Received: 1 March 2023 | Revised: 11 May 2023 | Accepted: 25 May 2023

DOI: 10.1111/ppl.13940

ORIGINAL RESEARCH

Physiologia Plantarum

Vessel development in compound buds of interspecific hybrid grape induced by artificial deacclimation treatments

Jun Kasuga | Ayumi Kawase | Rika Kamigaki | Yuka Mihara | Daisuke Kondoh

Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

Correspondence
Jun Kasuga, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho 2-11, Obihiro, Hokkaido 080-8555, Japan.
Email: jkasuga@obihiro.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Number: 21K05555

Edited by E. Pesquet

Abstract

Grape compound buds adapt to subfreezing temperatures in winter by supercooling, but the supercooling ability is thought to be lost upon formation of xylem connections between canes and buds. It was reported that compound buds of the *Vitis vinifera* variety 'Chardonnay' lack xylem cells in mid-winter, and that vessels differentiate during deacclimation. However, the pattern of vessel formation in compound buds may differ in cold-hardy *Vitis* species and interspecific hybrid varieties grown in colder regions. We investigated vessel formation in compound buds of the interspecific hybrid variety 'Yamasachi', which were harvested in mid-winter, during artificial deacclimation treatments. Before these treatments, 'Yamasachi' buds had a high supercooling ability (approx. −30°C) and contained cells with characteristics of vessel elements, that is, secondary wall thickening and lignification, at the basal parts. However, the cells still contained organelles and did not have a hydraulic conductivity function. Xylem continuity between the canes and buds was established from day 7 of deacclimation at 20°C. The different pattern of seasonal vessel formation in compound buds of 'Yamasachi' from that of *V. vinifera* may reflect the rapid development traits of *Vitis* species growing in cold regions with short growing seasons.

1 | INTRODUCTION

Low temperature is one of the major environmental factors that restrict the geographical distribution and productivity of plants (Parker, 1963; Sakai & Larcher, 1987). In temperate and boreal zones, perennial woody plants acclimate to cold temperatures and freezing conditions during autumn and winter in response to short daylength and/or cold temperatures. They acquire maximum freezing resistance in mid-winter, and then lose this resistance via a deacclimation process in response to increasing temperatures and/or daylength (Kalberer et al., 2006; Arakawa et al., 2018). The physiological changes that occur in deacclimating plants involve preparations for the resumption of growth in the next growing season as well as a decrease in freezing resistance (Pagter et al., 2017).

Winter compound buds of *Vitis* species adapt to subfreezing temperatures through freezing avoidance mechanisms (Quamme, 1986; Kasuga et al., 2020). A typical grape compound bud has three embryonic shoots inside, referred to as the primary, secondary, and tertiary buds. Each of them maintains a supercooled state individually under subfreezing temperatures. To maintain the supercooled state, each embryogenic shoot has to avoid ice propagating from ice crystals outside the bud. Suberin-coated bud scales are one likely barrier against external snow and frost (Jones et al., 2000). Ice crystals also exist in apoplastic spaces of cane tissues under subfreezing conditions (Horiuchi et al., 2021). Thus, another structural barrier should exist between the bud embryogenic shoots and ice crystals in canes. Previously, it was shown that the supercooling ability of dormant grape buds is lost or severely weakened by the elimination of nodal tissue beneath bud tissues (Quamme, 1986; Wolf & Pool, 1987), suggesting that some tissue near the xylem-bud boundary could act as a barrier against ice propagation from the cane apoplast into buds. The deposition of waxy substances such as suberin has not been observed at the tissues near the xylem-bud boundary (Jones et al., 2000). For the tissue to have a barrier function, therefore, the microcapillaries connecting apoplastic ice crystals and supercooled water in buds must be narrow enough to depress the melting point of water inside at the temperature in the

tissue (Ashworth & Abeles, 1984). According to Ashworth and Abeles (1984), the microcapillary diameter has a large effect on melting point when the diameter is smaller than 7 nm. A study on the compound buds of *Vitis* species detected a decreased dye permeability from canes to buds during winter (Jones et al., 2000).

When the water requirements of trees' buds for their development in spring exceed the supply capacity of non-vascular pathways, water is supplied via pipe-like water conduits, vessels, or tracheids that connect the cane xylem with the new shoots (Savage & Chuine, 2021). However, such water conduits with a large diameter can act as ice propagation pathways across the xylem-bud boundary. Thus, it has been proposed that the establishment of xylem continuity between cane xylem and buds leads to the loss of the supercooling ability of winter buds in some tree species (Ashworth, 1984; Callan, 1990; Ashworth et al., 1992; Kader & Proebsting, 1992; Xie et al., 2018). In flower buds of *Prunus* species that adapt to subfreezing temperatures by supercooling, only procambial cells are present in dormant floral primordia, and xylem development progresses during deacclimation (Ashworth, 1984; Callan, 1990; Kader & Proebsting, 1992). A similar pattern of xylem development was also reported for dormant compound buds of *Vitis vinifera* cv. 'Chardonnay' in relation to their supercooling ability (Xie et al., 2018). During mid-winter, no xylem cells are present in the buds and the junction regions between buds and canes. Cells with properties of vessel elements are present in buds that are starting to swell, before the establishment of a xylem connection.

