Carbohydrate Changes in Peach Shoot Tissues and Their Relationship to Cold Acclimation and Deacclimation

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This study was performed to examine differences in cold hardiness, carbohydrate content, and β-amylase gene expression during cold acclimation and deacclimation in shoots of two peach cultivars (Prunus persica ‘Daewol’ and ‘Kiraranokiwami’). During cold acclimation (from September to November 2011), cold hardiness of the cultivars as determined by electrolyte leakage analysis increased dramatically. Maximum cold hardiness was reached at the end of December 2011 in both cultivars. During deacclimation (late January to April 2012), the cold hardiness of both cultivars decreased gradually. According to the temperatures at which 50% injury occurred (LT₅₀), there was a noticeable difference in cold hardiness between ‘Daewol’ and ‘Kiraranokiwami’ during the deacclimation period from the end of January 2012. Cold hardiness was significantly associated with total soluble sugar, sucrose, and β-amylase gene expression in both cultivars. Sorbitol, the most abundant soluble sugar, was not associated with cold hardiness in either cultivar. Glucose and fructose contents in ‘Daewol’, which was more freezing-tolerant, were approximately double those in ‘Kiraranokiwami’ from January to February 2012. These results suggest that the conversion of sucrose to glucose and fructose might be correlated with the difference of cold hardiness during deacclimation in these peach cultivars.

Key Words: β-amylase, cold hardiness, freezing injury, Prunus persica, soluble sugars.

Introduction

Peach trees (Prunus persica), which are known to possess insufficient tolerance to freezing (Flore, 1994), have been damaged by winter freezes and/or spring frosts combined with unseasonably warm spells—irregular dynamics that result from global climate change—for the past few years (Johnson and Howell, 1981; Kim et al., 2012; Pagter et al., 2011a). Thus, there is a greater need for cultivars that are adaptable to irregular changes in temperature than for very hardy cultivars that tolerate extremely low temperatures.

Freezing temperatures can cause significant losses in the productivity of overwintering temperate trees and can limit their geographical distribution and growth. As temperatures decrease below 0°C, freezing injury results primarily from severe cellular dehydration (Levitt, 1980; Steponkus and Webb, 1992) and involves freeze-induced denaturation of proteins, precipitation of various molecules, and direct physical damage caused by the accumulation of intercellular ice (Thomashow, 1998).

The ability of most trees to survive freezing temperatures is improved by gradual exposure to low, non-freezing temperatures, a phenomenon known as cold acclimation (Thomashow, 1999). Cold acclimation involves multiple mechanisms, such as changes in membrane lipid composition, accumulation of hydrophilic polypeptides, and production of sucrose and other sugars, which stabilize membranes against freezing temperatures (Arora and Wisniewski, 1994; Guy et al.,...
1980; Steponkus et al., 1993). Deacclimation, the reverse process of cold acclimation, is mainly driven by warm temperatures and occurs more rapidly than it (Kalberer et al., 2006).

Previous studies in sibling deciduous and evergreen peach genotypes confirmed transitions in cold hardiness during cold acclimation and deacclimation, focusing mainly on cold acclimation (Arora and Wisniewski, 1994; Arora et al., 1992). However, as a result of global climate change, temperature patterns in temperate regions during late winter and spring have become increasingly irregular, with unseasonably warm spells (Pagter et al., 2011a), and research on deacclimation in temperate perennial plants has become of increasing interest.

