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Comparison of Mid-winter Cold Hardiness in Highbush Blueberry (*Vaccinium corymbosum*) Cultivars and Its Changes during Deacclimation

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ABSTRACT

Cold hardiness and soluble sugar contents were compared in shoots of twenty-one highbush blueberry cultivars (*Vaccinium corymbosum* cvs. Berkeley, Bluecrop, Bluegold, Bluehaven, Bluejay, Burlington, Chippewa, Collins, Dixi, Duke, Herbert, Jersey, Nelson, Northblue, Northland, Polaris, Rancocas, Sharpblue, Sierra, Spartan, Sunrise) in mid-winter. Relationships between cold hardiness and soluble sugar contents were also examined. The level of cold

hardiness was expressed as temperature representing 50% injury occurred (LT_{50}) and temperature at maximum rate of injury (T_{max}) determined by electrolyte leakage analysis at various freezing temperatures. According to cold hardiness levels based on LT_{50}, twenty-one highbush blueberry cultivars were ranked as 'Jersey' > 'Northblue' > 'Berkeley' = 'Sierra' > 'Northland' > 'Dixi' > 'Bluejay' > 'Chippewa' > 'Burlington' > 'Bluegold' > 'Spartan' > 'Bluecrop' > 'Sunrise' > 'Duke' = 'Rancocas' > 'Herbert' > 'Polaris' > 'Collins' > 'Bluehaven' > 'Sharpblue' > 'Nelson'. T_{max} was significantly correlated with LT_{50} ($r^2 = 0.96$***). The cold hardiness was highly correlated with total soluble sugar contents ($r^2 = –0.74$*** and $r^2 = –0.63$*** for LT_{50} and T_{max}, respectively). Of the detected soluble sugars, fructose and glucose contents were significantly associated with cold hardiness in the shoots of highbush blueberry cultivars, but sucrose and raffinose contents were not associated with cold hardiness. Seven highbush blueberry cultivars of 'Bluecrop', 'Jersey', 'Rancocas', 'Sharpblue', 'Sierra', 'Spartan', and 'Sunrise' were selected depending on mid-winter cold hardiness and growth condition. And then, changes of their cold hardiness were examined during deaccliamation. According to the cold hardiness levels based on LT_{50}, all cultivars except 'Sharpblue' maintained their cold hardiness until February 20 and then steeply decreased until March 5. However, 'Sharpblue' sharply decreased its cold hardiness until March 5. Since March 5, all cultivars except 'Sharpblue' reacclimated as air temperature decreased. Degree of cold hardiness increased by –4.2, –3.5, –3.4, –2.5, –2.3, and –2.2°C for 'Rancocas', 'Jersey', 'Bluecrop',

‘Sierra’, ‘Sunrise’, and ‘Spartan’, respectively on March 12. ‘Bluecrop’ and ‘Sunrise’ regained cold hardiness by –3.6 and –2.0°C, respectively, on March 26. ‘Jersey’ also increased its cold hardiness slightly on April 2. Changes of cold hardiness based on T_{max} were also similar. Although ‘Spartan’ was more cold-hardy in mid-winter, it lost cold hardiness earlier and faster than ‘Bluecrop’, ‘Sunrise’, and ‘Rancocas’. Thus, deacclimation resistance was not always correlated with mid-winter cold hardiness. Furthermore, the water contents in the shoots tended to increase with decreasing cold hardiness. However, they were not always significantly correlated with the cold hardiness levels during deacclimation in all cultivars examined.

***Key words*:** blueberry, cold hardiness, deacclimation, LT_{50}, reacclimation, soluble sugars, T_{max}, water content, woody perennials

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INTRODUCTION

Blueberry is a popular fruit shrub originated from the North America. Blueberry is classified as highbush (*Vaccinium corymbosum*), lowbush (*V. angustifolium*), and rabbiteye blueberries (*V. ashei*). For commercial and practical purposes, the highbush blueberry is the most popular species due to large fruit size and early ripening. However, lack of cold hardiness and susceptibility to spring frosts are the most critical problems for current highbush blueberry cultivars (Ehlenfeldt et al., 2006; Moore, 1993).

Freezing is a major environmental stress, leading to economic damage on horticultural crops and limiting the distribution of both wild and crop species (Pearce, 2001). Freezing causes membrane disintegration, intracellular ice formation, cell dehydration due to extracellular ice formation, xylem embolism, and sunscald due to frost subsequent pathogen attacks (Morin et al., 2007).

Survival of woody perennials at freezing temperatures is dependent on process of cold acclimation in response to environmental stimuli such as short photoperiod and non-freezing low temperature (Chinnusamy et al., 2007; Thomashow et al., 2001; Xin and Browse, 2000). Cold acclimation is a complex process with several contributing factors. It involves gene regulation, membrane modification, increase in antioxidant and compatible solutes, decrease in water content, and growth inhibition (Wisniewski et al., 2003; Xin and Browse, 2000).

For successful overwintering, woody perennials require not only sufficient

maximum freezing tolerance, but proper timing and rates acclimation and deacclimation (Suojala and Lindén, 1997). Cold deacclimation is a process reducing cold hardiness which was originally attained through a cold acclimation process (Kalberer et al., 2006). Deacclimation is strongly dependent on temperature and occurs much faster than cold acclimation (Kalberer et al., 2007a). The rate of deacclimation is not a linear response but may change as deacclimation progress. In addition, the rate and/or timing of deacclimation may vary with species, cultivars, ecotypes, etc., due to genetic variability for deacclimation kinetics and genetic adaption to the local climate (Kalberer et al., 2007a). Deacclimation response is important for reproductive success in woody perennials because late winter or early spring warm spells followed by hard freezes can cause severe injury (Rowland et al., 2005). For this reason, deacclimation kinetics and reacclimation capacity have intensively studied (Arora et al., 2004; Kalberer et al., 2007a; Lenahan et al., 2010; Pagter et al., 2011a, b; Rowland et al., 2005).

In this study, mid-winter cold hardiness levels of twenty-one highbush blueberry (*V. corymbosum*) cultivars of 'Berkeley', 'Bluecrop', 'Bluegold', 'Bluehaven', 'Bluejay', 'Burlington', 'Chippewa', 'Collins', 'Dixi', 'Duke', 'Herbert', 'Jersey', 'Nelson', 'Northblue', 'Northland', 'Polaris', 'Rancocas', 'Sharpblue', 'Sierra', 'Spartan', and 'Sunrise' were compared. In addition, sugar contents were measured to examine the relationship with cold hardiness. On the basis of mid-winter cold hardiness and growth conditions, seven highbush

blueberry cultivars of ‘Bluecrop’, ‘Jersey’, ‘Rancocas’, ‘Sharpblue’, ‘Sierra’, ‘Spartan’, and ‘Sunrise’ were selected to monitor the changes of cold hardiness and water content during deacclimation. This study will provide practical information for deacclimation kinetics and ultimately for breeding and cultivation of highbush blueberries.

LITERATURE REVIEW

Plant freezing and damage

Freezing temperatures induce the ice formation in the inter- or intra-cellular spaces and cell walls of plant tissues. The plasma membrane is the primary site of freezing injury (Guy, 2003; Xin and Browse, 2000).

Ice formation occurs in two markedly distinct locations within the tissues of most plants depending on cooling rate (Guy, 1990). If cooling is slow, intercellular ice formation occurs firstly because the intercellular fluid has a higher freezing point than the intracellular fluid (Rodrigo, 2000; Thomashow, 1999; Xin and Browse, 2000). Furthermore, the intercellular fluid commonly contains various ice nucleating agents, such as dust and ice nucleation active bacteria (Brush et al., 1994; Xin and Browse, 2000). Although intercellular ice formation is not lethal to plant cells (Levitt, 1980), it drops water potential outside the cell (Xin and Browse, 2000). Subsequently, water from the cytoplasm moves through the plasma membrane by osmosis, leading to cellular dehydration. The dehydration damages the membrane structure and function. And then water, various solutes, and electrolytes are leaked out through the damaged plasma membrane (Xin and Browse, 2000). If cooling is rapid, on the contrary, ice forms inside the cell. The intracellular freezing is considered instantaneously lethal because intracellular ice crystal disrupts the integrity of the protoplasm (Burke et al., 1976; Guy, 1990). Level of freezing damage is also affected by timing or duration of freezing,

reached minimum temperature, repeated freeze-thaw cycles, nucleation temperature, water content, and nutrition status (Guy, 2003).

