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The possible role of chilling in floral and vegetative bud dormancy release in *Pyrus pyrifolia*

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Abstract

The role of chilling in bud dormancy release and biochemical changes in different organs were evaluated in stem cuttings of pear (*Pyrus pyrifolia*) cv. Cuiguan selected at the leaf fall. The cuttings were exposed to 5 °C for 0, 100, 200, 300, 400, 500, 600, and 700 chilling hours (named positive chill units; PCU). A 50 % bud break was observed in floral and vegetative bud cuttings at 300 and 600 PCU, respectively. A mean time to bud break was inversely proportional to the chilling treatment. The low-temperature stimulated starch hydrolysis accompanied with sucrose accumulation in all organs. Sucrose and sorbitol content increased substantially peaking at 100, 400, and 100 PCU in floral buds, vegetative buds, and bark, respectively, thereafter decreased when buds approached chilling satisfaction (300 and 600 PCU for the floral and vegetative buds, respectively), and then increased again up to 700 PCU. Hexoses (glucose and fructose) accumulated constantly in the buds from 0 to 700 PCU. In bark, glucose and fructose content increased up to 400 PCU, and then gradually decreased. Total amylolytic and α-amylase activities increased in all organs, especially in the floral and vegetative buds up to 100 PCU and then decreased in the floral and vegetative buds before increasing again after endo-dormancy release. Invertase activity remained high in the buds during chilling satisfaction possibly because of translocation of sucrose to the buds which functioned as a strong sink. The results suggest that a low availability of hexoses may be the cause of limited bud breaks due to lack of chilling. Chilling satisfaction of the buds may increase the content of soluble sugars and acid invertase activity, and decrease the starch content, which may correlate with improved bud breaks.

Additional key words: amylase, fructose, glucose, invertase, pear, sorbitol, starch, stem cuttings, sucrose.

Introduction

Temperate deciduous fruit trees, including pears, annually pass through the physiological phase of development known as dormancy. The dormancy is defined as the temporary suspension of visible growth of any plant structure containing a meristem (Lang *et al.* 1987). The dormancy helps to protect plant tissues from harsh environmental conditions and to secure their proper architecture. To resume growth, buds require a specific amount of chilling, which is genetically controlled and varies among genotypes (Balandier *et al.* 1993, Egea *et al.* 2003). The number of chilling hours quantifies the exposure to temperatures < 7.2 °C required to break dormancy and induce floral and vegetative bud break. Artificial means of breaking dormancy are needed to maintain economic production of pears in particular regions that lack sufficient natural chilling (Teng *et al.* 2014).

Researchers commonly use accumulated positive chill units (PCU) to calculate bud break requirement. Under field conditions, it is almost impossible to determine accurately the chilling requirements for breaking bud dormancy because other environmental factors cannot be controlled (Dennis 2003). Thus, excised shoots are often used for measurement of the dormancy depth and duration (Dennis 2003). The physiological changes that occur in different organs and tissues during the

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Abbreviations: CR - chilling requirements; HPLC - high-performance liquid chromatography; MTB - mean time to bud break; PCU - positive chill units.
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dormancy period include sugar metabolism (Marquat *et al.* 1999, Maurel *et al.* 2004, Bonhomme *et al.* 2005, González-Rossia *et al.* 2008), water content (Trejo-Martínez *et al.* 2009), respiration rate (Young *et al.* 1987), and growth regulation (Powell 1987). However, our knowledge of the processes leading to dormancy release and of an order in which they occur in different organs and tissues remains limited (Halaly *et al.* 2008).

During dormancy, notable changes are observed in the content of sugars, nucleic acids, amino acids, and organic acids, which might be associated with bud break and flowering time (Wang *et al.* 1987). The sugar content in vegetative and reproductive tissues change significantly in fruit trees submitted to chilling deprivation compared with those grown under natural chilling conditions (Bonhomme *et al.* 2005). Sugars are the main source of energy for the metabolic changes that occur during dormancy release. Sugar accessibility is likely to be important for the control of bud growth and development during dormancy induction and release (Sherson *et al.* 2003). Starch is accumulated in reserve tissues during the preceding summer and is converted to sucrose and other soluble sugars during the dormant phase. The effects of chilling on changes in content of both starch and sugars may be attributable to an increased amylase activity (Elle and Sauter 2000). Soluble sugars are important signaling molecules involved in many processes of the plant life-cycle including dormancy (Sheen *et al.* 1999, Gibson 2000, Smeekens 2000). However, a bud break pattern appears to be more correlated with the capacity of buds to use soluble sugars than with sugar abundance in dormant tissues (Leite *et al.* 2004, Bonhomme *et al.* 2005).

In response to a low temperature during winter, starch degraded by amylases is used for sucrose synthesis by sucrose-6-phosphate synthase. In reserve tissues, sucrose is produced and then transported *via* the xylem to buds where sucrose is hydrolyzed to glucose and fructose to produce energy and carbonic precursors (Yoshioka *et al.* 1988). Two enzymes, sucrose synthase (EC 2.4.1.13) and invertase (EC 3.2.1.26), are involved in sucrose metabolism. Sucrose synthase catalyzes a reversible reaction that degrades sucrose to UDP-glucose and fructose, whereas invertase catalyzes the irreversible reaction of sucrose cleavage to glucose and fructose. In plants, there are different types of invertases, for example, alkaline invertases, such as cytoplasmic invertases, and acidic invertases, such as cell wall and soluble vacuolar invertases. Invertases play a vital role in growth, sink initiation, and cell expansion, whereas sucrose synthase is linked with metabolism for storage, fruit maturation, and polysaccharide synthesis (Koch 2004).

The objective of the present work was to study the sequence of events leading to endo-dormancy release of dormant pear floral and vegetative buds. To better understand the physiology of dormancy, we studied the quantitative changes caused by artificial chilling on sugar content and amylase and invertase activities during dormancy in floral and vegetative buds and bark tissue of *Pyrus pyrifolia*.

