

カキの耐凍性における休眠覚醒と温度環境との関係について

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Cold Hardiness of Persimmon (*Diospyros kaki* Thunb.) Buds in Relation to Dormancy Release and Temperature Conditioning

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Summary

Using persimmon (*Diospyros kaki* cv. Hiratanenashi) buds collected in Yamagata, Kyoto, and Kochi Prefectures in Japan having different climate, the effect of bud dormancy and temperature conditionings (high temperature, 25°C; low temperature, 4°C) on the bud cold hardiness was studied. Additionally, the same temperature conditionings were applied to 'Hiratanenashi' and 'Fuyu' buds whose dormancy release was delayed with 100 ppm gibberellic acid (GA₃). Evaluation of cold hardiness was conducted on the buds using microcomputer-based thermal analysis.

The dormancy release of 'Hiratanenashi' bud from Yamagata Prefecture, where mean air temperature during the winter was 2 to 3°C lower than Kyoto and Kochi Prefectures, was completed in late November, whereas that of the other locations occurred in late December. Irrespective of locations, the buds showed the same seasonal pattern, being hardest in late January. Neither the low nor the high temperature conditionings had an effect on the bud cold hardiness during the deep dormant period at all locations. After the release from bud dormancy was induced, the high temperature conditioning decreased the bud cold hardiness by a maximum of 4°C, whereas the low temperature conditioning increased the bud cold hardiness by a maximum of 1.5°C. Two foliar applications of GA in the fall to 'Hiratanenashi' and 'Fuyu' delayed the release of bud dormancy 12 and 8 days, respectively. As with untreated 'Hiratanenashi' buds from different locations, GA-treated 'Hiratanenashi' and 'Fuyu' buds responded to the temperature conditionings only after the buds were released from dormancy. Likewise, potted 'Hiratanenashi' trees in Kyoto, subjected to the temperature conditionings, did not respond to cold hardiness conditionings until the bud dormancy release was completed.

Key Words: cold hardiness, *Diospyros kaki*, dormancy, temperature conditioning, thermal analysis.

Introduction

The dormant flower buds of many woody deciduous species avoid freezing injury by deep supercooling (Quamme, 1978). The ability to deep supercool changes seasonally during the annual cycle, increasing as winter progresses and decreasing with the increase in temperature in spring (Andrews et al., 1983b; Kaku et al., 1981). These seasonal changes result from a series of processes called cold acclimation and deacclimation, respectively. Therefore, chilling injury can be caused by very low temperatures during dormancy or untimely freezing temperatures before acclimation in fall or after deacclimation in spring (Ashworth and Wisniewski, 1991; Quamme, 1978). Consequently, low temperature limits the production and distribution area of fruit crops (Ashworth and Wisniewski, 1991; Gogoleva et al., 1994). Persimmon which is the major commercial species of the genus *Diospyros* has been cultivated in temperate regions such as China, Italy, Japan and Korea, and its response to freezing temperature was variable in different environmental conditions (Leng et

al., 1993; Sharpe, 1966). In Korea, seasonal fluctuations in cold hardiness of persimmon have limited its production and cultivation area (Kim et al., 1988). Hence, in planning an orchard, not only the maximum level of the cold hardiness of the plants but also the physiological and environmental aspects as related to its seasonal changes are important factors to consider.

The seasonal change in cold hardiness of plant is well related to changes of intensity of bud dormancy (Fuchigami et al., 1982; Harada, 1985; Proebsting, 1963). In addition, the relationship between the cold hardiness and environmental temperature in the acclimation and deacclimation processes has also been extensively studied in bud dormancy (Fuchigami et al., 1982; Tanino et al., 1989). The acclimation process is mainly affected by lowering the temperature and is completed by the end of the dormant period when the maximum level of cold resistance is reached (Weiser, 1970). The deacclimation process can occur in the dormant period, but the response to warm spells is increased mainly after the dormant stage. During the short warm spells cold resistance is maintained at a definite level until the resumption of bud growth (Gogoleva et al., 1994; Proebsting et al., 1980).

