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Acclimation responses of *Tamarix chinensis* seedlings related to cold stress

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Abstract

The purpose of this study was to investigate the acclimation responses of *Tamarix chinensis* to cold stress. We evaluated the acclimation responses by measuring biomass, daily elongation rate, chlorophyll content, and total soluble carbohydrate content. The plant samples comprised leaves from seedlings of 2 different ages (8 and 12 weeks); the leaves were collected 0, 2, and 4 weeks after cold treatment. We found that the cold-treated samples showed reduced daily elongation rates and chlorophyll content. Further, these samples showed more than 8-fold increase in the total soluble carbohydrate content. However, the seedling ages did not have a significant influence on the growth of cold-treated seedlings. On the basis of these findings, we can conclude that *T. chinensis* seedlings aged less than 1 year old show acclimation to cold stress by accumulating soluble carbohydrates. This study may help us understand how *T. chinensis* seedlings acclimatize to their first cold season.

Key words: abiotic stress, cold acclimation, dormancy, physiological ecology, seedling recruitment, *Tamarix chinensis*, total soluble carbohydrate

INTRODUCTION

The composition of vegetation at a site is determined by the environmental and biotic factors, which constantly change and interact with each other. Environmental factors such as low temperature, shade, and flooding can affect growth, recruitment, and survival of the plants (Weiser 1970). Because cold can limit the geographical distribution of plants, most overwintering plants have freezing tolerance, which is also known as cold acclimation (Sasaki et al. 1996). In particular, woody plants achieve extreme cold acclimation in a sequential manner (Weiser 1970).

Cold acclimation, which affords plants resistance to cold stress, is a seasonal process (Pagter et al. 2008) that is induced by both short day-length and low temperature (Welling et al. 2002). Induction of cold acclimation is a complex process that can cause biochemical changes, such as changes in the levels of total carbohydrate, free proline, and soluble protein (Alberdi et al. 1993). Plants show a great ability to adjust their respiratory rate, which is associated with excessive accumulation of sugars (Sasaki et al. 1996, Lambers et al. 1998). Therefore, the total soluble carbohydrate content of plants has been considered to be one of the most important factors contributing to freezing tolerance.

Tamarix chinensis L. is the only woody plant with a different ecological niche from other non-woody plants in the salt marshes in Korea. *T. chinensis* plants are distributed along the west coast of South Korea, including sites such as the Shihwa Lake, Sudokwon Landfill, and Youngjong Island (Min et al. 2005, Kim 2010, Song 2010). These

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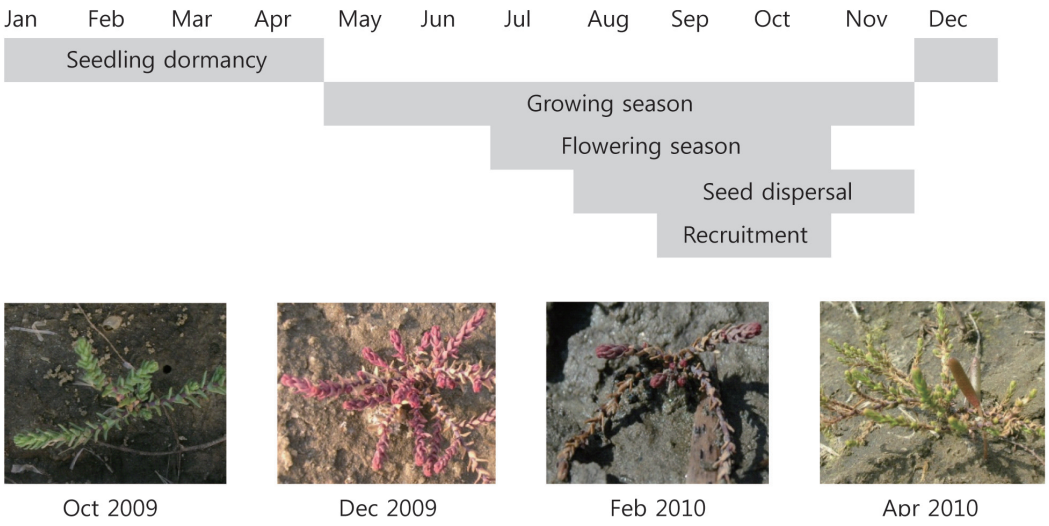