Various biochemical and molecular analyses have demonstrated changes in soluble carbohydrates and differential expression during cold acclimation and deacclimation in herbaceous and woody plants (Anderson et al., 2005; Pagter et al., 2008; Schrader and Sauter, 2002; Yoshida et al., 1998). In fall, starch produced during the summer is hydrolyzed into soluble carbohydrates that accumulate in cells (Schrader and Sauter, 2002). In spring, starch is resynthesized and then mobilized during bud break (Pagter et al., 2011a). In freezing-tolerant tissues, the protective function of sugars involves stabilizing membranes and proteins during freeze-induced dehydration (Crowe et al., 1998). Sugars are believed to depress the nucleation temperature in cells undergoing deep supercooling (Kasuga et al., 2007). Sucrose is probably the most studied soluble carbohydrate in relation to cold hardiness (Pagter et al., 2008), but glucose and fructose have also frequently been found to accumulate during cold acclimation (Améglio et al., 2004; Wong et al., 2003). González-Rossia et al. (2008) reported that sorbitol, fructose, and glucose were the main sugars in bark tissues, while contents of sucrose and raffinose were lowest in all *Prunus* spp. studied. In addition, sorbitol and starch contents decreased whereas glucose and fructose contents increased at low temperature (3°C) (González-Rossia et al., 2008). Of the various enzymes and genes related to carbohydrate metabolism, the primary function of β-amylase in plants is to break down starch, and this function is induced by low temperature (Kaplan and Guy, 2004; Kossmann and Lloyd, 2000). The expression and activity of β-amylase are regulated by abiotic stressors including osmotic, salt, and cold stresses (Dreier et al., 1995; Nielsen et al., 1997). Most previous studies in peach focused on associations between cold hardiness and the expression of dehydrin proteins and genes (Arora and Wisniewski, 1994; Arora et al., 1992; Bassett et al., 2009; Wisniewski et al., 2006). Very little information is available on changes in carbohydrate metabolism and related genes during cold acclimation and deacclimation in peaches.

The objectives of this study were to (1) estimate seasonal changes in cold hardness, and (2) determine seasonal changes in carbohydrate content and related gene expression during cold acclimation and deacclimation in two peach cultivars (*Prunus persica* ‘Daewol’ and ‘Kiraranokiwami’). Our findings should facilitate further research on deacclimation and reacclimation related to recent irregular climatic changes.

**Materials and Methods**

**Plant materials**

Two peach cultivars (*Prunus persica* ‘Daewol’ and ‘Kiraranokiwami’) were selected according to the result of a preliminary experiment. We had performed a preliminary experiment to select the longest chilling-required cultivar and the shortest one among 10 peach cultivars (*P. persica* ‘Aikawanakajima’, ‘Daewol’, ‘Izumi Hakuto’, ‘Janghowon Hwangdo’, ‘Kiraranokiwami’, ‘Mihong’, ‘Misshong’, ‘Soomee’, ‘Suhong’, and ‘Sun Gold’). Branch cuttings from 10 cultivars of 7- to 8-year-old deciduous peach trees in a peach orchard in Cheongju, Korea (latitude 36°39′03″N, longitude 127°24′53″E), were placed in a greenhouse [kept under an 11-h photoperiod at 105 μmol·m⁻²·s⁻¹ and 25/19°C (day/night)] on January 15 to 25, 2011, at 5-d intervals to force floral bud break. After 14 d, the chilling requirement of each cultivar was considered to be satisfied when more than 50% of the floral buds on the branch cuttings opened. More than 50% of ‘Kiraranokiwami’ floral buds placed in a greenhouse on January 15 and more than 50% of ‘Daewol’ floral buds placed on January 25 opened within 14 days (data not shown).

Current-year shoots from two cultivars of 7- to 8-year-old deciduous peach trees were collected at the end of each month from August 31, 2011, to April 30, 2012, in a peach orchard in Cheongju, Korea (latitude 36°39′03″N, longitude 127°24′53″E). Thirty-two or thirty-five samples were randomly collected from four to five trees of each cultivar. Samples were pooled for each cultivar and packed on ice, brought to the laboratory, and appropriately processed for the experiments, as described below.