Cold hardiness in woody perennials

Cold hardiness is quite diverse ranging from whole plant level to cellular adaptations that include specific metabolites and changes in membrane structure for adapting and withstanding low temperature (Gusta and Wisniewski, 2013). The development of cold hardiness in woody perennials can be divided into five categories: 1) the initiation time of cold acclimation, 2) the rate of cold acclimation, 3) the degree of freezing tolerance attained, 4) the maintenance of freezing tolerance during winter, and 5) the rate of loss of freezing tolerance upon resumption of spring growth (Wisniewski et al., 2003).

Cold hardiness is a complex trait with several contributing factors and a dynamic process changing with time (Wisniewski et al., 2003). Woody perennials have cold hardiness to survive harsh winter. Commonly, they have freezing avoidance and tolerance strategies to resist sub-freezing temperatures (Burke and Stushnoff, 1979; Levitt, 1980).

Freezing avoidance is typically associated with supercooling and glass formation (Gusta and Wisniewski, 2013). Supercooling is the ability of cells of entire organs to retain cellular water in a liquid phase at sub-freezing temperatures due to the lack of nucleating substances required for ice initiation (Jones et al., 2000; Wisniewski et al., 2003). In general, the supercooling occurs in xylem ray

cells of hardwood, flower buds of angiosperms, and both shoots and floral primodia of conifers (Bañuelos et al., 2008). Glass formation is a unique metastable condition for survival of plant tissues to dry state induced by dehydration (Buitink and Leprince, 2004; Welling and Palva, 2006). Aqueous glasses are extremely viscous, brought by a high solute concentration at a sufficiently low temperature (Wisniewski et al., 2003). Their high viscosity may stop almost all chemical reactions that require molecular diffusion (Burke, 1986; Crowe et al., 1998).

On the other hand, freezing tolerance is a mechanism that allows after extracellular formation (Wisniewski et al., 2003). It is associated with various reactions such as compositional changes in membranes, compatible solute concentration, osmotic adjustments, plant hormone regulation, and antioxidant defense system (Gusta and Wisniewski, 2013). The freezing tolerance is achieved and developed through sequential stages of cold acclimation (Gray et al., 1997).

Cold hardiness varies among species, genotypes, provenances, and even different parts of the same plant. Generally lowbush blueberries (*V. angustifolia*) are more cold-hardy than highbush blueberries (*V. corymbosum*). The highbush blueberries are more cold-hardy than rabbiteye blueberries (*V. ashei*) (Ehlenfeldt et al., 2007; Morin et al., 2007). Mid-winter cold hardiness among twenty-five rabbiteye blueberry cultivars was evaluated ranged from –24.9 to –13.7°C (Ehlenfeldt et al., 2006).

Cold acclimation

Cold acclimation in woody perennials is triggered by a short photoperiod and non-freezing low temperature (Kozlowski and Pallardy, 2002; Thomashow, 1999; Weiser, 1970). Short photoperiod is primary signal to induce cold acclimation as well as growth cessation and dormancy development (Bañuelos et al., 2008). Subsequent exposure to non-freezing low temperature is required to increase freezing tolerance (Bañuelos et al., 2008).

Compatible solutes such as proline, betaine, polyols, and soluble sugars accumulate during cold acclimation (Kishitani et al., 1994; Koster and Lynch, 1992; Sasaki et al., 1996; Tao et al., 1998). Especially, soluble sugars are essential for cold acclimation. In general, starch stored in stems and buds is converted to soluble sugars (Ashworth et al., 1993; Morin et al., 2007). The soluble sugars protect cell membranes from freezing injury through interacting with the lipid bilayer (Ma et al., 2009). The soluble sugars also serve as osmoprotectants, cryoprotectants, hormone-like signal messengers, and nutrients (Ma et al., 2009; Xin and Browse, 2000). The major soluble sugars associated with cold hardiness differ among between different species. Sucrose is a major sugar associated with cold hardiness in hydrangea (Pagter et al., 2008) and red-raspberry (Palonen et al., 2000). Glucose and fructose are the major sugars in highbush blueberry (Lee et al., 2012) and oak (Morin et al., 2007), and raffinose family oligosaccharides such as raffinose and stachyose are the major sugars in forsythia (Flinn and Ashworth, 1995), honeyberry (Imanishi et al., 1998), and red osier dogwood (Ashworth et al.,

1993). The raffinose family oligosaccharides have commonly been found to accumulate during cold acclimation and associated with cold hardiness in woody perennials (Cox and Stushnoff, 2001).

In addition, numerous physiological and biochemical changes occur during cold acclimation (Li et al., 2004; Xin and Browse, 2000). They include reduction or cessation of growth, decrease in tissue water content (Levitt, 1980), transient increase in abscisic acid levels (Chen et al., 1983), and changes in membrane lipid composition (Lynch and Steponkus, 1987; Uemura and Steponkus, 1994).

Cold deacclimation and reacclimation

The abilities of plants to resist deacclimation during transient warm spells and to reacclimate when cold temperatures return are significant for survival in late winter or early spring (Kalberer et al., 2006). Deacclimation is strongly dependent on temperature and occurs much faster than cold acclimation (Arora et al., 1992; Kalberer et al., 2007a; Leinonen et al., 1997). Cold acclimation requires large amounts of energy to change cellular structure and function. Deacclimation, however, is relatively less energy-intensive process, in which gene expression and associated metabolisms are down-regulated rather than up-regulated (Kalberer et al., 2006). Deacclimation upon exposure to warm temperatures is as important as acclimation for survival of woody perennials. However, high resistance to deacclimation is not always associated with high mid-winter cold hardiness. Although ‘Concord’ grape (*Vitis labrusca*) is more cold-hardy than ‘Cabernet

Sauvignon' grape (*V. vinifera*) in mid-winter, 'Concord' was deacclimated more rapidly (Wolf and Cook, 1992). In blueberry cultivars, 'Tifblue' buds had higher cold hardiness than 'Legacy' ones, but the former lost their cold hardiness mostly for 1 day of deacclimation (Arora et al., 2004). Moreover, the rates of cold acclimation and deacclimation were not correlated in potato species (Vega et al., 2000). In addition, the rate and/or timing of deacclimation vary among species, cultivars, and ecotypes as reported in blueberry (Arora et al., 2004; Rowland et al., 2005) as well as American snowbell (Lenahan et al., 2010), azalea (Kalberer et al., 2007a, b), and hydrangea (Pagter et al., 2011a, b).

Reacclimation is a process that regains some or most cold hardiness lost upon temporary exposure to cold temperature during deacclimation. The reacclimtion is common in many overwintering woody perennials, but not always possible (Kalberer et al., 2006). Level of regained cold hardiness varies with species and timing of reacclimation. Reacclimation capacity of *Rhododendron viscosum* var. *montanum*, a colder climate variety, was higher than that of *R. viscosum* var. *serrulatum*, a warmer climate variety, at 1 and 3 day of deacclimation. However, the *serrulantum* variety had 3-times higher reacclimation capacity than the *montanum* variety at 8 day of deacclimation (Kalberer et al., 2007a). In addition, the capacity and/or timing of reacclimation in diverse plant species is affected by degree or duration of warm air temperature and deacclimation advances (Kalberer et al., 2007a, b). These deacclimation resistance and reacclimation capacity in late winter or early spring are important factors

contributing to survival of sudden or severe cold temperature during deacclimation in woody perennials (Kalberer et al., 2007a).