In cold climate regions where economically viable wine grape production is not possible with *V. vinifera* varieties, cold-hardy interspecific hybrid varieties, which are products of crosses between *V. vinifera* and native Asian or North American *Vitis* species, are often grown for winemaking (Fennell, 2004; Riesterer-Loper et al., 2019). For grapevines grown in cold regions, an early bud breaking trait is desirable so that the fruit has time to mature during the cool growing season (Fuller & Telli, 1999; Hamed et al., 2000). Thus, the manner of xylem development in winter buds of cold-hardy interspecific hybrid varieties may differ from that in *V. vinifera* varieties growing in relatively warm regions. In this study, we investigated changes in the supercooling ability of, and xylem development in, winter compound buds during artificial deacclimation in the cold-hardy interspecific hybrid grape variety 'Yamasachi'. Based on the results, we discuss the role of vessel formation in the decrease in the supercooling ability of 'Yamasachi' buds during deacclimation.

2 | MATERIALS AND METHODS

2.1 | Plant materials

Canes were harvested from approximately 20-year-old 'Yamasachi' vines growing in the vineyard of the Tokachi-Ikeda Research Institute for Viticulture and Enology (42°56′N, 143°22′E) in Hokkaido, Japan, in early February 2021 and 2022. The canes were collected in the morning, cut into lengths of approximately 20 cm, and stored in a plastic bag with crushed ice at −7°C until use.

2.2 | Deacclimation treatments

Cane pieces (approximately 6 cm in length), each containing a compound bud, were cut from stored canes, and the lower cut ends were inserted into rock wool blocks in water-filled plastic trays (Figure S1). The cuttings were deacclimated in incubators (i-CUBE FCI-280G; AS ONE) set at 4°C or 20°C for 1 to 9 days in darkness.

2.3 | Determination of supercooling ability of primary buds

The supercooling ability of primary buds in grape compound buds was determined by differential thermal analysis. Compound buds with small cane pieces (1.5 cm in length) were cut from non-deacclimated (non-DA) and deacclimated canes with a pair of pruning shears. A junction of a T-type thermocouple (36 AWG) was fixed on the surface of each bud by wrapping with parafilm (Bemis, Oshkosh). These buds were maintained at 4°C for 1 h in a programmable freezer constructed from a deep freezer (MDF-C8V1-PJ; Panasonic Healthcare), a fan heater (TNK-FH100; Tanaka), and a temperature control unit (TNK-TS100; Tanaka), and then cooled to −40°C at a rate of 5°C/h. Eight buds were tested per each condition. Differences in thermal responses between experimental bud samples and oven-dried reference samples were recorded with a multi-channel data logger (LR8400; Hioki). Grape buds generate two kinds of exothermal responses during the cooling process: One exothermal peak generated at temperatures higher than −10°C (high-temperature exotherm: HTE) originates from the freezing of water outside the bud primordia; and one or more small sharp peaks generated at lower temperatures than the HTE (low-temperature exotherm: LTE) originate from the freezing of supercooled water in the bud (Quamme, 1986; Kasuga et al., 2020). Multiple LTEs from a compound bud are indicative of independent freezing of supercooled water in primary, secondary, and tertiary buds. In this study, we recorded the initiation temperature of the LTE at the highest temperature to evaluate the supercooling ability of primary buds.

2.4 | Microscopic observations

To observe cells with secondary walls in basal parts of compound buds, buds with small pieces of cane tissues were cut with a razor blade and fixed overnight in 3% (v/v) glutaraldehyde in 60 mM phosphate buffer solution (pH 7.0) at 4°C. The buds were subsequently washed with phosphate buffer solution, dehydrated using a graded alcohol series,

and then embedded in paraffin (Paraplast Plus; Sigma-Aldrich). Cross sections of buds were cut to a thickness of 10 µm at 50 µm intervals with a steel blade mounted on a rotary microtome (RM2125RT; Leica). After deparaffinization, sections were double-stained with 1% (w/v) safranin (Merck) in 50% ethanol and 1% (w/v) light green SF yellowish (Merck) in 100% ethanol. Cells with secondary walls were identified by polarized optical microscopy. Five buds were used for the observation per each condition. Secondary walls with birefringent properties appear bright between crossed polarizing elements, whereas the background remains dark (Schweingruber, 2007). Prepared sections of basal parts of buds 100 to 200 µm away from the xylem-bud boundary were observed under a polarized optical microscope (PL-213; AS ONE). The numbers of vascular bundles and cells with secondary walls in the sections were counted, and the mean number of cells with secondary walls in a vascular bundle was calculated for each bud.

Lignified cells in compound buds were visualized by phloroglucinol-HCl staining, according to Lee et al. (2017). Longitudinal sections were cut by hand from non-DA compound buds with a razor blade. The sections were immersed in 10% (w/v) phloroglucinol in 95% ethanol for 10 min, and then an equal volume of concentrated HCl was added. After 2 min, the sections were rinsed with MilliQ water and observed under a light microscope (DM2500; Leica). Five non-DA buds were used for this observation.

Small tissue blocks (2 × 2 × 2 mm) containing boundaries between basal parts of primary buds and the cane xylem and their surroundings were cut from non-DA buds with a razor blade for transmission electron microscopy. The blocks were double-fixed with 3% (v/v) glutaraldehyde in 60 mM phosphate buffer solution (pH 7.0) at 4°C overnight and subsequently with 1% (w/v) osmium tetroxide in the phosphate buffer solution at room temperature for 2 h. After washing three times with phosphate buffer solution, the blocks were dehydrated using a graded alcohol series and embedded in epoxy resin (Plain resin; Nisshin-EM) according to the manufacturer's instructions. Ultrathin sections (70 nm thick) were placed on copper grids and examined under a transmission electron microscope (HT7700; Hitachi) with neither uranyl acetate nor lead citrate staining. Five buds were used for the electron microscopic observation. The brightness and contrast of the obtained TIFF data were adjusted by Photoshop CS6 (Adobe).