Recently, thermal analysis (or differential thermal analysis) has been used in determining cold hardiness of flower buds in woody species (Biermann et al., 1979; Quamme, 1978) on account of its simplicity and high resolution. In thermal analysis, low temperature exotherm (LTE) due to ice formation within the flower primordia is used as a sign of bud death (Andrews et al., 1983a; Kaku et al., 1981). In persimmon trees, Leng et al. (1995) conducted differential thermal analysis on the stem tissues of 2 *Diospyros* species and observed high temperature exotherm (HTE) and LTE. However, we (Kang et al., 1997) observed that the excised persimmon buds had only one exotherm in buds without nodal tissue and that the buds were injured when it occurs with or without the nodal tissue. Hong and Hwang (1980) and Kang et al. (1997) reported that the persimmon bud tissue was less cold hardy than stem tissue. Thus, we suggest that cold hardiness of persimmon trees can be evaluated by thermal analysis on excised buds.

The objective of our study was to determine the relationship between release of bud dormancy and temperature conditioning as related to bud cold hardiness by using persimmon buds having different dormancy conditions, naturally or artificially induced.

Materials and Methods

Plant source.

From the university orchards in Yamagata, Kyoto, and Kochi Prefectures in Japan, one-year-old twigs from over 40-year-old 'Hiratanenashi' persimmon trees showing stable productivity were harvested on October 30, November 21, December 27, 1995, and January 23, 1996. Comparable twig samples from Yamagata and Kochi were sent to Kyoto University where the experiments were conducted. Seasonal changes in air temperature of these locations are shown in Figure 3D. To delay the release of bud dormancy, foliar applications of 100 ppm gibberellic acid (GA) were made in late September and mid October 1995 to 'Hiratanenashi' and 'Fuyu' trees growing at the experimental orchard of Kyoto University. Then, the twigs were sampled on November 4, December 6, and January 11 in 'Hiratanenashi', and November 21 and December 23 in 'Fuyu'. To estimate the response of whole trees to temperature conditioning, four-year-old potted 'Hiratanenashi' trees in Kyoto were also examined in late October, late November, late December 1995, and late January 1996.

Temperature conditioning.

The twigs were kept in polyethylene bags within another polyethylene bag and kept moist with a wet towel. The samples were exposed to 25°C (high temperature conditioning) or 4°C (low temperature conditioning) in the dark for 1 to 3 weeks. The potted plants

were placed in a growth chamber set at 4°C in the dark or 25°C under natural day length for 4 weeks, except the January sample which was conditioned for only 2 weeks. The control pots were kept outdoors in Kyoto University; pots of the high temperature treatment and control pots were irrigated regularly.

Thermal Analysis (TA).

TA was performed by using thermoelectric modules (Komatsu Electronics, Tokyo) in a programmable freezer (Kang et al. 1997). The temperature of the freezer and measurement data were recorded with a micro-computer (Fig. 1). The excised bud sample was mounted on one side of a thermoelectric module and placed in a glass jar which served as a sample chamber. The chamber was then placed in freezer and cooled at a rate of 6°C/hr after preconditioning at a temperature of 0°C for 30 minutes. The temperature within the jar was monitored with a copper-constantan thermocouple at 4-second intervals. Silicone grease was used to facilitate contact between the thermoelectric module and the buds. TA was performed on an excised apical bud without its nodal tissue. Before and after temperature conditionings, changes in bud cold hardiness were measured. Ten samples were estimated for each measurement.

A typical profile of a TA is shown in Figure 2. In our previous report (Kang et al., 1997), bud without nodal tissue showed only one exotherm whose temperature was coincident with bud death. Even though moist filter papers were applied to cut faces of persimmon buds to induce a high temperature exotherm, they did not change the exothermic profiles of the buds. LTE of bud with nodal tissue was derived from nodal tissue. Thus, in all these experiments, TA was conducted on a bud without its nodal tissue; moist filter paper was omitted.

