Original Paper

Effect of soil moisture conditions during the period from late autumn to early spring on the freezing tolerance of the Japanese chestnut (*Castanea crenata Sieb.* et *Zucc.*)

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Summary

This study was conducted to determine the effect of soil moisture during the period from late autumn to early spring on the seasonal change in freezing tolerance of the Japanese chestnut cultivar 'Porotan'. Two-year-old trees planted in pots filled with gray lowland soil were used in this study. Soil moisture, based on the volumetric water content in the pots, was maintained at 15%, 25% and 40% by irrigation using amplitude domain reflectometry. The advance of cold hardening in autumn and delay of cold dehardening in spring were induced by the 15% and 25% soil moisture treatments under natural temperature conditions. At the same time, the highest total sugar content in the current season's shoots (CSSs) in the 25% soil moisture treatment was correlated with a delay of cold dehardening in spring. High soil moisture (40%) resulted in an increase in the water content of CSSs and a rapid decrease in freezing tolerance during the cold dehardening period. These results suggested that dry soil was responsible for the high sugar content and low water content in CSSs of chestnut trees in winter and resulted in the prevention of freezing injury. Our results also indicated that the effective soil moisture conditions for preventing freezing injury of 'Porotan' Japanese chestnut might be below 25% in the case of gray lowland soil.

Key words: freezing injury, sugar content, water content

Introduction

In Japanese chestnut (*Castanea crenata Sieb.* et *Zucc.*), freezing injury often occurs in young trees with a high mortality rate. Freezing injury occurs mainly in two to four- year-old trees, but occasionally occurs in five to ten-year-old trees (Horimoto and Araki, 1999 A). Several studies have investigated the factors leading to freezing injury and methods to prevent freezing injury. Seasonal and annual variations in freezing tolerance were studied as major factors contributing to freezing injury in twigs and stems of Japanese chestnuts (Yasunobu, 1968; Yamamoto and Sakai, 1977). Soil moisture is an important environmental factor that influences the low temperature survival of plants (Paquin and Mehuys, 1980). Recently, freezing injury in young chestnut trees occurred frequently in an orchard converted from a paddy field using gray lowland soil (Mizuta et al., 2014). Acceleration of cold hardening in autumn and a delay of cold dehardening in spring were induced by dry soil moisture

(Received 24 December 2014, Accepted 1 April 2015)
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conditions (Yasunobu, 1963; Sawano, 1980). In fact, very large amounts of rainfall during the winter induce the occurrence of freezing injury (Kamio and Mizuta, 2013). During the winter, cold hardiness is positively correlated with sugar content in chestnut trees (Sawano, 1965). From these observations, restricting water uptake from roots by root pruning, root loosening and application of molasses to the soil root zone were developed as effective methods to mitigate freezing injury of Japanese chestnut (Horimoto and Araki, 1999 B). In previous reports, cut current season's shoots (CSSs) or stems were mainly used to evaluate freezing tolerance, and rarely were whole trees used. Also, to our knowledge, there have been no studies on how soil moisture conditions affect the seasonal patterns of the sugar content of the CSSs of Japanese chestnut in the period from late autumn to early spring.

In this study, we investigated the effect of soil moisture conditions from late autumn to early spring on the seasonal patterns of freezing tolerance, water content and sugar content of the Japanese chestnut 'Porotan'. Based on the results of these experiments, we discuss a potential mechanism for how soil moisture could affect cold hardiness of Japanese chestnut trees.

Materials and Methods

Plant materials

Two-year-old potted trees of the Japanese chestnut 'Porotan' (*Castanea crenata Sieb.* et *Zucc.*) grown at the NARO Institute of Fruit Tree Science (Tsukuba, Japan), located at 36° 3'N and 140° 8'E, were used for all experiments. Gray lowland soil was used to fill one hundred 7L pots, and a two-year-old 'Porotan' tree was planted in each pot in March. In mid-April, 5 g of readily available chemical fertilizer [N-P_2O_5-K_2O = 10%-10%-10%] was applied to each pot. All potted trees were managed according to the ordinary cultural practices used in Tsukuba (Ibaraki Prefecture) in a rain shelter house. Air temperature data were obtained from the nearest weather station (Japan Meteorological Agency) located about 1.5 km west of the experimental orchard.

