Carbohydrate Metabolism in 'Housui' Japanese Pear Floral Buds Exposed to Different Temperatures During Endodormancy

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Abstract - To elucidate the effects of temperature on carbohydrate metabolism under mild winter conditions, Japanese pear (Pyrus pyrifolia Nakai) shoots were exposed to temperatures of 0, 6 and 12 °C for 600 hours during endodormancy, and subsequent bud dormancy release and carbohydrate metabolism were examined. Lateral floral buds were collected after 0, 200, 400, and 600 hours under the treatments, and after the accumulation of 2000, 4000, 6000, and 8000 growing degree hours (GDH) under heat accumulation, for carbohydrate and enzyme activities analysis. The bud burst was earliest for the 12 °C treatment, followed by the 6 and 0 °C treatments. Sucrose concentration under heat accumulation decreased early in buds from the 12 °C treatment. The sorbitol and sucrose concentration in the buds increased under 0 °C during the endodormancy. In contrast, glucose and fructose concentrations tended to increase under heat accumulation. Sucrose synthase (SS) activity increased early under heat accumulation in buds from the 6 and 12 °C treatments, but in 0 °C buds it increased more slowly, from 2000 GDH onwards. Similarly, soluble acid invertase (AI) activity increased markedly in all treatments from the middle of the heat accumulation period. We therefore suggest that a temperature of 12 °C during endodormancy may advance bud burst as the result of earlier conversion of sucrose into hexoses.

Key words – Dormancy. Bud break. Sugar. Starch.

Resumo - Para elucidar os efeitos da temperatura no metabolismo de carboidratos em condições de inverno ameno, as gemas de pereira japonesa (Pyrus pyrifolia Nakai) foram expostas sob temperaturas de 0, 6 e 12 °C por 600 horas durante a endodormência, e a subsequente quebra de dormência das gemas e o metabolismo de carboidratos foram examinados. Gemas florais foram coletadas após o acúmulo de 0, 200, 400 e 600 horas de frio (CH-chilling hours) e após o acúmulo da soma térmica de 2000, 4000, 6000 e 8000 (GDH-growing degree hours) para análise de carboidratos e atividade enzimática. A brotação de gemas ocorreu mais cedo para o tratamento sob temperatura de 12 °C, seguido pelos tratamentos sob temperaturas de 6 e 0 °C. A concentração de sacarose durante o acúmulo de calor diminuiu rapidamente em gemas sob temperatura de 12 °C. A concentração de sorbitol e sacarose aumentou sob temperatura de 0 °C durante a endodormência. No entanto, as concentrações de glicose e frutose tendeu-se a aumentar durante o acúmulo de calor. A atividade da sacarose sintase (SS) aumentou mais cedo durante o acúmulo de calor em gemas dos tratamentos sob temperaturas de 6 e 12 °C, porém sob temperatura de 0 °C a atividade aumentou lentamente a partir de 2000 GDH. De modo semelhante, a atividade da invertase ácida solúvel (AI) aumentou acentuadamente em todos os tratamentos a partir da metade do período de acúmulo de calor. Portanto, sugere-se que a temperatura de 12 °C durante a endodormência pode promover a brotação devido a rápida conversão da sacarose em hexoses.

Palavras-chave – Dormência. Quebra de dormência. Açúcar. Amido.

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1 INTRODUCTION

Dormancy is defined as a period of inability to initiate growth under favorable conditions (ROHDE; BHALERAO, 2007), and is a complex event that enables plants to survive long periods of adverse conditions (ARORA; ROWLAND; TANINO, 2003). Lang *et al.* (1987) classified dormancy into three different types: paradormancy, when growth is influenced by other plant parts, such as apical buds; endodormancy, when growth is controlled by a physiological process within the bud; and ecodormancy, when growth is prevented by environmental factors. The accumulation of time below a certain chilling temperature is the primary factor involved in the release of endodormancy in deciduous fruit trees (LANG, 1996). In mild winter regions, where chilling requirements are not fully satisfied under natural conditions, dormancy may be a serious problem in commercial fruit orchards (DOZIER *et al.*, 1990; WALTON; CLARK; BOLDINGH, 1991; DOOKOOZLIAN *et al.*, 1995; BEN MOHAMED *et al.*, 2012).