MATERIALS AND METHODS

Plant materials

Twenty-one highbush blueberry cultivars (Table 1) were used in this study. ‘Bluegold’, ‘Duke’, ‘Northblue’, ‘Sharpblue’, ‘Sierra’, ‘Spartan’, and ‘Sunrise’ were grown at the experimental orchard of Seoul National University, Suwon (37° 17’ E, 127° 00’, N), Korea. ‘Berkeley’, ‘Bluecrop’, ‘Bluehaven’, ‘Bluejay’, ‘Burlington’, ‘Collins’, ‘Chippewa’, ‘Dixi’, ‘Herbert’, ‘Jersey’, ‘Nelson’, ‘Northland’, ‘Polaris’, and ‘Rancocas’ were from the National Institute of Horticultural and Herbal Science, Suwon, Korea. Shoots of the highbush blueberry cultivars were collected to examine their cold hardiness from December 26 to 30, 2011 when they had maximum cold hardiness (data not shown). Among them, seven cultivars of ‘Bluecrop’, ‘Jersey’, ‘Rancocas’, ‘Sharpblue’, ‘Sierra’, ‘Spartan’, and ‘Sunrise’ were selected depend on mid-winter cold hardiness and growth condition to examine changes of their cold hardiness and water contents during deacclimation, from February 6 to April 23, 2012.

Meteorological condition

Daily maximum, minimum, and mean air temperatures at the experimental site from February to April, 2012 were provided from Korea Meteorological Administration.

Table 1. Pedigrees of twenty-one highbush blueberry cultivars used in this study.

Type	Cultivar	Pedigree
Northern highbush	Berkeley	Stanley × (Jersey × Pioneer)
	Bluecrop	(Jersey × Pioneer) × (Stanley × June)
	Bluegold	Bluehaven × (Ashworth × Bluecrop)
	Bluehaven	Berkeley × 19-H
	Bluejay	Berkeley × (Pioneer × Taylor)
	Burlington	Rubel × Pioneer
	Collins	Stanley × Weymouth
	Dixi	(Jersey × Pioneer) × Stanley
	Duke	(Ivanhoe × Earliblue) × (E-30 × E-11)
	Herbert	Stanley × (Jersey × Pioneer)
	Jersey	Rubel × Grover
	Nelson	Bluecrop × (F-72 × Berkeley)
	Rancocas	(Brooks × Russell) × Rubel
	Sierra	US-169 × G-156
	Spartan	Earliblue × US-11-93
	Sunrise	G-180 × ME-US-6620
Half highbush	Chippewa	(G-65 × Ashworth) × US-3
	Northblue	(G-65 × Ashworth) × U-53
	Northland	Berkeley × 19-H
	Polaris	(G-65 × Ashworth) × Bluetta
Southern highbush	Sharpblue	FL61-5 × FL62-4

Cold hardiness determination through Electrolyte Leakage (EL) analysis

Cold hardiness was estimated by EL analysis using the methods described by Arora et al. (1992), Morin et al. (2007), and Pagter et al. (2011b) with slight modifications. Twenty-four shoots were excised to 7-cm-long from upper position of the blueberry shrub. The excised shoots were rinsed under running cold distilled water. After rinsing, the samples were placed in 50-mL test tubes containing 1 mL of distilled water. They were incubated in a bath circulator (RW-1040G and RW-2040G, Jeio Tech, Seoul, Korea) equipped with a temperature controller (UP351E, Yokogawa Electric Korea Co., Ltd., Seoul, Korea) and cooled at a rate of 4°C/h to the target temperature, and then maintained for 2 h at each target temperature. The test tubes were taken out and thawed at 4°C. Four target temperatures were selected in ranges of –10 to –40°C in December, 2011; –8 to –30°C in March, 2012; –2 to –25°C in April, 2012. The temperatures were monitored every second with a data logger (CR-10X, Campbell Scientific, Inc., Logan, UT, USA) using a copper-constantan thermocouple.

After the freezing-thawing treatment, buds were removed from shoots. The shoots were cut into 1-cm-long, placed in 15 mL test tubes containing 8 mL of distilled water, and then vacuum-infiltrated for 1 min. The test tubes were shaken at 125 rpm on an orbital shaker (Supertech^{TM} Orbital shaker, SeouLin Bioscience, Seoul, Korea) at room temperature for 24 h and electrical conductivity (EC) of the aliquots were measured using an EC meter (Model 1461-81, Cole-Parmer, Vernon

Hills, IL, USA). After autoclaving at 120°C, 17 PSI for 30 min, EC was measured again. Then, percent injury was calculated according to the method of Arora et al. (1992):

$$\% \text{ Injury} = (\% \text{ EL}_{(t)} - \% \text{ EL}_{(4°C)})/(100 - \% \text{ EL}_{(4°C)}) \times 100$$

Where % $EL_{(t)}$ and % $EL_{(4°C)}$ are percentages of initial EC to total EC for the each target temperature and unfrozen control, respectively. The percent injury data were adjusted using the method of Lim et al. (1998). Samples treated at –65°C were representing 100% freeze-injured samples by extreme freezing, and the percent injury was then transformed by following equation;

$$\% \text{ Adjusted injury} = (\% \text{ Injury}_{(t)}/\% \text{ Injury}_{(-65°C)}) \times 100$$

With these percent adjusted injury data, two quantitative estimates of shoot cold hardiness, temperature at which 50% injury occurred (LT_{50}) and temperature at maximum rate of injury (T_{max}), were calculated using the Gompertz function (Lim et al., 1998).

Soluble sugar analysis

Soluble sugars were analyzed using the method described by González-Rossia et al. (2008) with slight modifications. Shoots from twenty-one highbush

blueberry cultivars were immediately frozen in liquid nitrogen to stop metabolism, then lyophilized, ground using a mill (Thomas Wiley® Mini Mill 3383-L10, Thomas Scientific, Swedesboro, NJ, USA) with 60-mesh sieve. One hundred milligrams of the powder were put in a 2-mL test tube containing 1 mL of 80% ethanol. After incubating at 85°C for 15 min and centrifuging at 6,300 g for 5 min, the supernatant was collected in a 50-mL test tube and the pellet was re-extracted twice as above. The collected supernatant was evaporated using a nitrogen evaporator (N-EVAP™, Organomation Associates, Inc., West Berlin, MA, USA) at 60°C. The solution of sugar extracts was dissolved in 3 mL of distilled water, passed through 0.45 μm nylon filter (Acrodisc® 13mm syringe filter, Pall Co., Washington, NY, USA) and C18 Sep-Pak cartridge (Waters Associates, Milford, MA, USA). Sugars were analyzed using an HPLC (UltiMate 3000, Dionex, Sunnyvale, CA, USA) connected to a Shodex RI-101 detector (Showa Denko K.K., Kawasaki, Japan). The filtered extracts of 10 μL were injected in a Sugar-Pak column which temperature was kept at 75°C and distilled water was used as a solvent at a flow rate of 0.5 $mL \cdot min^{-1}$.

Water content

Water content of shoots was determined by the method described by Pagter et al. (2011a). Excised shoots of 10-cm-long were weighed before and after drying at 80°C. The water content was calculated as the following equation:

Water content (%) = [(fresh weight – dry weight)/fresh weight] × 100

Statistical analysis

Statistical differences were determined via analysis of variance with the SAS 9.2 software package (SAS Institute Inc., Cary, NC, USA). Graph plotting, and correlation coefficient analysis were by SigmaPlot 10.0 program (Systat Software, Inc., San Jose, CA, USA).

RESULTS AND DISCUSSION

Estimation LT_{50} and T_{max}

The percent EL from unfrozen samples was 19.8% for 'Rancocas' (Table 2). The EL values in the unfrozen samples indicate the amount of electrolytes located in extracellular space and these electrolytes were leaked from the cutting plane due to cell destruction. The percent injury values of the samples treated at –65°C did not reach at 100% (Table 2). Since the cell wall containing various ions was also broken besides the plasma membrane and organellar membranes during the autoclave for extruding total electrolytes.