Soil moisture management

Experiments were conducted from November to March in 2012/2013 and 2013/2014. Soil moisture, based on the volumetric water content in the pots, was maintained at 40% by an automatic irrigation system (DIK-6563-S150, Daiki Rika Kogyo Co., Ltd.) with amplitude domain reflectometry (ADR) from planting to October. In 2012/2013, potted trees were randomly divided into two groups. The soil moisture in one of the groups was gradually reduced to 25% during the first 10 days in November. The soil moisture of the second group of plants was maintained at 40% during the experimental period. In 2013/2014, potted trees were randomly divided into three groups. In addition to the 40% soil moisture (control) group, the soil moisture in two of the three groups was gradually reduced to 25% or 15% during the first 22 days in November. The soil moisture setting of each treatment was maintained until the freezing tolerance of the potted trees was analyzed in the spring. Sawano (1980) reported that the effective soil moisture conditions for preventing the freezing injury of chestnut trees are above a soil moisture retention value (pF) of 3.0. Accordingly, in this study, pF values for each treatment were 1.2 (wet), 2.1 (moderate) and 3.5 (dry) on 22 January, 2014, corresponding to 40%, 25% and 15% soil moisture, respectively, controlled by an automatic irrigation system. The pF values were calculated from an approximation formula of pF-ADR soil moisture.

Measurement of water content and sugar content in CSSs

We selected three CSSs having 5 - 10 buds on each 30 - 40 cm long shoot per treatment. A CSS was collected from each of three potted trees in each soil moisture treatment at about three-week intervals starting in early November. The water content of the CSSs was calculated by measuring the dry matter weight after drying for 24 hours at 80 ℃ (water weight as a percentage of fresh weight). Dried CSS samples of each treatment were used for extraction and analyses of soluble sugars (sorbitol, sucrose, fructose and glucose) by high-performance liquid chromatography (HPLC) as described by Ito et al. (2012).

Determination of freezing tolerance

Freezing tolerance experiments using cut CSSs were conducted in the middle of December, 2013. A CSS was collected from each of three potted trees representing each soil moisture treatment. The experiment was carried out using an environmental test chamber (SU-642, ESPEC, Co.). Three CSSs with leaf buds were collected from each treatment, wrapped with polyethylene bags and precooled for 3 hr at 0 ℃. After leaving the CSSs at selected temperatures, i.e., −8, −12, −16 or −20 ℃, for 16 hr, the frozen samples were transferred to 0 ℃ for 3 hr and then 5 ℃ for 5 hr. To evaluate the viability of leaf buds after freezing, cut CSSs were placed in plastic containers with Rockwool cubes submerged in distilled water in a chamber set to 20 ℃ under continuous dark conditions for two weeks. Thereafter, freezing injury was measured by the visual browning test as described by Kuroda et al. (1990). Fifteen to twenty-five leaf buds were analyzed for each treatment. Freezing tolerance was defined as the temperature at which half of the trees were killed and was expressed as the lethal temperature 50 (LT50).

Freezing tolerance tests using whole trees were conducted in the middle of February and the middle of March, 2013, and the middle of February and late March, 2014, respectively. These experiments were carried out using a programmable freezer (TH-250, OHNISHI NETSUGAKU CO., LTD.). Three to four potted trees in each treatment were sprayed with water entirely to ensure ice nucleation, and then each was wrapped with polyvinylchloride film to prevent desiccation. The freezer temperature was initially set to 0 ℃ for 90 min and was gradually lowered by 2 ℃ every 30 min until reaching the desired temperature demonstrated by Yasunobu (1968). The potted trees were kept in the freezing treatments for 3 hr at the following temperatures, i.e., −10, −13 or −16 ℃. After the freezing treatment, pots were maintained at 5 ℃ for 90 min, and then transferred to a greenhouse at 15 ℃ to 20 ℃ for forcing. The conditions of the trees after the freezing treatment were observed at 2–4 d intervals to judge freezing injury, the degree of which was indicated by the number of withering trees.

Statistical analyses

Statistical analysis of the water content and total sugar content data for CSSs were evaluated by *t*-test (2012/2013) and the Tukey-Kramer test (2013/2014).