Materials and methods

Plants and treatments: *Pyrus pyrifolia* Nakai cv. Cuiguan grafted on *P. calleryana* Decne rootstocks were grown in the experimental pear orchard of the Zhejiang University, Hangzhou, Zhejiang province, China (36°13′ N, 120°12′ E, an elevation of 41.7 m). One-year-old shoots (*ca*. 120 cm long) were randomly collected from adult trees during the leaf fall (1 November 2012) and cut into segments (*ca*. 60 cm long) comprising seven buds, obtaining 800 shoot cuttings for each floral and vegetative buds, divided into two groups for low-temperature treatment. Each set of shoots was further divided into groups, covered with paper, placed in plastic bags and exposed to a constant temperature of 5 °C in a cold room for 0, 100, 200, 300, 400, 500, 600, and 700 h to simulate chilling accumulation to different extent. Each hour of the cold treatment was the equivalent of 1 positive chill unit (PCU), as stated by Linsley-Noakes *et al.* (1995). The chilling requirement of cv. Cuiguan was previously estimated to be 304 and 704 PCU for floral and vegetative bud breaks, respectively, using the Utah Model (Liu 2013). Some differences have been recorded in chilling requirements (CR) for floral and vegetative buds among cultivars and environmental conditions (Liu 2013). After each chilling treatment, a group of treated shoots (for both floral and vegetative buds) was selected and divided into two sets. In one set of shoots, bud break was forced to assess the effect of chilling on the bud break. The second set of shoots, which consisted of three replicates, was used for biochemical analysis. Floral and vegetative buds and bark tissue were sampled, immediately frozen in liquid N_2, and stored at -80 °C.

Assessment of chilling effect on floral and vegetative bud break: After each chilling treatment period, three replicates of both floral and vegetative bud cuttings were placed with the shoot base in a vial containing water and transferred in a phytotron with a 12-h photoperiod, an irradiance of 320 μmol m^{-2} s^{-1}, day/night temperatures of 25 ± 1/18 ± 1 °C, and an air humidity of 75 %. The shoot base was cut and water in the vials replaced daily to avoid clogging xylem vessels caused by algal growth. Buds that reached the green tip stage were recorded as open and bud break percentages were determined after one month following the dormancy status classification of pear by Liu *et al.* (2012) with some modifications. When 50 % of buds on a shoot were at the green tip stage, we considered that endo-dormancy had been broken. However, if less

than 50 % of buds on a shoot had opened, the buds were considered to be in the endo-dormancy phase. The results are expressed as the percentage of bud breaks.

A mean time to bud break (MTB) of both floral and vegetative buds was calculated after the chilling treatment of the shoots. The results (the arithmetic means of each of two groups of nine excised shoots) are expressed as the MTB in days.

Determination of sugar and starch content: Sucrose, glucose, fructose, sorbitol, and starch were extracted using the method described by Huang *et al.* (2009) with slight modifications. Approximately 1 g of a frozen sample was homogenized in 10 cm^3 of 80 % (v/v) ethanol at 80 °C for 10 min. The extract was centrifuged at 12 000 *g* for 12 min. The extraction was repeated three times and the supernatants were collected and pooled. To remove phenolic compounds, 5 % (m/v) polyvinylpolypyrrolidone was added to the combined extracts and left overnight. The pellets were saved and stored at -40 °C for further starch analysis. The combined extracts were centrifuged at 3 000 *g* for 15 min, and the supernatant was evaporated to dryness under vacuum below 40 °C until the ethanol was removed. The volume was adjusted to 1 cm^3 with distilled water for analysis of sucrose, glucose, fructose, and sorbitol content using high-performance liquid chromatography (HPLC; *LC-20T*, *Shimadzu*, Kyoto, Japan). The *System Gold* software (*LC-20T*, *Shimadzu*) was used to run the HPLC and to process results. The content and composition of sugars were determined using the method of Komatsu *et al.* (1999) with some modifications. A 20 mm^3 aliquot solution was injected into a 5.0-μM NH_2 (4.6 × 250 mm) column (*Dalian Sipore Co*., Dalian, China). Eluted peaks were detected with a refractive index detector *RID-10A* (*LC-20AT*, *Shimadzu*). Acetonitrile:water (80:20, v/v) was used as mobile phase with a flow rate of 1 cm^3 min^{-1}. Sugars were quantified from a standard peak using sugar standards (*Sangon Biotech*, Shanghai, China).

Starch content was determined using the perchloric acid method of Rose *et al.* (1991). The residue remaining in the tubes after sugar extraction was further extracted three times with 5 cm^3 of 35 % (m/v) perchloric acid with continuous shaking at a low speed for 15 min. The extracts were pooled and centrifuged at 10 000 *g* for 5 min. The supernatants were collected in graduated tubes and diluted to 20 cm^3 with distilled water. For colorimetric determination, a 1 cm^3 aliquot of the extract was mixed with 5 cm^3 of an anthrone reagent (0.175 %, m/v, in 75 %, m/v, cold sulfuric acid) in the tube and briefly mixed. The mixture was placed in a boiling water bath for 12 min and then kept on ice. Absorbance of the mixture was read at 620 nm using an *Eppendorf Biospectrometer* (*Eppendorf*, Hamburg, Germany). Glucose standards from 0 to 100 g cm^{-3} were used for calibration.

Determination of enzyme activities: Total amylolytic, α-amylase, acid invertase, and alkaline invertase activities were determined using a spectrophotometric method in accordance with Ben Mohamed *et al.* (2010). Briefly, 1 g of frozen tissue was crushed into 2 cm^3 of ice-cold Tris-HCl buffer (50 mM, pH 7.5) containing Na_2CO_3 (4 mM), $CaCl_2$ (6 mM), 2 % (m/v) insoluble PVPP, and ascorbic acid (1 g dm^{-3}). The extracts were centrifuged on 10 000 *g* for 12 min at 4 °C, and the supernatants were used to determine the enzyme activities.

Total amylolytic activity was calculated by the sum of amylopectin 6-glucanohydrolase, β-amylase, α-glucosidase, and α-amylase activities. The enzyme extracts were heated at 70 °C for 12 min to inactivate all heat sensitive enzymes for α-amylase assay. The enzymatic assays were performed at 47 °C and 40 °C for α-amylase and total amylase respectively.

Alkaline invertase was assayed by adding of 0.6 cm^3 of 50 mM Tris-HCl buffer (pH 7.5) and 0.2 cm^3 of 0.1 M sucrose solution to the extracts. The reaction was processed at 40 °C for 60 min, while stopped by the addition of 2 cm^3 of dinitrosalicylic acid reagent. The tubes containing solution were kept in boiling water for 5 min then cooled to room temperature and lastly diluted with 5 cm^3 of bi-distilled water. The absorbance values were recorded at 540 nm. Acid invertase assay was same as alkaline invertase assay, only acetate buffer (0.1 M; pH 4.5) was used instead of Tris-HCl buffer. The absorbance values were shown in glucose equivalents using a normal calibration curve.