**Determination of cold hardness**

Cold hardiness was determined by the electrolyte leakage (EL) method described by Arora et al. (1992) and Pagter et al. (2008), with slight modification. We randomly collected 18–21 shoot segments (5-cm length of xylem with bark) with similar diameters from the upper portion of current-year shoots of four to five trees of each cultivar. The shoot segments were rinsed under cold running tap water for 15 s and then 3 shoot segments were each placed in a 50-mL conical tube containing 1 mL of distilled water to initiate ice formation. The tubes were incubated in a refrigerating bath circulator (RW-2040G; Jeio Tech, Daejeon, Korea) equipped with a temperature controller and cooled at a rate of
5°C/h until the target temperature was reached. The starting temperature was 5°C for the freezing test. The target temperature was maintained for 2 h, after which the tubes were withdrawn and thawed at 4°C. Four or five target temperatures, which had been confirmed in our previous experiment (Shin et al., unpublished data), were selected as follows: 5, −5 to −20, and −80°C (shoots were kept in a deep freezer for 2 h to induce maximum EL) from August to October 2011, and April 2012; and 5, −15 to −35, and −80°C from November 2011 to March 2012.

After the freezing treatment, 1 shoot was cut into 1-cm-long sections that were placed in 15-mL conical tubes containing 8 mL of distilled water. The tubes were shaken at 125 rpm on a shaker (Green Seriker VS-202D; Vision Scientific, Daejeon, Korea) at room temperature for 20 h and electrical conductivity (EC) of the aliquots was then measured using an EC meter (TetraCon 325; WTW GmbH, Germany). After samples had been autoclaved at 120°C for 30 min, EC was measured again, and percent injury was calculated according to the method of Pagter et al. (2008). Quantitative values of shoot cold hardness, estimated as temperatures that resulted in 50% injury (LT50), were calculated by the Gompertz function, an asymmetric sigmoid curve that is commonly used to analyze data on plant responses to temperature stress (Lim et al., 1998).

Carbohydrate analysis

Seven shoots were randomly collected monthly from August 2011 to April 2012 from the same trees used for the cold-hardiness determination, to analyze carbohydrate contents. Shoot segments collected from both cultivars were immediately frozen in liquid N2, lyophilized, ground using a mill (Cyclotec 1093; Foss Tecator Technology, Höganäs, Sweden) with a 60-mesh sieve, and stored as powder at −80°C until use.

Soluble sugars were extracted using the method described by González-Rossia et al. (2008) with slight modifications. Ground powder (200 mg) was placed in a 15-mL conical tube containing 10 mL of 80% ethanol and incubated using an ultrasonic extractor (WUC-D22H; Daihan Scientific, Seoul, Korea) at 25°C for 1 h. After centrifugation at 1500 × g for 10 min at 4°C, the supernatants were collected and evaporated using a rotary evaporator (Eyela N-1000; Rikakikai, Tokyo, Japan) at 60°C; pellets were saved for further starch analysis. The ethanolic solution of sugar extracts was dissolved in 2 mL of HPLC water and passed through a C18 Sep-Pak cartridge (Waters Associates, Milford, MA, USA) and a 0.45-μm nylon syringe filter (Noble Biosciences, Hwaseong, Korea). Sugars were analyzed using a high-performance liquid chromatograph (Alliance-2795 HPLC; Waters Associates) connected to a detector (2420-ELSD; Waters Associates). Filtered extracts (10 μL) were injected into a Sugar-Pak column (Shodex NH2P-50; Showa Denko, Tokyo, Japan) with temperature maintained at 30°C; distilled water was used as the solvent at a flow rate of 0.5 mL·min⁻¹.

Starch in the remaining pellet was quantified by the dinitrosalicylic acid method (Araújo et al., 2004; Miller, 1959). The pellet was dissolved in 2 mL of distilled water, autoclaved to gelatinize starch granules, and then incubated at 55°C for 2 h for hydrolysis with 1 mL of 0.2 M sodium acetate (pH 5.5) buffer, 30 U amyloglucosidase (A7095; Sigma-Aldrich, Yongin, Korea), and 10 U β-amylase (A3404; Sigma-Aldrich). The supernatants were collected after centrifugation at 13000 × g for 10 min. Dinitrosalicylic acid reagent (0.2 mL) was added to 0.1 mL of supernatant. The mixtures were heated for 5 min in a boiling water bath. After cooling to room temperature, 0.9 mL of distilled water was added, and absorbance at 525 nm was measured using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan).