Temperature is the most important environmental factor involved in the processes of dormancy initiation and termination (PÉREZ; LIRA, 2005). Temperatures may inhibit or release bud dormancy in the Japanese pear (*Pyrus pyrifolia* Nakai) (SUGIURA; HONJO, 1997). Temperatures below 12 °C may cause dormancy induction in apple and pear (HEIDE; PRESTRUD, 2005), while temperatures of 0 to 6 °C are most effective for endodormancy release in the Japanese pear (SUGIURA; HONJO, 1997). Although many studies have been conducted to determine the effects of temperatures on endodormancy release, the effects of different temperatures on carbohydrate metabolism during dormancy in Japanese pear remains poorly understood.

Carbohydrate dynamics have been studied in peaches (MARQUAT *et al.*, 1999; MAUREL *et al.*, 2004; BONHOMME *et al.*, 2005), grapes (BEN MOHAMED *et al.*, 2012), and the Japanese pear (MARAFON *et al.*, 2011; ITO; SAKAMOTO; MORIGUCHI, 2012) during the dormancy phase. Bonhomme *et al.* (2005) reported significant changes in the carbohydrate content under chilling deprivation. Moreover, carbohydrate availability, uptake and utilization capacity are essential for normal bud development subsequent to the release of dormancy (ITO; SAKAMOTO; MORIGUCHI, 2012).

In many Rosaceae species, such as *Malus* (apples), *Pyrus* (pears) and *Prunus* (stone fruit), sorbitol is the primary photosynthetic product and accounts for 60–90% of carbon exported from the leaf (LOESCHER, 1987). In the Japanese pear, sorbitol is the most abundant sugar molecule, and carbohydrate is transported in this form from source to sink during endodormancy (ITO; SAKAMOTO; MORIGUCHI, 2012). In addition, sorbitol concentration has been shown to increase in the xylem sap of the Japanese pear during the progression of endodormancy (ITO; SAKAMOTO; MORIGUCHI, 2013).

Starch is the primary source of reserved energy in plants, and is made up of homopolymers of glucose. Starch is converted into soluble sugars in response to low temperatures during dormancy (ELLE; SAUTER, 2000). Yoshioka *et al.* (1988) suggested that starch is degraded under low temperatures by amylase and metabolized into sucrose by sucrose-6-phosphate synthase and sucrose synthase (SS; EC 2.4.1.13). In contrast, the enzymatic cleavage of sucrose is catalyzed by invertase (EC 3.2.1.26) or SS. Invertase converts sucrose into fructose and glucose, whereas SS converts sucrose and uridine-5′ -diphosphate (UDP) into fructose and UDP-glucose (KOCH, 2004).

To elucidate the cause of problems encountered during dormancy of Japanese pear trees grown in areas with mild winter conditions, it is essential to determine the physiological changes occurring during endodormancy phase under different temperatures. Thus, this study aims to investigate the effects of different temperatures on carbohydrate metabolism in the Japanese pear floral buds during dormancy.

2 MATERIAL AND METHODS

2.1 Plant material

One year 'Housui' Japanese pear shoots with at least 6 lateral floral buds (approximately 80 cm in length) were collected from the orchard of the Agricultural and Forestry Research Center,

University of Tsukuba, Japan (36°N, 140°E) before chilling was initiated (30 October 2012). To assess heat accumulation in the phytotron, the number of growing degree hours (GDH) was calculated by subtracting 4.5 °C from each hourly temperature, and then summing these values (RICHARDSON *et al.*, 1975). Chilling unit (CU) values were calculated using the Utah Model (RICHARDSON; SEELEY; WALKER,. 1974).

The experiment was conducted using a completely randomized design, with three temperature treatments and three replications (six shoots per replication). The shoots were placed in bottles containing distilled water and kept in separate growth chambers set at 0, 6, or 12 °C. After 600 hours under these temperature conditions, the shoots were placed under forcing conditions by moving them to a phytotron set at 25 ± 1 °C, with a 14 hours photoperiod. Tips of the water-immersed shoots were cut-off and water was changed every three days during the experimental period. Lateral floral buds were collected after 0, 200, 400, and 600 hours under the temperature treatments and at 2000, 4000, 6000, and 8000 GDH, frozen in liquid nitrogen and stored in an ultra-freezer (–80 °C) until further analysis.