Bud dormancy and water content.

The bud dormancy release was determined as a percent budbreak after placing twig cuttings with 3 or 4 buds in a vase with water for 3 weeks at 25°C. With potted trees, several twigs were cut from the tree to determine the bud dormancy release. A high percentage budbreak was indicative of weak dormancy intensity. For measurement of the water content, 5 apical buds were weighed before and after being oven-dried for 48 hours at 80°C. The water content was expressed as percentage of tissue fresh weight.

Results

Monthly mean air temperature of three locations during winter season is shown in Fig. 3D. Buds of 'Hiratanenashi' collected from 3 locations showed a same seasonal change, being hardest in late January (Fig. 3A). The buds from Yamagata Prefecture had the lowest exotherm temperatures, followed by those from Kyoto and Kochi Prefectures throughout the sampling

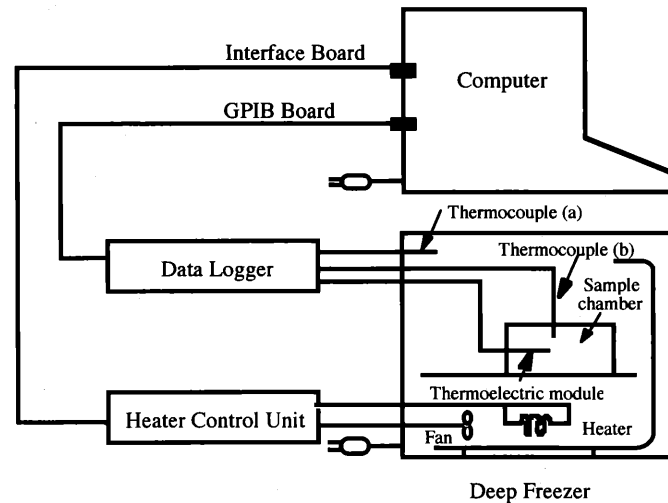


Fig. 1. Schematic illustration of data acquisition and thermal analysis system. The temperature of deep freezer was regulated by a heater controlled by a signal from a thermocouple (a) in the freezer through a microcomputer. The exotherm reactions of samples were detected by a thermoelectric module, and the pattern of exotherm was recorded on a computer through the logger. The temperature of the sample chamber was also monitored by a thermocouple (b) and its data was stored automatically in the computer.

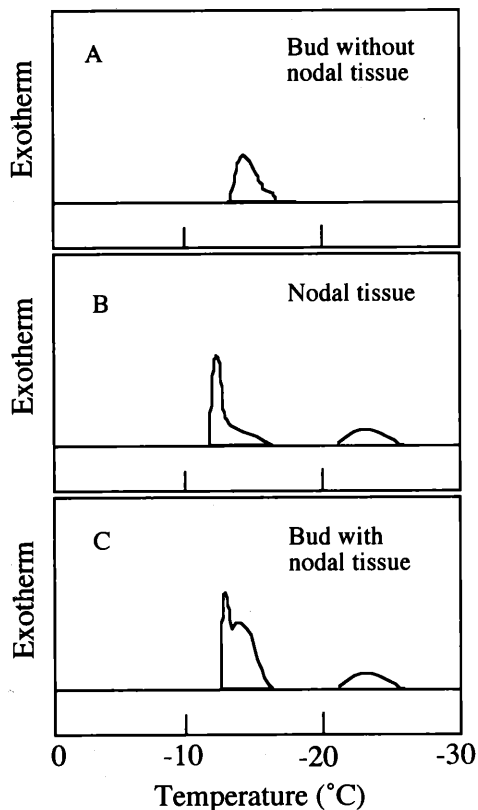


Fig. 2. Typical profiles of thermal analysis in (A) apical bud without nodal tissue, (B) nodal tissue and (C) apical bud with nodal tissue of 'Hiratanenashi' persimmon in mid winter. The bud without nodal tissue was prepared by excising it at its base.

dates, except for the first samples on October 30.