Fig. 1. Life cycle and dormant seedling of *Tamarix chinensis* in Korea on the basis of field observation from May 2009 to November 2010.

plants flower in late June and produce seeds until October (Fig. 1). Because their seeds can germinate without vernalization, most seedlings are established in the same year. Central Korea experiences sub-zero temperatures during the winter. Therefore, increasing freezing tolerance in autumn is important for seedling recruitment in the next year. Several studies have been performed on the physiological traits of tamarisk plants in cold environments (Sexton et al. 2002, Friedman et al. 2008); however, little is known about the physiological responses of tamarisk seedlings to environmental factors such as cold stress.

In this study, to identify cold acclimation in *T. chinensis* plants during autumn, we examined cold acclimation in seedlings that were of different ages and received cold treatment for different durations. Because new tamarisk seedlings can grow for a maximum period of 12 weeks (Fig. 1) before winter dormancy sets in, we selected 8-week-old and 12-week-old seedlings. Moreover, to determine the effect of the duration of cold treatment, we treated the seedlings to cold stress for 0, 2, and 4 weeks. Seedling cold acclimation was measured by determining changes in biomass, daily elongation length, chlorophyll content, and total soluble carbohydrate content. Our objectives were to confirm the cold acclimation patterns of newly established *T. chinensis* seedlings as well as to study the effects of age and cold-treatment duration on cold acclimation responses in these seedlings.

MATERIALS AND METHODS

Plant material and growth conditions

Mature seeds of *T. chinensis* were collected from Youngjong Island, Incheon City, Korea on September 15, 2009. The geographical coordinates of the study site were 37°29′17″ N and 126°24′17″ E. During the winter, from December to February, this region is cold and dry under the dominant influence of the Siberian air mass. In contrast, the summer, from June to August, is hot and humid with frequent, heavy rainfall. The climate is mild and serene during the spring and fall with fairly periodic passages of the transient cold and warm air currents. The annual mean temperature is 11.9℃, and the annual precipitation at Youngjong Island for the past 10 years has been about 1,200 mm.

The sampled seeds were dried at room temperature after harvesting and stored in a cold room at 4℃ to maintain their viability (Merkel and Hopkins 1957). Seeds were grown in 32-cell trays (cell size 60 × 60 × 60 mm). The germinated seedlings were transplanted into plastic pots (height, 18 cm; diameter, 10 cm) with bed soil (vermiculite, 60%; cocopeat, 20%; zeolite, 6%; loess, 6%; peatmoss, 5%) in the growth chamber.

Seedling growth and biomass

We conducted transplant experiment to test the effects of age and duration of cold treatment on seedling responses. Seedlings of *T. chinensis* were grown for 8 weeks (G8) and 12 weeks (G12) and received cold treatment for a duration of 0 (C0, control), 2 (C2), and 4 weeks

(C4) (Table 1).

Control seedlings were grown in controlled environmental chambers, and cold stress-treated seedlings were grown in a controlled cold room (Table 2). Each cold treatment was performed on seedlings of 2 ages and on 7 replicates for each age (a total of 42 seedlings were used in our experiment). The seedlings were watered daily throughout the experimental period. To determine daily elongation rates, seedling lengths were measured at the first and last day of the experiment. On April 8, 2010 we harvested all the treated seedlings in growth chambers. Harvested seedlings were separated into the root and shoot parts, and the seedling root and shoot biomasses were calculated subsequently.