2.2 Determination of bud burst (%)

The bud burst (%) was determined by examining shoots with single lateral floral buds. The buds were examined every three days, and bud burst was considered to have occurred when the buds reached the phenological stage C3 (CALVET; GUIRBAL, 1979; COUTANCEAU, 1971).

2.3 Determination of sugar concentration

The sugar content was determined using approximately 300 mg of frozen lateral floral buds (approximately 3 to 4 buds) that were ground in liquid nitrogen in a mill (ForceMill, Osaka Chemical Co., Osaka, Japan) and extracted twice with 6 mL of 80% ethanol. After 1 mL of pentaerythritol (1%) as an internal standard was added, the extract was evaporated in a vacuum at 40 °C. Three milligrams of polyvinylpolypyrrolidone (PVPP) was added to 1 mL aliquots of the extract to remove phenolic compounds, centrifuged at 14.000 rpm for 10 min and then filtered using a Millipore filter (0.45 mm) attached to a syringe. Afterwards, 20 µL samples were injected into a high-performance liquid chromatography (HPLC) equipped with an auto-sampler, pre-column (Shin-pack SPR-Ca, Shimadzu, Japan), a packed column (SC-1011, SHODEX, Showa Denko K.K., Tokyo, Japan), and a refractive index detector (RI-101, SHODEX, Denko K.K., Tokyo, Japan). The equipment was set to a flow rate of 1.0 mL min^{-1} and a column temperature of 80 °C. For the mobile phase, ultra-pure water (18 mΩ) was used. Sugar concentration was quantified based on the calculations of peak areas obtained from regression curves of sugar standards.

2.4 Determination of starch concentration

A total starch kit (AOAC Official Method 996.11, Megazyme International Ireland Ltd. Bray, Ireland) was used in order to perform starch quantification. After the ethanol extraction for sugar determination the remaining pellet was oven-dried overnight at 60 °C. The dried samples were stirred using 0.2 mL of 80% (v/v) ethanol and a vortex mixer, 3 mL of thermostable α-amylase diluted in 50 mM MOPS buffer (pH 7.0) was added, and then the samples were incubated in boiling water for 12 min. The tubes were placed in a water bath at 50 °C, 4 mL of sodium acetate buffer was added, followed by 0.1 mL of amyloglucosidase, and incubated for 30 min. Before centrifugation at 3000 rpm, the volume of the samples was adjusted to 10 mL using deionized water. Duplicated 0.2 mL aliquots of the diluted solution were transferred to glass tubes (16 × 100 mm), and 3 mL of glucose oxidase/peroxidase (GOPOD) reagent was added, followed by incubation at 50 °C for 20 min. Absorbance at 510 nm was determined using a spectrophotometer (V-550, Jasco Co., Tokyo, Japan). D-glucose was used as the standard, and deionized water as the blank.

2.5 Enzyme extraction

Enzyme extraction was performed according to Yamaki and Ishikawa (1986) with slight modifications. Samples were homogenized in liquid nitrogen in a chilled mortar with 3 mL of 0.2 M K-phosphate buffer (pH 7.0) containing 10 mM Na ascorbate, 20 mM 2-mercaptethanol, 0.1% Triton-X, and 10% PVPP. The homogenate was filtered through one layer of Miracloth, followed by centrifugation at 12,000 × *g* for 20 min, and the supernatant was immediately desalted by applying pre-packed PD-10 columns containing Sephadex G-25 equilibrated with 0.2M K-phosphate buffer (pH 7.0) containing 20 mM 2-mercaptethanol. The eluted fraction was used for the determination of SS and AI activities.

2.6 Determination of sucrose synthase (SS) and soluble acid invertase (AI)

The SS activity was determined according to Nomura and Akazawa (1973) and AI was estimated with the modification of Yamaki and Ishikawa (1986). The determination of SS activity was conducted in reaction mixtures containing 20 mM Hepes-KOH buffer (pH 8.5), 20 mM UDP-glucose, 20 mM fructose, 5 mM $MgCl_2$, 20 mM NaF and the enzyme extract. Mixtures were incubated at 30 °C for 30 min, and incubation was terminated by the addition of 60 µL of 30% KOH. Sucrose content was determined according to the method reported in Roe (1934). Soluble acid invertase (AI) was assayed using the Somogyi-Nelson method (NELSON, 1944), by determining the amounts of glucose produced. The reaction was conducted for 30 min at 30 °C in a reaction mixture composed of 60 mM acetic buffer (pH 5.0) and 70 mM sucrose. It was stopped by adding 2.5 M tris(hydroxymethyl) aminomethane. Enzyme activity was expressed as nanomoles of sucrose (SS) or glucose (AI) produced per minute per gram of bud fresh weight.