All buds, irrespective of locations, showed the lowest water content in late January (Fig. 3C); those from Kochi Prefecture had the highest water content throughout the sampling dates. In trees of Yamagata Prefecture, bud dormancy release was already completed in November 21 with 100% budbreak, whereas those in Kyoto and Kochi Prefectures exhibited about 90% budbreak on December 27 (Fig. 3B).

The buds from Yamagata Prefecture collected in late November responded to both high and low temperature conditionings; the high temperature conditioning decreased bud cold hardiness, whereas the low temperature conditioning made them hardier (Fig. 4). The buds from Kyoto and Kochi Prefectures, however, did not respond until December 27 to reach the same response stage as buds from Yamagata. The degree of bud cold hardiness usually increased with the length of the conditioning period. The high temperature conditioning of 3 weeks reduced the cold hardiness by a maximum of 4°C, whereas the low temperature conditioning of 3 weeks increased the bud cold hardiness by a maximum of 1.5°C.

No changes in bud water content occurred with the low or the high temperature conditionings (data not shown).

With the GA application in September and October to leaves of mature 'Hiratanenashi' and 'Fuyu' trees, bud dormancy release was delayed about 12 days in 'Hiratanenashi' (Fig. 5B) and 8 days in 'Fuyu' (data not shown). In addition, GA application prolonged the de-

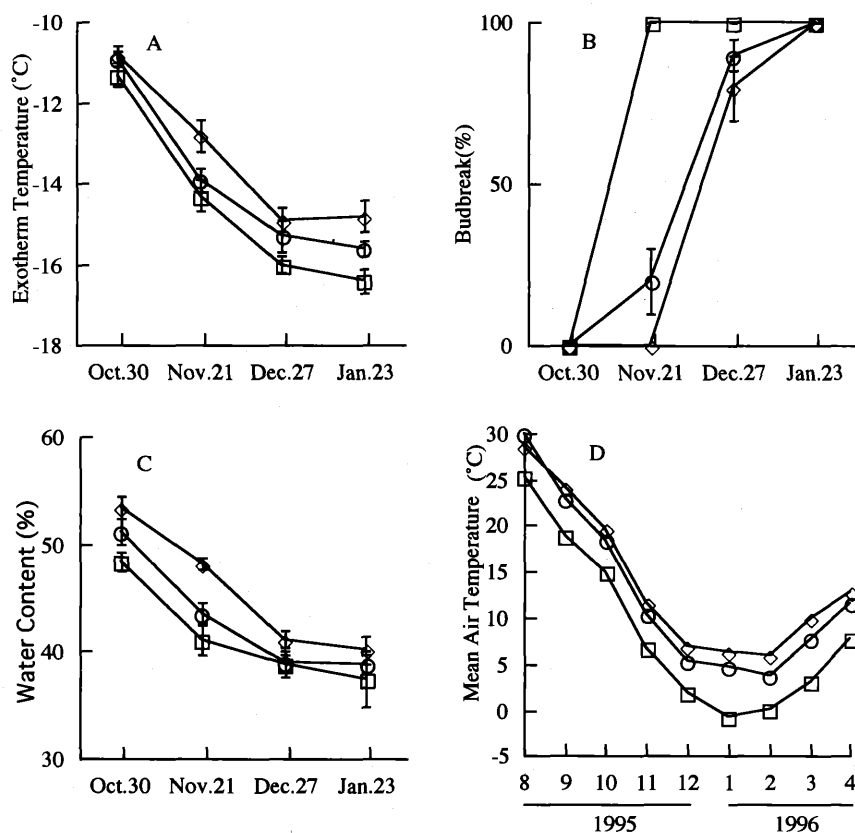


Fig. 3. Exotherm temperature (A), intensity of bud dormancy (B) expressed as budbreak after 3 weeks incubation, water content (C) of 'Hiratanenashi' persimmon buds during the winter season 1995-1996, and monthly mean air temperature (D) (Monthly report of the Japan Meteorological Agency, 1995-1996) during the winter season 1995-1996 in Yamagata, Kyoto, and Kochi Prefectures, Japan. Vertical bars indicate standard error. Symbols: □ Yamagata, ○ Kyoto, ◇ Kochi

