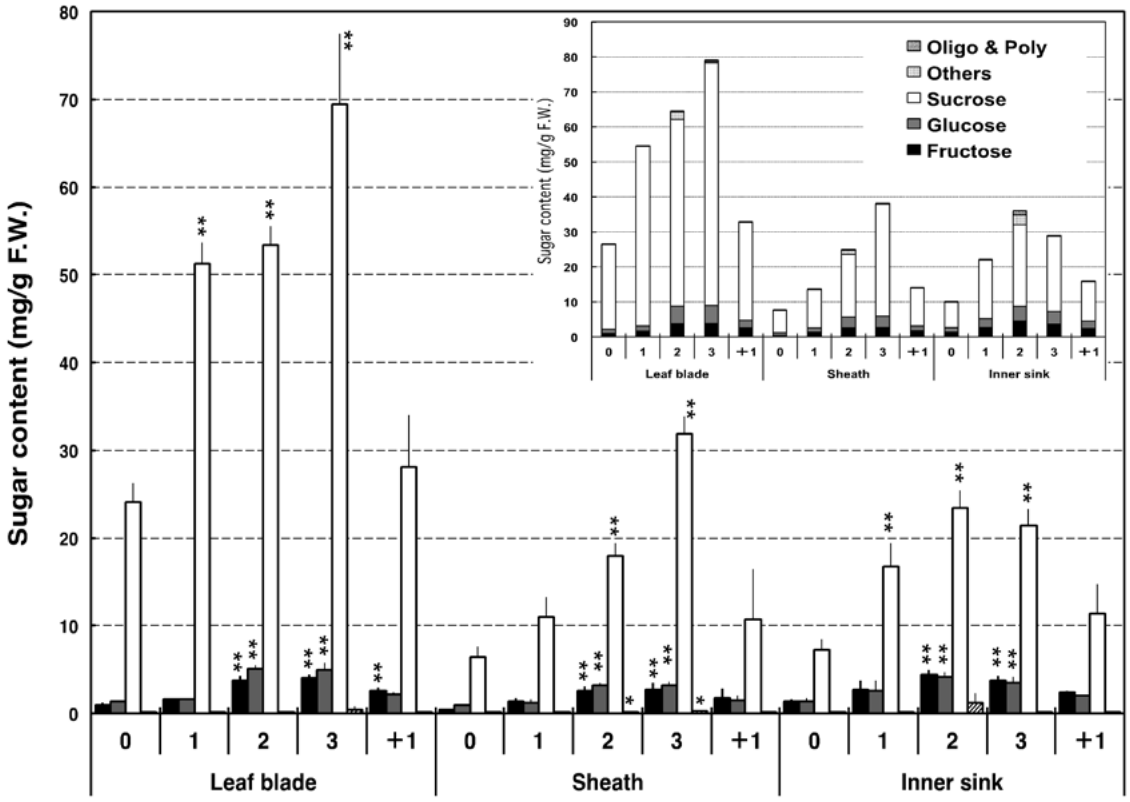

Fig. 2. Changes in the amounts of fructose, glucose, sucrose, and oligo- and polysaccharides in the 6th leaf blade and its sheath and in the inner sink tissue of approximately 6-week-old rice plants (cv. Yukihikari) before (day 0), during (days 1, 2 and 3), and after (day +1) chilling treatment (12°C).
The inset shows changes in total sugar content and its constituent components (fructose, glucose, sucrose, other simple sugars, oligo- and polysaccharides). Data were obtained from three independent plants. Bars indicate standard deviations (n = 3). Significant differences compared to day 0 are indicated by $*P < 0.05$ or $**P < 0.01$.

that before chilling, although less than half of that in the leaf blades. The sucrose content in the inner sink tissues was about double and triple that before the treatment, on the first and second days of chilling, respectively. The content was similar to the levels found in sheaths. A continuous increase in sucrose content in the inner sink tissues was not detected on the third day of chilling treatment.