2.7 Statistical analysis

The means and standard errors were calculated from the replicates. The data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests at 5% probability in Graphpad Prism 7.0 (GraphPad Software). All percentage data were arc-sine transformed before analysis. Graphics were produced using Graphpad Prism 7.0 (GraphPad Software).

3 RESULTS AND DISCUSSION

To simulate a mild winter condition, the shoots were exposed to 600 hours under different temperatures, which represents 80% of the 750 hours (≤ 7.2 °C) needed to release endodormancy in Japanese pear (NISHIMOTO; KISAKI; FUJISAKI, 1995). Temperatures of 0, 6 ºC and 12 ºC were used in the study because the first two have been reported to be effective in releasing endodormancy, while the last was considered a warm temperature ineffective in completing endodormancy in Japanese pear (SUGIURA; HONJO, 1997). The shoots from the 12 °C treatment exhibited the most advanced bud burst among all treatments (Fig. 1). Bud burst in shoots exposed to 12 °C was higher than 0 and 6 ºC on day 15 under forcing conditions. In addition, 12 °C exposed shoots exhibited a higher bud burst than shoots exposed to 0 ºC at day 18 under forcing conditions. Similarly, Chavarria (2009) observed that mild temperatures (10 and 15 °C) during the endodormancy phase were more effective in advancing flowering and leafing of peaches than colder temperatures (5 °C). All shoots eventually reached 100% of bud burst, which is consistent with the findings of Couvillon (1995) and Rakngan, Gemma and Iwahori (1996), that a temperature of 12 °C was as effective as lower temperatures in contributing to endodormancy release. However, the traditional chilling model considers 7.2 °C to be the maximum efficient temperature for bud break (WEINBERGER, 1950), and temperatures of 0 to 6 °C are reported to be the most effective for releasing endodormancy (SUGIURA; HONJO, 1997). The calculated CU values of shoots exposed to 0 °C, 6 °C and 12 °C were 0 CU, 600 CU and 300 CU, respectively. Although shoots exposed to 0 and 12 °C exhibited lower CU values than 6 °C, bud burst occurred in all treatments. It is important to emphasize that dormancy induction depends on autumn temperatures (HEIDE; PRESTRUD, 2005), and bud dormancy state of the collected shoots may also influence bud burst rates. Furthermore, it is known that chilling requirement differs among species, cultivars, bud types and position of buds (HAUAGGE; CUMMINS, 1991). The reasons why the obtained bud burst (%) did not correlate some models are possibly related to the factors described above. Our results suggest that the condition of 12 °C for 600 hours is effective to advance bud burst in the lateral flower buds in 'Housui' Japanese pear.

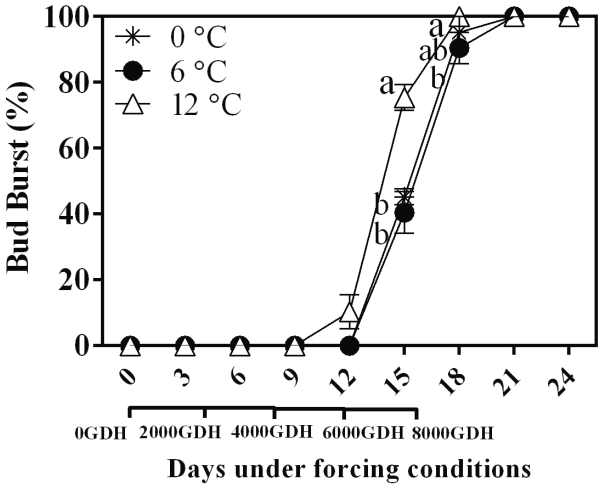

Figure 1 - Bud burst (%) after forced in phytotron in excised shoots subjected to 0, 6 and 12 °C for 600 hours during endodormancy in 'Housui' Japanese pear lateral floral buds. Different letters indicate significant differences between treatments at each sampling time. Means compared by Tukey's multiple comparison tests at 5% probability, ± SE (n=3).

