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RESEARCH ARTICLE

Cold Hardiness of 8 Hybrid Poplar Clones for the Introduction to Arid and Semi-Arid Areas

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ABSTRACT Endodormancy is a key determinant of cold and freezing hardiness in plant cycles. Short plant growth periods and increasing frequencies of frosting caused by increasing temperatures are major environmental challenges faced by trees in arid areas of central Mongolia. In the present study, the primary aim was to determine an effective method for cold hardiness with the use of six introduced and two Mongolian poplar clones. The secondary aim was selecting clones suitable for afforestation in Mongolia. Year old branches were subjected to four temperature treatments to induce cold hardiness. Electrolyte leakage, 2,3,5-triphenyltetrazolium chloride (TTC) reduction, leaf sprouting, and leaf browning rates were compared. High rates of electrolyte leakage and browning rates were observed along with low leaf sprouting at a low-temperature of −30℃. Temperatures between −25℃ and −30℃ damaged certain clones more than others. TTC reduction rate method for determining cold hardiness was considered effective in this case. In addition, Mongolian poplar *P. sibirica* differed distinctly from other poplar clones owing to the difference in dormancy-breaking whereas DN 247 and DN sim were better adapted to cold hardiness based on TTC reduction rate. These findings suggest that factors such as plant dormancy depth and physiological differences might significantly affect productivity and performance among plants. Evidently, further studies are required using other plant parts for selecting suitable poplar clones.

Keywords Endodormancy, Freezing treatment, Electrolyte leakage, TTC reduction rate, *Populus sibirica*

Introduction

Endodormancy is a result of physiological changes that plants implement to inhibit growth when the season's shift to a period unfavourable for plant growth (Horvath *et al*. 2013). When plants enter the dormancy period, acclimation is induced by low temperatures. Consequently, cold hardiness of plants increases and they are able to survive in winter (Thomashow 1999). The absence of low temperatures during winter season induces a break in endodormancy (Mohamed *et al*. 2010; Atkinson *et al*. 2013; Takemura *et al*. 2013). Mean temperature increases in spring reducing cold hardiness in plants, and they begin to deacclimate and resume normal growth processes (Vitasse *et al*. 2014). Plant phenology is accelerating because of the recent temperature rise, and cold hardiness of plants is lost rapidly in early spring. The risk of plant damage caused by exceptionally low temperatures and frost during the early spring period continues to increase (Kalberer *et al*. 2006).

The climate in dry areas of central Mongolia is characterized by the remarkably short duration of the plant growth period, which lasts only from May to August.

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During extraordinary low temperatures in early spring (mid-May in reference to Mongolia), frost, strong winds, hail, and damages due to climate change have been reported in other areas (Kolářová *et al*. 2014).

In Mongolia, research related to cold hardiness of native and hybrid poplar clones planted as windbreak only empirically estimated the general cold hardiness based on survival rate data. In this context, the goal of this study was to assess, various methods to determine cold hardiness of 6 hybrid poplar clones of *Populus deltoides* W.Bartram ex Marshall x *Populus nigra* var. pyramidalis Spach (DN 002, DN 247, DN 270, DN 034, DN sim), *Populus tremula* var. davidiana (Dode) Schneider x *Populus nigra* var. *pyramidalis* Spach (TN 074) and 2 Mongolian poplar species (*Populus sibirica* Hort. *ex* Tausch and *Populus simonii* Carrière) for selection of suitable species that would assist in the prevention of deforestation in Mongolia.

Generally, the viability of tissue after a freeze-thaw cycle is evaluated by measuring the primary injuries in the plant membranes. The aims of the study, type and physiological state of tested plant materials and the availability of equipment determine the choice of evaluation methods. More than one method can be utilized at one time to confirm the results obtained. By using the visual observation method in addition to TTC reduction rate, electrolyte leakage measurements were also conducted. This is due to bud and stem cell damage of cuttings and the rate of leaf sprouting after cold injury has a significant effect on the overall pant growth in the current year (Ouyang *et al*. 2019; Wu *et al*. 2019).