2. Analysis of the expression levels of *OsSUTs* in young rice plants subjected to chilling

Figure 3 shows changes in the expression levels of *OsSUT1*, *OsSUT2* and *OsSUT4*. The expression levels of *OsSUT3* and *OsSUT5* are not shown in Fig. 3 because both were very low in the tissues tested. The expression level of *OsSUT1* was higher in the sheath than in the leaf blade and sink tissue under normal conditions. The expression levels of *OsSUT1* in the sheath tended to decrease during the chilling treatment but recovered to the levels before chilling upon the return to normal temperatures. In contrast, the expression levels of *OsSUT1* in the 6th leaf blade increased for the first two days during the chilling treatment and decreased thereafter. The expression of *OsSUT2* and *OsSUT4* responded similarly to chilling, although the expression level of *OsSUT4* was low (Fig. 3). Both genes were up-regulated in the leaf blades on the first day of chilling treatment. The expression levels decreased thereafter. One day after the completion of chilling, the expression levels returned to the levels before chilling. A similar trend was observed to a lesser extent in the sheaths and not significantly in the sink tissues.

3. Changes in soluble sugar contents in booting-stage rice plants exposed to chilling

We examined changes in soluble sugar content in the panicles and other tissues of two booting-stage japonica cultivars (Yukihikari and Oborozuki) subjected to the same chilling treatment. The two cultivars showed similar responses (Fig. 4). The relative amounts of sucrose within total sugars decreased in sink tissues, while the relative contents of fructose and glucose increased toward the inner sink tissues. Sucrose levels were high in the penultimate leaf blade and showed a rapid increase after the start of chilling treatment; the levels decreased after the return to normal temperatures. The sucrose content in the sheaths of the penultimate leaf and in the flag leaf also increased during chilling treatment. The highest rate of increase in sucrose was observed in the sheaths of the penultimate leaf, in which sucrose content on the fourth day of chilling treat-