RNA extraction and β-amylase gene expression analysis

Seven shoots of each cultivar were randomly collected, immediately frozen in liquid N2, ground using a mortar and pestle, and stored at −80°C until use. Total RNA was extracted according to the protocol described by Chang et al. (1993) and Gasic et al. (2004). Finely ground samples were placed into 2.0-mL sample tubes. Then, 900 μL of RNA extraction buffer [2% cetlytrimethylammonium bromide (CTAB), 2% polyvinylpyrrolidone (PVP, K-30), 100 mM Tris-HCl (pH 8.0), 25 mM EDTA (pH 8.0), 2 M NaCl, 0.05% spermidine, 2% β-mercaptoethanol] and an equal volume of chloroform:isoamylalcohol (24:1) were added, and the mixture was stirred thoroughly. Then, the sample tubes were centrifuged at 13000 × g at room temperature for 15 min. The supernatant was moved to a new sample tube and 125 μL of 8 M LiCl was added and mixed. RNA was precipitated overnight at 4°C and harvested by centrifugation at 13000 × g and 4°C for 30 min. After the settled pellet was confirmed, the supernatant was discarded. The pellet was dissolved in 500 μL of SSE [1 M NaCl, 0.5% sodium dodecyl sulfate (SDS), 10 mM Tri-HCl (pH 8.0), 1 mM EDTA (pH 8.0)] and an equal volume of chloroform:isoamylalcohol (24:1), and the mixture was stirred thoroughly. Then, the sample tubes were centrifuged at 13000 × g at room temperature for 15 min and the supernatant was moved to a new tube. Two volumes of ethanol were added to the supernatant and the sample tubes were precipitated at −80°C for at least 30 min. Then, the sample tubes were centrifuged at 13000 × g and 4°C for 30 min. The supernatant was discarded after the settled pellet was confirmed. Finally, the pellet was dried and dissolved in 40 μL of RNase-free water (Qiagen, Dusseldorf, Germany).

Complementary DNA (cDNA) synthesis of 2 μg of total RNA was performed using a QuantiTect reverse transcription kit (Qiagen). Quantitative real-time RT-PCR was performed using a 2× QuantiMix SYBR Kit...
(PhileKorea, Seoul, Korea) with a Rotor-Gene 6000 Real-Time Cycler (Corbett Research, Mortlake, Australia). PCR conditions included a pre-denaturing step at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 50°C for 15 s, and extension at 72°C for 30 s. Relative expression of RNA was calculated using Rotor-Gene 6000 Real-Time Rotary Analyzer 1.7 software and the delta delta Ct method (Corbett Research, Mortlake, Australia). Relative gene expression values were calculated in comparison with gene expression levels measured in ‘Daewol’ in December 2011, expressed as 1. Three technical replicates were performed.

β-amylase (GenBank accession No. JQ911593) was used as a target gene and its primers were obtained from Lee et al. (2012) as follows: forward 5'-GGA ATG CTG CAA GAG TCT CC-3' and reverse 5'-CGA TGA ATG CCA GTT TGA TG-3'. A BLAST search against the GenBank EST database permitted the nucleotide sequences of peach corresponding to the β-amylase gene to be found. The similarity level between Vaccinium corymbosum and the peach EST was 90% for 207 nucleotides. RNA polymerase II (GenBank accession No. AT2G15430) was used as a housekeeping gene and its primers were designed according to Tong et al. (2009) as follows: forward 5'-TGA AGC ATA CAC CTA TGA TGA AG-3' and reverse 5'-CTT TGA CAG CAC CAG TAG ATT CC-3'.