foliation of both persimmon cultivars by about 2 weeks. Bud cold hardiness in the control and GA-applied trees increased as the winter progressed (Fig. 5A). With 'Hiratanenashi', buds were in deep dormancy on November 4 whether or not they were GA-treated (Fig. 5B), and they did not respond to the temperature conditionings (Fig. 6). However, on December 6 when control buds were released from dormancy, the temperature conditionings were effective on bud cold hardiness of control buds, but not on GA-treated ones. Furthermore, on January 11, when the GA-applied buds completed their dormancy release, those buds lost some cold hardiness and responded to temperature conditionings like the control buds (Fig. 6). The same trend was observed in GA-treated 'Fuyu' buds (data not shown). During the dormant period, the temperature conditionings had no effect on the bud cold hardiness. After completion of dormancy release, however, the high and the low temperature conditionings induced a decrease and an increase in bud cold hardiness, respectively.

The potted persimmon trees subjected to the temperature conditionings also had no changes in bud cold hardiness until bud dormancy release was completed

(Fig. 7). After completion of dormancy release, however, the high and low temperature conditionings induced a decrease and an increase in bud cold hardiness, respectively.

Discussion

Cold hardiness of persimmon buds expressed as their exotherm temperatures varied in different climate locations, but showed same seasonal fluctuations, increasing as the winter progressed (Fig. 3A) as reported by Leng et al. (1995) and Hong and Hwang (1980). Bud water content also decreased with increase in cold hardiness (Fig. 3C). In woody plants of temperate climates, the seasonal changes in cold hardiness is a universal phenomenon and has been reported in innumerable species and variety. It results from a series of processes called acclimation and deacclimation (Weiser, 1970). The two processes are well correlated with the water content in many plant species. In plants which acclimate, the water content almost invariably decreases with increasing hardiness (Burke et al., 1976).

Correlation between bud dormancy and cold hardiness has also been extensively studied (Fuchigami et al.,