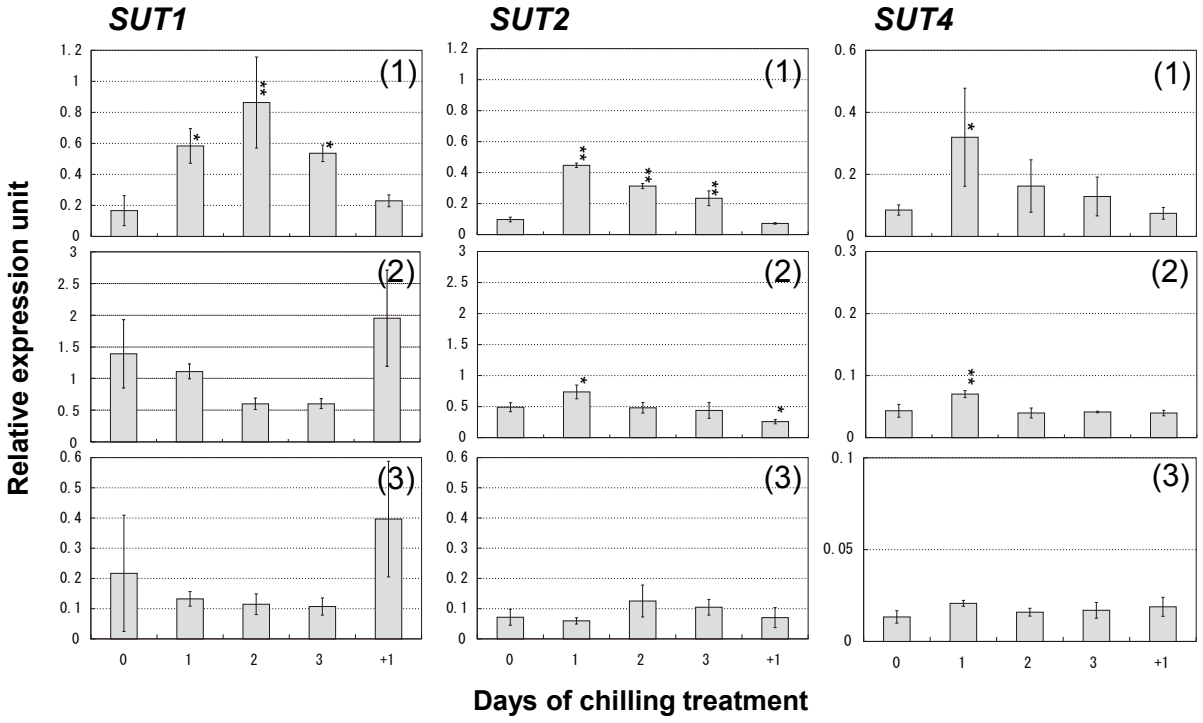

Fig. 3. Changes in mRNA levels of *OsSUT1*, *OsSUT2* and *OsSUT4* genes in the 6th leaf blade (1), its sheath (2), and inner sink tissue (3) of approximately 6-week-old rice plants (cv. Yukihikari) before (day 0), during (days 1, 2 and 3) and after (day +1) chilling treatment (12°C).
The mRNA levels of OsSUT genes were normalized against those of a ubiquitin gene. Data were obtained from three independent plants. Bars indicate standard deviations (n = 3). Significant differences compared to day 0 are indicated by **P* < 0.05 or ***P* < 0.01.