Results

Effect of soil moisture conditions on water and sugar contents of CSSs

The seasonal changes in water content of the CSSs subjected to each soil moisture treatment are shown in Fig. 1. In general, lower temperatures were accompanied by a decrease in the water content of the CSSs and vice versa. In mid-February, there was no significant difference in the water content of CSSs harvested from

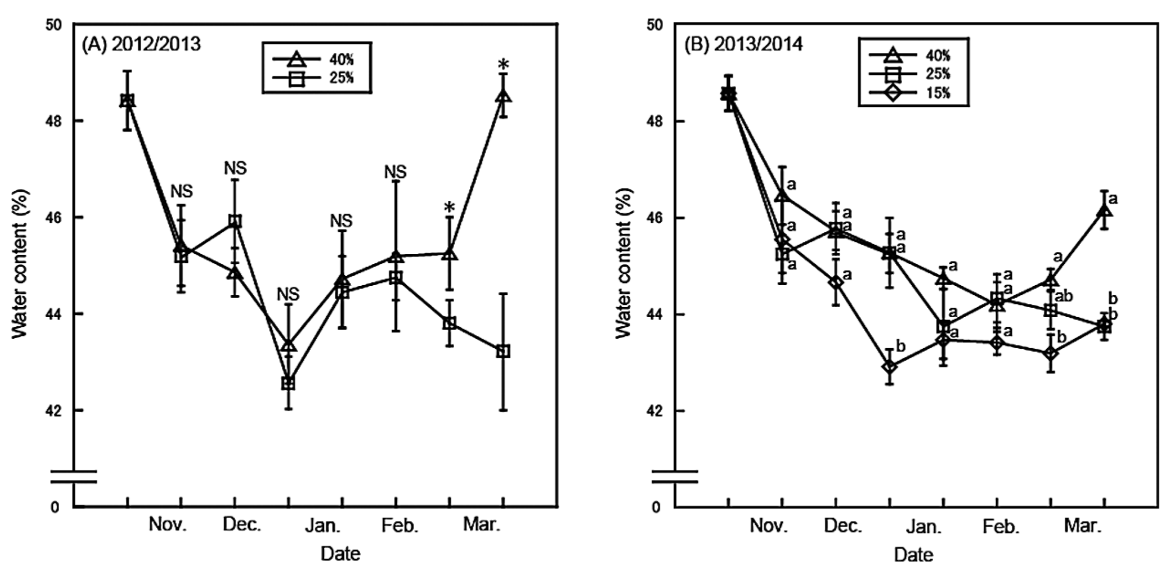

Fig. 1. The effect of soil moisture on the water content of CSSs of Japanese chestnut 'Porotan' in 2012/2013 (A), 2013/2014 (B). Soil moisture treatments were 40%; (△), 25%; (□), 15%; (◇). Vertical bars indicate the SE (n = 3). * and NS: significance at p = 0.05 and non-significance by *t*-test in 2012/2013, respectively. Different letters mean significance at the 5% level by the Tukey-Kramer test in 2013/2014.

trees held at a soil moisture level between 25% and 40%; however, the water content of CSSs from trees held at 25% soil moisture was significantly lower than that from the 40% soil moisture treatment by 28 March in the first year of our analysis, 2012/2013 (Fig. 1A). During this period in the second year of study, 2013/2014, the water content of the CSSs tended to be high when trees had been subjected to a 40% soil moisture condition. In particular, the CSS water content in the 15% soil moisture treatment was significantly lower than that from the 40% treatment on 6 January, 6 March and 28 March. The water content of CSSs from the 25% soil moisture treatment was significantly lower than that at the 40% treatment on 28 March (Fig. 1B).

The total sugar content of CSSs increased rapidly, reached a maximum in January and subsequently decreased sharply toward early spring (Fig. 2). In 2012/2013, the total sugar content of CSSs treated at 25% soil moisture was significantly higher than that in the 40% treatment on 21 November (Fig. 2A). In 2013/2014, the total sugar content in CSSs treated at 25% soil moisture was the highest among all treatments, whereas the lowest sugar content was measured in CSSs subjected to the 40% soil moisture treatment on 28 March (Fig. 2B).