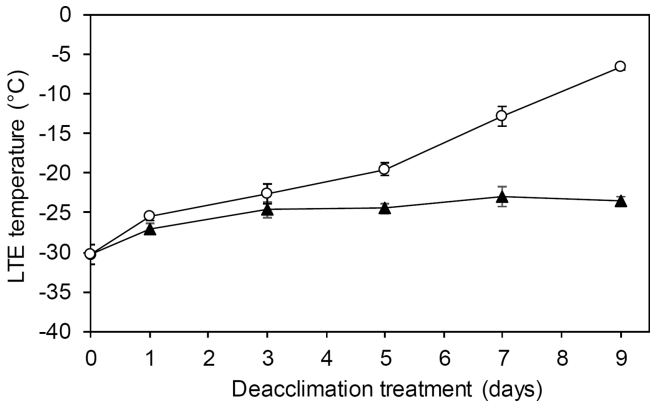

FIGURE 1 Changes in freezing temperatures of primary buds induced by artificial deacclimation treatments. Triangles: deacclimation at 4°C. Circles: deacclimation at 20°C. Values are means ± SD ($n = 8$ buds from different plants).

2.5 | Evaluation of xylem continuity between twig and buds

Xylem continuity between twig and buds was examined in a dye-uptake experiment. Compound buds were excised with a 1 mm section of cane attached. The cut surfaces of canes were submerged in 0.01% (w/v) fluorescein sodium salt (F6377; Sigma-Aldrich; Stokes-Einstein radius = approx. 0.45 nm) in 10 mM potassium phosphate buffer (pH 5.8) at 20°C for 16 h to allow the buds to take up the dye. The buds were then rinsed with MilliQ water and longitudinal sections

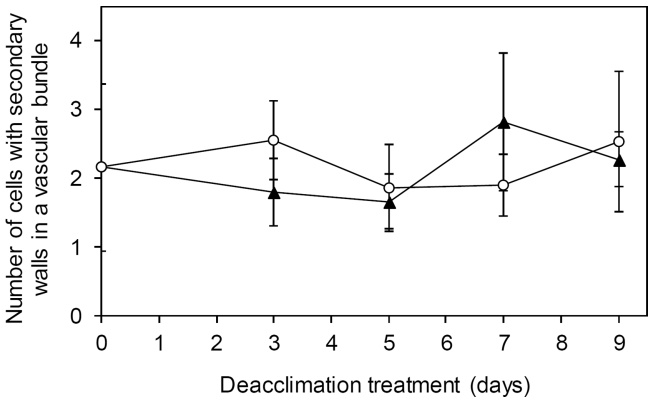

FIGURE 2 Changes in numbers of cells with secondary walls in a vascular bundle in basal parts of primary buds during deacclimation treatments. Triangles: deacclimation at 4°C. Circles: deacclimation at 20°C. Values are means ± SD ($n = 5$ buds from different plants). No significant differences were observed among data points (one-way analysis of variance, $p = 0.19$ for deacclimation at 4°C and $p = 0.54$ for deacclimation at 20°C, respectively).

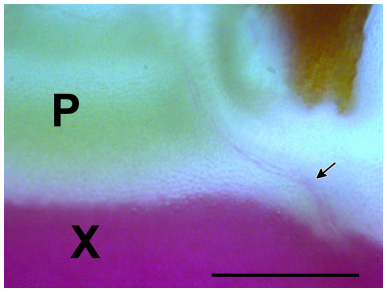

FIGURE 3 Lignified cells in basal part of a non-deacclimated 'Yamasachi' bud. Figure shows a longitudinal section of a compound bud stained with phloroglucinol-HCl to visualize lignified cells. Linear structures composed of stained cells were located across the basal part of the bud (arrow). P: primary bud; X: xylem tissue of cane. Scale bar, 500 µm.

were cut by hand using a razor blade. Distribution of fluorescein was examined under a fluorescence microscope (THUNDER 3D; Leica). Eight buds were tested per each condition.

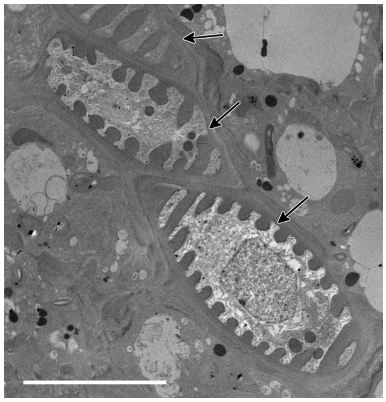

FIGURE 4 Transmission electron micrograph of developing vessel elements in a non-deacclimated primary bud. This image shows a part of bud tissue approximately 200 μm away from the xylem-bud boundary. Arrows indicate xylem cells having walls with annular or spiral secondary wall thickenings. Some organelles were present in these cells. Scale bar, 10 μm.

