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Sorbitol-Facilitated Preconditioning Improves Desiccation Resistance of Douglas-fir and Western Hemlock Seedlings

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Abstract. A hypertonic solution of sorbitol was used to precondition Douglas-fir and Western hemlock plug seedlings to improve desiccation resistance. Seedlings were preconditioned by soaking their root balls in water, -0.75 or -1.50 MPa sorbitol solution for 22 hr, and then exposed to desiccation conditions for 8 days. During the desiccation period, a transpirational water loss was significantly reduced by the sorbitol preconditioning, with its effect positively depending on concentration. This preconditioning-induced reduction in water loss was mainly caused by the decline in needle stomatal conductance. Sorbitol-induced stomatal control was more closely associated with reduction in plant water potential, rather than increase in abscisic acid concentrations. After rehydration of stressed-plants, most of the preconditioned seedlings with sorbitol were survived, while only 35% of Douglas-fir and 28% of Western hemlock seedlings treated with water were alive. The post-growth was significantly greater in the preconditioned seedlings than only water-treated seedlings. These results suggested that the earlier stomatal control with sorbitol-facilitated preconditioning could play a role in improving desiccation resistance of evergreen woody plants at transplanting in the field where water supply is limited or dry conditions are prevailing.

Key words : *Pseudotsuga menziesii, Thuga heterophylla*, abscisic acid, osmoticum, stomatal conductance, transpirational water loss

Introduction

Water deficit can cause serious losses in most crops. Water stress can affect plant processes such as cell growth (Hsio, 1973), ABA synthesis (Davies and Mansfield, 1983), stomatal movement (Henson et al., 1989), and CO_2 assimilation (Robinson et al., 1988).

Many plants develop either morphological and/or physiological features that enable them to resist water stress. Exogenous means have also been developed to improve water stress resistance. There are largely two methods available. In one method, stomata are physically plugged with surface-applied large organic polymers, i.e., film-forming antitranspirants (Englert, 1992; Murakami et al., 1990; Remmick, 1995). These compounds can be difficult to apply for complete coverage and are frequently toxic to the leaf tissue. In a second method, water loss can be reduced by introducing metabolically-active compounds such as abscisic acid (ABA) into the plant, physiologically causing stomatal closure. ABA has inhibited transpiration in a wide range of species (Davies and Mansfield, 1983). However, it is expensive to synthesize, and can be rapidly inactivated by sunlight and metabolized in the plant (Davies and Mansfield, 1983). Often, its effect is also temporary and/or inconsistent.

Recently, a stomatal control method using osmotica has been suggested as a tool to improve desiccation resistance of plants (Guak, 1998). This method, as sorbitol was used as an osmoticum, was devised to soak the plant root system in the hypertonic solution of sorbitol before transplanting, and proved to be effective in reducing plant water loss via lowering leaf turgor potential and thus causing stomatal closure, with its effect dependent upon concentration and species. Generally herbaceous plants require lower concentration than woody plants to induce stomatal closure but without an evident phytotoxic effect.

In this study, the above method was evaluated with Douglas-fir and Western hemlock seedlings. Douglas-fir is now commercially important as a Christmas tree in North America, while Western hemlock is widely planted for reforestation. I tested the hypothesis that soaking root system with a hypertonic solution of sorbitol can increase desiccation resistance of those trees exposed to the conditions of limited water supply or drought at transplanting.

Materials and Methods

Plant materials

One-year old dormant Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] and Western hemlock [*Thuga heterophylla* (Raf.) Sarg.] seedlings were obtained from the Rochester nursery (Weyerhauser Co., Rochester, WA, USA). The seedlings had been grown in plugs (40 holes with 3.5 cm diameter ´ 14.0 cm depth) in the greenhouse.

Treatments and desiccation resistance tests

Sorbitol was used as an osmoticum for preconditioning the seedlings. This compound was provided by Great Lakes Chemical Corporation (West Lafayette, IN, USA) that patented it as an osmotic agent "GLK-8924" whose major ingredient is sorbitol. Based on preliminary tests, two rates of sorbitol solution (–0.75 and –1.50 MPa chemical potentials) were selected to use, in consideration of the effectiveness of stomatal closure and of the possibility of phytotoxicity to the plant. The chemical potential of these solutions was determined with a Wescor Vapor Pressure Depression Osmometer (Model 5100C, Wescor Inc., Logan, UT, USA).