Statistical analysis: Statistical analyses of data were performed using analysis of variance with the *Data Processing System v. 7.05* (Zhejiang University, Hangzhou, China). A completely randomized design with three replications was used. The Duncan's multiple range test was used to compare the significance of differences among means at $\alpha = 0.05$.

Results

Pear cuttings collected during the leaf fall and immediately placed in a controlled environment showed 7.4 and 2.1 % floral and vegetative bud breaks, respectively, indicating that the buds were in a deep endo-dormant phase. The percentage of both floral and vegetative bud breaks increased gradually with the duration of chilling applied before forcing (Fig. 1). Floral and vegetative bud breaks attained 50 % after at least 300 and 600 PCU, respectively (Fig. 1). The MTB values of both types of the buds decreased with an increase in PCU (Fig. 2). In shoots treated for 0 to 400 PCU, floral bud break occurred earlier than vegetative bud break. After 500 PCU, the MTB for both floral and vegetative buds was identical. However, at 600 and 700 PCU, the MTB

was lower for vegetative buds compared with floral buds. A point at which the MTB values for floral and vegetative buds were equal was defined as the point of critical chilling accumulation (Fig. 2).

The starch content decreased significantly in floral buds in response to exposure to artificial chilling (Fig. 3*A*). The amount of starch in floral buds was at its maximum of 77.7 mg g^{-1}(f.m.) at the leaf fall (*i.e.*, 0 PCU) and at its minimum of 27.8 mg g^{-1}(f.m.) at 300 PCU. The chilling treatment initially induced a rapid decline in starch content and then a gradual decrease up to 300 PCU, but thereafter the starch content increased slowly up to 700 PCU (Fig. 3*A*). Initially, there was a rapid increase in sucrose content concomitant with a rapid decrease in starch content at 100 PCU (Fig. 3*A*,*B*). The chilling of floral buds induced a gradual increase in content of sucrose, glucose, fructose, and sorbitol which attained their highest values of 18.5, 14.7, 15.2, and

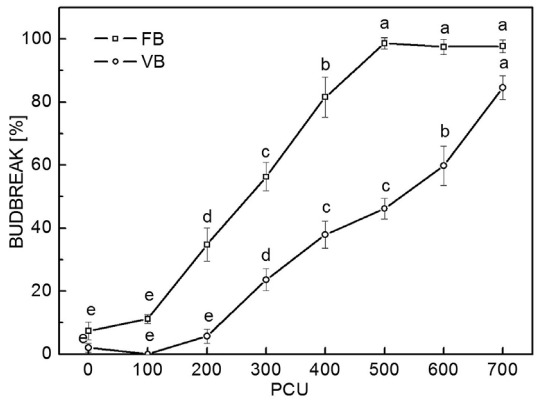

Fig. 1. The effect of artificial chilling on floral bud (FB) and vegetative bud (VB) breaks in excised shoots of pear. PCU - positive chill units. Means ± SE of three replicates. Values followed by the same letter are not significantly different at $P \geq 0.05$.

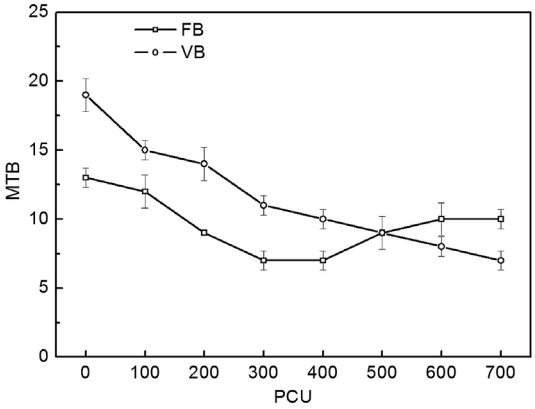

Fig. 2. The effect of chilling on a mean time to floral bud (FB) and vegetative bud (VB) breaks (MTB). PCU - positive chill units. Means ± SE of three replicates.

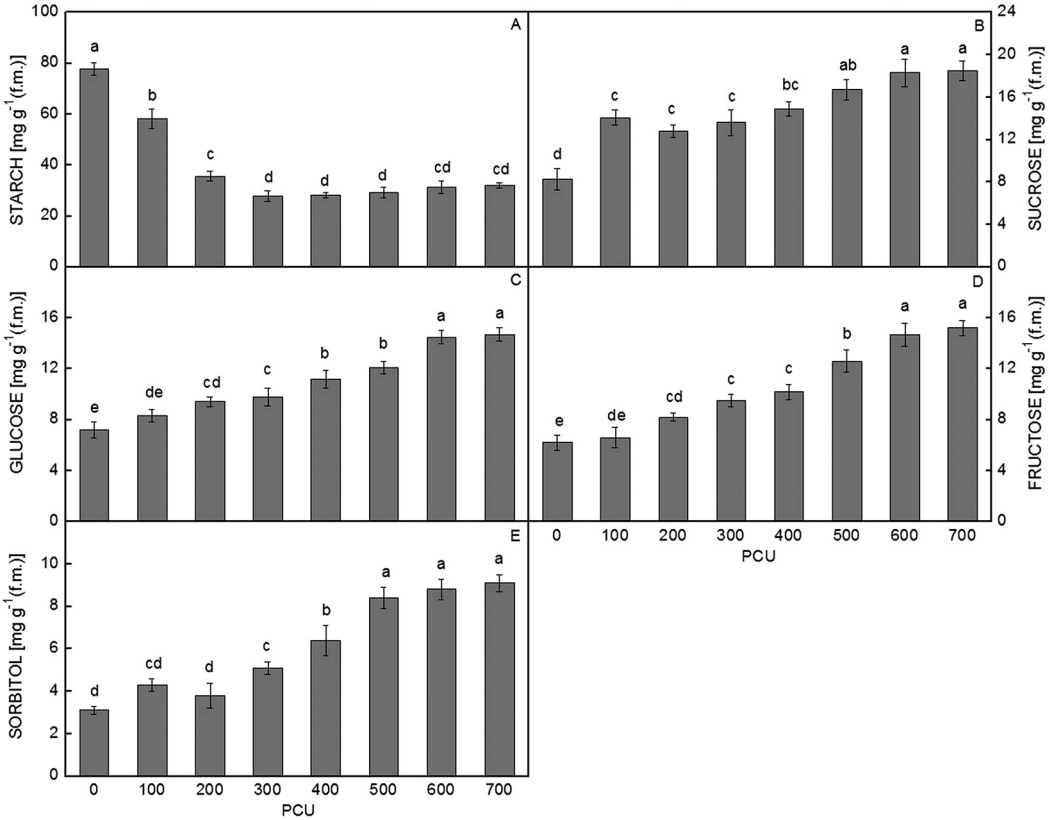

Fig. 3. Changes in starch (*A*), sucrose (*B*), glucose (*C*), fructose (*D*), and sorbitol (*E*) content in floral buds during a low-temperature treatment. Means ± SE of three replicates. Values followed by the same letter are not significantly different at $P \geq 0.05$.