The Gompertz function had a great fit with the percent adjusted injury data at target temperatures; $r^2 = 0.98^{***}$ (Fig. 1). Thus, the percent adjusted injury obtained through EL analysis at target temperature. LT_{50} and T_{max} representing cold hardiness in the highbush blueberry cultivars were estimated using EL analysis.

Comparison of cold hardiness levels

LT_{50} and T_{max} representing cold hardiness were estimated during mid-winter to compare among twenty-one highbush blueberry cultivars. LT_{50} and T_{max} were ranged from –31.5 to –40.1°C and from –29.6 to –35.9°C, respectively. According to cold hardiness levels based on LT_{50}, the twenty-one highbush blueberry cultivars were ranked as 'Jersey' > 'Northblue' > 'Berkeley' = 'Sierra'

Table 2. An example for calculation of percent adjusted injury at various temperatures through electrolyte leakage analysis in the shoots of 'Rancocas' highbush blueberry on December, 2011.

Treatment Temp. (°C)	Initial EC[z] (μs)	Total EC(μS)	% EL[y]	% Injury[x]	% Adjusted injury[w]
4	32.5	167.5	19.8	-	-
–12	35.8	169.4	21.2	1.6	3.1
–20	34.4	158.3	21.7	2.3	4.4
–28	39.4	130.4	30.3	13.0	25.1
–38	82.2	181.2	45.2	31.6	61.9
–65	140.5	228.0	61.4	51.9	100.0

[z]Raw data of electrical conductivity (EC).

[y](Initial EC/total EC) × 100.

[x](% $EL_{(t)}$ – % $EL_{(4°C)}$)/(100 – % $EL_{(4°C)}$) × 100.

[w](% $Injury_{(t)}$/% $injury_{(–65°C)}$) × 100.

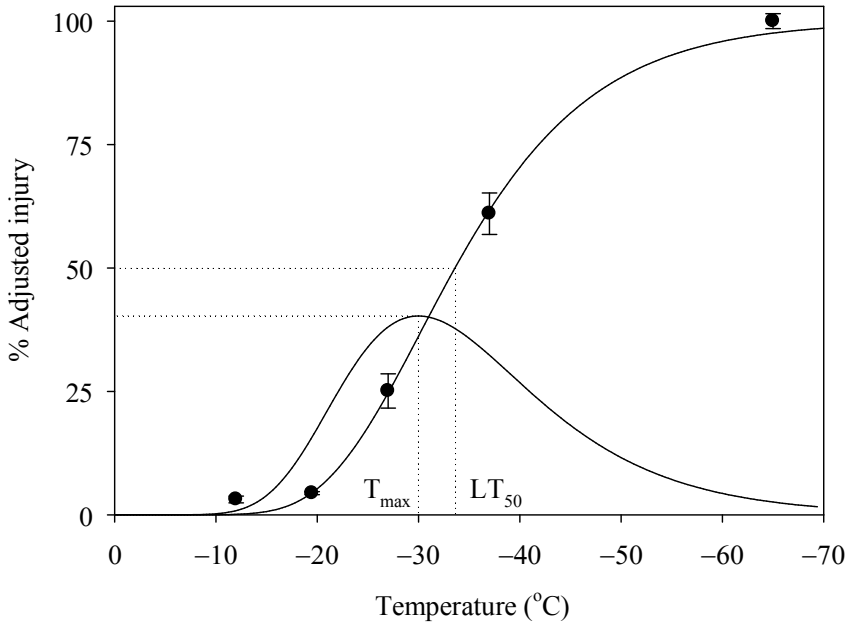

Fig. 1. Estimation of the temperature representing 50% injury occurred (LT_{50}) and the temperature at maximum rate of injury (T_{max}) in the shoots of 'Rancocas' highbush blueberry using the Gompertz function fitted to means of the percent adjusted injury data per treatment temperature on December, 2011. Vertical bars are the standard deviations of the means.

> ‘Northland’ > ‘Dixi’ > ‘Bluejay’ > ‘Chippewa’ > ‘Burlington’ > ‘Bluegold’ > ‘Spartan’ > ‘Bluecrop’ > ‘Sunrise’ > ‘Duke’ = ‘Rancocas’ > ‘Herbert’ > ‘Polaris’ > ‘Collins’ > ‘Bluehaven’ > ‘Sharpblue’ > ‘Nelson’ (Table 3). In other hands, cold hardiness levels based on T_{max} were ‘Jersey’ > ‘Northblue’ > ‘Northland’ > ‘Dixi’ > ‘Sierra’ > ‘Berkeley’ = ‘Chippewa’ > ‘Bluejay’ > ‘Burlington’ > ‘Bluegold’ > ‘Bluecrop’ = ‘Sunrise’ > ‘Spartan’ > ‘Duke’ = ‘Herbert’ > ‘Polaris’ > ‘Collins’ > ‘Bluehaven’ > ‘Sharpblue’ > ‘Rancocas’ > ‘Nelson’ (Table 3). Most of the cultivars except ‘Rancocas’, were ranked similarly based on either LT_{50} or T_{max}. T_{max} was significantly correlated with LT_{50} ($r^2 = 0.96$***) (Fig. 2). Thus, both LT_{50} and T_{max} values can be utilized as indicators of cold hardiness. Cold hardiness of ‘Jersey’ was the strongest as –40.1 and –35.9°C for LT_{50} and T_{max}, respectively. Furthermore, half highbush blueberry (*V. corymbosum* × *V. angustifolia*) cultivars, ‘Chippewa’, ‘Northblue’, and ‘Northland’ were generally cold-hardy, except ‘Polaris’. On the other hand, ‘Sharpblue’, southern highbush blueberry cultivar, was cold-sensitive as –32.5 and –30.2°C for LT_{50} and T_{max}, respectively (Table 3).

Cold hardiness and soluble sugar contents

Fructose, glucose, sucrose, and raffinose were detected in the shoots of twenty-one highbush blueberry cultivars. Ranges of fructose, glucose, sucrose, and raffinose contents were 13.5-28.8, 14.1-29.5, 6.2-21.2, and 2.2-5.6 $mg \cdot g^{-1}$ dry weight (DW), respectively. Fructose, glucose, and sucrose were in major quantities, while, raffinose was in minor (Table 4). Soluble sugar contents in

Table 3. Comparison of cold hardiness levels in the shoots of twenty-one highbush blueberry cultivars based on LT_{50} and T_{max}.

Cultivar	LT_{50} (°C)	Cultivar	T_{max} (°C)
Jersey	–40.1	Jersey	–35.9
Northblue	–37.9	Northblue	–34.5
Berkeley	–37.1	Northland	–34.2
Sierra	–37.1	Dixi	–34.1
Northland	–37.0	Sierra	–33.9
Dixi	–36.7	Berkeley	–33.6
Bluejay	–36.5	Chippewa	–33.6
Chippewa	–36.4	Bluejay	–33.4
Burlington	–36.3	Burlington	–33.1
Bluegold	–35.5	Bluegold	–32.4
Spartan	–34.5	Bluecrop	–31.6
Bluecrop	–34.2	Sunrise	–31.6
Sunrise	–33.9	Spartan	–31.2
Duke	–33.6	Duke	–31.1
Rancocas	–33.6	Herbert	–31.1
Herbert	–33.4	Polaris	–31.0
Polaris	–33.3	Collins	–30.4
Collins	–32.8	Bluehaven	–30.2
Bluehaven	–32.7	Sharpblue	–30.2
Sharpblue	–32.5	Rancocas	–30.0
Nelson	–31.5	Nelson	–29.6