Statistical analysis
Statistical differences were assessed via analysis of variance (ANOVA) with the SAS 9.1 software package (SAS Institute Inc., Cary, NC, USA). The data analysis was performed using SigmaPlot 8.0 (Systat Software, Inc., San Jose, CA, USA). The correlation coefficients between LT<sub>50</sub> values and content of different carbohydrates or relative expression of β-amylase were examined using Pearson’s correlation coefficient (PROC CORR).

Results
Seasonal changes in air temperature
Daily air temperatures at the experimental site decreased consistently from August and reached the minimum temperature (−15.2°C) at the beginning of February (Fig. 1). Daily minimum temperatures were < 0°C for 94 days. The pattern of change in winter air temperature was not significantly different from the usual. However, the daily temperature range from February to March 2013 was greater than that during midwinter.

Seasonal changes in cold hardiness
Minimum cold hardiness in the cultivars occurred at the end of August 2011 (LT<sub>50</sub> = −4.1 and −1.4°C for ‘Daewol’ and ‘Kiraranokiwami’, respectively) (Fig. 2). During cold acclimation (September to November 2011), cold hardiness increased dramatically in both cultivars. Maximum cold hardiness was reached at the end of December 2011 (LT<sub>50</sub> = −61.1 and −55.1°C for ‘Daewol’ and ‘Kiraranokiwami’, respectively). During deacclimation (from the end of January to April 2012), cold hardiness decreased gradually in both cultivars. LT<sub>50</sub> values in April were −7.3 and −7.7°C for ‘Daewol’ and ‘Kiraranokiwami’, respectively, suggesting that cold-acclimated hardiness had been completely lost. Differences in cold hardiness of ‘Daewol’ and ‘Kiraranokiwami’, as represented by LT<sub>50</sub> values, were obvious throughout the experimental period, with the exceptions of November 2011 and April 2012.
Seasonal changes in carbohydrate content

At the beginning of the experiment, the level of starch temporarily increased in both cultivars (Fig. 3A). Thereafter, starch content decreased steadily to January 2012. However, starch content appeared to be relatively steady throughout the experimental period. Total soluble sugar content (including sucrose, sorbitol, glucose, and fructose) in shoots of the two peach cultivars increased during cold acclimation, coinciding with a steady decrease in starch content, and decreased during deacclimation (Fig. 3B). Soluble sugar contents varied to different extents depending on the sugar and the cultivar. Sucrose and sorbitol were the main sugars in shoots of both cultivars during cold acclimation, while glucose and fructose were present at low levels. Sucrose contents in ‘Daewol’ and ‘Kiraranokiwami’ were 4.9 and 2.7 mg·g\(^{-1}\) dry weight, respectively, at the beginning of cold acclimation, and reached a maximum in December 2011 (26.1 and 26.6 mg·g\(^{-1}\) dry weight, respectively) (Fig. 3C). Sorbitol was the most abundant sugar in the shoots, but significant seasonal patterns were not observed for it (Fig. 3D). Glucose and fructose contents showed slight changes during cold acclimation in both cultivars (Fig. 3E, F); both sugars accumulated dramatically at the beginning of deacclimation (January–February 2012) in both cultivars and those in ‘Daewol’ were approximately double those in ‘Kiraranokiwami’.

Correlation between cold hardiness and carbohydrate content

Seasonal changes in cold hardiness (represented by LT\(_{50}\) values) were negatively related to changes in total soluble sugar and sucrose in both cultivars (\(P \leq 0.001\)) (Table 1). Although increasing soluble sugar content and the development of cold hardiness coincided with decreasing starch content in both cultivars, indicating starch-to-sugar conversion, the correlation between cold
hardiness and starch content was not statistically significant. Sorbitol and glucose contents were not correlated with cold hardiness in either cultivar. Fructose content was negatively related to cold hardiness in ‘Daewol’ ($P < 0.01$).