MATERIALS AND METHODS

Experiment materials

Six hybrid poplar clones (DN 002, DN 247, DN 270, DN 034, DN sim, and TN 074) and 2 Mongolian poplar species (*P. sibirica* and *P. simonii*) were used in this study, these clones are currently growing in the tree nursery at the Dongguk University research forest in Ungil Mountain, Republic of Korea.

Low-temperature treatment

One-year-old branches exhibiting consistent growth were sampled on February 28; this is when the seedlings entered the deacclimation phase. The cuttings were dried for 24 hours at room temperature and were cut at 20 cm lengths. A Topsin paste was applied over the branch cuttings at both ends. The temperatures for cold hardiness treatment were set at 4 levels, including −15, −20, −25, and −30℃, and the temperature was configured to fall 10℃ in 3 hours (−10℃/3 hours). The cuttings that reached the treatment temperature were exposed for 9 hours. The temperature was increased using the +10℃/3 hours condition to 0℃ upon treatment. The cuttings were then stored at 0℃ for 24 hours. Electrolyte leakage, 2,3,5-Triphenyltetrazolium chloride (TTC) reduction rate, leaf sprouting rate, and browning rates of stem and buds were determined. To assess cold hardiness according to the treatment temperatures, 5 cuttings were surveyed 3 times for each condition.

Electrolyte leakage assay

Of the cuttings which underwent low-temperature treatment, the middle of the branch without buds was used for electrolyte leakage assay. The cut samples were quantified to 5 g, and were soaked in glass tubes containing 40 mL of distilled water (Sigma 25 mm × 150 mm); after which, they were cultured at 20℃ for 15 hours. The electrolyte leakage (C1) of the cultured solution was measured using a conductivity meter (pH/LF 12, SCHOTT, Germany). The solution was then double boiled at 95℃ for 30 minutes to destroy tissues. This was incubated at 20℃ for 15 hours, and the electrolyte leakage (C2) was measured again. The electrolyte leakage rate was calculated by the formula (C1/C2) × 100 (Kim *et al*. 2007).

TTC reduction

The branch bark treated with low-temperature treatment was quantified to 0.5 g and was soaked in a 0.1% TTC solution at 25℃ for 15 hours. The bark was then washed with distilled water twice and soaked in 10 mL of 70℃ anhydrous alcohol for 30 minutes. The absorbance of the crimson triphenylformazan (TF) extract was measured

using a spectrometer (Multiskan GO, Thermo Scientific, USA) at 530 nm. The result was calculated as a percentage relative to the temperature and the control group without the treatment (Kim *et al*. 2007).

Survey of leaf sprouting rate and browning rate of stem and buds

One-year-old branch cuttings of 20 cm with more than 3 buds were used for testing the leaf sprouting rate were cut diagonally to allow water absorption at their tips and were planted in glass tubes (Sigma 25 mm × 150 mm). Observations were recorded every 3 days in the incubation room, where a photoperiod of 16 hours was provided at 25℃. Approximately 2 mm of a protruding bud was considered a sprouting leaf (Leng *et al*. 1993). The browning rate of stem and bud were assessed by examining the browning on the longitudinal and cross-section of the branches and the longitudinal section of the buds with the naked eye (Mckenzie *et al*. 1974).

Statistical treatment

The data from this experiment are presented as means ± standard deviation obtained from three or more repetitions, excluding electrolyte leakage. A two-way analysis of variance (ANOVA) was performed to assess the changes between the poplar clones and the experimental group treated with each temperature condition. If the assumption of normality was not satisfied, a one-way ANOVA was performed to examine the difference between the poplar clones according to temperature. The differences between these groups were compared using Duncan's multiple range tests at the 0.05 significance level. All statistical calculations were performed using SPSS Version 23 (IBM Corp. USA).

Results

An overall high electrolyte leakage and leaf browning rates were demonstrated for the treatment group at −30℃. Conversely, the absorbance value and the leaf sprouting rates were low.

Electrolyte leakage assay

The electrolyte leakage measurement results (Table 1) for each poplar clone according to the treatment showed that the amount of leakage generally increased as the temperature of the treatment decreased. Leakage in DN 034, DN sim, TN 074, *P. sibirica*, and *P. simonii* dramatically increased between −25℃ and −30℃. The Mongolian poplars *P. sibirica* and *P. simonii* demonstrated a high leakage rate of 33.53% and 50.76%, respectively at −30℃.