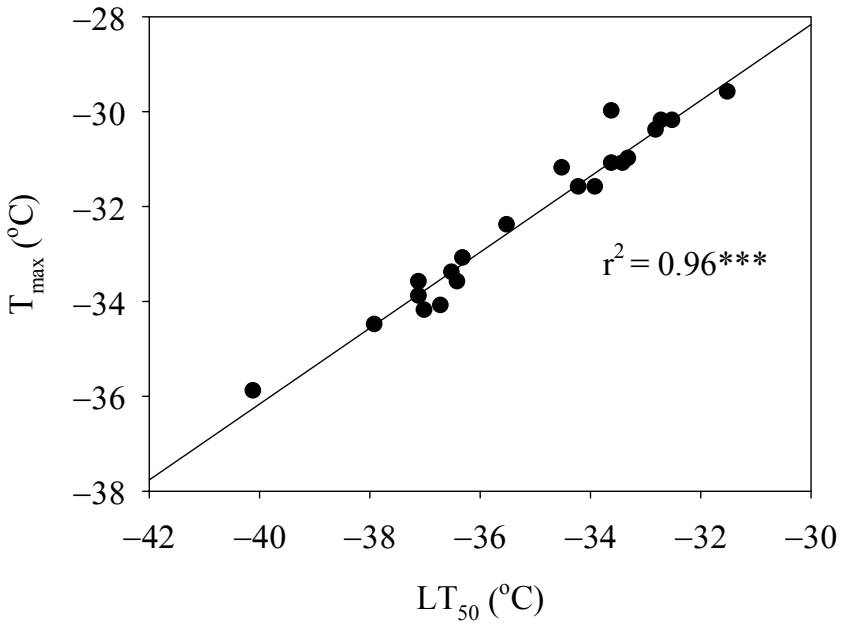

Fig. 2. Relastionship between LT_{50} and T_{max} in the shoots of twenty-one highbush blueberry cultivars.

Table 4. Fructose, glucose, sucrose, raffinose, and total soluble sugar contents in the shoots of twenty-one highbush blueberry cultivars. Values are the means with standard deviations.

	Soluble sugar ($mg \cdot g^{-1}$ DW)				
Cultivar	Fructose	Glucose	Sucrose	Raffinose	Total
Berkeley	22.9±0.9	24.7±1.5	15.5±1.4	3.6±0.4	66.7±3.9
Bluecrop	23.3±0.8	23.9±1.4	8.9±0.4	2.5±0.3	58.7±2.9
Bluegold	23.0±1.0	21.2±1.3	10.9±0.8	3.5±0.2	58.6±3.1
Bluehaven	16.5±0.4	15.5±0.5	19.5±1.5	3.1±0.8	54.7±3.0
Bluejay	28.0±0.5	26.6±0.8	6.8±0.6	4.8±0.6	66.2±2.4
Burlington	22.5±0.6	24.1±0.5	14.6±0.9	4.1±0.4	65.3±2.1
Chippewa	18.6±0.6	19.8±0.6	10.6±0.7	5.5±0.8	62.3±3.7
Collins	17.5±1.2	17.4±0.7	19.6±1.0	4.8±0.4	59.3±3.2
Dixi	23.0±1.1	23.2±1.7	10.6±0.7	5.5±0.8	62.3±3.7
Duke	19.8±1.5	19.2±1.2	14.4±1.5	2.2±0.5	55.6±4.7
Herbert	19.7±0.8	21.1±0.4	14.8±1.1	4.7±1.0	60.3±3.2
Jersey	28.8±2.3	29.5±0.5	14.5±0.5	2.5±0.2	75.2±1.4
Nelson	16.2±1.4	17.1±1.3	10.9±1.0	3.3±0.4	47.7±4.1
Northblue	27.9±0.5	28.3±0.6	16.7±0.4	4.5±0.2	77.6±0.8
Northland	24.1±0.2	24.9±0.3	9.5±0.2	2.7±0.2	61.3±0.6
Polaris	20.9±0.9	20.4±0.6	6.2±0.4	3.0±0.1	50.6±1.9
Rancocas	20.5±1.0	20.1±0.7	18.8±1.2	3.4±0.3	62.8±3.1
Sharpblue	13.5±0.7	14.1±0.4	21.2±0.6	2.7±0.4	51.5±1.8
Sierra	25.1±1.1	23.3±1.0	9.2±0.6	5.6±0.8	63.1±3.5
Spartan	21.5±0.6	20.5±0.4	16.2±0.5	3.0±0.1	61.2±1.6
Sunrise	19.7±0.5	17.4±0.2	16.9±0.4	4.1±0.2	58.2±1.1

'Jersey', the cold-hardiest cultivar, were highest in quantity as 75.2±1.4 $mg \cdot g^{-1}$ DW. Total soluble sugar contents closely correlated with cold hardiness. They tended to increase with decreasing LT_{50} or T_{max} (Fig. 3). Especially, LT_{50} was negatively correlated with fructose and glucose contents, but not with sucrose and raffinose contents in the shoots of highbush blueberry cultivars (Fig. 4). This tendency was also similar with the relationship of T_{max} and individual soluble sugar contents (Fig. 5). Besides, the relationship with fructose and glucose contents was highly significant (Table 5). Similarly, correlations between soluble sugar contents and cold hardiness have been often found in many woody perennials such as European oak (Morin et al., 2007), forsythia (Flinn and Ashworth, 1995), highbush blueberry (Lee et al., 2012), honeyberry (Imanish et al., 1998), hydrangea (Pagter et al., 2008, 2011a, b), red osier dogwood (Ashworth et al., 1993), and red raspberry (Palonen et al., 2000).

Raffinose and stachyose have been reported to be the major sugar associated with cold hardiness in several species such as forsythia (Flinn and Ashworth, 1995), honeyberry (Imanishi et al., 1998), and red osier dogwood (Ashworth et al., 1993). Although, raffinose and stachyose were commonly accumulated during cold acclimation, raffinose was in much lower quantity than fructose, glucose, and sucrose, and stachyose was not detected. Thus, these results indicate that fructose and glucose are the major soluble sugars associated with cold hardiness in the shoots of highbush blueberry.

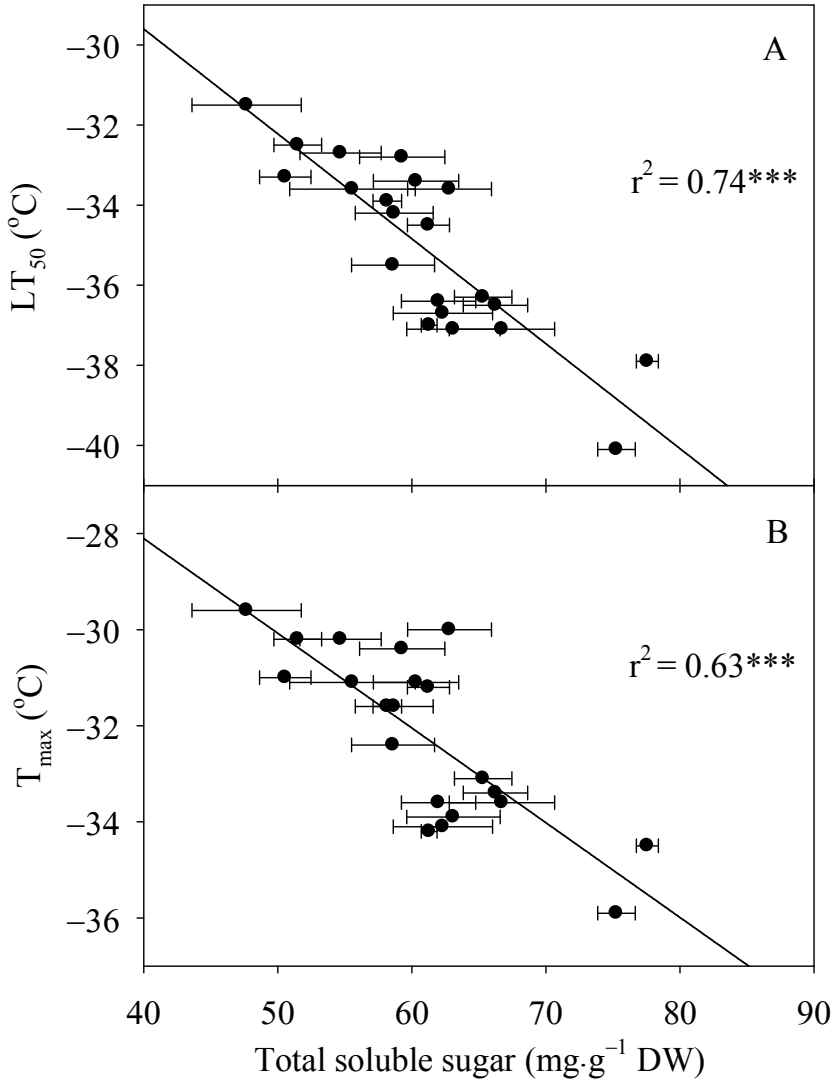

Fig. 3. Relationship between total soluble sugar contents and cold hardiness estimated LT_{50} (A) and T_{max} (B) in the shoots of twenty-one highbush blueberry cultivars. Horizontal bars are the standard deviations of the means.