Effect of soil moisture on freezing tolerance

The effect of soil moisture on the freezing tolerance of 'Porotan' potted trees during the cold hardening and dehardening periods is shown in Tables 1-3. In 2013/2014, the freezing tolerance of CSSs growing in 40% soil moisture was −8 ℃, and those in 15% or 25% soil moisture were freezing tolerant to −12 ℃ in mid-December (Table 1). Cold hardening of CSSs in December was delayed in the 40% soil moisture treatment compared to the 15% and 25% soil moisture treatments. In 2012/2013, the freezing tolerance (LT_{50}) of potted trees treated at a soil moisture of 40% was below −16 ℃ in mid-February and −13 ℃ in mid-March. The freezing tolerance of potted trees growing in 25% soil moisture was below −16 ℃ in mid-February and mid-March (Table 2). The freezing tolerance of potted tree in all soil moisture conditions was below −16 ℃ in mid-February as observed in 2013/2014. In contrast, potted trees in the 40% soil moisture treatment were tolerant to −10 ℃ and potted trees grown in 15% or 25% soil moisture were tolerant to below −16 ℃ in late March (Table 3). Also, the survival rate of the 40% soil moisture treatment was inferior to other treatment at test temperatures ranging from −8 ℃ to −20 ℃ at mid-December and mid or late-March (Tables 1, 2 and 3).

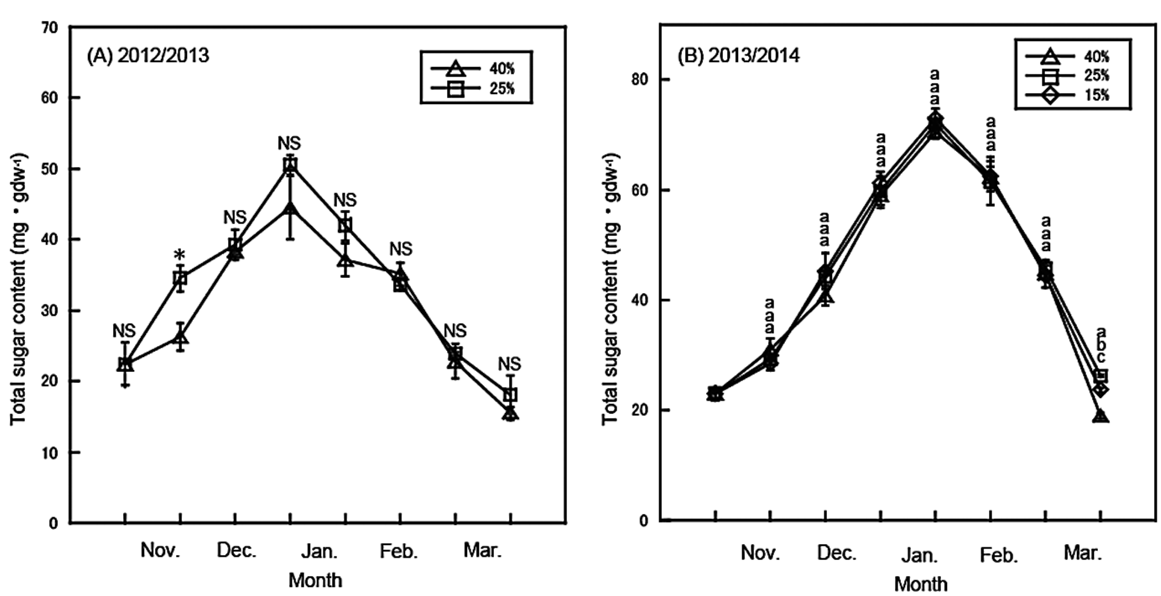

Fig. 2. The effect of soil moisture on the total sugar content of CSSs of Japanese chestnut 'Porotan' in 2012/2013 (A), 2013/2014 (B). Soil moisture treatments were 40%; (△), 25%; (□), 15%; (◇). Vertical bars indicate the SE ($n=3$). * and NS: significance at $p=0.05$ and non-significance by t-test in 2012/2013, respectively. Different letters mean significance at the 5% level by the Tukey-Kramer test in 2013/2014.

Table 1 The effect of soil moisture during the cold hardening period on the freezing tolerance of 'Porotan' current season's shoots (n = 3) in 2013

Date	Soil moisture (%)	Freezing temperature (℃)				total (survival rate (%))
		− 8	− 12	− 16	− 20	
17 December	40	3/3[z]	0/3	0/3	0/3	3/12 (25%)
	25	3/3	3/3	0/3	0/3	6/12 (50%)
	15	3/3	2/3	0/3	0/3	5/12 (42%)

[z] Number of surviving trees / Number of samples

Table 2 The effect of soil moisture during the cold dehardening period on the suvival rate of 'Porotan' potted trees (n = 3–4) in 2013

