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Hydrogen cyanamide enhances MRI-measured water status in flower buds of peach (*Prunus persica* L.) during winter

Suravoot Yooyongwech[1,2], Akemi K Horigane[3], Mitsura Yoshida[3], Yoshihiro Sekozawa[1], Sumiko Sugaya[1], Suriyan Cha-um[4], Hiroshi Gemma[1*]

[1]Pomology Laboratory, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan
[2]Department of Agricultural Science, Mahidol University, Kanchanaburi Campus, Kanchanaburi, Thailand
[3]Molecular Structure and Dynamics Laboratory, National Food Research Institute, Tsukuba, Japan
[4]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Pathumthani, Thailand

***Corresponding author: gemma@sakura.cc.tsukuba.ac.jp**

Abstract

Braking bud dormancy in temperate fruit species using physical and chemical agents is a challenge issue. In present study, we investigated the interaction between hydrogen cyanamide (HC) and temperature in breaking dormancy to gain a basic knowledge on water status in peach (*Prunus persica* L.) floral buds. The water status, relaxation time (T_2) and apparent diffusion coefficient (ADC), in the upper part (flower primordia and bud scales), basal part, and bud trace of flower buds were determined using the magnetic resonance imaging (MRI). Bulk water, which might flow through the bud trace, increased the water content in the basal part, and water molecular mobility as reflected by an increase in T_2 in HC-treated buds after 1 week at 5°C. The HC triggers the influx of water into the bud, where the basal part exhibited the highest level of water accumulation. The study provides significant insights into the status of water in floral buds and highlights the role of HC to solve the problems of produce in temperate fruits.

Keywords: apparent diffusion coefficient (ADC), hydrogen cyanamide (HC), MRI, peach flower bud, relaxation time (T_2), water status.
Abbreviations: ADC_apparent diffusion coefficient; CU_chilling units; HC_hydrogen cyananmide; MRI_magnetic resonance imaging; NMR_nuclear magnetic resonance; SNF_sucrose non-fermenting; T_2_relaxation time.

Introduction

Bud dormancy in temperate plants is a well-studied phenomenon at the physiological level (Chao et al., 2007; Rohde and Bhalerao, 2007). It is a complex process and buds may fail to grow because of interacting developmental and physiological processes (Horvath, 2009). Bud dormancy has been separated into three developmental stages: paradormancy, endodormancy and ecodormancy (Lang et al., 1987; Horvath et al., 2003). It is generally conditioned by environmental cues, such as low temperature; and dormancy is broken at the completion of endodormancy in the temperate perennials after they have been exposed to adequate chilling temperature (Arora et al., 2003). Development of buds often reduced growth rates relative to non-dormant buds when the plant was placed in growth-conducive environments, particularly during endodormancy (Horvath, 2009). The prospect of warmer winters as a result of global climate change is a concern for temperate fruit production because of the potential interference with dormancy mechanisms and economic production (Cook and Jacobs, 2000; Houghton, 2005). To date, elucidation of the mechanisms of shoot development and bud-break have remained a daunting task (Walton et al., 2009). Hydrogen cyanamide (HC) has been found to be a dormancy-breaking agent that can overcome a lack of natural chilling in several temperate fruit plants, such as peach, grape and blueberry (Dozier et al., 1990; Siller-Cepeda et al., 1992; Or et al., 2000; Williamson and Maust, 2001; Lombard et al., 2006; Halaya et al., 2008). Previously, the timing of application and concentration were critical in determining the extent of HC-caused bud-break in 'Redhaven' peach buds (Siller-Cepeda et al., 1992). The response of peach buds to HC apparently depends on the stage of bud development at the time of treatment (Erez et al., 1971). Furthermore, a transcript encoding enzyme in fermentative respiratory pathway, sucrose non-fermenting (SNF)-like protein kinase, is involved in the perception of a bud break signal induced by HC in grape buds (Or et al., 2000). However, further insights into the physiological aspects linked to the role of HC in breaking bud dormancy are lacking. Dormancy, an essential event in the overwintering processes of woody plants, is closely related to changes in water transport (Welling and Palva, 2006). Yooyongwech et al. (2008a) opined that the information of the changes in water transport within buds in different phases of dormancy should help to optimize cultivation conditions for temperate fruit trees. Magnetic resonance imaging (MRI) is a useful technique for evaluating the biophysical aspects of water status in tissues in a non-destructive manner and provides information on the morphology and dynamics in a developmental analysis (van der Toorn et al., 2000). In MRI, spin–spin relaxation time (T_2) is easy motivated by the molecular motion of water; apparent diffusion coefficient (ADC) gives detail on water diffusion within a tissue area; and