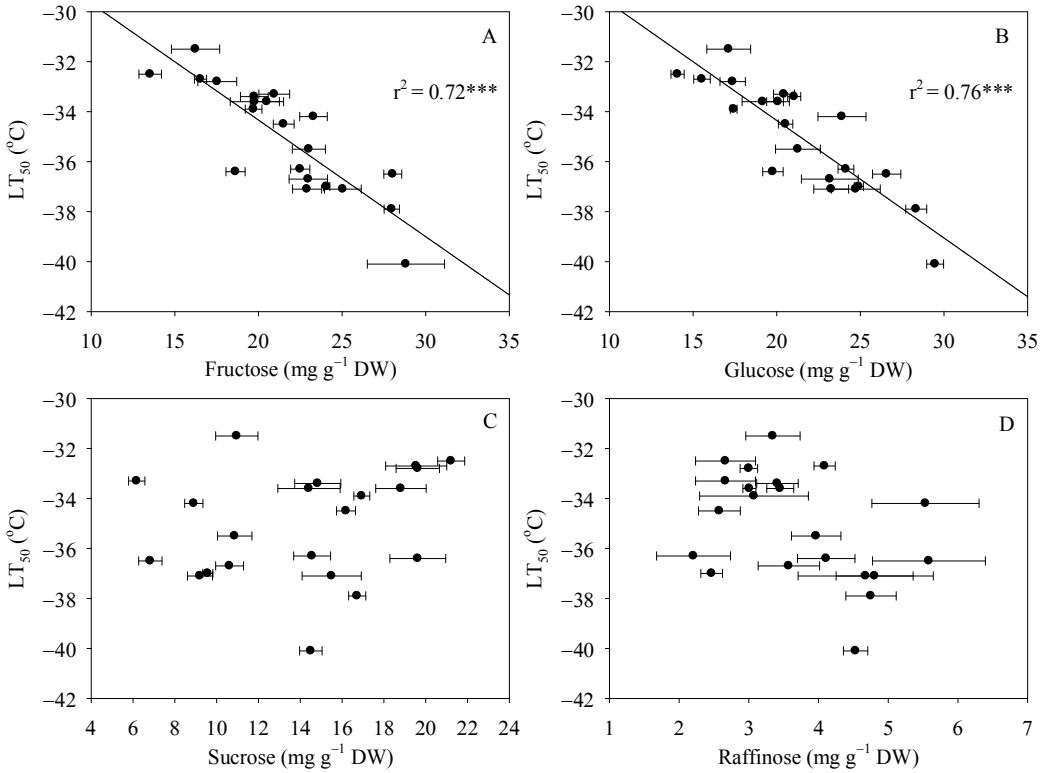

Fig. 4. Relationship of LT_{50} with fructose (A), glucose (B), sucrose (C), and raffinose (D) contents in the shoots of twenty-one highbush blueberry cultivars. Horizontal bars are the standard deviations of the means.

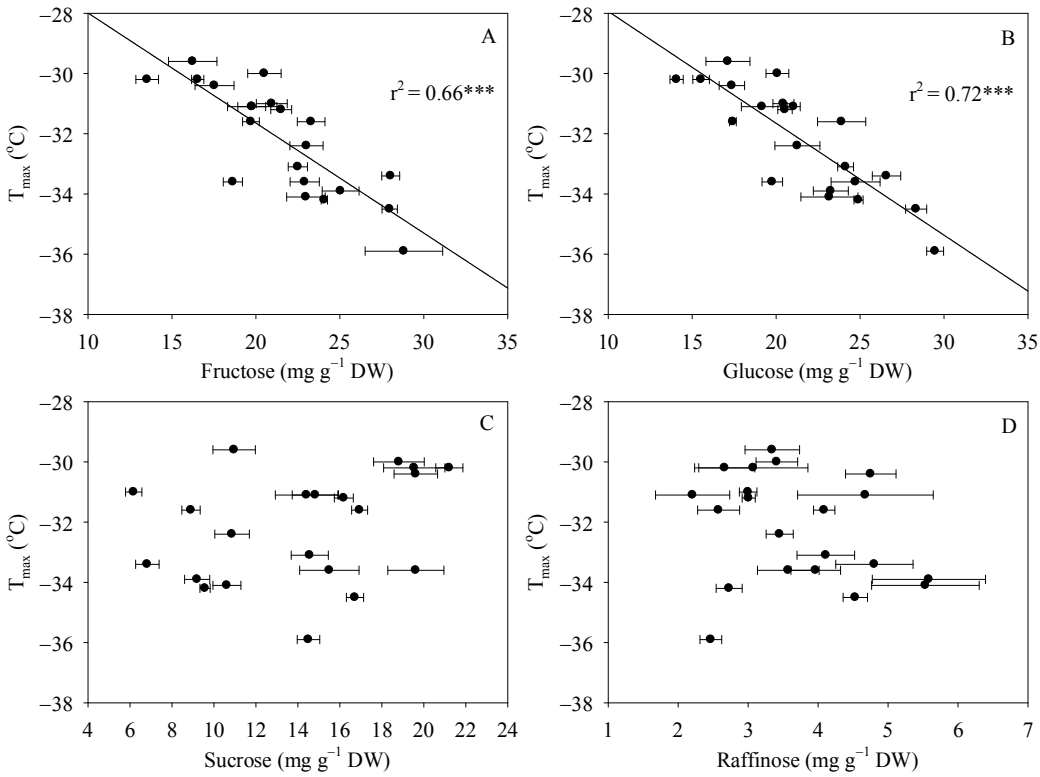

Fig. 5. Relationship of T_{max} with fructose (A), glucose (B), sucrose (C), and raffinose (D) contents in the shoots of twenty-one highbush blueberry cultivars. Horizontal bars are the standard deviations of the means.

Table 5. Multivariate correlation coefficient of LT_{50}, T_{max}, fructose, glucose, sucrose, raffinose, and total soluble sugar contents in the shoots of twenty-one highbush blueberry cultivars.

	Correlation coefficient (r)					
Variable	T_{max}	Fructose	Glucose	Sucrose	Raffinose	Total soluble sugar
LT_{50}	0.98***	–0.85***	–0.87***	0.22^{ns}	$–0.21^{ns}$	–0.86***
T_{max}		–0.81***	–0.85***	0.28^{ns}	$–0.26^{ns}$	–0.79***
Fructose			0.96***	$–0.52^{ns}$	0.22^{ns}	0.80***
Glucose				$–0.46^{ns}$	0.17^{ns}	0.83***
Sucrose					$–0.10^{ns}$	0.06^{ns}
Raffinose						0.29^{ns}

^{ns,} ***Nonsignificant or significant at $P = 0.001$, respectively.

Change of cold hardiness during deacclimation

Air temperature at experimental site increased steadily with slight fluctuations from February to April, 2012 (Fig. 6). Cold hardiness changed coincidentally with the changes of air temperature during deacclimation.