On receiving the seedlings of two species, they were extracted from plugs before preconditioning, with root system and media intact. The seedlings were treated with either water, –0.75 or –1.5 MPa of sorbitol solution by soaking the root system and medium of 10 plants in a 4 L plastic container containing 1.5 L of the above solution for 22 hr in a greenhouse set at 25°/18°C (day/night) temperature under natural light. After soaking root system, excess solution was permitted to drain.

Desiccation stress was induced by placing treated seedlings into 5.0 cm ´ 17.0 cm individual plugs (one seedling per plug) and left on the bench in the greenhouse (25°/18°C for day/night) for 8 days. During stress period, those plugs remained unfilled with media. After stress, those plugs were filled with artificial medium (perlite:vermiculite = 1:1 v/v) and fully watered to allow seedlings to grow, with a caution of overwatering.

During stress period, relative weight changes in the seedling + medium were measured at 2 to 4 days intervals. The extent of seedling recovery from stress was evaluated in 90 days after rewatering, by measuring stem diameter (at 5 cm above the soil), plant height, and survival rate.

Stem water potential and stomatal conductance measurements

In both species, the needle stomatal conductance (g_s) was measured in 4 hr or 22 hr of preconditioning with sorbitol, using a Li-Cor 1600 steady state porometer equipped with a chamber specifically designed for conifers with needles (LI-COR, Inc., Lincoln, NE). Quantum flux densities during each measurement period averaged 750 and 800 mol · m^{-2} · s^{-1}, respectively. Stem xylem water potential was also determined with a pressure chamber (PMS Instruments, Corvallis, OR), as described by Scholander et al. (1965).

Xylem sap collection and ABA analysis

Immediately after water potential determination, xylem sap was collected by the pressure chamber. Approximately 3 cm of bark was removed from the cut end of the stem to prevent contamination of xylem and phloem sap. The apical portion of the stem was placed in the pressure chamber with the basal end of the stem protruding. The pressure applied was around 0.5 MPa. Approximately 200-300 µl of sap was extracted from a plant (n = 4). To prevent contamination from damaged surface cells the first few hundred mls were discarded. The sap was collected in 1.5 ml eppendorf vials in shade, then frozen in liquid nitrogen, and stored at –80°C. Just before use, the sap was thawed and microcentrifuged at 12,000 ´ g for 2 min to obtain clean sap.

ABA content of the xylem sap was determined by RIA (Vernieri et al., 1989) using a monoclonal antibody to ABA from Sigma (Sigma Chemical Co. St. Louis, MO). Briefly, a PBS buffer (50 mM sodium phosphate and 100 mM NaCl, pH 7.0) was used. Approximately 2.5 TBq · $mmol^{-1}$ [G-^{3}H]-(RS)-Abscisic acid (Nycomed Amersham Place, Buckinghamshire, England, UK) in ethanol was diluted to 0.27 ml · ml^{-1} in PBS containing 5.0 mg · ml^{-1} bovine γ-globulin (Sigma), a co-precipitant with the antibody. The antiserum (Sigma), developed in rabbit with abscisic acid-HAS as the immunogen, was reconstituted and diluted in PBS containing the co-precipitant, per supplier's directions. Standard ABA solutions were prepared with (±) cis, trans-ABA (Sigma).

Experimental design and data analysis

Completely randomized experimental designs with 10 (desiccation resistance tests), 5 (stomatal conductance), and 4 (sap ABA analysis) replications were used in this study. Means were separated with Duncan's multiple range test at $p = 0.05$ level.

Results

Initial responses to the preconditioning treatments

Preconditioning treatment, by soaking the root system in the sorbitol solution, rapidly reduced g_s in both species, as measured in 4 hr of preconditioning (Tables 1 & 2). This reduction was proportional to sorbitol concentrations. In Douglas-fir, the reduction in g_s was 32% in the −0.75 MPa solution treatment, while 49% in the solution of −1.5 MPa. In Western hemlock, it was 38% in −0.75 MPa, while 58% in −1.5 MPa. These initial reductions of g_s were closely associated with those of stem xylem water potential (Ψ_w), rather than xylem sap ABA concentrations (Tables 1 & 2). Soaking the root system in the solution of −1.5 MPa, for example, reduced stem Ψ_w of Douglas-fir by 32% (from −0.64 MPa to −0.86 MPa), while Ψ_w of Western hemlock was reduced by 55% (from −0.56 MPa to −0.87 MPa). However, xylem sap ABA concentrations did not change significantly after 4 hr of preconditioning in both species. A significant increase was evident after 22 hr of preconditioning. Stomatal conductance and Ψ_w were further reduced with increasing preconditioning periods in both treatment rates and species (Tables 1 & 2).