3 | RESULTS

3.1 | Changes in supercooling ability of 'Yamasachi' buds during deacclimation

In this study, 'Yamasachi' buds were artificially deacclimated at 4°C and 20°C (Figure 1). Before deacclimation, primary buds in 'Yamasachi' compound buds were able to supercool to approximately −30°C as reported in Kasuga et al. (2020). The supercooling ability of grape primary buds started to decrease soon after the initiation of deacclimation treatments. After deacclimation for 1 day at 4°C and 20°C, the supercooling abilities decreased to −27.0°C and − 25.5°C, respectively. The pattern of the decrease in supercooling ability differed depending on the deacclimation temperature. The supercooling ability of primary buds became nearly constant at approximately −24°C from day 3 to day 9 of the deacclimation treatment at 4°C. In contrast, the supercooling ability continued to decrease until day 9 of the deacclimation treatment at 20°C, when it reached −6.6°C. There was little change in the appearance of 'Yamasachi' buds by day 9 of deacclimation at 20°C, but distinct bud swelling was visible on day 11 (Figure S1).

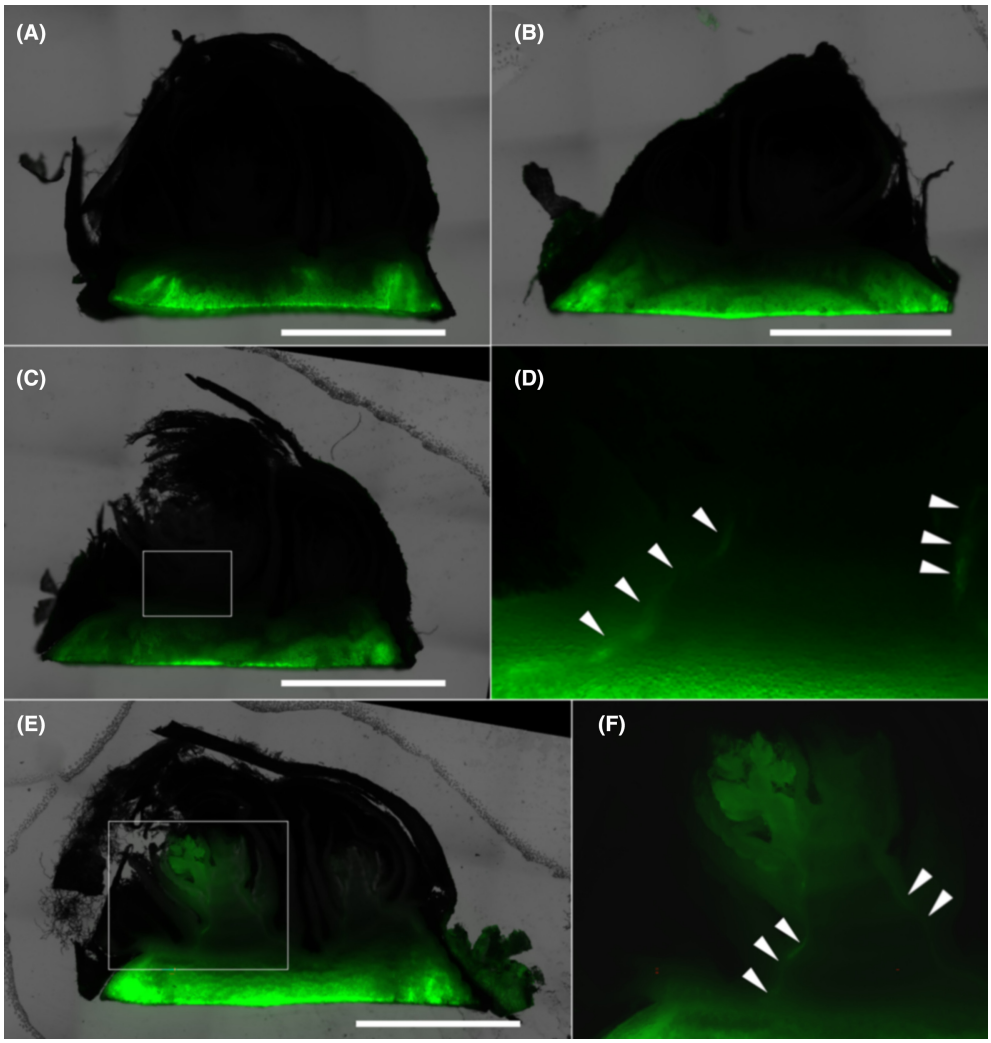

FIGURE 5 Acquisition of vessel function in 'Yamasachi' buds during deacclimation treatments. Fluorescein was taken up at the cut surfaces of cane xylem. (A) Deacclimation at 4°C for 9 days; (B) deacclimation at 20°C for 5 days; (C) deacclimation at 20°C for 7 days; (D) magnified image of region marked by white square in (C); (E) deacclimation at 20°C for 9 days; (F) magnified image of region marked by white square in (E). Arrowheads in (D) and (F) indicate the fluorescence from vessels. Fluorescein signal spread into primordial tissues in (E) and (F). Scale bars in (A), (B), (C), and (E), 3 mm.

3.2 | Cells with characteristics of vessel elements in basal parts of compound buds

We calculated the mean number of cells with secondary walls in a vascular bundle in each bud as an indicator of the progress of secondary wall formation (Figure 2). Cells with secondary wall thickening were present even in non-DA buds. On average, each vascular bundle contained approximately two cells with secondary wall thickening. This number showed little increase during the 9-day deacclimation treatments at both 4°C and 20°C. No significant differences were detected among the data points in Figure 2.

Phloroglucinol-HCl staining revealed the presence of lignified cells in non-DA 'Yamasachi' buds (Figure 3). Lines of stained cells ran from the xylem of canes to the inside of buds.