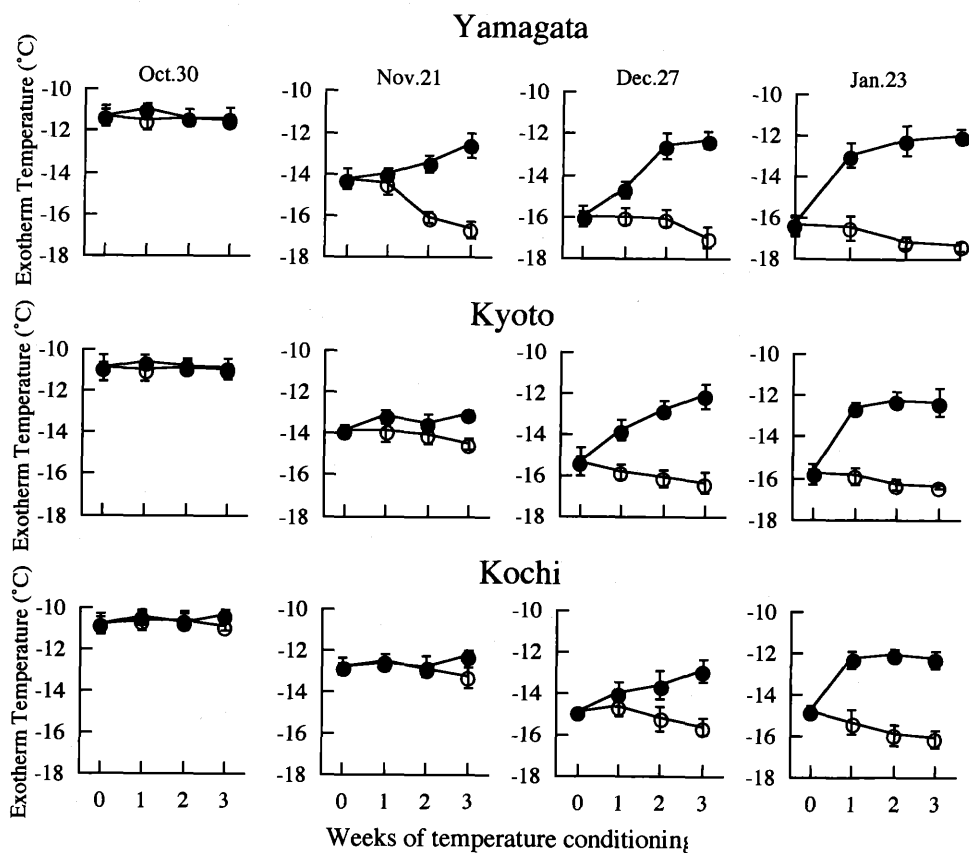


Fig. 4. Effect of high (HT, 25°C) and low (LT, 4°C) temperature conditionings on the exotherm temperature in 'Hiratanenashi' persimmon buds collected from Yamagata, Kyoto, and Kochi Prefectures, Japan, during winter season 1995-1996. Vertical bars indicate standard error. Symbols: ● HT, ○ LT

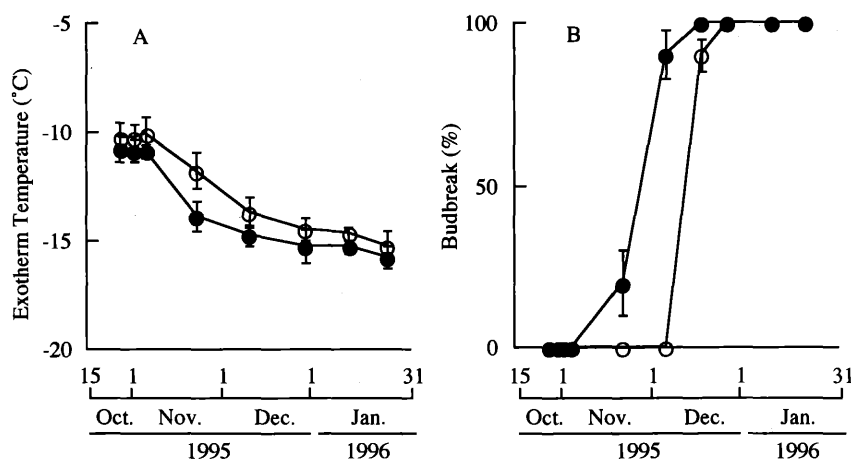


Fig. 5. Exotherm temperature (A) and intensity of bud dormancy (B) in 'Hiratanenashi' persimmon buds as influenced by gibberellic acid. Bud dormancy was expressed as budbreak 3 weeks after incubation. Gibberellic acid (100 ppm) was applied to leaves in Sept. and Oct. of 1995. Vertical bars indicate standard error. Symbols: ● Control, ○ GA