Table 1. Electrolyte leakage rate of poplar clones at different levels of low-temperature treatment.

Clone	Electrolyte leakage (%)			
	−15 (℃)^{ns}	−20 (℃)^{ns}	−25 (℃)^{ns}	−30 (℃)^{ns}
DN002	9.99 ± 0.47	14.09 ± 1.40	16.73 ± 1.94	17.44 ± 0.52
DN247	18.25 ± 2.31	20.65 ± 2.49	21.79 ± 0.41	21.37 ± 0.86
DN270	8.52 ± 3.68	10.40 ± 2.02	15.16 ± 1.63	17.78 ± 2.82
DN034	15.83 ± 2.63	17.84 ± 0.97	18.96 ± 2.25	23.89 ± 2.37
DNsim	18.73 ± 2.87	20.17 ± 0.72	22.17 ± 2.40	28.28 ± 5.02
TN074	11.77 ± 0.89	12.71 ± 0.77	16.52 ± 0.86	24.46 ± 1.46
P. sibirica	17.88 ± 3.24	19.00 ± 1.88	24.79 ± 1.57	33.53 ± 2.17
P. simonii	25.89 ± 1.29	30.77 ± 0.98	32.50 ± 2.41	50.76 ± 4.36
Mean*	15.86 ± 5.65^{y}	18.20 ± 6.29^{y}	21.08 ± 5.67^{yz}	27.19 ± 10.91^{z}
Clones		ns		
Temperature		*		
Clones × temperature		ns		

^{y,z}Temperature; means ± standard deviation (n = 3); *represents significance at the 0.05 probability level. The values followed by the same letter are not significantly different based on Duncan's multiple range test ($P < 0.05$).

Electrolyte leakage occurs because of cell membrane damage caused by stress (Dexter *et al*. 1932; Palta and Li 1978). The electrolyte leakage assay is an accurate, objective, and simple method of assessing plant cold hardiness (Burr *et al*. 1990; Lee *et al*. 2012) and is usually used for measuring the degree of tissue damage caused by low temperatures in woody plants (Wilner 1960; Green and Warrington 1978; Burr *et al*. 1990; Arora *et al*. 1992).

TTC reduction rate

The TTC test results (Table 2) revealed that the value of absorbance was significantly reduced in all poplar clones as the treatment temperature declined. Significant differences were clearly observed in the poplar clone cuttings based on the varying temperatures of the low-temperature treatment. DN 247, DN 270, DN 034, and *P. sibirica* demonstrated a considerable difference between the reduction value at −15℃ and −20℃. TTC reduction rate is a method used to determine tissue activity by measuring the degree of redness observed in the reduction of the TTC solution to TF by measuring the absorbance. Unlike other cold hardiness test methods, the TTC reduction rate results indicated a clear difference between the poplar clones at each treatment temperature.

Leaf sprouting rate and browning rate assessment

Leaf sprouting and browning rate of each poplar treated with low temperatures exhibited a decreasing pattern as the treatment temperature declined (Table 3). In particular, all clones showed low leaf sprouting rates of less than 60% at −30℃. No significant difference was observed in the poplar clones, except at −25℃. The browning rate dramatically increased to over 60% at −30℃, and DN 034 suffered the greatest recorded damage of 89.78 ± 10.72. No significant difference was observed in the poplar clones in relation to each temperature for the treatment.

DISCUSSION

Appropriate temperature and acclimation treatment durations vary according to species, province and season. This generally is determined during preliminary studies. Under simulation cooling to imitate natural frost conditions, the temperature is decreased at a rate of 1 to 2℃/hour. According to Haynes *et al*. (1992) a common approach is to use a fixed rate of 2 to 6℃/hour. In order to establish a thermodynamic equilibrium, the low-temperature exposure must be sufficient. Based on the studies by Larcher (1968) exposure of a minimum of 4 to 6 hours is considered

Table 2. TTC reduction rates of poplar clone branch cuttings at different levels of low-temperature treatment.