water protons diffusing in biological tissue reflect the size and membrane permeability of cells (Brandl and Haase, 1994; de Faÿ et al., 2000). Nuclear magnetic resonance (NMR) and MRI have been earlier used to investigate bud dormancy in temperate plants such as apple and peach (Millard et al., 1993; Erez et al., 1998). However, no study has been undertaken to investigate the relationship between the response of bud development to HC and water status at the onset of dormancy in the different parts of peach bud. Peach (*Prunus persica* L.) is a good model of temperate fruit species, which are well investigated the genetic diversity (Rahemi et al., 2010).
The present study measured the changes in water status, based on MRI parameters of T_2 and ADC, in different portions (upper part– scales and flower primordia, basal part, and bud trace) of the developmental dormant buds in response to HC.

Results

Bud-break and bud development

In December, no bud-break was observed in the control buds, whereas about 6% of HC-treated buds broke. The percentage of bud-break in HC-treated buds was the same as in the control buds in January, and much less than that in the control buds in February (Fig. 1). The low temperature period (October – December) directly influences bud brake dormancy. In addition, the HC pretreated floral bud is an alternative way to break the dormancy in peach. Primordial flower width (W_i) in HC-treated buds at 14-DAT (days after treatment) in December was significantly increased, whereas the control buds remained unchanged. Therefore, the W_i of HC-treated and control buds were similar at each measurement time in January (Fig. 1). The low temperature in winter season (October – December) is very important to regulate the W_i, which demonstrated in Fig. 1. Alternatively, the floral bud braking and W_i regulation in peach were practiced using HC treatment.

Effect of HC on water status under growth conducive conditions

MRI was used to observe the morphology and water status inside HC-treated and water-treated buds. The proton signals for T_2 and ADC maps were derived mainly from water protons and reflect the mobility of water molecules in the buds. However, due to forcing treatment at 23°C, the T_2 values were always higher in the upper and basal parts than in the bud trace in both the treatments (Fig. 2). In December, the T_2 values in the upper part increased after 1 day in both treatments. However, 7-DAT, T_2 values in the basal parts of the HC-treated buds increased slightly, whereas there was no increase in water-treated buds (Fig. 2). In HC-treated buds in January, the T_2 values in the upper and basal parts began to increase immediately after the start of forcing, whereas in the water-treated buds the increase in the T_2 values was detected on day 7 (Fig. 2). Changes in bud morphology and the T_2 maps in buds are shown in Figure 3, with the color gradient corresponding to the range of T_2 values. The ADC values were the highest in the basal part in HC-treated buds and water-treated buds in all periods. In December, the ADC values increased significantly 1-DAT in HC-treated buds, though a slight increase was also seen in the control buds. In January, the ADC values tended to increase in all the periods in all parts of the buds irrespective of treatment (Fig. 2). However, the ADC values were the highest values in the basal part (Fig. 3).

Effect of HC on water status under low temperature (5°C)

The T_2 values in the basal part of the HC-treated buds gradually increased under low temperature (5°C) but gradually decreased in water-treated buds. In the upper part, the T_2 values decreased at 1-WAT in both the treatments, recovered at 2-WAT in water-treated buds, and at 3-WAT in HC-treated buds (Fig. 4). The ADC values in the basal part of the HC-treated buds declined for up to 2 weeks, and thereafter ADC values increased. In the water-treated buds, the ADC value in the basal part changed little during the 3 weeks but appeared to increase slightly in the upper parts compared with the HC-treated buds (Fig. 4).