Date	Soil moisture (%)	Freezing temperature (℃)			total (survival rate (%))
		− 10	− 13	− 16	
18 - 22 February	40	3/3[z]	4/4	4/4	11/11 (100%)
	25	4/4	3/3	3/3	10/10 (100%)
11 - 14 March	40	4/4	4/4	1/4	9/12 (75%)
	25	4/4	4/4	3/4	11/12 (92%)

[z] Number of surviving trees / Number of samples

Table 3 The effect of soil moisture during the cold dehardening period on the freezing tolerance of 'Porotan' potted trees (n = 3–4) in 2014

Date	Soil moisture (%)	Freezing temperature (℃)			total (survival rate (%))
		− 10	− 13	− 16	
17 - 19 February	40	4/4[z]	4/4	4/4	12/12 (100%)
	25	4/4	4/4	4/4	12/12 (100%)
	15	4/4	4/4	3/3	11/11 (100%)
24 - 26 March	40	3/4	2/4	1/4	6/12 (50%)
	25	4/4	4/4	3/4	11/12 (92%)
	15	4/4	3/4	3/4	10/12 (83%)

[z] Number of surviving trees / Number of samples

Discussion

Generally, woody plants are tolerant to − 5 ℃ just after growth cessation and freezing tolerance gradually increases during winter and is the highest in midwinter; freezing tolerance then decreases toward early spring and is lost after leafing (Kuroda et al., 1985). Freezing tolerance in woody perennials can be induced by low temperature, water stress, short days, or combinations of these factors (Chen et al., 1978). In this study, cold hardening of CSSs in December was delayed in the 40% soil moisture treatment compared to the 15% and 25% soil moisture treatments in 2013/2014 (Table 1). The freezing tolerance of CSSs in all soil moisture conditions reached their maximum levels in February. In March, freezing tolerance decreased in all soil moisture conditions, and the reduction in freezing tolerance was earlier for CSSs in the 40% soil moisture treatment than in the other conditions (Table 2 and 3). From these results, it is clear that for trees grown in excess soil moisture, as seen in the 40% soil moisture treatment, the cold hardening is delayed, whereas the freezing tolerance in mid-winter is the same as that in the15% or 25% soil moisture treatments, and the cold dehardening period is advanced. For trees grown in deficient soil moisture conditions, as seen in the 15% or 25% soil moisture treatments, freezing tolerance during the cold hardening period is high, and the decrease in freezing tolerance during the cold dehardening period is delayed. On the other hand, dormancy often inhibits or prevents resumption of growth and the dehardening process (Kalberer et al., 2006). Dehardening resistance was high when grape buds were endodormant but dehardening occurred more readily as spring approached (Wolf and

Cook, 1992). Sakamoto et al. (2015) reported that the endodormancy completion date is mid - end of December in Japanese chestnut 'Porotan'. After that, the cold dehardening process progresses toward spring with the rise in temperature. In fact, the freezing tolerance of potted trees treated with excess soil moisture in March was lower than that in February in both years.

The reasons for these responses may be due to the influence of the growth cessation time of CSSs on the cold hardening that is processed in autumn (Kuroda et al., 1985). In fact, defoliation was accelerated by soil desiccation treatments in both years (data not shown). Thus, there is a possibility that soil desiccation promoted CSS growth cessation, resulting in early cold hardening of the trees. Cold hardening in autumn is also affected by the water content of CSSs and root growth in apple trees (Kuroda et al., 1985). Sawano (1980) reported that high soil moisture induced a rapid decrease in freezing tolerance in Japanese chestnut. In our study, there was no significant difference in the water content of CSSs between 40% and 25% soil moisture levels in either year with the exception of March (Fig. 1). The water content of CSSs in the 15% soil moisture treatment was significantly lower than that of other treatments in early-January and late-March in 2013/2014 (Fig. 1B). Also, the water content of CSSs in the 40% soil moisture treatment was significantly higher than that of all other treatments in late March in both years (Fig. 1). Therefore, we hypothesize that the water content of CSSs might be positively correlated with the degree of cold dehardening, but negatively correlated with the degree of cold hardening in Japanese chestnut trees.