**Relative expression of β-amylase**

Differences in the relative expression of β-amylase were confirmed by quantitative real-time RT-PCR in both peach cultivars ($P < 0.05$) (Fig. 4). Relative expression levels of β-amylase increased significantly during cold acclimation, showed a transient decrease in midwinter, and decreased during deacclimation. Relative expression of β-amylase was strongly correlated with cold hardiness ($r = −0.70$, $P < 0.001$ and $r = −0.76$, $P < 0.001$ for ‘Daewol’ and ‘Kiraranokiwami’, respectively).

**Discussion**

Cold hardiness in the peach cultivars ‘Daewol’ and ‘Kiraranokiwami’ increased dramatically during fall, reached a maximum in midwinter, and then gradually decreased (Fig. 2). Similar changes in cold hardiness have been observed in other studies of peach (Arora et al., 1992) and in *Hydrangea* species (Pagter et al., 2008). There was a significant increase in cold hardiness between September and October, at the early stage of cold acclimation. However, differences in cold hardiness between the cultivars were minimal during cold acclimation. Maximum cold hardiness of the two cultivars (LT$_{50} = −61.1$ and $−55.1^\circ C$ for ‘Daewol’ and ‘Kiraranokiwami’, respectively) was reached at the end of December 2011. Arora et al. (1992) reported that maximum LT$_{50}$ values of bark and xylem in deciduous peach were about $−50$ and $−36^\circ C$, respectively. Our cold hardiness data were lower than the data of the other peach (Arora et al., 1992), since the lowest temperature reached in the freezer was $−35^\circ C$ in our experiment and LT$_{50}$ values were induced from maximum EL values, determined by storage in a deep freezer for 2 h, in our samples (xylem with bark) from November 2011 to March 2012.

Cold hardiness began to decrease in January 2012 (Figs. 1 and 2). The two cultivars began to deacclimate at approximately the same time after the end of January when the ‘chilling requirement’ was satisfied (data not shown). Interestingly, during the deacclimation period (from the end of January to March 2012), LT$_{50}$ values of the more freezing-tolerant ‘Daewol’ indicated differences in cold hardiness above approximately $−8^\circ C$ compared with the more freezing-susceptible ‘Kiraranokiwami’ (Fig. 2). Although it is difficult to explain fully the deacclimation kinetics from the present experiment, ‘Daewol’ showed a slower rate of deacclimation than ‘Kiraranokiwami’ and significantly greater resistance to deacclimation. Deacclimation in spring is induced mainly by increased temperatures (Pagter et al., 2011b). Recent studies in other woody plants have also suggested that the rate of deacclimation is not linear (Kalberer et al., 2007; Pagter et al., 2011a; Rowland et al., 2005) and that there may be a lag phase during which exposure to warm temperatures does not instantly lead to deacclimation (Kalberer et al., 2006).

Seasonal changes in cold hardiness represented by LT$_{50}$ values were strongly correlated to total soluble sugar content in both cultivars (Table 1), as shown by many previous studies (Améglio et al., 2004; Morin et al., 2007; Palonen et al., 2000; Thomas et al., 2004). Sugars play an important role in plant growth and development under abiotic stress by regulating carbohydrate metabolism (Gupta and Kaur, 2005). The increase in total soluble sugar content during cold acclimation might have been a result of starch degradation, as reported for a wide range of woody plants (Ashworth et al., 1993; Levitt, 1980; Pagter et al., 2008; Sakai and Yoshida, 1968). Total soluble sugar content decreased rapidly during deacclimation and starch content increased slightly in February and March 2012. Starch is

### Table 1. Correlation coefficients between cold hardiness, estimated as the temperature at which 50% ion leakage (LT$_{50}$) occurred, and content of different carbohydrates in shoots of two peach cultivars (*Prunus persica* ‘Daewol’ and ‘Kiraranokiwami’) from August 2011 to April 2012.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>‘Daewol’</th>
<th>‘Kiraranokiwami’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Total soluble sugars</td>
<td>$-0.79^{***}$</td>
<td>$-0.66^{***}$</td>
</tr>
<tr>
<td>Sucrose</td>
<td>$-0.90^{***}$</td>
<td>$-0.87^{***}$</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Fructose</td>
<td>$-0.53^{**}$</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS Nonsignificant; ** significant at $P < 0.01$; *** significant at $P < 0.001$. 