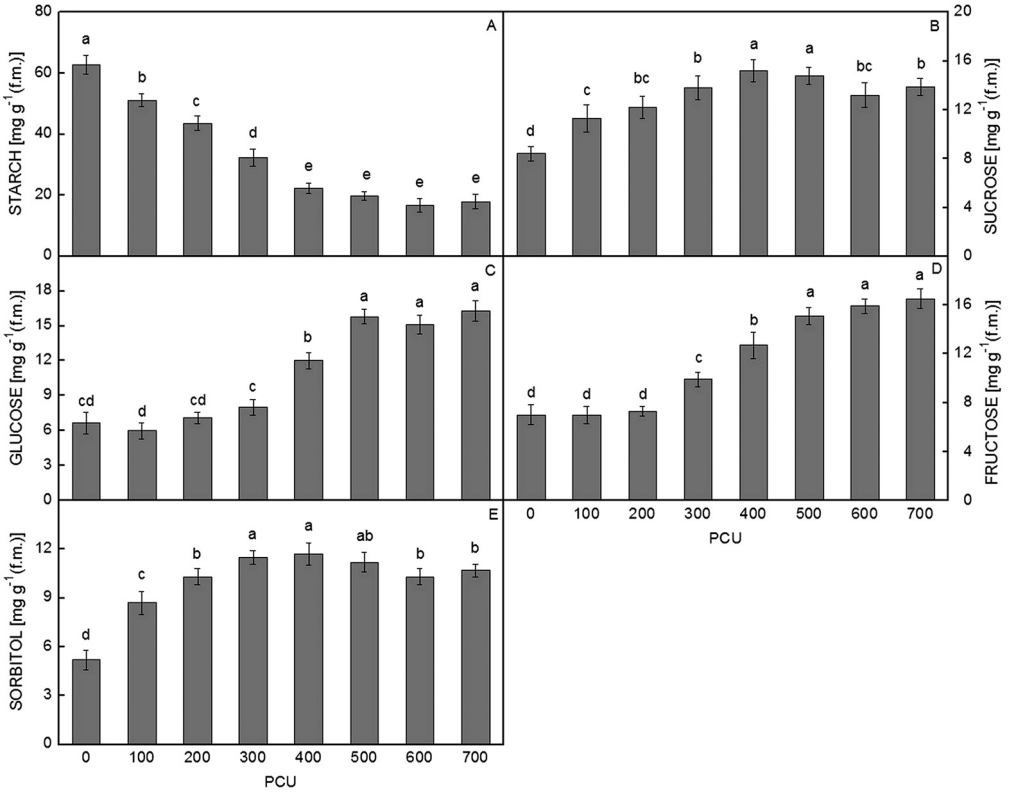

Fig. 4. Changes in starch (*A*), sucrose (*B*), glucose (*C*), fructose (*D*), and sorbitol (*E*) content in vegetative buds during a low-temperature treatment. Means ± SE of three replicates. Values followed by the same letter are not significantly different at $P \geq 0.05$.

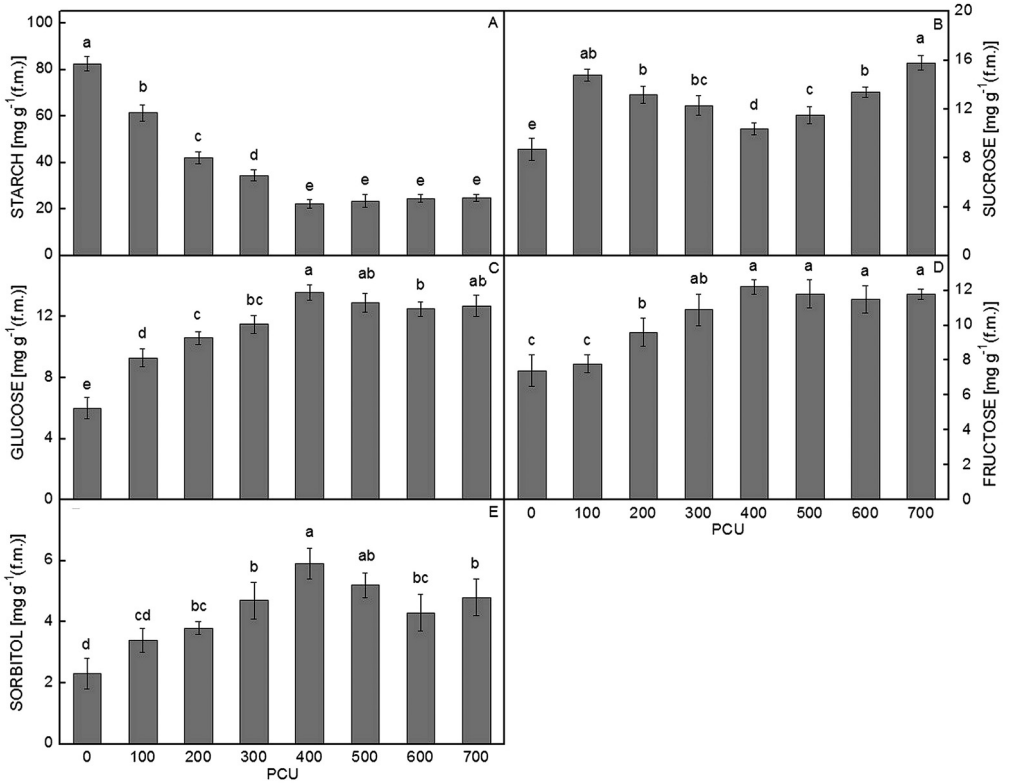

Fig. 5. Changes in starch (*A*), sucrose (*B*), glucose (*C*), fructose (*D*), and sorbitol (*E*) content in bark during a low-temperature treatment. Means ± SE of three replicates. Values followed by the same letter are not significantly different at $P \geq 0.05$.