Seven highbush blueberry cultivars of 'Bluecrop', 'Jersey', 'Rancocas', 'Sharpblue', 'Sierra', 'Spartan', and 'Sunrise' were selected depending on their mid-winter cold hardiness and growth condition. Changes of the cold hardiness were evaluated periodically from February 6 to April 23, 2012. According to the cold hardiness levels based on both LT_{50} and T_{max}, all cultivars except 'Sharpblue' maintained their cold hardiness until February 20 and then steeply decreased until March 5. However, 'Sharpblue' sharply decreased its cold hardiness until March 5. Since March 5, all cultivars except 'Sharpblue' increased cold hardiness (Fig. 7). This occurred coincidentally with decrease of air temperature. Degree of cold hardiness based on LT_{50} increased by –4.2, –3.5, –3.4, –2.5, –2.3, and –2.2°C for 'Rancocas', 'Jersey', 'Bluecrop', 'Sierra', 'Sunrise', and 'Spartan', respectively on March 12. 'Bluecrop' and 'Sunrise' regained cold hardiness by –3.6 and –2.0°C, respectively, on March 26. 'Jersey' also increased its cold hardiness slightly on April 2 (Fig. 7). Moreover, changes of cold hardiness based on T_{max} were also similar. They increased by –5.3, –3.7, –3.7, and –2.2°C for 'Spartan', 'Rancocas', 'Jersey', and 'Bluecrop', respectively, on March 12. 'Rancocas' regain its cold hardiness by –2.1°C on March 19. 'Rancocas' and 'Sunrise' were increased cold hardiness slightly on March 26. 'Spartan' also

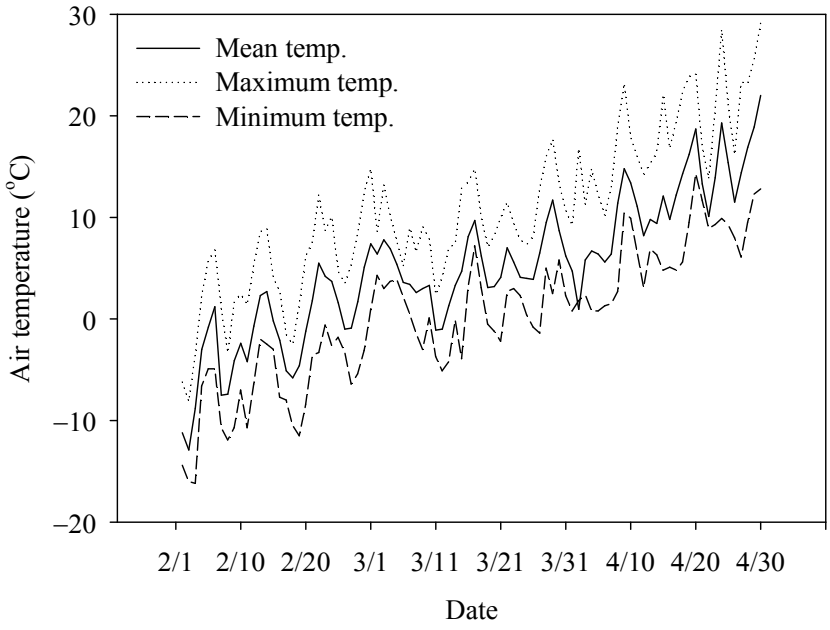

Fig. 6. Meteorological data at the experimental site (37° 17’ E, 127° 00’ N) from February to April, 2012.

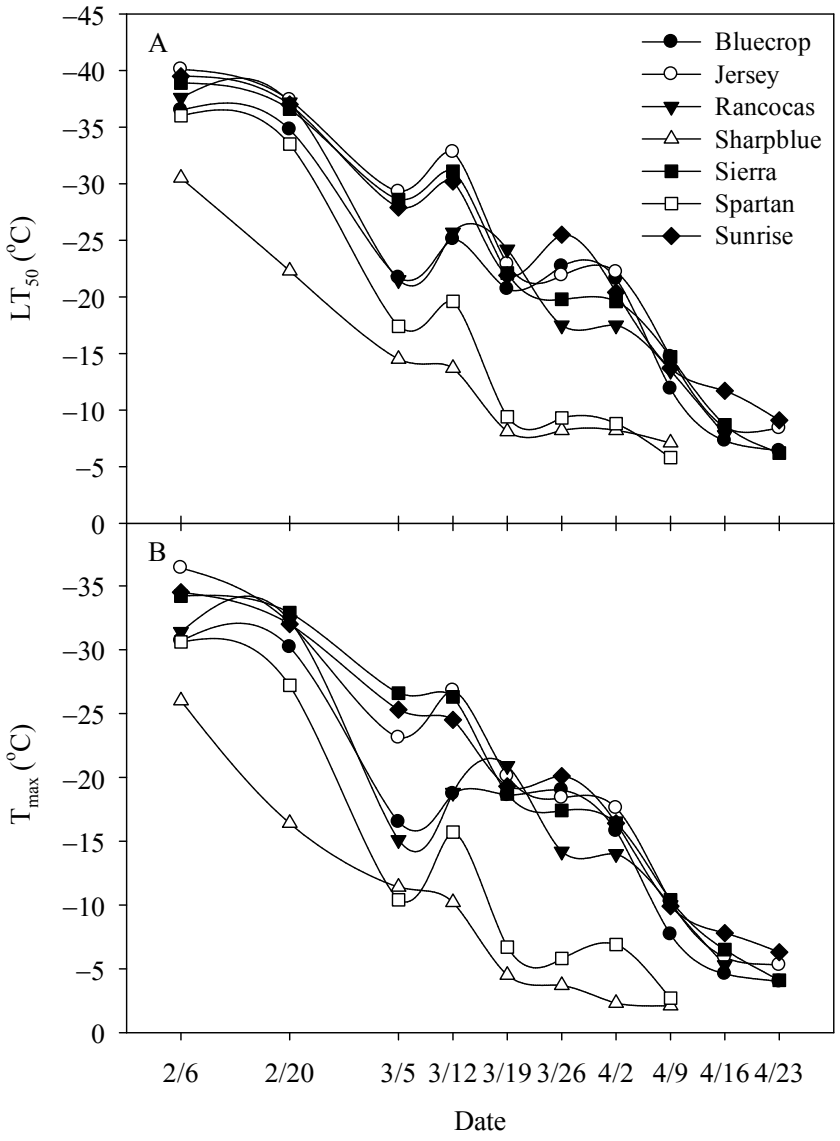

Fig. 7. Changes of cold hardiness estimated as LT_{50} (A) and T_{max} (B) in the shoots of 'Bluecrop', 'Jersey', 'Rancocas', 'Sharpblue', 'Sierra', 'Spartan', and 'Sunrise' highbush blueberry cultivars from February 6 to April 23, 2012.

regained its cold hardiness by −1.1°C on April 2 (Fig. 7). LT_{50} and T_{max} changed coincidentally with the changes of air temperature. T_{max} was significantly correlated with LT_{50} (r^2 = 0.99***) during deacclimation (data not shown). Generally, 'Sharpblue' and 'Spartan' lost their cold hardiness earlier and faster than the other cultivars (Fig. 7). This deacclimation resistance after exposure to warm temperatures is important factor determining frost hardiness as mid-winter cold hardiness. However, deacclimation resistance was not always associated with degree of mid-winter cold hardiness and rate or timing of cold acclimation in many plants such as azalea (Kalberer et al., 2007a, b), blueberry (Arora et al., 2004; Rowland et al., 2005, 2008), grape (Wolf and Cook, 1992), and hydrangea (Pagter et al., 2011a, b). Further studies are required to investigate changes of soluble sugars during deacclimation and to determine how cold hardiness is affected by cooling rate and duration at freezing temperature. They will help to understand to changes of soluble sugars and deacclimation kinetics as affected by various environmental conditions.