Discussion

The present study revealed that HC increased the percentage of bud-break in peach during December to January. HC-treatment correlated positively with the water status in buds transitioning paradormancy into endodormancy. At the end of endodormancy, the percentage of bud-break, primordial size, the levels of T_2 and ADC were similar to the control in January. These findings are supported by earlier report that chilling accumulation is required for HC to consistently result in the completion of the bud-break and bud development (Mohamed et al., 2010). In addition, the physiological development in peach flower bud coincided with changes of water status as indicated by spin-lattice relaxation time (Sugiura et al., 1995). It suggests that the levels of the water influx in both the treated buds were related to duration of the bud development and a number of interacting dormancy processes, which in turn was dependent on environmental signals, e.g., period of chilling. However, the T_2 and ADC values in the upper part (primordia) slightly increased in December (after 382 CU) at 23°C in the water-treated buds even though the primordia appeared undeveloped. Earlier, a similar observation was made by Yooyongwech et al. (2008b) who reported that T_2 value of water in the peach bud primordia increased during the cold deprivation period from October; however, the buds did not flower. Indeed, the water absorption in peach trees was almost linearly related to increase in (day time) temperature (Simonneau et al., 1993). The strong effect of high temperature (forcing condition) was a dominant factor in changing the state of water in a chilling dormant bud of peach (Erez et al., 1998). Our result confirms that the level of water status, T_2 and ADC, under the forcing condition increased in the bud despite the water-treated bud. The results suggested that temperature would be one of the cues reflecting the water status in the bud during the growth conducive conditions in HC and control treatments during the winter. It is still unclear how the water status is directly induced by the dormancy-breaking agent. Nevertheless, in a related study, authors avoided the effects by applying a high-temperature treatment on peach buds during development under growth conducive temperature from both control and HC-treated buds. This experiment has been conducted by using non-freeze-temperature treatment in ecodormant buds to enhance the effect of HC cyanamide. The result showed that the water molecular mobility, as indicated by T_2, did not increase suddenly in the bud under the control; while T_2 increased in the basal part of the HC-treated buds within 1 week at 5°C despite the decline in the water diffusion (Fig. 4).
This study concluded that the water content is enriched in the basal part. Earlier, Reinoso et al. (2002) and Yooyongwech et al. (2008a) implied that cells in the basal part of peach buds have high concentrations of metabolic substances, and the levels of these substances may affected to the high molecular

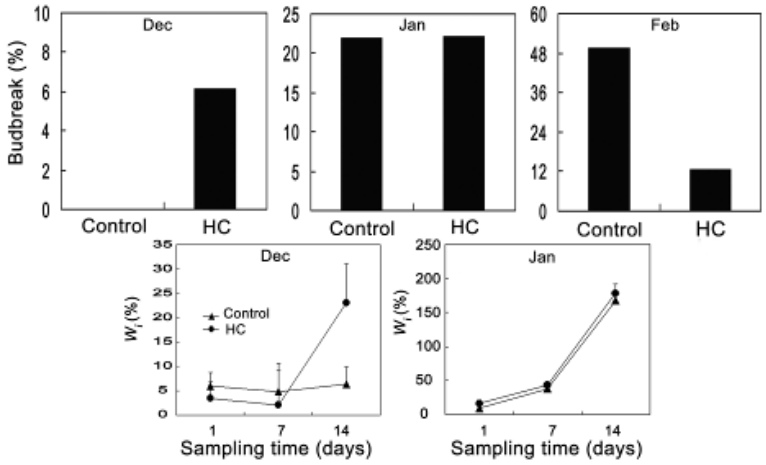

Fig 1. Flower bud break at 15 days after treatment (DAT) with water (control) and hydrogen cyanamide (HC) under forcing conditions during December, January, and February (Upper panel). Increase in width (W_i) of primordial flower in peach trees treated with either water (control) or HC in December 2006 and January 2007 (Lower panel) and kept at forcing temperature (23°C). Values are means ± SE (n = 4).

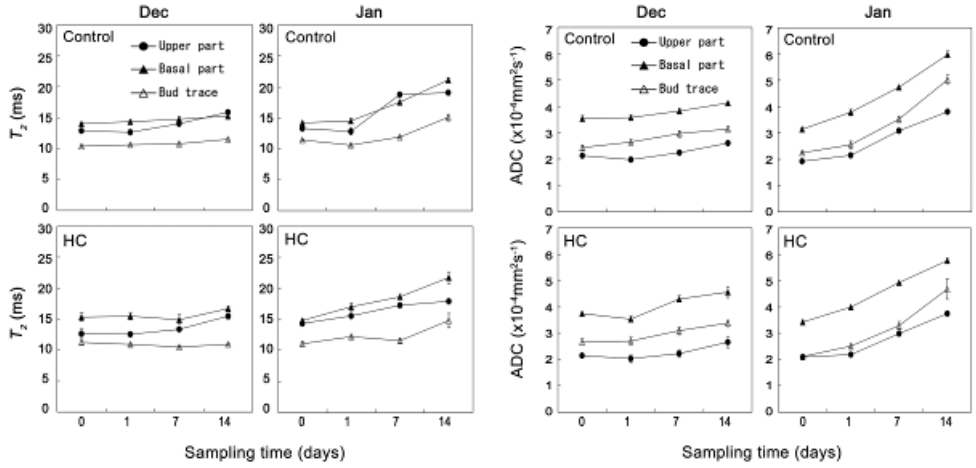

Fig 2. T_2 (left) and ADC values (right) at 0, 1, 7, 14 days after water (control) and hydrogen cyanamide (HC) treatment under forcing conditions for the upper part (scales and flower primordia), basal part and bud trace in December 2006 and January 2007. Values are means ± SE (n = 4).