![Graph](image_url)
the primary source of carbon for the growth and metabolism of developing buds in spring (Wong et al., 2003). Although starch provides a major reserve of carbohydrate in deciduous woody plants and can enhance cold hardiness via hydrolysis to soluble sugars (Morin et al., 2007), starch content in the shoots was not correlated with seasonal changes in cold hardiness in either cultivar (Table 1).

Analyses of the composition of xylem sap in peach trees indicate that hexoses (glucose and fructose) are present in addition to sorbitol and sucrose (Maurel et al., 2004a). González-Rossia et al. (2008) reported that sorbitol, fructose, and glucose were the main sugars induced in bark tissues by artificial chilling, whereas sucrose was present at the lowest quantities in various Prunus species. However, in our study, in which bark and xylem were not separated, sucrose and sorbitol were the primary soluble sugars in shoots during cold acclimation, while the quantities of glucose and fructose were low.

In freezing-tolerant tissues, the accumulation of sucrose and other simple sugars that typically occurs with cold acclimation stabilizes and protects membranes and proteins from freezing and dehydration (Crowe et al., 1998; Steponkus, 1984; Thomashow, 1999). Sucrose is probably the most studied soluble carbohydrate in relation to cold hardiness (Pagter et al., 2008) and has been associated with freezing tolerance in other woody plants (Cox and Stushnoff, 2001; Pagter et al., 2008; Palonen et al., 2000; Renaut et al., 2004). In our experiment, sucrose content was closely related to seasonal changes in cold hardiness (Fig. 3E, F). On the other hand, the glucose and fructose contents in ‘Daewol’, which was more freezing-tolerant, were approximately double those in ‘Kiraranokiwami’ from January to February 2012, when the lag phase and the maximum differences in cold hardiness between the two cultivars occurred. Our results imply that the dramatic accumulation of glucose and fructose during deacclimation might have been a result of the hydrolysis of sucrose into glucose and fructose. That might be characteristic of a hardy cultivar. Palonen (1999) reported similar results in canes and buds of red raspberry cultivars. In addition, glucose and fructose contents increased during bud break in April 2012. Maurel et al. (2004b) demonstrated that hexose contents in peach exhibited seasonal changes with strong accumulation before bud break.

Relative expression of β-amylase in the two peach cultivars increased during cold acclimation and decreased during deacclimation (Fig. 4). Although we observed the steady decline in starch contents in shoots of ‘Daewol’ and ‘Kiraranokiwami’ from fall to winter, relative expression of β-amylase decreased temporarily in midwinter (Figs. 3 and 4). β-amylase is an exoamylase that hydrolyzes the 1,4-glycosidic linkage of polyglucan chains at the non-reducing end to produce maltose (Kaplan and Guy, 2004). The primary function of β-amylase, which is induced by low temperature, is to participate in starch breakdown in plants (Kossmann and Lloyd, 2000). Although we found that β-amylase expression temporarily decreased in midwinter, changes in gene expression were strongly related to changes in cold hardiness (P < 0.001). Similar changes in β-amylase expression have been observed in blueberry cultivars (Lee et al., 2012). However, our results indicate that starch content was not correlated with seasonal changes in cold hardiness and starch content was not obviously correlated with sugar contents (Table 1; Fig. 3). In addition, because sucrose and glucose contents rather than starch were obviously related to deacclimation, the candidates for gene expression studies would be sucrose metabolism-related enzymes such as sucrose synthase and invertase, in addition to β-amylase.

In conclusion, the results presented here suggest that cold hardiness in peaches is associated with total soluble sugar content, sucrose content, and β-amylase gene expression, and that the conversion of sucrose into glucose and fructose might be correlated with deacclimation resistance in peach cultivars. Further research is required to obtain additional information about deacclimation and reacclimation during late winter and spring.

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