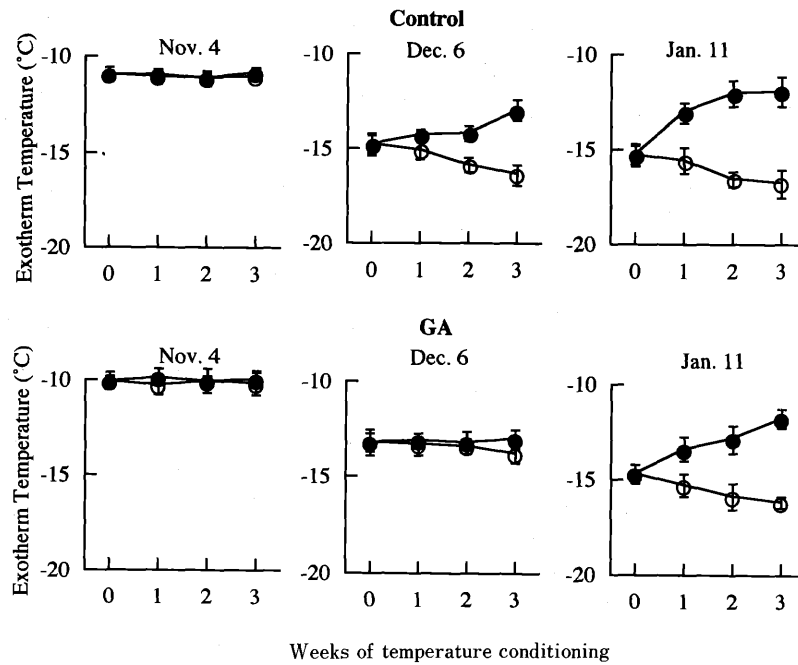


Fig. 6. Effect of high (HT, 25°C) and low (LT, 4°C) temperature conditionings on the exotherm temperature in 'Hiratanenashi' persimmon buds having various dormancy condition due to gibberellic acid. Gibberellic acid (100 ppm) was applied to leaves in Sept. and Oct. of 1995. Vertical bars indicate standard error. Symbols: ● HT, ○ LT

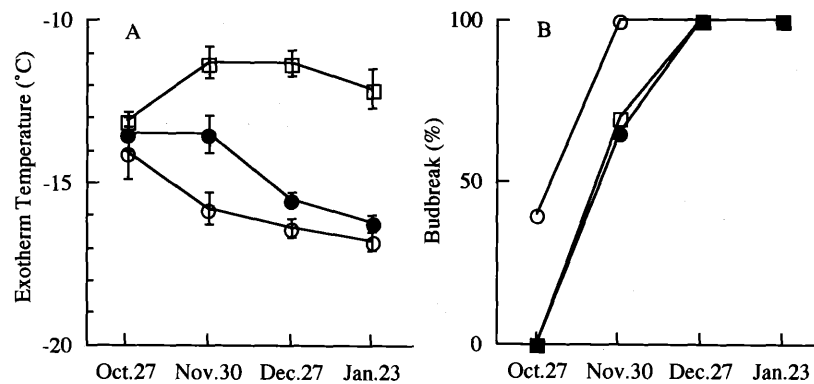


Fig. 7. Exotherm temperature of buds (A) and intensity of bud dormancy (B) in potted 'Hiratanenashi' persimmon as influenced by the low (4°C) or the high (25°C) temperature conditionings. The temperature conditionings lasted for 4 weeks starting from the indicated dates, except for January sample which was conditioned for only 2 weeks. The measurements were made after these conditionings. Bud dormancy was expressed as budbreak 3 weeks after incubation of the twigs cut from the potted trees. Vertical bars indicate standard error. Symbols: ● Control, □ HT, ○ LT

1982; Harada, 1985; Proebsting, 1963). Under natural condition, the onset of cold hardiness in red-osier dogwood occurs at the beginning of bud dormancy (Kobayashi et al., 1982), and the maximum cold hardiness is acquired after the dormant period is completed (Kobayashi and Fuchigami, 1983). The same relationship between dormancy and cold hardiness was re-

ported in grape bud (Schnabel and Wample, 1987). However, no relationship between them was reported in two woody species, *Viburnum plicatum tomentosum* and *Acer negundo* (Irving and Lanphear, 1967). The persimmon buds from different climate locations differed in their timing of dormancy release (Fig. 3B); their bud cold hardiness usually increased with a de-

crease in an intensity of dormancy, and reached maximum levels at or after completion of dormancy release (Fig. 3A) as reported by Harada (1985). Harada showed that the cold hardiness of buds in 10 persimmon varieties increased with a decrease in their intensity of dormancy. These results suggest that dormancy release in persimmon trees may be an important factor for attaining maximum cold hardiness. However, the buds from different locations expressed different degrees of cold hardiness even when their dormancy release was already completed (Fig. 3A,B). These differences are probably due to differences in air temperature between locations (Smithberg and Weiser, 1968), or to different sensitivity to warm temperature (Wolf and Cook, 1992).