motion of water in the basal cells. Based upon the observation, we assume that HC brings about a rapid increase in water-uptake and accumulation in water content in the bud, especially in the basal part. Further, it is reasonable to indicate that the HC is important to rapidly facilitate the bulk water uptake into the dormant flower bud.

In conclusion, HC may enhance an influx of water to the dormant bud, leading to an accumulation of water in the basal part of peach flower bud and thus help in bud dormancy break. Such studies hold immense potential in solving the problems of produce in temperate fruits owing to climate change.

Materials and methods

Plant materials

Plants of one-year-old grafted 'Hikawa Hakuho' peach (*Prunus persica* L.) were transferred into pot (diameter 20 cm) containing 2 kg clay soil (EC = 2.687 dS m^{-1}; pH = 5.5; organic matter = 10.36%; total nitrogen = 0.17%; total phosphorus = 0.07%; total potassium = 1.19%) in spring season (March to May 2006). All of the 'Hikawa Hakuho' plants were grown at the Agricultural and Forestry Research Center, University of Tsukuba, Japan (Latitude 36°06'40"N, Longitude 140°05'60"E). After flower bud initiation in 2007 season, the 'Hikawa Hakuho' cultivar of peach was treated with hydrogen cyanamide (HC).

Hydrogen cyanamide (HC) treatment under growth conducive condition

The plants that initiated the bud in 2007 season were sprayed with 1% (*v/v*) hydrogen cyanamide (H_2CN_2, Nippon Carbide Co. Ltd, Tokyo, Japan) and distilled water (as control) during three phases of dormancy; transition of endodormancy phase in December (after 382 chilling units, CU), endodormancy phase in January (after 920 CU), and the end of endodormancy phase in February (after 1,221 CU) (Dozier et al, 1990; Yooyongwech et al., 2008a). Immediately after

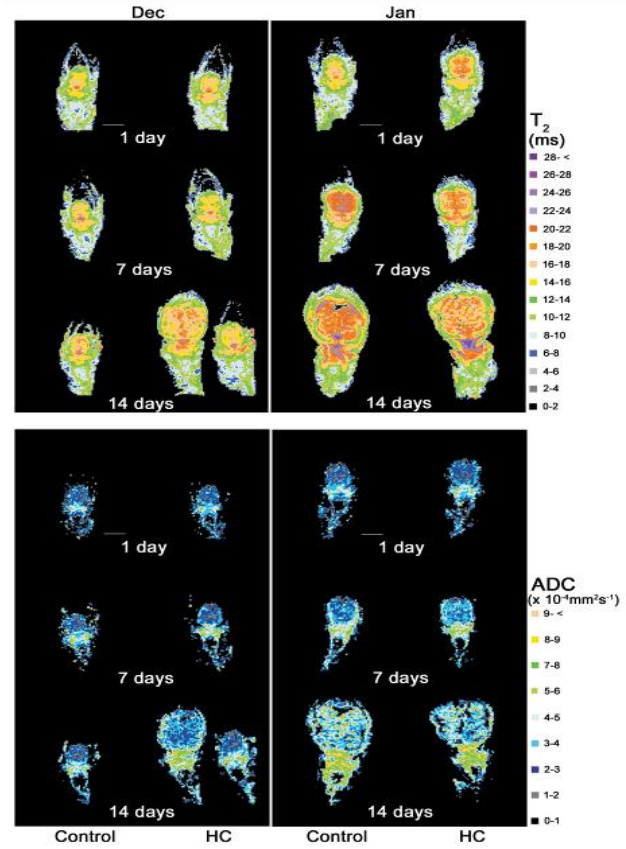

Fig 3. T_2 and ADC images of water- (control) and hydrogen cyanamide (HC)-treated buds at 1, 7, 14 days after treatment in December 2006 and January 2007. The color scale indicates T_2 values in steps of 2 ms and ADC color scale indicates values in steps of 1×10^{-4} mm^2 s^{-1} (scale bar = 1 mm).