Transmission electron microscopy analyses provided further evidence for the presence of cells with developed secondary walls in non-DA 'Yamasachi' buds. Figure 4 shows a part of bud tissue 200 μm away from the boundary between the xylem and the bud. The non-DA buds contained some cells with walls with annular or spiral wall thickenings. This characteristic thickening is formed as the innermost layer of the secondary walls of xylem cells. Organelles were present in these cells, suggesting that programmed cell death, autolysis of the cell contents, and subsequent acquisition of the hollow tube-like structure of vessel elements had not yet occurred in non-DA buds of 'Yamasachi'.

3.3 | Establishment of xylem continuity in compound buds during deacclimation treatments

To clarify when vessels in 'Yamasachi' buds acquire their hydraulic conductivity function during deacclimation, we conducted dye-uptake experiments. Fluorescein did not penetrate into non-DA buds and buds deacclimated at 4°C (Figure 5A). In contrast, although fluorescein did not penetrate into buds by day 5 of deacclimation at 20°C (Figure 5B), weak fluorescence of the dye was detected from the vessels in two out of eight tested buds on day 7 of deacclimation at 20°C (Figure 5C,D). By day 9 of deacclimation at 20°C, fluorescence was detected not only from vessels but also from primordial tissues in all tested buds (Figure 5E, F). These results indicated that vessels in 'Yamasachi' buds start to function from around day 7 of deacclimation at 20°C.

4 | DISCUSSION

It was surprising for us to observe secondary-wall-deposited and lignified cells in non-DA 'Yamasachi' buds (Figures 2 and 3) because it has been reported previously that supercooled winter compound buds of the grape variety 'Chardonnay' lack vessel elements (Xie et al., 2018). The lack of cells with characteristics of vessel elements during the dormant season has also been reported in supercooled flower buds of *Prunus* species (Ashworth, 1984) and apical buds of Norway spruce (*Picea abies*) (Lee et al., 2017). In these buds, vessels differentiate during deacclimation. If a water conduit across the junction of the bud and cane forms as a hollow pipe with a diameter larger than several micrometers, then freezing in canes will easily give rise to freezing in the buds through the conduit. The differentiation of xylem cells, including vessel elements, occurs in an overlapping series of events after cell division: primary wall synthesis, cell elongation, secondary wall synthesis dominated by polysaccharide production, secondary wall maturation dominated by lignification, and programmed cell death (Meents et al., 2018). In addition, partial degradation of primary walls occurs on end walls and at pits in vessel elements (Butterfield & Meylan, 1982). In the secondary xylem of oak (*Quercus rubra*) and poplar (*Populus nigra* var. *italica*), the degradation of the end walls occurs at about the same time as the disintegration of the protoplasm of vessel elements (Murmanis, 1978; Benayoun et al., 1981). Our electron microscopic observations revealed the presence of organelles in vessel elements in non-DA 'Yamasachi' buds (Figure 4). This result indicates that the vessel elements were still alive and had end walls in the non-DA buds. We expect that the end walls of vessel elements effectively function as a barrier against ice propagation from the canes. Very recently, Villouta et al. (2022) reported that cranberry (*Vaccinium macrocarpon*) buds that exhibited extraorgan freezing had tracheids at the base during winter. Although the authors did not test the viability of the tracheid cells, they expected that the tracheid cells were not fully functional during bud formation in the late summer and would remain in this state through the winter.

In a previous study, dye-uptake experiments on 'Chardonnay' revealed that hydraulic cells became functional in primordial tissues around the end of March when most buds lost their scales (Xie et al., 2018). In contrast, in this study, the initiation of fluorescein dye uptake into vessels of 'Yamasachi' buds occurred at day 7 of a deacclimation treatment at 20°C (Figure 5). At this time point, swelling of the buds was barely detectable based on their external appearance (Figure S1). Although we have to consider the differences in the deacclimation conditions between the two studies, these results suggest that, in addition to vessel formation, the initiation of the establishment of xylem continuity occurs earlier in 'Yamasachi' buds than in 'Chardonnay' buds in spring. Kovaleski et al. (2018) suggested that the growth of *Vitis riparia* was earlier and the deacclimation rates of *V. riparia* and *Vitis amurensis* were faster at low above-freezing temperatures compared with those of *V. vinifera* varieties. The earlier bud growth of hardy species might be explained by the evolutionary necessity for rapid development during short growing seasons (Ferguson et al., 2014; De Rosa et al., 2021). 'Yamasachi' was developed by crossing 'Seibel 13053' as the seed parent with a local wild mountain grape (probably *V. amurensis*) as the pollen parent. The pedigree of the seed parent 'Seibel 13053' includes some North American *Vitis* species, including *V. riparia* (Fisher, 1980). Earlier development of vessel elements in winter buds might be a characteristic of cold-hardy *Vitis* species and interspecific hybrid varieties, and may be related to their earlier bud growth compared with that of *V. vinifera* varieties.

Although xylem continuity had been completely established in all tested 'Yamasachi' buds by day 9 of deacclimation at 20°C (Figure 5),

LTE peaks from the buds were clearly separated from HTE peaks in profiles of differential thermal analyses at this time point (Figure S2). This result indicates that some weak but functional ice barriers still existed in the water transport pathway. The reproductive shoots of an alpine woody shrub *Calluna vulgaris* can supercool to below −20°C, even though xylem continuity is already established because they have ice barrier tissues at the base of the pedicels (Kuprian et al., 2016). In the barrier tissues, only tracheids are present as conducting xylem cells, and the structural characteristics of pits of the tracheids are thought to prevent ice propagation into supercooled reproductive shoots. So far, the mechanisms that prevent ice propagation into grape buds after the establishment of xylem continuity remain unclear. However, the supercooling events observed during the bud break of grape (Hamed et al., 2000) support the possibility that there are barrier structures in the water transport pathway in buds in the early stages of bud break.