Bud cold hardiness may fluctuate quite rapidly in response to changes in ambient temperature (Proebsting, 1970; Stergios and Howell, 1977) as well as bud water content. In persimmon, the tree survive low temperatures of -15 to -20°C (Hong and Hwang, 1980; Leng et al., 1993); but in regions where winters are mild, the tree can be injured by a 3 hours exposure to -7°C (Sharpe, 1966). In our studies, persimmon buds from different climate locations possessed different levels of cold hardiness until late January (Fig. 3A), unlike the report of Harada (1985) who found no differences in January. These results suggest that the degrees of hardening and dehardening are related to the temperature conditions to which the plants are exposed (Stergios and Howell, 1977; Young and Peinado, 1965).

The temperature conditionings did not always influence bud cold hardiness, at least in persimmon trees. Before the completion of dormancy release, high temperature conditioning had no adverse effect on bud cold hardiness. But once the dormancy release was complete, it decreased bud cold hardiness in mid winter (Fig. 4, 6, 7). This conditioning effect was observed in both detached twigs and potted trees which suggests that untimely warm temperature after completion of dormancy release could subsequently subject the persimmon buds to chilling injury. Similar results were reported in *Prunus* species (Proebsting, 1963), grape (Proebsting et al., 1980), and dogwood plants (Tanino et al., 1989). Like high temperature conditioning, low temperature conditioning had no effect on the bud cold hardiness when applied during the deep dormant period in persimmon. After the dormancy release was complete, bud cold hardiness was altered according to the previous conditioning treatment, suggesting that dormancy release may have an important role in the acclimation and deacclimation of persimmon trees in environmental temperature conditions.

The change in bud cold hardiness was to a lesser degree in the low temperature conditioning than in the high temperature conditioning (Fig. 4, 6, 7) which we attribute to the fact that 4°C used in our experiment was not low enough for acclimation of persimmon buds (Tanino et al., 1989; Weiser, 1970).

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カキの耐凍性における休眠覚醒と温度環境との関係について

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摘 要

異なる栽培地域のカキ‘平核無’および秋季のGA (100 ppm) の葉面散布処理により休眠打破を遅延させた‘平核無’と‘富有’を用いて耐凍性における休眠覚醒と高温 (25°C) および低温 (4°C) 環境との関係を検討した。また、鉢植えの4年生‘平核無’についても同様の温度処理実験を行った。耐凍性の測定には熱分析法を用いた。

‘平核無’の休眠覚醒時期は、山形では11月下旬で、冬季の平均気温が2~3°C高い京都、高知では12月下旬であった。同じ時期の‘平核無’の芽の耐凍性は山形で最も強く、次に京都、高知の順であった。

秋季のGAの葉面散布処理により‘平核無’と‘富有’の休眠覚醒は各々12日と8日遅れた。これらの休眠状態が異なるカ

キに与えた低温および高温処理の影響は休眠状態によって異なり、休眠の深い時期には高温、低温ともにほとんど影響がなかった。これに対して、休眠が醒めた時期の高温処理では芽の耐凍性が減少し、低温処理では、芽の耐凍性がやや増加した。温度処理の効果は温度処理期間に比例し、4週間の高温または低温処理で耐凍性は各々最大4°Cの減少と1.5°Cの増加が得られた。鉢植えのカキにおける高温および低温処理でも同様に、休眠覚醒後の処理のみに耐凍性の増減の効果が見られた。

以上の結果から、カキにおける冬季の温度環境は耐凍性の維持にとって重要であり、特に休眠覚醒後の高温は耐凍性の維持を困難にすると考えられた。