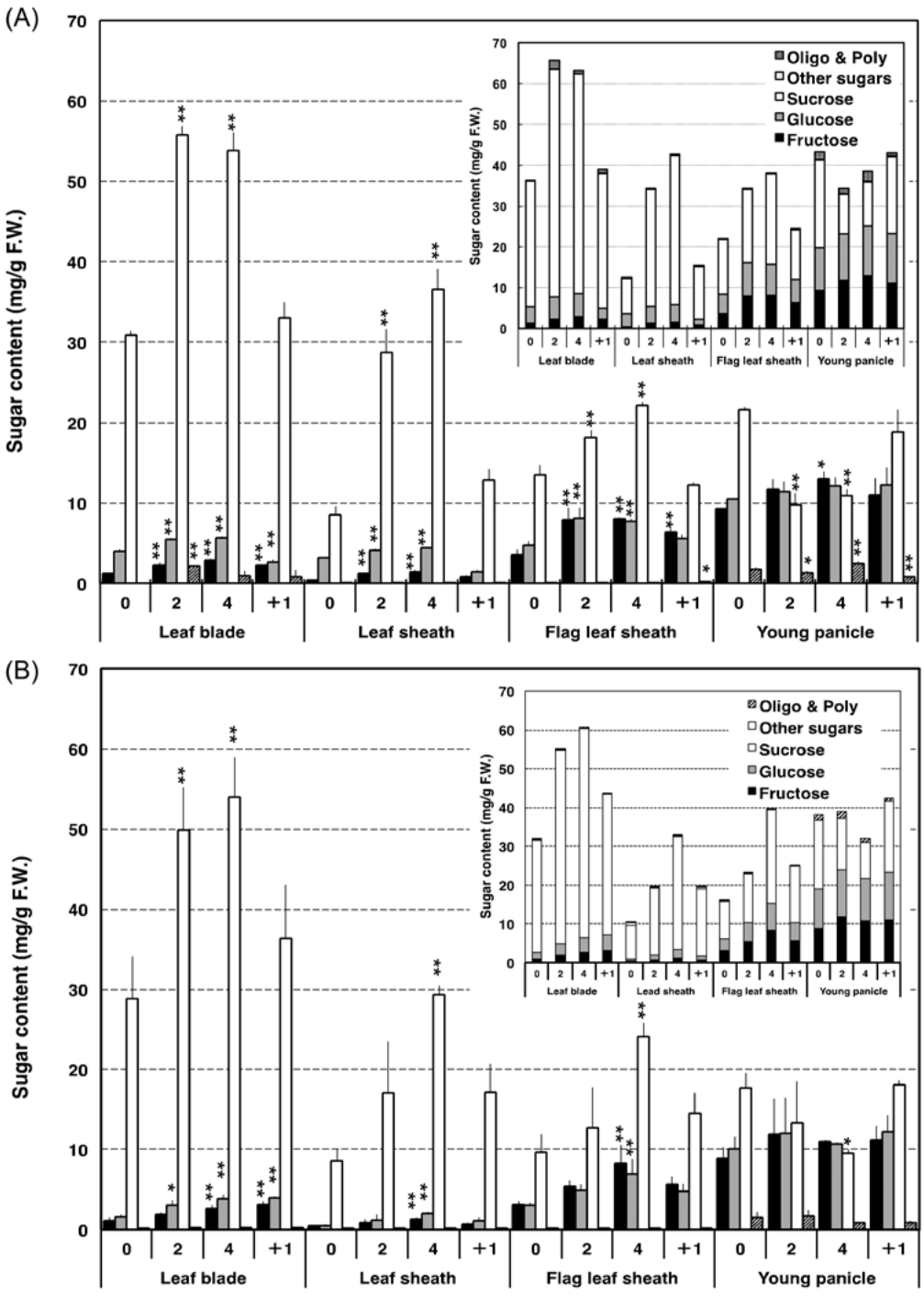

Fig. 4. Changes in the amounts of fructose, glucose, sucrose, and oligo- and polysaccharides in the penultimate leaf blade and its sheath (leaf sheath), the flag leaf sheath, and the young panicles of booting-stage rice plants (A: Yukihikari, B: Oborozuki) before (day 0), during (days 2 and 4) and after (day +1) chilling treatment (12°C).

The data for Yukihikari and Oborozuki were derived from independent examinations in different seasons. Data were obtained from three independent plants. Bars indicate standard deviations (n = 3). The inset shows changes in total sugar content and its constituent components (fructose, glucose, sucrose, other simple sugars, oligo- and polysaccharides). Significant differences compared to day 0 are indicated by $*P < 0.05$ or $**P < 0.01$.

ment was about four times higher than that under normal conditions. In contrast, the sucrose content only tended to decrease in young panicles during the chilling treatment whereas glucose and fructose did not decrease in the same tissues.

4. Effect of chilling on expression of *OsSUTs* in rice plants at the booting stage

The effect of chilling treatment on the expression of *OsSUT1*, *OsSUT2* and *OsSUT4* was determined in various tissues of two rice cultivars (Yukihikari and Oborozuki) at the booting stage (Fig. 5). Overall, the responses to chilling of these genes in the two cultivars were quite similar except for the responses of *OsSUT2* in sheath tissues. The transcript levels of *OsSUT3* and *OsSUT5* were very low in the tissues tested (data not shown). The expression responses of *OsSUT1* and *OsSUT2* to chilling treatment in the penultimate leaf, its sheath, and sheath of the flag leaf except for the responses of *OsSUT2* in sheath tissues of Oborozuki (Fig. 5) were similar to those in the leaf blade and its sheath, and in the inner sink tissue of young rice plants (Fig. 3). Expression levels of *OsSUT1* in the sheath decreased during the chilling treatment and then recovered upon the return to normal temperatures. In contrast to young plants, the expression pattern of *OsSUT4* in the sheath was similar to that of *OsSUT1* (Fig. 5). In young panicles, the transcript levels of *OsSUT1* continued to increase regardless of treatment, whereas the transcript levels of *OsSUT2* and *OsSUT4* decreased during the chilling treatment and recovered after the return to normal temperatures (Fig. 5).