Sucrose tended to accumulate in buds exposed to the 0 and 6 °C treatments (Fig. 2), with the former exhibiting the highest concentrations, and remained low during the dormancy phase in those in the 12 °C treatment. Sucrose concentration during heat accumulation decreased early in the buds from the 12 °C treatment, followed by those from the 6 °C and 0 °C treatments. The buds exposed to 12 °C exhibited the lowest sucrose concentration during the endodormancy phase and under heat accumulation. This may be because chilling deprivation reduces the capacity for sucrose synthesis in wood tissues and sucrose import by floral buds of the Japanese pear (MARAFON *et al.*, 2011). In contrast, buds exposed to 0 °C, exhibited an earlier accumulation of sucrose and attained a higher sucrose concentration. Similarly, Guy, Huber and Huber (1992) found that sucrose is the most commonly accumulated soluble sugar under low temperatures. Such conditions may strongly control the sugar co-transporters involved in carbohydrate uptake (BONHOMME *et al.*, 2010), and accumulation of carbon reserves during dormancy release is essential for bud burst induction (MARQUAT *et al.*, 1999). Yoshioka *et al.* (1988) suggested that sucrose plays an important role in conferring freezing tolerance to apple trees during the winter, and that it provides energy for growth in spring. Considering that SS might convert sucrose into UDP-glucose and fructose *in vivo* (WINTER; HUBER, 2000), the decrease in sucrose concentrations under forcing conditions might be related to the increase in AI and SS activities.

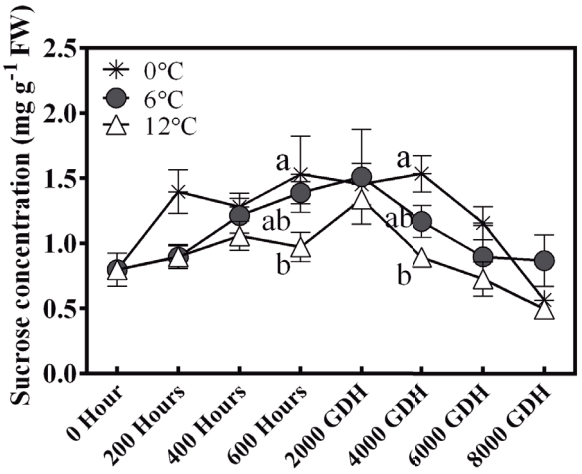

Figure 2 - Sucrose concentration in 'Housui' Japanese pear lateral floral buds subjected to 0, 6 and 12 °C for 600 hours during endodormancy and a subsequent heat accumulation period expressed as GDH (growing degree hours). Different letters indicate significant differences between treatments at each sampling time. Means compared by Tukey's multiple comparison tests at 5% probability, ± SE (n=3).

The glucose concentration remained stable during the temperature treatments and tended to increase under heat accumulation (Fig. 3). The concentration of glucose was higher in 6 and 12 °C exposed shoots than 0 °C during 8000 GDH under forcing conditions. In contrast, fructose levels remained stable during endodormancy and increased shortly after the onset of heat accumulation (Fig. 4). Fructose concentrations in buds was lower in shoots exposed to 0 °C than 6 °C during 8000 GDH under forcing conditions. Glucose and fructose concentrations tended to increase in all treatments under forcing conditions. Indeed, hexose concentrations are known to increase during spring as a result of high metabolic activity and resumption of growth during this period (RICHARDSON *et al.*, 2010). Marafon *et al.* (2011) suggested that fructose and glucose produced by AI activity in non-photosynthetic tissues could be stored in vacuoles or used for adenosine triphosphate (ATP) production during the respiration process, and that they may also be converted into starch. Thus, the increase in hexoses in buds may provide carbon and energy for the synthesis of various compounds necessary for the resumption of growth following the release of bud endodormancy.