9.1 mg g^{-1}(f.m.), respectively, at 700 PCU (Fig. 3*B*-*E*). The trends of the sucrose and sorbitol content were similar but amounts different. In vegetative buds, the initial starch content was 62.8 mg g^{-1}(f.m.) and decreased gradually with the increasing chilling duration up to 600 PCU when a minimum of 16.6 mg g^{-1} (f.m.) was attained (Fig. 4*A*). The sucrose and sorbitol content increased in response to chilling and attained a maximum of 15.2 and 11.7 mg g^{-1}(f.m.), respectively, at 400 PCU, but thereafter decreased. The glucose and fructose content in vegetative buds increased during chilling accumulation and reached maximum values of 16.3 and 16.5 mg g^{-1} (f.m.), respectively, at 700 PCU (Fig. 4*C*,*D*). It was hypothesized that starch hydrolysis was responsible for the increase in sucrose content because minimal changes were observed in hexoses (glucose and fructose) content in floral buds (Figs. 3,4). Both sucrose and sorbitol content decreased at the chilling treatment of 200 PCU in floral buds, whereas fructose and glucose started to accumulate at the chilling treatments of 100 PCU in floral buds. With a prolonged chilling treatment from 300 to 600 PCU, the starch content remained stable, whereas the content of sucrose, glucose, fructose, and sorbitol increased and attained their maximum values in both floral and vegetative buds.

In bark tissue, an initial starch content of 82.5 mg g^{-1}(f.m.) was higher than in floral and vegetative buds (Fig. 5*A*). In response to the chilling treatment, the starch content decreased. The content of all the sugars increased concomitantly with the decline in starch content. The sucrose content increased at 100 PCU, then decreased up to 400 PCU, and thereafter increased again up to 700 PCU. A maximum content of glucose and fructose of 13.6 and 12.2 mg g^{-1}(f.m.), respectively, were attained at 400 PCU (Fig. 5*C*,*D*). With the increased chilling duration, changes in starch, sucrose, and sorbitol content followed similar patterns as those in floral buds, whereas the glucose and fructose content did not increase. Approaching chilling satisfaction, vegetative buds contained a higher and floral buds lower glucose and fructose content compared with bark tissue.

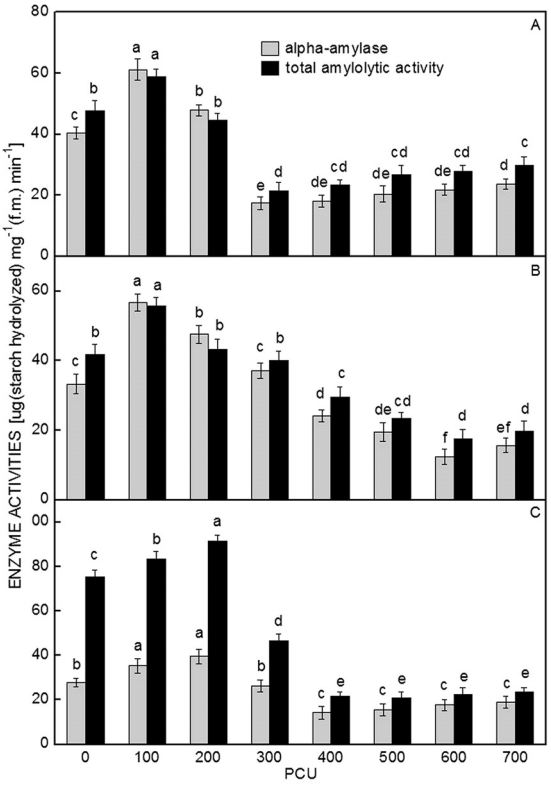

Fig. 6. Total amylolytic and α-amylase activities in floral buds (*A*), vegetative buds (*B*), and bark (*C*) during a low-temperature treatment. Both total amylolytic and α-amylase activities were statistically analyzed individually. Means ± SE of three replicates. Values followed by the same letter are not significantly different at $P \geq 0.05$.

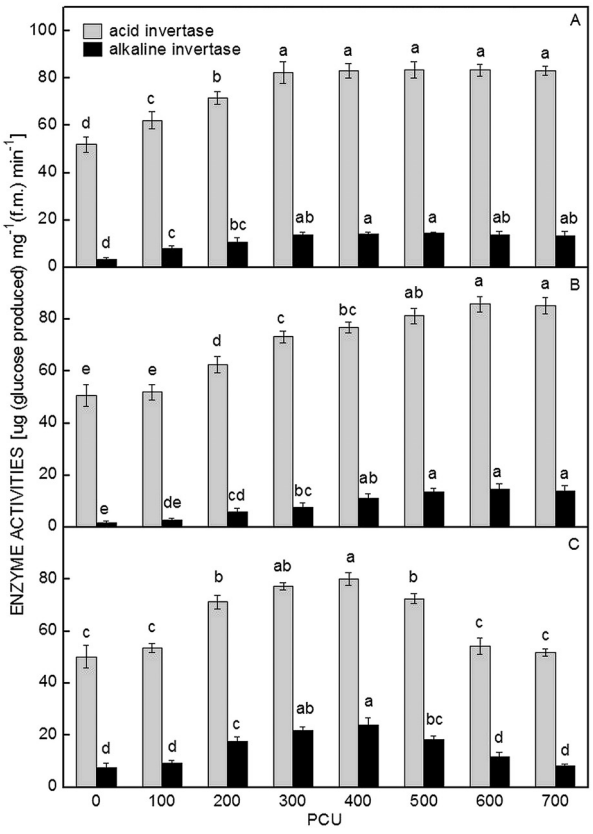

Fig. 7. Acid and alkaline invertase activities in floral buds (*A*), vegetative buds (*B*), and bark (*C*) during a low-temperature treatment. Both acid and alkaline invertase activities were statistically analyzed individually. Means ± SE of three replicates. Values followed by the same letter are not significantly different at $P \geq 0.05$.