Water loss

In both species, water loss during desiccation stress period occurred much slowly in sorbital-treated seedlings in comparison to non-treated control seedlings, with its effect being greater with the higher rate of treatment (Figs. 1 & 2). It was noted that the seedlings treated with −1.5 MPa sorbitol showed a symptom of wilting immediately after treatment. Such an initial wilt was recovered within 6 hr, without any damage on needles (data not shown).

Plant survival and growth

Assessed 90 days after the desiccation-stressed seedlings were rehydrated, most of the sorbital-preconditioned seedlings in both species were survived, while in non-preconditioned seedlings only 35% of Douglas-fir and 28% of Western hemlock were survived (Tables 3 & 4). The extent of growth that occurred for 90 days after stress, expressed in plant height and stem diameter, was significantly greater in the preconditioned seedlings of both species than only water-treated seedlings. During 90 days, the preconditioned seedlings were almost caught up with the well-watered (not stressed) controls in their growth, even though −1.5 MPa treatment appeared to reduce the growth.

Discussion

The preconditioning of Douglas-fir and Western hemlock seedlings by soaking the root system into the hypertonic solutions of sorbitol (−0.75 or −1.5 MPa) was found to be effective in reducing water loss during 8 days of desiccation stress periods, thus improving survival rate. Water loss reduction in the preconditioned seedlings was caused mainly by the earlier stomatal closure than in non-treated ones. In the Douglas-fir seedlings preconditioned with −1.5 MPa sorbitol solution, for example, g_s determined after 4 hr of preconditioning was about 51% of the well-watered control, and further decreased to 16% when measured after 22 hr of preconditioning (Table 1).

This initial reduction in g_s was believed to be largely due to the concurrent decline in needle Ψ_w or turgor potential

(Ψ_p). Although osmotic potential (Ψ_s) was not determined in this study, it was expected that initial reduction in Ψ_w could result in a subsequent decline in Ψ_p in that Ψ_s is known to change much more slowly than does Ψ_w or Ψ_p in response to water stress (Hsio, 1973). So, based on a popular water relation equation $\Psi_p = \Psi_w - \Psi_s$ (Nobel, 1999), turgor decline caused by the preconditioning with a sorbitol solution was mainly attributed to the reduction in Ψ_w. In fact, stem Ψ_w of Douglas-fir seedlings decreased from −0.64 MPa to −0.86 MPa after 4 hr of preconditioning with −1.5 MPa sorbitol solution (Table 1). Robinson and Barritt (1990) reported that PEG-induced water stress resulted in a rapid reduction in midday Ψ_p, paralleling the severity of the stress. The positive correlation between plant water status and g_s was also reported in sunflower (Sadras et al., 1993), sorghum (Sarig et al., 1988), and soybean (Bennett et al., 1987).

The results of this study indicated that the initial reduction in g_s of Douglas-fir and Western hemlock seedlings in response to preconditioning may have not been associated with changes in abscisic aicd concentrations. The reason is because xylem sap ABA concentrations little changed during the first 4 hr of preconditioning period, whereas a substantial increase in ABA concentrations was observed after 22 hr of preconditioning (Tables 1 & 2). These results suggest that, although ABA is generally considered to be the primary chemical involved in activating the 'signal' (Schurr et al., 1992; Zhang and Davies, 1989), the initial decline in g_s occurring during the early period of preconditioning could be related to hydraulic signals, rather than chemical messengers (e.g., ABA) originating in the roots. Saliendra et al. (1995) maintained that any treatment or event that changed soil water potential or hydraulic conductance resulted in hydraulic signals from the roots, and that these signals were received much earlier than chemical messengers carried in the transpiration stream. The positive correlation between g_s and hydraulic conductance of soil-root-shoot pathway was reported in woody species (Kuppers, 1984) and in sugarcane (Meinzer and Grantz, 1990).

In the preconditioned and stressed seedlings, the extent of growth that occurred for 90 days post stress was similar to (non-stressed) well-watered controls (Tables 3 & 4), even though well-watered controls were expected to grow more than stressed ones. The lack of difference between two treatments could be possibly due to the improper growth conditions such as a limited size of growth media and insufficient nutrients which allowed the stressed but preconditioned seedlings to catch up with well-watered seedlings.