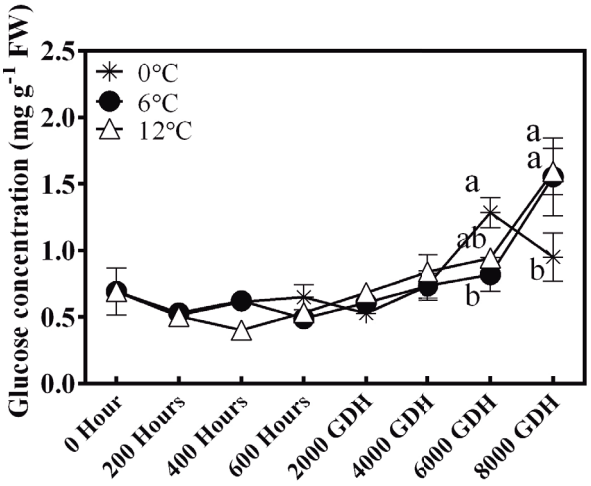

Figure 3 - Glucose concentration in 'Housui' Japanese pear lateral floral buds subjected to 0, 6 and 12 °C for 600 hours during endodormancy and a subsequent heat accumulation period expressed as GDH (growing degree hours). Different letters indicate significant differences between treatments at each sampling time. Means compared by Tukey's multiple comparison tests at 5% probability, ± SE (n=3).

The sorbitol concentration in buds was the highest among all soluble sugars (Fig. 5). The sorbitol in buds exposed to 6 and 12 °C remained low during the endodormancy phase, but tended to increase in buds exposed to 0 °C. The concentration of sorbitol

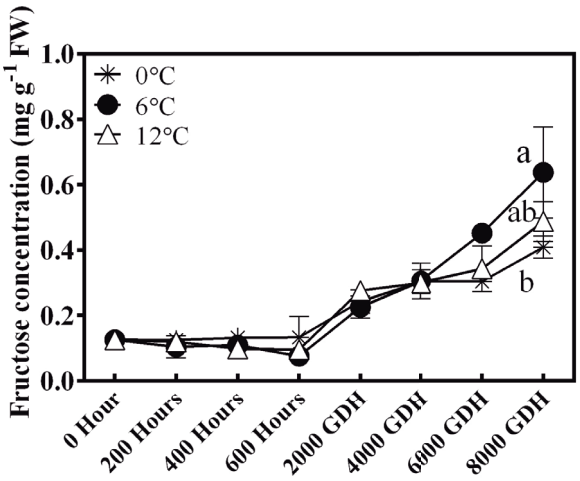

Figure 4 - Fructose concentration in 'Housui' Japanese pear lateral floral buds subjected to 0, 6 and 12 °C for 600 hours during endodormancy and a subsequent heat accumulation period expressed as GDH (growing degree hours). Different letters indicate significant differences between treatments at each sampling time. Means compared by Tukey's multiple comparison tests at 5% probability, ± SE (n=3).

in buds from the 12 °C treatment decreased rapidly under heat accumulation and was consistently lower than that in buds from the 0 °C treatment during 4000 and 6000 GDH. High sorbitol and sucrose levels were observed in apple shoots exposed to cold temperatures (RAESE; WILLIAMS; BILLINGSLEY, 1977), and the accumulation of soluble carbohydrates is positively correlated with cold hardiness (WAMPLE; BARY, 1992). According to Ito, Sakamoto and Moriguchi (2013), the xylem sap of the Japanese pear has high sorbitol content during winter, as a result of highly active sugar transport under low temperatures. The high sorbitol concentration we observed in the buds from the 0 °C treatment may thus have originated from the high accumulation of sorbitol in the xylem sap. Sorbitol is the primary translocated carbohydrate (LOESCHER; EVERARD, 1996) and the most abundant sugar molecule in the buds of the Japanese pear during the endodormancy phase (ITO; SAKAMOTO; MORIGUCHI, 2012). Furthermore, sorbitol is catabolized via enzymes, such as NAD-dependent sorbitol dehydrogenase and NADP-dependent sorbitol dehydrogenase, into fructose and glucose, respectively (YAMAKI; MORIGUCHI, 1988). This correlates the result obtained by Ito, Sakamoto and Moriguchi (2013) where they observed an increase in the activity of NAD-dependent sorbitol dehydrogenase during winter (mid- to late January) in Japanese pear flower buds. Although we could not find significant differences on the concentration of hexoses, it is possible that the highly accumulated sorbitol under 0 °C may have supported the demand of these sugars during bud burst under forcing conditions. Although Maurel *et al.* (2004) suggested that sorbitol availability was not associated with bud break capacity in peach vegetative buds, Nosarzewski and Archbold (2011) found that sorbitol dehydrogenase genes were expressed during emerging apple flower buds suggesting their role in bud break. Thus, it is clear that sorbitol is an important sugar and may play an important function during dormancy of Japanese pear flower buds. In the current study, low temperatures (0 °C) affected the sorbitol dynamics during endodormancy, which may be crucial in enhancing freeze tolerance under this condition. However, further studies are needed to clarify its involvement on endodormancy release of Japanese pear buds.