Chilling increased the total amylolytic activities in all analyzed tissues. Maximum amylolytic activities were attained at 100 PCU in floral buds and vegetative buds, and 100 and 200 PCU in bark tissue (Fig. 6*A*-*C*). The increases in total amylolytic activity might have been due to α-amylase activity which was enhanced by 51, 70, and 42 % in floral buds, vegetative buds, and bark tissue, respectively. The total amylolytic activity was higher in bark tissue than in both types of buds. Later, the total

amylolytic activity decreased rapidly and remained low in all the organs except for its slight increase in floral buds after 500 PCU. These results indicate that the activities of starch-degrading enzymes were inversely proportional to the starch content.

The acid invertase activity was higher than that of alkaline invertase, although the two enzymes showed similar activity patterns (Fig. 7*A*-*C*). In all tissues, the acid invertase activity was low during the first 100 PCU, and then increased gradually with increasing PCU, whereas in bark tissue, its activity decreased from 500 to 700 PCU. The acid invertase activity rose from 52.1, 50.7, and 50.2 μg(glucose produced) g^{-1} (f.m.) min^{-1} at the leaf fall to 83.6, 85.8, and 80.2 μg(glucose produced) g^{-1} (f.m.) min^{-1} in floral buds, vegetative buds, and bark tissue after exposure to 500, 600, and 400 PCU, respectively. The invertase activity changed inversely to the change in sucrose content with the chilling treatment up to 300 PCU in floral buds and bark, and up to 500 PCU for vegetative buds; thereafter, the observed sucrose accumulation coincided with the highest invertase activity. Low activities of acid and alkaline invertases were observed at ≥ 200 PCU, and increased activities with exposure to 300 and 500 PCU in bark tissue.

Discussion

In this study, we explored the effect of artificial chilling on floral and vegetative bud breaks and on biochemical changes in different organs of pear cv. Cuiguan during exposure of excised shoots to a controlled environment. Endo-dormancy release requires a specific duration of chilling, which is genetically controlled. A low temperature is the key stimulus for endo-dormancy release (Balandier *et al.* 1993). Lack of chilling is the main factor causing abnormal patterns of bud break, development, and flower bud abortion in temperate fruit trees grown under mild winters (Mauget and Rageau 1988, Klinac and Geddes 1995, Petri and Herter 2002, Petri *et al.* 2002, Oh and Klinac 2003). The present results reveal that the chilling treatment at 5 °C for at least 300 h (for floral buds) and at least 600 h (for vegetative buds) induced more than 50 % bud break (Fig. 1). This finding is in accordance with those of González-Rossia *et al.* (2008), who reported that an artificial chilling treatment increased a bud break percentage in dormant shoots of *Prunus* sp. Much less than 50 % bud break was observed in both types of buds on shoots collected at the leaf fall (without a chilling treatment), which suggests a maximum endo-dormancy at that time. Similar responses to artificial chilling have been reported in previous studies on dormant shoots of peach and grape (Balandier *et al.* 1993, González-Rossia *et al.* 2008, Ben Mohamed *et al.* 2010). During endo-dormancy release, different biochemical changes occur, therefore it is essential to determine the dormancy status of buds during an artificial chilling treatment. As suggested previously by many researchers (*e.g.*, Cook and Bellstedt 2001, Dennis 2003, Ben Mohamed *et al.* 2010, Marafon *et al.* 2011, Liu *et al.* 2012, Hussain *et al.* 2015), excised shoots containing floral and vegetative buds, as used in the present experiment, should only illustrate the impact of endo-dormancy and not apical dominance on physiological processes leading to bud dormancy release.

In the current study, the chilling treatment increased the frequency of floral and vegetative bud breaks, which was accompanied with changes in sugar metabolism. A rapid hydrolysis of starch in floral and vegetative buds and bark tissue was observed in response to chilling. Starch degradation could be divided into two stages. During the first stage (0 - 300 PCU in floral buds and bark tissue, and 0 - 400 PCU in vegetative buds), a rapid decrease in starch content occurred (Fig. 3*A*, 4*A,* 5*A*), which might be due to the strong increase in amylolytic activity, especially α-amylase activity, in all the tissues (Fig. 6*A*-*C*). The sharp decrease in starch content seemed to be an early reaction to a low-temperature exposure. Similar effects of low temperature were reported in grapes (Ben Mohamed *et al.* 2010), Japanese pear (Marafon *et al.* 2011), poplar (Elle and Sauter 2000), and walnut (Ameglio *et al.* 2005). Elle and Sauter (2000) stated that a rapid increase in α-amylase activity induced by low temperature may be a thermal effect. The synthesis and activity of α-amylase are promoted by gibberellic acid (Galet 1993) and/or decreased by abscisic acid (Koussa *et al.* 2005). The present results indicate that α-amylase was the key enzyme involved in starch mobilization in both types of buds but not in bark tissue (Fig. 6*A*-*C*). The differences in α-amylase activity in the different organs might be due to the presence or absence of meristematic tissues. For example, during seed germination, α-amylase activity is promoted by gibberellic acid which originates in the embryo. We speculate that pear buds exhibit a similar phenomenon because buds show a higher meristematic activity than bark tissue. It has been suggested previously that buds show a higher meristematic activity than internodes in grapes (Ben Mohamed *et al.* 2010). The accumulation of sucrose and sorbitol was associated with the decrease in starch content in all underlined tissues/organs (Figs. 3-5). These results are in accordance with previous works by Sauter (1988) and Ben Mohamed *et al.* (2010) who proposed that starch-sucrose conversion may be induced by low temperature. It can be assumed that low temperature stimulates the mechanism by which starch molecules are degraded to sucrose. Low glucose and fructose content might be responsible for the low bud break percentage during 400 and 200 PCU in both floral and vegetative buds respectively. Nevertheless, the absence of observable bud growth might be

predominantly caused by the inability of buds to use available soluble sugars at this stage.