In this study, a decrease in the supercooling ability of 'Yamasachi' buds was detected from day 1 of deacclimation treatments at both 4°C and 20°C (Figure 1). However, the establishment of xylem continuity into the buds only occurred from day 7 of a deacclimation treatment at 20°C (Figure 5). Therefore, the decreased bud supercooling ability detected at day 9 of the deacclimation treatment at 4°C and day 5 of the deacclimation treatment at 20°C were not directly induced by the establishment of xylem continuity in buds. This raises the question as to which factors are involved in changing the supercooling ability of buds.

It has been proposed that quantitative and qualitative changes in pectin in the cell walls of the barrier tissues are involved in the functional decline of the ice barrier of supercooled plant cells or tissues during deacclimation (Panter et al., 2020; Takahashi et al., 2021). In the xylem parenchyma cells of peach (*Prunus persica*), which adapt to subfreezing temperatures by deep supercooling, swelling of an amorphous layer in the vicinity of pit membranes was observed in water-soaked twigs during a deacclimation treatment (Wisniewski & Davis, 1989). A treatment of peach twigs with a pectinase-rich enzyme mixture, macerase, resulted in extensive structural modifications of both the pit membrane and amorphous layer and the disappearance of LTE peaks from the differential thermal analysis profiles (Wisniewski et al., 1991). Those findings suggested that modifications in pectin around pit membranes are involved in the decrease in the supercooling ability of the xylem parenchyma during deacclimation. In dormant buds of Norway spruce, a short-day treatment induced an increase in the un-esterified pectin content in crown tissues, which act as an ice barrier against extraorgan ice crystals, and a subsequent long-day treatment resulted in decreased un-esterified pectin content (Lee et al., 2017). Seasonal changes in un-esterified pectin contents were also detected in the bud axis subtending the floral primordium in floral buds of peach (Wisniewski & Davis, 1995). A monoclonal antibody against un-esterified pectins, JIM5, labeled intercellular spaces in the bud axis in samples harvested in winter, but not in those harvested in summer. Cross-linking between un-esterified pectin and calcium ions might reduce the microcapillary size in ice barrier tissues. Lee et al. (2017) detected callose deposition at the plasmodesmata in crown cells in dormant buds of Norway spruce and a reduction in callose deposition induced by a long-day treatment. The opening of plasmodesmata by the degradation of callose might contribute to the enlargement of ice propagation pathways. In addition, ongoing degradation of primary walls at end walls and pits (Murmanis, 1978; Benayoun et al., 1981) can weaken the barrier function of tissues near the xylem-bud boundary.

The breakdown of the supercooled state of water is induced not only by ice propagation from external ice but also by spontaneous ice nucleation in supercooled water. Deacclimation processes might increase the possibility of intracellular ice nucleation events. The strength of osmolality of a solution affects the spontaneous ice nucleation temperatures colligatively, as well as affects the melting point (Rasmussen & MacKenzie, 1972; Charoenrein & Reid, 1989). During deacclimation of grape buds, the water content increases (Ershadi et al., 2016; Meitha et al., 2018; Xie et al., 2018) and there are decreases in the contents of soluble carbohydrates (Jones et al., 1999; Grant & Dami, 2015; Ershadi et al., 2016) and proline (Ershadi et al., 2016). These physiological changes would cause a reduction of osmolality of the intracellular solution in grape buds.

In this study, we attempted to clarify the deacclimation mechanisms of winter buds in an interspecific hybrid grape variety, focusing on vessel formation. Our results indicate that there are complex mechanisms underlying the decrease in the supercooling ability of grape compound buds during deacclimation. Several transcriptomic studies have focused on deacclimating grape buds (Meitha et al., 2018; Kovaleski & Londo, 2019), although more studies have focused on the cold acclimation process (Xin et al., 2013; Fennell et al., 2015; Kim et al., 2017; Ma et al., 2022). Such comprehensive analyses may shed light on the factors contributing to the reduction in supercooling ability as well as dormancy release and growth resumption in grape buds. Further studies are required to explore the mechanisms of seasonal changes in the supercooling ability of grape buds, including acclimation in fall and deacclimation in spring.

AUTHOR CONTRIBUTIONS

Jun Kasuga designed the study, conducted the experiments, and lead the writing of the manuscript. Ayumi Kawase and Yuka Mihara contributed to the experiments related to light microscopy. Rika Kamigaki performed the experiments related to electron microscopy. Daisuke Kondoh assisted the microscopic observations. All authors have read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors thank Tokachi-Ikeda Research Institute for Viticulture and Enology for providing grape samples. We thank Jennifer Smith, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript. This work was supported by partly supported by the JSPS KAKENHI (21K05555).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jun Kasuga https://orcid.org/0000-0002-3173-618X

REFERENCES

Arakawa, K., Kasuga, J. & Takata, N. (2018) Mechanism of overwintering in trees. In: Iwaya-Inoue, M., Sakurai, M. & Uemura, M. (Eds.) *Survival strategies in extreme cold and desiccation: adaptation mechanisms and their application*. Singapore: Springer Nature Singapore Pte Ltd., pp. 129–147.