Change of water content in shoots

From March 5 to April 16, water contents in the shoots of highbush blueberry cultivars tended to increase with increasing air temperature. As deacclimation progressed, the shoots were rehydrated. The water content of 'Sierra' was the most as 46.4%, while that of 'Rancocas' was the least as 20.0% on March 5 (data not shown). However, relative water content of 'Rancocas' was increased the most as

41.9%, while ‘Spartan’ was the least as 9.9% (Fig. 8). In all cultivars, the water contents of the shoots were increased as their cold hardiness lost. However, they were not always significantly correlated with the cold hardiness levels during deacclimation in all cultivars examined. The water contents in the shoots of ‘Bluecrop’, ‘Jersey’, ‘Rancocas’, ‘Spartan’, and ‘Sunrise’ were significantly correalated with cold hardiness, but those of ‘Sharpblue’ and ‘Sierra’ were not significant (Table 6). In general, water content is known to be negatively correlated with cold hardiness (Pagter et al., 2011a). However, relationship between water content and cold hardiness was difficult to be determined during deacclimation because water content varies from shoot to shoot.

To conclude, mid-winter cold hardiness of twenty-one highbush blueberry cultivars was ranked on LT_{50} and T_{max}. Fructose and glucose were the major sugars associated with the cold hardiness in the highbush blueberry cultivars. Furthermore, deacclimation resistance was not always associated with mid-winter cold hardiness. Although ‘Spartan’ was more cold-hardy in mid-winter, it lost cold hardiness earlier and faster than ‘Bluecrop’, ‘Sunrise’, and ‘Rancocas’. This study will provide practical information for breeding and cultivation of highbush blueberries.

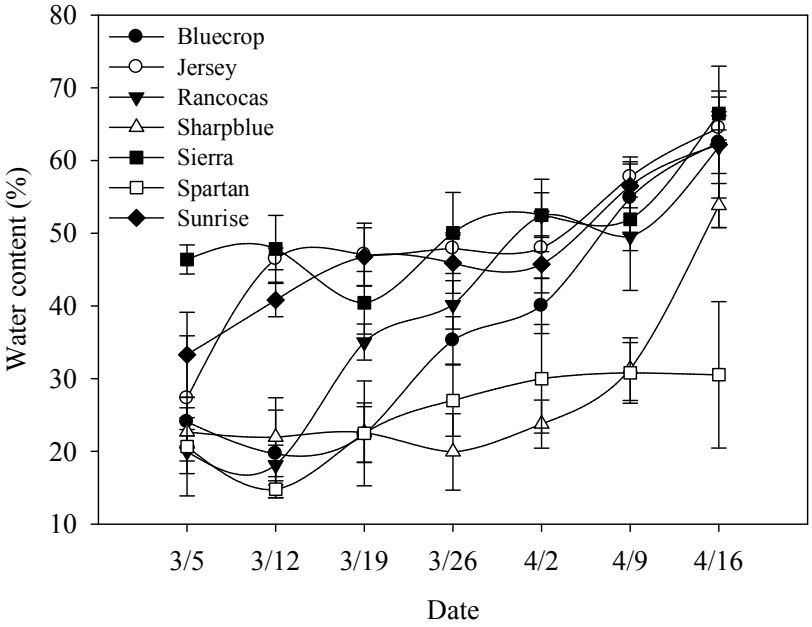

Fig. 8. Changes of water contents in the shoots of 'Bluecrop', 'Jersey', 'Rancocas', 'Sharpblue', 'Sierra', 'Spartan', and 'Sunrise' highbush blueberry cultivars from March 5 to April 16, 2012. Vertical bars are the standard deviations of the means.

Table 6. Correalation coefficient between water content and cold hardiness estimated by LT_{50} and T_{max} in the shoots of 'Bluecrop', 'Jersey', 'Rancocas', 'Sharpblue', 'Sierra', 'Spartan', and 'Sunrise' highbush blueberry cultivars.

	Correlation coefficient (r)	
Cultivar	LT_{50}	T_{max}
Bluecrop	0.91**	0.92**
Jersey	0.81*	0.79*
Rancocas	0.88**	0.76*
Sharpblue	0.37^{ns}	0.40^{ns}
Sierra	0.74^{ns}	0.72^{ns}
Spartan	0.91*	0.91*
Sunrise	0.92**	0.96***

^{ns,} *, **, ***Nonsignificant or significant at $P = 0.05$, 0.01, or 0.001, respectively.

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ABSTRACT IN KOREAN

본 연구는 하이부시 블루베리 21품종(Berkeley, Bluecrop, Bluegold, Bluehaven, Bluejay, Burlington, Chippewa, Collins, Dixi, Duke, Herbert, Jersey, Nelson, Northblue, Northland, Polaris, Rancocas, Sharpblue, Sierra, Spartan, Sunrise)을 대상으로 한겨울철 가지의 내한성과 가용성 당 함량을 비교하였다. 다양한 빙점 이하의 저온을 처리한 후 전해질 유출에 따른 전기전도도의 측정을 통해 내한성을 측정하였다. 내한성은 50%의 피해가 발생하는 온도인 LT_{50}과 피해율이 최대가 되는 온도인 T_{max}로 나타내었다. LT_{50}을 기준으로 비교한 하이부시 블루베리 21품종의 내한성은 'Jersey' > 'Northblue' > 'Berkeley' = 'Sierra' > 'Northland' > 'Dixi' > 'Bluejay' > 'Chippewa' > 'Burlington' > 'Bluegold' > 'Spartan' > 'Bluecrop' > 'Sunrise' > 'Duke' = 'Rancocas' > 'Herbert' > 'Polaris' > 'Collins' > 'Bluehaven' > 'Sharpblue' > 'Nelson'순이었다. LT_{50}과 T_{max}는 서로 밀접한 상관관계를 보였으며(r^2 = 0.96***), T_{max}에 의한 내한성 서열도 유사하게 나타났다. 이러한 내한성은 총가용성 당 함량과 고도로 유의하였다. 특히, fructose와 glucose의 함량은 내한성과 밀접한 상관 관계를 갖는 반면에 sucrose와 raffinose의 함량은 유의하지 않았다. 탈순화 기간 동안의 하이부시 블루베리의 내한성의 변화를 알아보기 위해, 한겨울철 내한성과 나무의 생육 상태를 고려하여 하이부시 블루베리 7품종('Bluecrop', 'Jersey', 'Rancocas', 'Sharpblue', 'Sierra', 'Spartan', 'Sunrise')을 선발하였다. LT_{50}에 의한 품종별 내한성을 보면 'Sharpblue'는 3월 5일까지 내한성이 급격히

감소한 반면, ‘Sharpblue’를 제외한 모든 품종은 내한성을 2월 20일까지 어느 정도 유지하다가 그 후로는 소실하였다. 뿐만 아니라 3월 5일 이후에는 기온이 일시적으로 내려감에 따라 ‘Sharpblue’를 제외한 모든 품종에서 잃었던 내한성을 어느 정도 다시 회복하는 재순화 현상을 관찰할 수 있었다. 3월 12일에 ‘Rancocas’, ‘Jersey’, ‘Bluecrop’, ‘Sierra’, ‘Sunrise’, ‘Spartan’은 각각 –4.2, –3.5, –3.4, –2.5, –2.3, –2.2°C 정도 내한성이 증가하였다. 특히, ‘Bluecrop’과 ‘Sunrise’는 3월 26일에 각각 –3.6, –2.0°C만큼 내한성이 증가하였으며 ‘Jersey’의 경우도 4월 2일에 약간의 내한성 증가를 보였다. T_{max}에 의한 내한성의 변화 역시 유사한 양상을 보였다. ‘Spartan’의 경우 ‘Bluecrop’, ‘Sunrise’, ‘Rancocas’보다 한겨울철 내한성이 컸지만 탈순화 기간 동안에는 내한성을 더 빠르게 잃는 것으로 미루어 보아 한겨울철의 내한성은 탈순화 저항성과 항상 상관 관계를 보이지는 않았다. 품종별 블루베리 가지의 수분 함량은 기온이 증가하고 내한성을 잃을수록 증가하는 경향을 보였으나 모든 품종에서 유의하지는 않았다.