Clone	Absorbance rate (%)			
	−15 (℃)**	−20 (℃)**	−25 (℃)**	−30 (℃)**
DN002	70.33 ± 2.84^{c}	70.33 ± 3.21^{a}	65.50 ± 1.80^{a}	56.67 ± 1.26^{c}
DN247	82.33 ± 2.25^{a}	55.83 ± 1.76^{c}	53.83 ± 2.08^{d}	33.67 ± 1.61^{f}
DN270	75.67 ± 2.36^{b}	65.83 ± 3.82^{b}	63.67 ± 1.26^{bc}	56.83 ± 1.15^{c}
DN034	75.67 ± 1.04^{b}	67.50 ± 1.32^{ab}	65.83 ± 2.75^{ab}	59.33 ± 0.76^{b}
DNsim	52.00 ± 0.87^{e}	50.67 ± 1.53^{e}	48.17 ± 0.58^{e}	41.17 ± 0.58^{e}
TN074	61.17 ± 1.26^{d}	57.50 ± 1.00^{c}	49.67 ± 1.15^{e}	48.17 ± 0.76^{d}
P. sibirica	78.33 ± 3.51^{b}	71.00 ± 0.00^{a}	67.00 ± 1.00^{a}	66.33 ± 0.58^{a}
P. simonii	71.39 ± 0.58^{b}	66.00 ± 2.00^{b}	62.00 ± 1.00^{c}	55.33 ± 0.58^{c}
Mean**	71.39 ± 9.74^{x}	63.08 ± 7.34^{y}	59.83 ± 7.84^{y}	52.19 ± 10.14^{z}
Clones		**		
Temperature		**		
Clones × temperature		**		

^{a,b,c,d,e,f}Clones; ^{x,y,z}temperature; means ± standard deviation (n = 15); **represents significance at the 0.01 probability level. The values followed by the same letter are not significantly different based on Duncan's multiple range test ($P < 0.05$).

Table 3. Sprouting and Browning rate of poplar clone branch cuttings at different levels of low-temperature treatment.

Clone	Sprouting rate (%)				Browning rate (%)			
	−15 (℃)^{ns}	−20 (℃)^{ns}	−25 (℃)*	−30 (℃)^{ns}	−15 (℃)^{ns}	−20 (℃)^{ns}	−25 (℃)^{ns}	−30 (℃)^{ns}
DN002	93.33 ± 11.55	100.00 ± 00.00	93.33 ± 11.55^{a}	41.67 ± 14.43	6.67 ± 11.55	16.67 ± 14.43	35.00 ± 8.66	63.89 ± 12.73
DN247	93.33 ± 11.55	93.33 ± 11.55	87.78 ± 10.72^{a}	47.02 ± 17.16	6.67 ± 11.55	13.33 ± 11.55	31.11 ± 10.18	66.27 ± 8.94
DN270	91.67 ± 14.43	88.89 ± 19.24	86.00 ± 12.77^{a}	50.00 ± 10.00	8.33 ± 14.43	22.22 ± 9.62	33.33 ± 16.67	71.67 ± 10.41
DN034	93.33 ± 11.54	94.43 ± 9.64	94.43 ± 9.64^{a}	60.00 ± 24.04	6.67 ± 11.55	5.56 ± 9.520	23.33 ± 8.82	89.78 ± 10.72
DNsim	95.83 ± 7.22	94.44 ± 9.62	86.11 ± 12.73^{a}	58.33 ± 17.56	4.17 ± 7.22	11.11 ± 19.24	33.33 ± 16.67	80.00 ± 20.00
TN074	100.00 ± 00.00	100.00 ± 00.00	87.83 ± 11.26^{a}	55.19 ± 5.01	0.00 ± 0.00	16.99 ± 2.87	23.60 ± 4.45	71.11 ± 7.70
P. sibirica	100.00 ± 00.00	83.33 ± 14.43	60.00 ± 17.32^{b}	31.67 ± 16.07	6.67 ± 11.55	20.83 ± 7.22	34.44 ± 15.03	63.33 ± 20.21
P. simonii	95.83 ± 7.22	75.55 ± 13.50	56.11 ± 21.11^{b}	32.78 ± 7.52	12.50 ± 12.50	17.78 ± 1.92	31.67 ± 16.07	73.89 ± 6.73
Mean***	95.42 ± 8.43^{x}	91.25 ± 12.57^{x}	81.45 ± 18.28^{y}	47.08 ± 16.39^{z}	6.45 ± 9.69^{w}	15.56 ± 10.50^{x}	30.73 ± 11.59^{y}	72.24 ± 13.56^{z}
Clones	ns	ns	*	ns	ns			
Temperature	***				***			
Clones × temperature	ns				ns			