The increase in acid invertase activity observed in floral and vegetative buds might contribute to strengthening the sink (buds) and thus attracting sucrose from other underlined tissues such as bark. Sugars are transported in grapes mainly in the form of sucrose to nourish buds (Swanson and El-Shishiny 1958, Ben Mohamed *et al.* 2010). Furthermore, acid invertase activity shows a good concordance with sink expansion and control sucrose accumulation by catabolizing sucrose in the sink organ/tissue (Strum 1996, Koch 2004). It was observed in previous studies, that buds utilize starch reserves to synthesize soluble sugars during peach and Japanese pear dormancy (Marquat *et al.* 1999, Ben Mohamed *et al.* 2010, Marafon *et al.* 2011). Subsequently, the bud ability to attract exogenous sucrose from other tissues increases during dormancy release. Moreover, the notable increase in sucrose content in all the three tissues during the second phase could be explained in terms of sucrose translocation from other tissues as well as reduced invertase activity. The current results show that the maximum invertase activity in floral and vegetative buds appeared to be responsible for glucose and fructose accumulation. It was observed that the presence of glucose and fructose during bud break is well associated with bud growth capacity (Maurel *et al.* 2004). The accumulation of glucose and fructose in the buds during chilling satisfaction can have two advantages: it gives bud cells with carbon and energy required for synthesis of different compounds which needed for growth, and it also decreases water potential inside the bud and therefore attracts water which is required for cell expansion. Sugars function not only as source of nutrients and energy, but also control bud growth and regulate physiology, metabolism, and development of a plant (Arroyo *et al.* 2003, Roitsch and González 2004, Azymi *et al.* 2012). Therefore, bud break can be considered to be a continuous process starting throughout chilling treatment, whereas bud growth is initiated when endo-dormancy release and temperature become suitable. The present results indicate that the sharp decrease in starch content enables organs/tissues to survive the initial period of low temperature and aids the development of sink strength, whereas the increased content of hexoses may assist with endo-dormancy release.

In conclusion, the exposure of the excised shoots to low temperature caused a significant increase in the frequency of floral and vegetative bud breaks and affected the sugar content and enzyme activities in floral and vegetative buds and bark tissue of pear cv. Cuiguan. The floral and vegetative bud break percentages wre enhanced by increasing the chilling duration. We observed that the starch content decreased after the initiation of chilling, whereas the sucrose and sorbitol content increased. A high content of hexoses was observed in all the tissues at the time of bud endo-dormancy release. A high activity of acid invertase and accumulation of soluble sugars were observed during initiation of the growth resumption phase and might be directly responsible for the high bud break percentage.

References

Ameglio, T., Alves, G., Decourteix, M., Poirier, M., Bonhomme, M., Guilliot, A., Sakr, S., Brunel, N., Petel, G., Rageau, R., Cochard, H., Julien, J.L., Lacointe, A.: Winter biology in walnut tree: freezing tolerance by cold acclimation and embolism repair. - Acta Hort. **705**: 241-249, 2005.

Arroyo, A., Bossi, F., Finkelstein, R.R., Leon, P.: Three genes that affect sugar sensing (abscisic acid insensitive 4, abscisic acid insensitive 5 and constitute triple response 1) are differentially regulated by glucan in *Arabidopsis*. - Plant Physiol. **133**: 231-242, 2003.

Azymi, S., Sofalian, O., Jahanbakhsh, G.S., Khomari, S.: Effect of chilling stress on soluble protein, sugar and prolin accumulation in cotton (*Gossypium hirsutum* L.) genotypes. - Int. J. Agr. Crop Sci. **4**: 825-830, 2012.

Balandier, P., Bonhomme, M., Rageau, R., Capitan, F., Parisot, E.: Leaf bud endo dormancy release in peach trees: evaluation of temperature models in temperate and tropical climate. - Agr. Forest. Meteorol. **67**: 95-113, 1993.

Ben Mohamed, H., Vadel, A.M., Geuns, J.M.C., Khemira, H.: Biochemical changes in dormant grape vines shoot tissues in response to chilling: possible role in dormancy release. - Scientia Hort. **124**: 440-447, 2010.

Bonhomme, M., Regeau, R., Lacointe, A., Gendraud, M.: Influences of cold deprivation during dormancy on carbohydrate contents of vegetative and floral primordial and nearby structures of peach buds (*Prunus persica* L. Batch). - Scientia Hort. **105**: 223-240, 2005.

Cook, N.C., Bellstedt, D.U.: Chilling response of 'Granny Smith' apple lateral buds inhibited by distal shoot tissues. - Scientia Hort. **89**: 299-308, 2001.

Dennis, F.G.: Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. - Hort. Sci. **38**: 347-350, 2003.

Egea, J., Ortega, E., Martínez-Gómez, P., Dicenta, F.: Chilling and heat requirements of almond cultivars for flowering. - Environ. exp. Bot. **50**: 79-85, 2003.

Elle, D., Sauter, J.J.: Seasonal changes of activity of a starch granule bound endoamylase and of a starch phosphorylase in poplar wood (*Populus* × *canadensis* Moench ‹robusta›) and their possible regulation by temperature and phytohormones. - J. Plant Physiol. **156**: 731-740, 2000.

Galet, P.: Précis de Viticulture. 6th Ed. - Dhean Press, Montpelier 1993. [In French]

Gibson, S.I.: Plant sugar-response pathways: part of a complex regulatory web. - Plant Physiol. **124**: 1532-1539, 2000.

González-Rossia, D., Reig, C., Dovis, V., Gariglio, N., Agustí, M.: Changes on carbohydrates and nitrogen content in the bark tissues induced by artificial chilling and its relationship with dormancy bud break in *Prunus* sp. - Scientia Hort. **118**: 275-281, 2008.

Halaly, T., Pang, X., Batikoff, T., Crane, O., Keren, A.,

Venkateswari, J., Ogrodovitch, A., Sadka, A., Lavee, S., Or, E.: Similar mechanisms might be triggered by alternative external stimuli that induce dormancy release in grape buds. - Planta **228**: 79-88, 2008.

Huang, C., Yu, B., Teng, Y., Su, J., Shu, Q., Cheng, Z., Zeng, L.: Effects of fruit bagging on coloring and related physiology, and qualities of red Chinese sand pears during fruit maturation. - Scientia Hort. **121**: 149-158, 2009.

Hussain, S., Liu, G., Liu, D., Ahmed, M., Hussain, N., Teng, Y.: Study on the expression of dehydrin genes and activities of antioxidative enzymes in floral buds of two sand pear (*Pyrus pyrifolia* Nakai) cultivars requiring different chilling for budbreak. - Turkish J. Agr. Forest. doi:10.3906/tar-1407-164, 2015.

Klinac, D.C., Geddes, B.: Incidence and severity of the floral 'budjump' on nashi grown in the Waikato region of New Zealand. - New Zeal. J. Crop hort. Sci. **23**: 185-190, 1995.