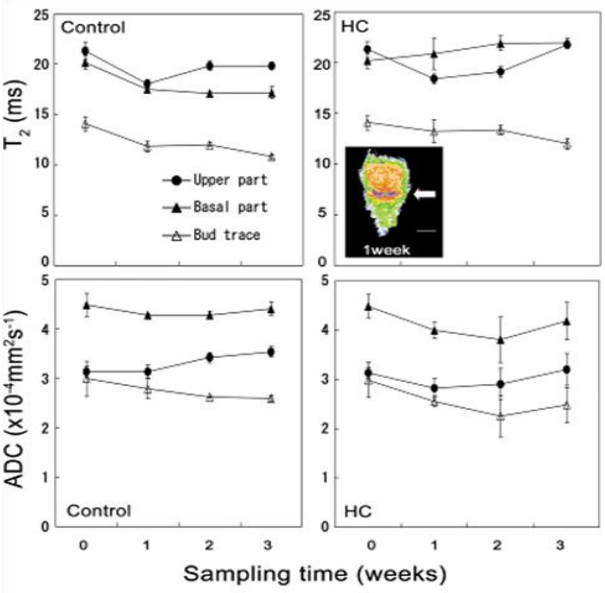

Fig 4. T_2 and ADC values in the upper part (scales and flower primordia), basal part, and bud trace in buds of water (control) and hydrogen cyanamide (HC) treatments in shoots excised in February 2007 and held at 5°C for 3 weeks. Values are means ± SE (n = 3). The T_2 map showed an increased signal after 1 week in the basal part of HC-treated buds as indicated by the arrow (scale bar = 1 mm).

treatments (HC and control), these plants were transferred to a glasshouse at 23°C under natural light conditions. Four buds of each shoot were collected as replicates for bub break in December to February, and water status observation at 1-, 7-, and 14- days after treatments (DAT).

HC treatment under low temperature

Shoots from the grafted plants were excised in February after adequate chilling and treated with 1% HC and distilled water for control. These shoots were placed in water and kept low temperature in growth chambers (MLR 350, Sanyo, Osaka, Japan) under 16 h d^{-1} photoperiod of 84.9 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) provide by fluorescence lamps with ambient temperature at 5°C. Buds from the shoots were subjected to Magnetic Resonance Imaging (MRI) measurement for water-status analysis at 1-, 2-, and 3-weeks after the treatment (WAT).

Observations of bud-break

The whole plants treated with hydrogen cyanamide or distilled water (control) were kept in the control temperature glasshouse at 23±2°C under the natural light conditions (500-1,200 µmol m^{-2} s^{-1} PPFD) with 10 h d^{-1} photoperiod throughout the winter season (December–February) for observation of flower bud-break. The bud-break percentage was calculated by counting burst buds until 15 days when kept under optimal growth conditions.

Bud size analysis

The widths (W_t) of flower primordia sampled at 1-, 7-, and 14-DAT were measured using MRI with a measurement tool provided in the software package of MRI (ParaVision®, ver. 3.0.2, Bruker BioSpin, Karlsruhe, Germany). The percentage enlargement in primordia width (W_i) was calculated relative to the initial width (W_0) just before treatment using the following equation: W_i (%) = $[(W_t - W_0)/ W_0] \times 100$, where W_i = increase of primordia width, W_0 = initial primordia width, and W_t = primordia width at specific time.

MRI measurement

MRI was used to observe the water status inside dormant buds. MRI measurement was done with an NMR spectrometer (DRX300WB, Bruker BioSpin, Karlsruhe, Germany) supplied a magnetic field of 7.1 Tesla (proton resonance frequency: 300 MHz) as per Yooyongwech et al. (2008a). NMR data was analyzed with ParaVision® and XWIN-NMR programs. T_2 image was obtained by the spin-echo-based pulse program of MRI, whereas the ADC image was carried out by spin-echo diffusion. T_2 and ADC values were calculated by using the ParaVision software.

The T_2 value was estimated by the equation:

$M = M_0 e^{(-t/T2)}$

where M = signal intensity at echo time t, M_0 = signal intensity at echo time 0, and T_2 = spin-spin relaxation time.

The ADC values were estimated from the equation:

$M = M_0 \exp^{(-D \times B)}$,

where M = signal intensity at b-value B, M_0 = signal intensity at gradient strength 0, and D = the apparent diffusion coefficient.

Three areas of flower bud in the longitudinal section were fixed manually. These were: the upper part (scales and

primordial flower), the basal part, and the bud trace (Yooyongwech et al. 2008a). A total of three replicate samples were collected from shoots, with a maximum of two buds per shoot.

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