In many woody plants, there is a positive correlation between sugar content and freezing tolerance (Sakai, 1962). For example, freezing tolerance in the cold dehardening period is closely associated with the sugar content of apple tree CSSs (Kuroda et al., 1985). In this study, the total sugar content of CSSs increased early, reached a maximum in January and subsequently decreased sharply toward early spring (Fig. 2). In 2012/2013, there was no significant difference in dry weight between the 25% soil moisture treatment and the 40% soil moisture treatment in late March 2013 (Fig. 2A). Calculated on fresh weight basis, however, the sugar content was about 1.3 times as high in the 25% treatment compared with the 40% treatment in late March 2013 as well as in late November 2013. Also, the total sugar content was the highest in the 25% soil moisture treatment and the lowest in the 40% soil moisture treatment in late March 2014 (Fig. 2B). From these results, we suggest that there is a positive correlation between sugar content and cold dehardening in Japanese chestnut trees as documented for other woody plants.

The acceleration of cold hardening in autumn and the delay of cold dehardening in spring are induced at dry soil conditions (Yasunobu, 1963; Sawano, 1980). However, in our experiment, the freezing tolerance of CSSs in December and trees in February and March in the 15% soil moisture treatment was nearly identical to that of the 25% soil moisture treatment. In addition, the total sugar content of CSSs in the 15% soil moisture treatment was significantly lower than that in the 25% soil moisture treatment in late March 2014 (Fig. 2B). Kuroda et al. (1985) reported that excessive soil moisture desiccation at maturity (from June to November) was responsible for an early decrease in the sugar content during the cold dehardening period, an observation that might also be the case for this study.

Horimoto et al. (1999 B) reported that root loosening (cracking throughout the root zone soil with an excavator) in late January effectively suppressed the occurrence of freezing injury in young Japanese chestnut trees. In addition, Mizuta et al. (2013) found that a more suitable time to loosen the roots was in mid-November to prevent freezing injury during early winter. Our results showing that the increase of cold hardening in autumn and delay of cold dehardening in spring were promoted by the 15% and 25% soil moisture treatments from November support the idea that the root loosening in November is effective to prevent freezing injury in Japanese chestnut. In a combination of raised ridge planting and polyethylene nonwoven fabric-covered soil beginning in mid-September, the soil moisture was lower (about 30% using ADR methods) than that in an open field (control treatment) and resulted in less freezing injury (Kamio and Mizuta, 2014). Our data related to the adequate soil moisture conditions required to prevent freezing injury are coincident with their report.

In this study, only gray lowland soil was used. Further studies will be necessary to fully identify the influence of soil characters, including soil moisture, on the freezing tolerance of Japanese chestnut trees.

Acknowledgements

This study was supported financially by the Science and Technology Research Program for Agriculture, Forestry, Fisheries and Food Industry.

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ニホングリにおける晩秋期から早春期の土壌水分条件が耐凍性に及ぼす影響

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摘　要

近年，渋皮剥皮性が良いニホングリ‘ぽろたん’の登場により，クリの栽培面積が急増している．その一方で，水田転換園等で頻発する新植樹の凍害による枯死が，生産者の生産拡大意欲に大きなダメージを与えていることから，凍害防止対策が産地拡大における喫緊の課題となっている．これまでに，クリの凍害発生は土壌水分との関連が示唆されている．そこで，クリの凍害防止に有効な土壌水分含量を求める目的で，水田土壌の代表的な土壌である灰色低地土条件下で，晩秋期から早春期にかけて土壌水分含量を15%，25%および40%を目安に設定したポット植栽苗を用いて，耐凍性との関連が示唆されている新梢内の水分含量および全糖含量を調査するとともに，耐凍性増大期における耐凍性について一年生枝を，また，耐凍性減少期における耐凍性について樹全体を用いて試験を実施した．その結果，土壌水分を40%に設定した区は，15%および25%に設定した区に対し，秋の耐凍性獲得が遅れ，春には早期に耐凍性が低くなる傾向が認められた．糖含量については，耐凍性と相関が認められ，一年生枝の糖含量は，3月下旬では，土壌水分を25%に設定した区で最も高くなり，40%に設定した区で最も低くなった．一年生枝における含水率は，土壌水分を15%および25%に設定した区では，処理開始直後および3月以降，40%に設定した区に対し低く推移し，15%設定区では計測期間を通じ低く推移した．以上の結果より，灰色低地土園における晩秋期から早春期の土壌水分含量を25%以下にすることが，ニホングリ‘ぽろたん’の凍害発生の防止に有効である可能性が示唆された．