Discussion

1. Effect of changes in expression of *OsSUTs* on the sucrose content of rice tissues subjected to a chilling temperature

Of the various sugars, sucrose is present at very high levels in the leaf blade and sheath of the source tissues in rice. Our results showed that the sucrose content of these tissues increased during chilling treatment (Figs. 2 and 4), regardless of the plant growth stage. In contrast, the increase in sucrose content in the sink tissues of young plants was suppressed on the third day of chilling treatment (Fig. 2), and sucrose levels decreased in young panicles during chilling treatment at the booting stage (Fig. 4). This suggests that the amount of sucrose transport in the sheath decreased more toward the inner sink tissues that are far from the primary source leaves, though changes in the activities of invertase and/or sucrose synthase may also be involved among a number of related metabolisms. Our results showed that the expression level of *OsSUT1* in the sheath was higher than that in the leaf blade at normal temperatures. However, *OsSUT1* expression was repressed during chilling treatment in the sheath in young plants and in mature plants at the booting stage (Figs. 3 and 5), consistent with the observed increase in sucrose content in source tissues and decrease in young panicles (Figs. 2 and 4) as OsSUT1 may play a role in retrieval by loading leaked sucrose from the apoplasm back into phloem in the sheath as proposed by Scofield et al. (2007b). In young panicles, the transcript levels continued to increase regardless of temperature (Fig. 5). It was previously reported that *OsSUT1* expression in panicles and developing caryopses increased during heading (Aoki et al. 2003, Chen & Wang 2008, Hirose et al. 1999). The increase in *OsSUT1* expression at the booting stage observed in this study might also reflect a strong demand for sugars during this developmental phase (Fig. 5). The expression patterns of *OsSUT4* in the leaf and sheath at the booting stage were similar to those of *OsSUT1* (Fig. 5), though the transcript levels of *OsSUT4* in these tissues were lower than those of *OsSUT1*. Since OsSUT4 has been suggested to play a role in sucrose loading into phloem of the source leaf sheath, the down-regulated expression of *OsSUT4* in the sheath during chilling treatment is expected to have an effect on sucrose translocation from the leaves to the inner sink tissues, as does OsSUT1 in the same or a different manner.

The expression of *OsSUT2* also responded to chilling treatment. *OsSUT2* is in the same *SUT4* clade as *Arabidopsis SUT4* (*AtSUT4*), the expression of which is regulated by sucrose levels (Lundmark et al. 2006). The changes in expression of *OsSUT2* in rice tissues appeared to be related to changes in sucrose content of the tissues induced by chilling treatment. The OsSUT2 function of sugar export from leaf tissues has been proved (Eom et al. 2011). And the increase in the expression of *OsSUT2* in the leaf blade during chilling treatment was significant. It may induce an increase in sucrose export from the leaf blade to sheath, which is related to an increase in sucrose content in the sheaths observed in our study.

2. Relationship between cold tolerance and transport of sugars at the booting stage

In this study, we found that chilling caused a decrease in the sucrose content of young panicles at the booting stage. A number of candidate metabolic pathways might be involved in this change. Our results showing low-temperature repression of *OsSUT1* and *OsSUT 4* expression in rice sheath tissues (Fig. 5), which corresponds to the changes in total sucrose levels in sink tissues (Fig. 4), may support the explanation that the amount of sucrose translocation (including that by the symplastic pathway and by apoplastic loading) toward the panicles is down-regulated during chilling treatment. The young panicles examined in this study included various types of tissue. The disruption of *OsSUT1* by Tos17 or antisense suppression of *OsSUT1* expression