Ashworth, E. (1984) Xylem development in *Prunus* flower buds and the relationship to deep supercooling. *Plant Physiology*, 74, 862–865.

Ashworth, E.N. & Abeles, F.B. (1984) Freezing behavior of water in small pores and the possible role in the freezing of plant tissues. *Plant Physiology*, 76, 201–204.

Ashworth, E.N., Willard, T.J. & Malone, S.R. (1992) The relationship between vascular differentiation and the distribution of ice within forsythia flower buds. *Plant Cell & Environment*, 15, 607–612.

Benayoun, J., Catesson, A.M. & Czaninski, Y. (1981) A cytochemical study of differentiation and breakdown of vessel end walls. *Annals of Botany*, 47, 687–698.

Butterfield, B.G. & Meylan, B.A. (1982) Cell wall hydrolysis in the tracheary elements of the secondary xylem. In: Baas, P. (Ed.) *New perspectives in wood anatomy*. Dordrecht, Netherland: Springer, pp. 71–84.

Callan, N.W. (1990) Dormancy effects on supercooling in deacclimated 'meteor' tart cherry flower buds. *Journal of the American Society for Horticultural Science*, 115, 982–986.

Charoenrein, S. & Reid, D.S. (1989) The use of DSC to study the kinetics of heterogeneous and homogeneous nucleation of ice in aqueous systems. *Thermochimica Acta*, 156, 373–381.

De Rosa, V., Vizzotto, G. & Falchi, R. (2021) Cold hardiness dynamics and spring phenology: climate-driven changes and new molecular insight into grapevine adaptive potential. *Frontiers in Plant Science*, 12, 644528.

Ershadi, A., Karimi, R. & Mahdei, K.N. (2016) Freezing tolerance and its relationship with soluble carbohydrates, proline and water content in 12 grapevine cultivars. *Acta Physiologiae Plantarum*, 38, 2.

Ferguson, J.C., Moyer, M.M., Mills, L.J., Hoogenboom, G. & Keller, M. (2014) Modeling dormant bud cold hardiness and budbreak in twenty-three *Vitis* genotypes reveals variation by region of origin. *American Journal of Enology and Viticulture*, 65, 59–71.

Fennell, A. (2004) Freezing tolerance and injury in grapevines. *Journal of Crop Improvement*, 10, 201–235.

Fennell, A.Y., Schlauch, K.A., Gouthu, S., Deluc, L.G., Khadka, V., Sreekantan, L. et al. (2015) Short day transcriptomic programming during induction of dormancy in grapevine. *Frontiers in Plant Science*, 6, 834.

Fisher, K.H. (1980) Interspecific hybrids used in breeding wine grapes for southern Ontario, Canada (43° N latitude). In: *Proceedings of the third international symposium on grape breeding*. Davis, CA: University of California, pp. 12–20.

Fuller, M.P. & Telli, G. (1999) An investigation of the frost hardiness of grapevine (*Vitis vinifera*) during bud break. *Annals of Applied Biology*, 135, 589–595.

Grant, T.N.L. & Dami, I.E. (2015) Physiological and biochemical seasonal changes in *Vitis* genotypes with contrasting freezing tolerance. *American Journal of Enology and Viticulture*, 66, 195–203.

Hamed, F., Fuller, M.P. & Telli, G. (2000) The pattern of freezing of grapevine shoots during early bud growth. *CryoLetters*, 21, 255–260.

Horiuchi, R., Jitsuyama, Y., Arakawa, K., Kasuga, J. & Suzuki, T. (2021) Freezing resistance and behavior of winter buds and canes of wine grapes cultivated in northern Japan. *Cryobiology*, 101, 44–51.

Jones, K.S., McKersie, B.D. & Paroschy, J. (2000) Prevention of ice propagation by permeability barriers in bud axes of *Vitis vinifera*. *Canadian Journal of Botany*, 78, 3–9.

Jones, K.S., Paroschy, J., McKersie, B.D. & Bowlye, S.R. (1999) Carbohydrate composition and freezing tolerance of canes and buds in *Vitis vinifera*. *Journal of Plant Physiology*, 155, 101–106.

Kader, S.A. & Proebsting, E.L. (1992) Freezing behavior of *Prunus*, subgenus *Padus*, flower buds. *Journal of the American Society for Horticultural Science*, 177, 955–960.

Kalberer, S.R., Wisniewski, M. & Arora, R. (2006) Deacclimation and reacclimation of cold-hardy plants: current understanding and emerging concepts. *Plant Science*, 171, 3–16.

Kasuga, J., Tsumura, Y., Kondoh, D., Jitsuyama, Y., Horiuchi, R. & Arakawa, K. (2020) Cryo-scanning electron microscopy reveals that supercooling of overwintering buds of freezing-resistant interspecific hybrid grape 'Yamasachi' is accompanied by partial dehydration. *Journal of Plant Physiology*, 253, 153248.

Kim, S.A., Ahn, S.Y. & Yun, H.K. (2017) Transcriptomic changes in dormant buds of two grapevine cultivars following exposure to freezing temperature. *Horticulture, Environment, and Biotechnology*, 58, 152–161.

Kovaleski, A.P. & Londo, J.P. (2019) Tempo of gene regulation in wild and cultivated *Vitis* species shows coordination between cold deacclimation and budbreak. *Plant Science*, 287, 110178.