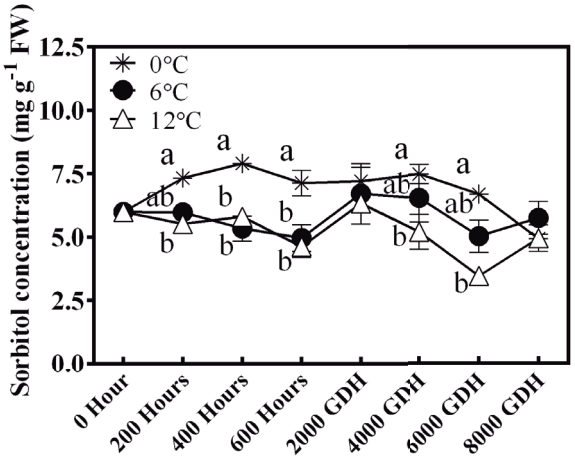

Figure 5 - Sorbitol concentration in 'Housui' Japanese pear lateral floral buds subjected to 0, 6 and 12 °C for 600 hours during endodormancy and a subsequent heat accumulation period expressed as GDH (growing degree hours). Different letters indicate significant differences between treatments at each sampling time. Means compared by Tukey's multiple comparison tests at 5% probability, ± SE (n=3).

The starch concentration remained stable and was similar among all three treatments during endodormancy, and tended to decrease under heat accumulation (Fig. 6). Furthermore, buds from the 12 °C treatment tended to decrease after 4000 GDH, and exhibited the lowest values thereafter (0.62 mg g^{-1} FW). The concentration of starch in buds of shoots exposed to 12 °C was lower compared to the shoots exposed to 0 °C in 4000 GDH, and shoots exposed to 6 °C in 8000 GDH. Low temperatures have been associated with starch degradation followed by increases in soluble sugar content in *Populus* (poplar) stems (SAUTER; ELLE; WITT, 1998) and *Juglans* (walnut) trees (LACOINTE *et al.*, 1993). The effects of chilling on changes in starch and sugar content are

correlated with the induction of amylase activity by cold temperatures (ELLE; SAUTER, 2000). It has been reported that other key enzymes, such as β-amylase and starch phosphorylase, occur even in the absence of cold temperatures (GUY *et al.*, 2008). However, our results showed that temperature during endodormancy did not affect starch concentration of buds. It is also important to mention that flower bud cushion is a significant reserve of starch and represents an essential source of sugars during bud burst (RODRIGUES *et al.* 2006). Moreover, accumulated starch is used for the resumption of growth in spring (GEMMA, 1995). The starch concentration in the buds, which changed only under heat accumulation, is important to provide soluble sugars for the resumption of growth.

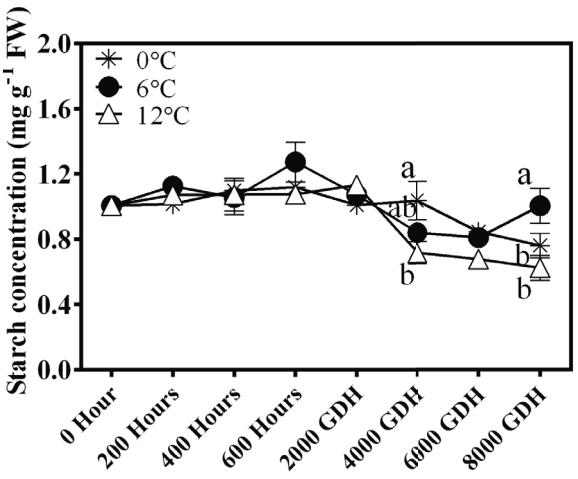

Figure 6. Starch concentration in 'Housui' Japanese pear lateral floral buds subjected to 0, 6 and 12 °C for 600 hours during endodormancy and a subsequent heat accumulation period expressed as GDH (growing degree hours). Different letters indicate significant differences between treatments at each sampling time. Means compared by Tukey's multiple comparison tests at 5% probability, ± SE (n=3).