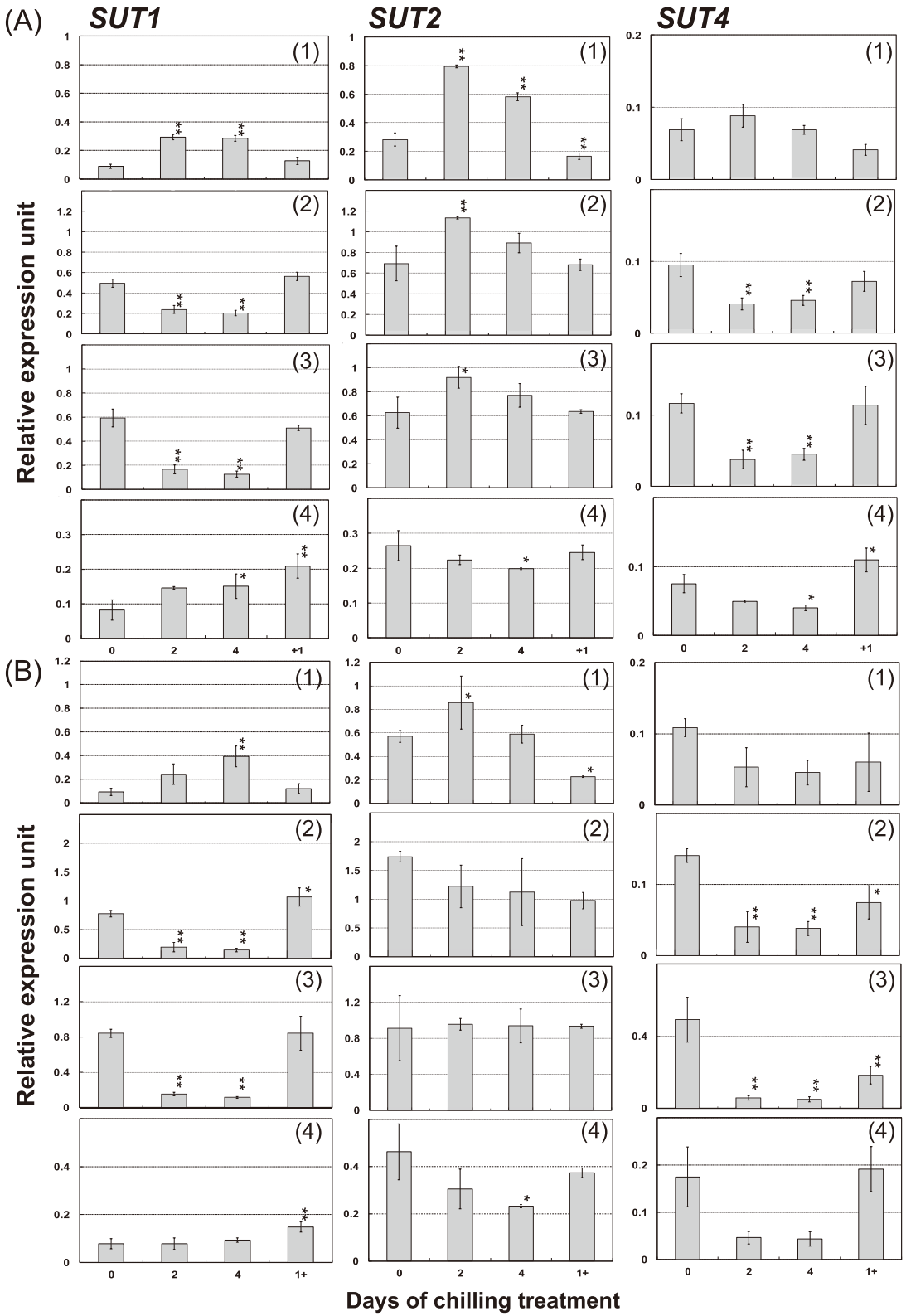

Fig. 5. Changes in mRNA levels of *OsSUT1*, *OsSUT2* and *OsSUT4* genes in the penultimate leaf blade (1), its leaf sheath (2), flag leaf sheath (3), and young panicles (4) of booting-stage rice plants (A: Yukihikari, B: Oborozuki) before (day 0), during (days 2 and 4) and after (day +1) chilling treatment (12°C).
The mRNA levels of SUT genes were normalized against those of a ubiquitin gene. The data for Yukihikari and Oborozuki were derived from independent examinations in different seasons. Data were obtained from three independent plants. Bars indicate standard deviations (n = 3). Significant differences compared to day 0 are indicated by $*P < 0.05$ or $**P < 0.01$.

has reportedly had effects on the pollen function (e.g., pollen germination) or on the growth and production of filled grain, resulting in decreased grain weight (Hirose et al. 2010, Ishimaru et al. 2001, Scofield et al. 2002). These reports also suggest that the decrease in expression levels of *OsSUT1* in rice sheaths at the booting stage observed in our study may affect the development of anthers, including pollens.