[a,b]Clones; [w,x,y,z]temperature; means ± standard deviation (n = 15); *,***represent significance at the 0.05, 0.001 probability levels, respectively. The values followed by the same letter are not significantly different based on the Duncan's multiple range test ($P < 0.05$); ns: not significant.

enough. Whereas longer exposure increases damage (Su *et al*. 1987).

The Mongolian poplar *P. sibirica* suffered extensive damage at −30℃ in the low-temperature treatment, showing the highest electrolyte leakage. Conversely, in the TTC reduction rate test, the poplar cuttings demonstrated the highest absorbance. The leaf browning rate was lowest, while at the same time the leaf sprouting rate reached 31.67%. The reason Mongolian poplar *P. sibirica* exhibited differences distinct from other poplar clones was due to the difference in dormancy breaking periods between the hybrid poplar clones and the Mongolian poplar clones. In general, January is the coldest period of the year in Korea hence, deciduous fruit trees in temperate zones are known to have their deepest dormancy during this period. A study by Chung *et al*. (2008) observed the physiological short-term cold hardiness was stronger and the plants exhibited a low leaf browning rate and a high leaf sprouting rate during this period. Plant dormancy depth and the dormancy breaking period vary among time periods. In relation to this, several research teams tested plant phenology models by measuring the dormancy depth of grapes at different times. They determined the frost damage risk based on the models and the lowest temperature data to estimate frost incidence (Kwon *et al*. 2006). Secondly, it is expected that greater changes were observed because of the differences in the methods used for the experiments. It is extremely difficult to establish standards for frost damage assessment that can be commonly applied to multiple species. An existing study reported that responses varied depending on different parts even within an individual plant (Kang and Oh 2004). In the case of *Pittosporum tobira*, a low-temperature treatment experiment using leaves suggested a reliable pattern for cold hardiness assessment. However, other cold hardiness tests based on the high-temperature treatment of leaves and high and low-temperature treatment of stems produced overestimated results (Kim *et al*. 2014).

Cold hardiness of woody perennials is usually tested using the whole plant (seedlings or rooted cuttings) or as severed plant parts (stem segments, buds, roots or leaves) collected from plants in the field. In addition, *in vitro* cultures can also be used as test materials (Caswell *et al*. 1986; Ambroise *et al*. 2019; Di *et al*. 2019). The use of severed plant parts provides detailed information on the level of cold hardiness in different tissues and organs. The results usually have been in accord with field observations under natural cold injury (Pellett *et al*. 1981; Dunne *et al*. 2019). Knowledge of the relative importance of different tissues and a plant's ability to recover is required in order to relate the data to whole plant survival. This study assessed the cold hardiness using one-year-old branches of poplar clones; therefore it is necessary to conduct additional experiments using leaves and other plant parts too. This additional data from various plant parts and organs will help relate to cold hardiness of the overall survival of the plant.

This study was conducted to assess the degree of damage to poplar clones from freezing injuries during the period when growth resumed after dormancy and the most suitable methods used to determine this. Frost on plants and their consequent death do not simply occur due to differences in external temperature but differ because of considerable differences in association with physiological conditions and plant responses. It would be unreasonable to make a hasty conclusion because it is an external change manifested as a result of the effects of various factors, including water content, intracellular sugar, and lipid content, and the properties of the protoplasm of the plants (Salisbury and Ross 1992). This can be seen through the results obtained specifically for DN247, DNsim based on their low TTC reduction rate.

Based on the different methods used for determining cold hardiness in various poplar species. TTC reduction rate method provides statistically reliable data. Other methods such as leaf sprouting and browning rate assessment could provide initial field data when assessing cold hardiness. However, for further statistically competent methods, TTC reduction rate can be utilized.

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