SS activity followed a similar trend to that of soluble AI: both remained low under endodormancy and tended to increase under heat accumulation (Fig. 7). SS activity increased rapidly in buds from the 6 and 12 °C treatments and more slowly in those from the 0 °C treatment, beginning its increase at 2000 GDH and peaking at 8000 GDH (Fig. 7A). SS activity in buds of shoots exposed to 12 °C was higher than shoots exposed to 0 °C in 4000 and 6000 GDH. Activity of soluble AI was more consistently low and more similar among treatments during endodormancy and the increase under heat accumulation was also in a more uniform manner across treatments (Fig. 7B). The marked increase began around the middle of heat accumulation period. Soluble AI and SS activity increased considerably under heat accumulation, contributing to the increase in the fructose and glucose concentrations. SS activity in buds from the 12 °C treatment increased rapidly, which may be related to the advance in bud dormancy release via sucrose catabolism. It has been reported that AI and SS activity increases in Japanese pear buds in early December, contributing to dormancy release through carbohydrate utilization (ITO; SAKAMOTO; MORIGUCHI, 2012). In our study, AI activity increased during heat accumulation period. However, high AI activity has also been observed in grapes during endodormancy release (BEN MOHAMED *et al.*, 2010). We therefore suggest that a temperature of 12 °C advanced bud burst as a result of earlier conversion of sucrose into hexoses.

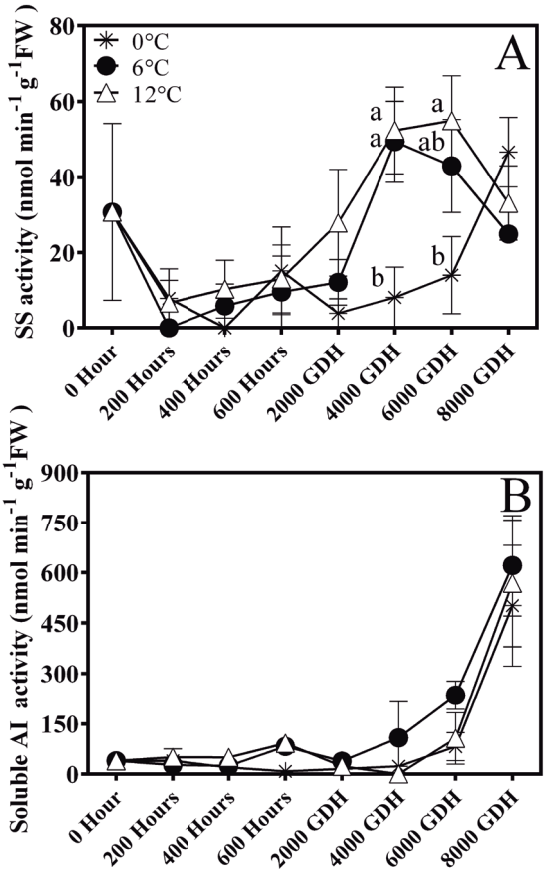

Figure 7 - SS (sucrose synthase) (A) and soluble AI (acid invertase) activity (B) in 'Housui' Japanese pear lateral floral buds subjected to 0, 6 and 12 °C for 600 hours during endodormancy and a subsequent heat accumulation period expressed as GDH (growing degree hours). Different letters indicate significant differences between treatments at each sampling time. Means compared by Tukey's multiple comparison tests at 5% probability, ± SE (n=3).

4 CONCLUSIONS

Sorbitol was found to be the major sugar in buds and its concentration increased in response to a chilling temperature of 0 °C during endodormancy.

Sucrose concentration increased in buds exposed to 0 °C during endodormancy and tended to decrease earlier during heat accumulation in buds exposed to 12 °C for 600 hours. Furthermore, 12 °C for 600 hours during endodormancy phase accelerated the bud burst of Japanese pear lateral floral buds compared to colder temperatures (0 and 6 °C). Therefore, we suggest that bud burst may have occurred faster in 12 °C followed by high sucrose catabolism.

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