Eom et al. (2011) reported that OsSUT2 is a tonoplast H^+-sucrose symporter that is localized in many organs including the spikelet, and that an *ossut2* mutant with no expression of *OsSUT2* showed repression of plant growth and grain weight, suggesting that OsSUT2 functions in sucrose uptake from the vacuole. This shows that the expression of *OsSUT2* is essential for the normal growth of reproductive organs. In our study, the expression of *OsSUT2* in young panicles decreased during chilling treatment. This decrease in *OsSUT2* expression suggests a decrease in sucrose supply from the vacuoles (tonoplast) in panicle cells to reproductive organs. Of these tissues, pollen is localized in the anther locule and lacks connections to other cells via plasmodesmata. It is known that tapetal hypertrophy (Nishiyama 1976, Oliver et al. 2005) and sucrose accumulation (Ito 1978, Kawaguchi et al. 1996) occur in anthers in rice exposed to chilling at around the young microspore stage. Eom et al. (2011) also showed that the decrease in sucrose export activity from the cells of *ossut2* resulted in sucrose accumulation in the leaf. Our results further imply that the coincident occurrence of the decrease in sucrose supply to anthers described above and the impairment of sucrose export from anther cells (such as tapetum cells) by decreased expression of *OsSUT2* leads to an imbalance of sugar concentration involved in osmotic pressure between cells and the apoplast in anthers.

It was recently shown that OsSUT4 was localized in various tissues in the spikelet during the pre-heading period, in the stigma and anther on the flowering day, and in germinating pollen (Chung et al. 2014). In that study, the expression levels of *OsSUT4* in anthers under the condition of 15/13°C treatment that impaired starch formation in pollen and anthers, were lower than those with 30/23°C treatment. This decrease in expression is consistent with our results on panicles. Sucrose exported from anther tissues into the locule is thought to be hydrolyzed by invertase (INV). The hexose produced may be transported to pollen by a monosaccharide transporter (MST). Chung et al. (2014) proposed that OsSUT4 might function in this process of pollen development. On the other hand, microarray analysis of rice anthers showed that the expression of an invertase and two sucrose synthase genes was down-regulated by chilling (Yamaguchi et al. 2004). It has also been reported that the expression of *OsINV4* and *OsMST8* (anther-specific genes) decreased in rice exposed to low temperatures, and that the decrease in the expression of *OsINV4* and *OsMST8* during chilling was associated with poor pollen wall formation, abnormal vacuolation and hypertrophy of the tapetum, and unusual starch accumulation in post-meiotic anthers (Mamun et al. 2006, Oliver et al. 2005, 2007). Thus, the changes in expression of *OsSUT1*, *OsSUT2* and *OsSUT4* accompanied by other genes related to sugar transport (Lemoine et al. 2013) should cause the repression in sugar supply to the anther/pollen via long- and short-distance sucrose transport from source tissues during chilling.

In conclusion, we showed decreases in the expression of *OsSUT1* and *OsSUT4* in rice sheaths during chilling treatment at the young and booting stages. The resulting decrease in long-distance sucrose transport from source to sink tissues seems related to the increase in sucrose contents in the sheaths of both young and mature rice plants, and the decrease in sucrose content in panicles at the booting stage. The expression of *OsSUT2* in panicles decreased during the chilling treatment. This is expected to induce a decrease in sucrose export from the panicle cells. Thus, these events may result in a decrease in sucrose supply to the apoplast in the anther that experienced chilling treatment. The chilling-induced decrease in *OsSUT4* expression in panicles observed in this study may also cause impaired of sucrose transport to anther locules followed by hexose supply to pollen. The down-regulation of OsSUT genes may lead to the fatal impairment of pollen development under the condition of chilling stress.

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