Koch, K.: Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. - Curr. Opin. Plant Biol. **7**: 235-246, 2004.

Komatsu, A., Takanokura, Y., Moriguchi, T., Omura, M., Akihama, T.: Differential expression of three sucrose-phosphate synthase isoforms during sucrose accumulation in citrus fruits. - Plant Sci. **140**: 169-178, 1999.

Koussa, T., Rifai, L.A., Cherrad, M.: Annual variations pf alpha-amylase and invertases activities in buds and internodes of grape vines and their relation with carbohydrates and abscisic acid content. - J. Int. Sci. Vigne. Vin. **39**: 129-136, 2005.

Lang, G.A., Early, J.D., Martin, G.C., Darnell, R.L.: Endo-para and eco-dormancy: physiological terminology and classification for dormancy research. - Hort. Sci. **22**: 371-377, 1987.

Leite, G.B., Bonhomme, M., Lacointe, A., Rageau, R., Sakr, S., Guilliot, A., Maurel, K., Pétel, G., Couto-Rodrigues, A.: Influence of lack of chilling on bud-break patterns and evolution of sugar contents in buds and stem tissues along the one-year old shoot of the peach trees. - Acta hort. **662**: 61-71, 2004.

Linsley-Noakes, G.C., Louw, M., Allan, P.: Estimating daily positive Utah chill units using daily maximum and minimum temperatures. - J. S. Afr. Soc. hort. Sci. **5**: 19-22, 1995.

Liu, G.: Studies on Molecular Mechanism of Pear Dormancy. PhD Thesis, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 2013.

Liu, G., Li, W., Zheng, P., Xu, T., Chen, L., Liu, D., Hussain, S., Teng, Y.: Transcriptomic analysis of 'Suli' pear (*Pyrus pyrifolia* white pear group) buds during the dormancy by RNA-Seq. - BMC Genomics **13**: 700, 2012.

Marafon, A.C., Citadin, I., Amarante, L., Herter, F.G., Hawerroth, F.J.: Chilling privation during dormancy period and carbohydrate mobilization in Japanese pear trees. - Sci. Agr. **68**: 462-468, 2011.

Marquat, C., Vandamme, M., Gendraud, M., Petal, G.: Dormancy in vegetative bud of peach: relation between carbohydrate absorption potentials and carbohydrate concentration in the bud during dormancy and its release. - Scientia Hort. **79**: 151-162, 1999.

Mauget, J.C., Rageau, R.: Bud dormancy and adaptation of apple tree in mild winter climates. - Acta hort. **232**: 101-108, 1988.

Maurel, K., Leite, G.B., Bonhomme, M., Guilliot, A., Rageau, R., Pétel, G., Sakr, S.: Trophic control of budbreak in peach (*Prunus persica*) trees: a possible role of hexoses. - Tree Physiol. **24**: 579-588, 2004.

Oh, S., Klinac, D.: Relationship between incidence of floral bud death and temperature fluctuation during winter in Japanese pear (*Pyrus pyrifolia*) cv. Housui under New Zealand climate conditions. - J. korean Soc. hort. Sci. **44**: 162-166, 2003.

Petri, J.L., Herter, F.G.: Nashi pear (*Pyrus pyrifolia*) dormancy under mild temperate climate conditions. - Acta hort. **587**: 353-361, 2002.

Petri, J.L., Leite, G.B., Yasunobu, Y.: Studies on causes of floral abortion in Japanese pear (*Pyris pyrifolia* Nakai) in southern Brazil. - Acta hort. **587**: 375-380, 2002.

Powell, L.E.: Hormonal aspects of bud and seed dormancy in temperate zone woody plants. - Hort. Sci. **22**: 845-850, 1987.

Roitsch, T., González, M.C.: Function and regulation of plant invertases: sweet sensations. - Trend Plant Sci. **19**: 606-613, 2004.

Rose, R., Rose, C.L., Omi, S.K., Forry, K.R., Dural, D.M., Bigg, W.L.: Starch determination by perchloric acid *vs*. enzymes: evaluating the accuracy and precision of six colorimetric methods. - J. Agr. Food Chem. **39**: 2-11, 1991.

Sauter, J.J.: Temperature-induced changes in starch and sugars in the stem of *Populus* × *canadensis* 'Robusta'. - J. Plant Physiol. **132**: 608-612, 1988.

Sheen, J., Zhou, L., Jang, J.C.: Sugars as signaling molecules. - Curr. Opin. Plant Biol. **2**: 410-418, 1999.

Sherson, S.M., Alford, H.L., Forbes, S.M., Wallace, G., Smith, S.M.: Roles of cell wall invertases and monosaccharide transporters in the growth and development of *Arabidopsis*. - J. exp. Bot. **54**: 525-531, 2003.

Smeekens, S.: Sugar-induced signal transduction in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **51**: 49-81, 2000.

Strum, A.: Molecular characterization and functional analysis of sucrose cleaving enzymes in carrot (*Daucus carota* L.). - J. exp. Bot. **47**: 1187-1192, 1996.

Swanson, C.A., El-Shishiny, E.D.H.: Translocation of sugars in the Concord grape. - Plant Physiol. **33**: 33-37, 1958.

Teng, Y., Chen, L., Cai, D., Wang, T., Huang, X.: Effect of reflective film on sugar accumulation and sucrose-metabolizing enzyme activities of 'Cuiguan' pear under plastic tunnel culture. - Acta hort. **1015**: 59-66, 2014.

Trejo-Martínez, M.A., Orozco, A., Almaguer-Vargas, G., Carvajal-Millán, E., Gardea, A.A.: Metabolic activity of low chilling grape vine buds forced to budbreak. - Thermochim. Acta **481**: 28-31, 2009.

Wang, S.Y., Ji, Z.L., Faust, M.: Metabolic changes associated with bud break induced by thidiazuron. - J. Plant Growth Regul. **6**: 85-95, 1987.

Yoshioka, H., Nagal, K., Aoba, K., Fukumoto, M.: Seasonal changes of carbohydrates metabolism in apple trees. - Scientia Hort. **36**: 219-227, 1988.

Young, E., Motomura, Y., Unrath, C.R.: Influence of root temperature during dormancy on respiration, carbohydrates, and growth resumption in apple and peach. - J. amer. Soc. hort. Sci. **112**: 514-519, 1987.