In conclusion, a sorbitol-facilitated preconditioning (by soaking a root system into the hypertonic solution of sorbitol) showed a possibility of being used as a tool for improving desiccation resistance in Douglas-fir and Western hemlock. The improvement of desiccation resistance was mainly acquired from stomatal control induced by preconditioning the seedlings. Seedling with sorbital. That is, soaking the root system into hypertonic solutions of sorbitol can affect water relations in the soil or plant via a reduction in water uptake by roots, which can in turn lead to a loss of turgor and thus decline in stomatal conductance. Although one considers that this preconditioning can be stressful to the plant, this practice can be advantageous to the evergreen woody plants that are vulnerable to the desiccation conditions at transplanting. Compared to other water reducing methods such as film-forming antitranspirants (surface-applied large organic polymers to physically plug stomata), this preconditioning method with sorbitol solution can be easier to practice and are less toxic to the leaf tissue. Since this technique was evaluated only for Douglas-fir and Western hemlock, however, any expansion of its use to other species should require further tests.

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Table 1. Effect of preconditioning of Douglas-fir seedlings by soaking the root system in sorbitol solutions for 22 hr on stem water potential, stomatal conductance, and xylem sap ABA concentrations, determined after 4 hr and 22 hr of preconditioning.

Treatment	Stem water potential (MPa)	Stomatal conductance (% of well-watered control)	Sap ABA conc. (μmolám^{-3})
		After 4 hr of preconditioning	
Well-watered control	-0.64 a^z	100 a	118.2 a
Water only	-0.67 a	102 a	157.4 a
-0.75 MPa sorbitol	-0.74 b	68 b	139.5 a
-1.50 MPa sorbitol	-0.86 c	51 c	168.3 a

		After 22 hr of preconditioning	
Well-watered control	-0.58 a	100 a	225.2 b
Water only	-0.65 a	98 a	136.8 b
-0.75 MPa sorbitol	-0.81 b	46 b	307.5 a
-1.50 MPa sorbitol	-1.04 c	16 c	352.6 a

[z] The same letters within a column for each measurement time mean no significant difference in means, separated by Duncan's multiple range test at $p = 0.05$.

Table 2. Effect of preconditioning of Western hemlock seedlings by soaking the root system in sorbitol solutions for 22 hr on stem water potential, stomatal conductance, and xylem sap ABA concentrations, determined after 4 hr and 22 hr of preconditioning.

Treatment	Stem water potential (MPa)	Stomatal conductance (% of well-watered control)	Sap ABA conc. (μmolám^{-3})
		After 4 hr of preconditioning	
Well-watered control	-0.56 a[z]	100 a	108.5 a
Water only	-0.61 a	97 a	132.8 a
-0.75 MPa sorbitol	-0.75 b	62 b	113.2 a
-1.50 MPa sorbitol	-0.87 c	42 c	159.3 a
		After 22 hr of preconditioning	
Well-watered control	-0.58 a	100 a	162.3 b
Water only	-0.66 a	97 a	123.1 b
-0.75 MPa sorbitol	-0.84 b	51 b	299.7 a
-1.50 MPa sorbitol	-1.10 c	18 c	389.2 a

[z] The same letters within a column for each measurement time mean no significant difference in means, separated by Duncan's multiple range test at $p = 0.05$.

Table 3. Recovery and post-growth of the desiccated Douglas-fir seedlings, assessed 90 days after rewatering. Just before desiccation treatment, seedlings were preconditioned by soaking their root systems into sorbitol solutions for 22 hr.

Treatment	Survival (%)	Height (cm)	Stem diameter (mm)
Well-watered control	100 a[z]	24.2 a	5.7 a
Water only	28 b	19.3[y] b	4.7[y] b
-0.75 MPa sorbitol	100 a	24.0 a	5.4 a
-1.50 MPa sorbitol	100 a	22.1 a	5.2 a

[z] The same letters within a column mean no significant difference in means, separated by Duncan's multiple range test at $p = 0.05$.

[y] Mean values of survived plants.

Table 4. Recovery and post-growth of the desiccated Western hemlock seedlings, assessed 90 days after rewatering. Just before desiccation treatment, seedlings were preconditioned by soaking their root systems into sorbitol solutions for 22 hr.

Treatment	Survival (%)	Height (cm)	Stem diameter (mm)
Well-watered control	100 a[z]	32.6 a	6.5 a
Water only	35 b	26.2[y] b	5.7[y] b
-0.75 MPa sorbitol	100 a	31.2 a	6.1 a
-1.50 MPa sorbitol	100 a	29.1 a	6.8 a

[z] The same letters within a column mean no significant difference in means, separated by Duncan's multiple range test at $p = 0.05$.

[y] Mean values of survived plants.