Kovaleski, A.P., Reisch, B.I. & Londo, J.P. (2018) Deacclimation kinetics as a quantitative phenotype for delineating the dormancy transition and thermal efficiency for budbreak in *Vitis* species. *AoB Plants*, 10, ply066.

Kuprian, E., Tuong, T.D., Pfaller, K., Wagner, J., Livingston, D.P., III & Neuner, G. (2016) Persistent supercooling of reproductive shoots is enabled by structural ice barriers being active despite and intact xylem connection. *PLoS One*, 11, e0163160.

Lee, Y.K., Karunakaran, C., Lahlali, R., Liu, X., Tanino, K.K. & Olsen, J.E. (2017) Photoperiodic regulation of growth-dormancy cycling through induction of multiple bud–shoot barriers preventing water transport into the winter buds of Norway spruce. *Frontiers in Plant Science*, 8, 2109.

Ma, X., Zhao, F., Su, K., Lin, H. & Guo, Y. (2022) Discovery of cold-resistance genes in *Vitis amurensis* using bud-based quantitative trait locus mapping and RNA-seq. *BMC Genomics*, 23, 551.

Meents, M.J., Watanabe, Y. & Samuels, A.L. (2018) The cell biology of secondary cell wall biosynthesis. *Annals of Botany*, 121, 1107–1125.

Meitha, K., Agudelo-Romero, P., Signorelli, S., Gibbs, D.J., Considine, J.A., Foyer, C.H. et al. (2018) Developmental control of hypoxia during bud burst in grapevine. *Plant, Cell & Environment*, 41, 1154–1170.

Murmanis, L.L. (1978) Breakdown of end walls in differentiating vessels of secondary xylem in *Quercus rubra* L. *Annals of Botany*, 42, 679–682.

Pagter, M., Alpers, J., Erban, A., Kopka, J., Zuther, E. & Hincha, D.K. (2017) Rapid transcriptional and metabolic regulation of the deacclimation process in cold acclimated *Arabidopsis thaliana*. *BMC Genomics*, 18, 731.

Panter, P.E., Panter, J.R. & Knight, H. (2020) Impact of cell-wall structure and composition on plant freezing tolerance. *Annual Plant Reviews Online*, 3, 607–642.

Parker, J. (1963) Cold resistance in woody plants. *The Botanical Review*, 29, 123–201.

Quamme, H.A. (1986) Use of thermal analysis to measure freezing resistance of grape buds. *Canadian Journal of Plant Science*, 66, 945–952.

Rasmussen, D.H. & MacKenzie, A.P. (1972) Effect of solute on ice-solution interfacial free energy; calculation from measured homogeneous nucleation temperatures. In: Jellinek, H.H.G. (Ed.) *Water structure at the water–polymer interface*. New York: Plenum Press, pp. 126–145.

Riesterer-Loper, J., Workmaster, B.A. & Atucha, A. (2019) Impact of fruit zone sunlight exposure on ripening profiles of cold climate interspecific hybrid winegrapes. *American Journal of Enology and Viticulture*, 70, 286–296.

Sakai, A. & Larcher, W. (1987) *Frost survival of plants: responses and adaptation to freezing stress*. Berlin: Springer-Verlag.

Savage, J.A. & Chuine, I. (2021) Coordination of spring vascular and organ phenology in deciduous angiosperms growing in seasonally cold climates. *New Phytologist*, 230, 1700–1715.

Schweingruber, F.H. (2007) *Wood structure and environment*. Berlin: Springer.

Takahashi, D., Willick, I., Kasuga, J. & Livingston, D., III. (2021) Responses of the plant cell wall to sub-zero temperatures: a brief update. *Plant & Cell Physiology*, 62, 1858–1866.

Villouta, C., Workmaster, B.A., Livingston, D.P., III & Atucha, A. (2022) Acquisition of freezing tolerance in *Vaccinium macrocarpon* Ait. is a multi-factor process involving the presence of an ice barrier at the bud base. *Frontiers in Plant Science*, 13, 891488.

Wisniewski, M. & Davis, G. (1989) Evidence for the involvement of a specific cell wall layer in regulation of deep supercooling of xylem parenchyma. *Plant Physiology*, 91, 151–156.

Wisniewski, M. & Davis, G. (1995) Immunogold localization of pectins and glycoproteins in tissues of peach with reference to deep supercooling. *Trees*, 9, 253–260.

Wisniewski, M., Davis, G. & Schaffer, K. (1991) Mediation of deep supercooling of peach and dogwood by enzymatic modifications in cell-wall structure. *Planta*, 184, 254–260.

Wolf, T.K. & Pool, R.M. (1987) Factors affecting exotherm detection in the differential thermal analysis of grapevine dormant buds. *Journal of the American Society for Horticultural Science*, 112, 520–525.

Xie, X., Forney, C.F. & Bondada, B. (2018) Renewal of vascular connections between grapevine buds and canes during bud break. *Scientia Horticulturae*, 233, 331–338.

Xin, H., Zhu, W., Wang, L., Xiang, Y., Fang, L., Li, J. et al. (2013) Genome wide transcriptional profile analysis of *Vitis amurensis* and *Vitis vinifera* in response to cold stress. *PLoS One*, 8, e58740.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kasuga, J., Kawase, A., Kamigaki, R., Mihara, Y. & Kondoh, D. (2023) Vessel development in compound buds of interspecific hybrid grape induced by artificial deacclimation treatments. *Physiologia Plantarum*, 175(3), e13940. Available from: https://doi.org/10.1111/ppl.13940