Photosynthetic pigment content

Chlorophyll content was measured to determine the effects of the duration of cold treatment and age on the levels of photosynthetic pigments. Extraction of the photosynthetic pigments from the *T. chinensis* seedlings was performed for 8 h in the dark by using dimethyl sulfoxide (Hiscox and Israelstam 1979, Tait and Hik 2003). The absorbance of the photosynthetic pigments was measured by using a UV/visible spectrophotometer (Spectramax Plus 384; Molecular Devices, Sunnyvale, CA, USA) at wavelengths of 665 nm and 649 nm. Comparing the chlorophyll a:b ratio is not direct test for plant photosynthesis, but seedlings of *T. chinensis* are very small and have narrow leaf area. Therefore, we measured chlorophyll a:b ratio for estimating photosynthetic efficiency. Photosynthetic pigment concentrations were calculated using the following equations (Wellburn 1994):

$$\text{Chlorophyll a} = 12.25A_{665\,nm} - 2.79A_{649\,nm}$$
$$\text{Chlorophyll b} = 21.50A_{649\,nm} - 5.10A_{665\,nm}$$

Table 1. Experimental design for the cold stress experiment

First week	After 4 weeks		From 13 weeks–16 weeks (cold treatment duration)
Germination of 12-week-old seedling (G12)	Germination of 8-week-old seedling (G8)	C0	Control temperature (4 weeks)
		C2	Control temperature (2 weeks) + Cold treatment (2 weeks)
		C4	Cold treatment (4 weeks)

Table 2. Conditions of control and cold treatment

	Control (C0)	Cold treatment (C2, C4)
Temperature (˚C) (day/night)	25/20	11/5
Photoperiod (hours) (day/night)	16/8	16/8
Photo intensity (μmol/s)	100-110	100-110

Total soluble carbohydrate content

We slightly modified the established method of determining the total soluble carbohydrate content (Liu et al. 2004, Renaut et al. 2005, Pagter et al. 2008). The leaf tissue samples (10 mg FW) were ground into a fine powder in liquid nitrogen, homogenized in 80% ethanol, and incubated for 30 min. After centrifugation (17,000 g, 10 min), the supernatant was collected and evaporated to dryness in a centrifugal vaporizer (CVE-100; EYELA, Tokyo, Japan). The dried residue was resuspended in 1 mL of double-distilled water and filtered using filter papers (No. 5; Whatman, Piscataway, NJ, USA). To measure the total soluble carbohydrate content, the filtrate samples (200 μL) was mixed with 1 mL of anthrone-sulphuric acid reagent (Van Handel 1968). The absorbance was measured with a UV/visible spectrophotometer (Spectramax Plus 384; Molecular Devices) at a wavelength of 620 nm.

Data analysis

The effects of age and cold-treatment duration on *T. chinensis* seedlings were examined using two-way ANOVA. The *t* test was used to assess the statistical significance of the initial lengths of the seedlings. Tukey's honestly significant difference (HSD) post hoc contrasts were used to determine the differences between the levels of the factors under study. Data were analyzed by using ANOVA in Proc GLM. SAS (SAS, 9.1.1 Enterprise Guide 4.1; SAS Institute Inc., Cary, NC, USA) was used for all the statistical analyses with a *P*-value of 0.05 for testing the hypothesis.

RESULTS

Seedling growth and biomass

Before cold treatment, we measured the initial length of 8-week-old and 12-week-old seedlings. The mean length of 12-week-old seedlings was 64.9%, greater than

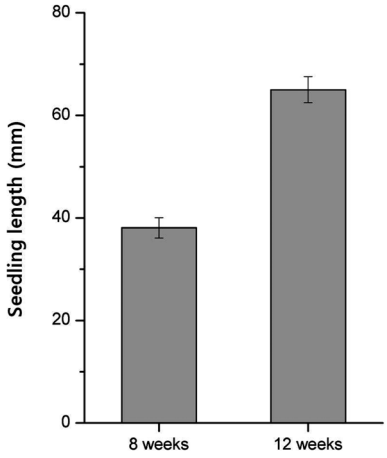

Fig. 2. Initial seedling length of seedlings with 2 different ages.

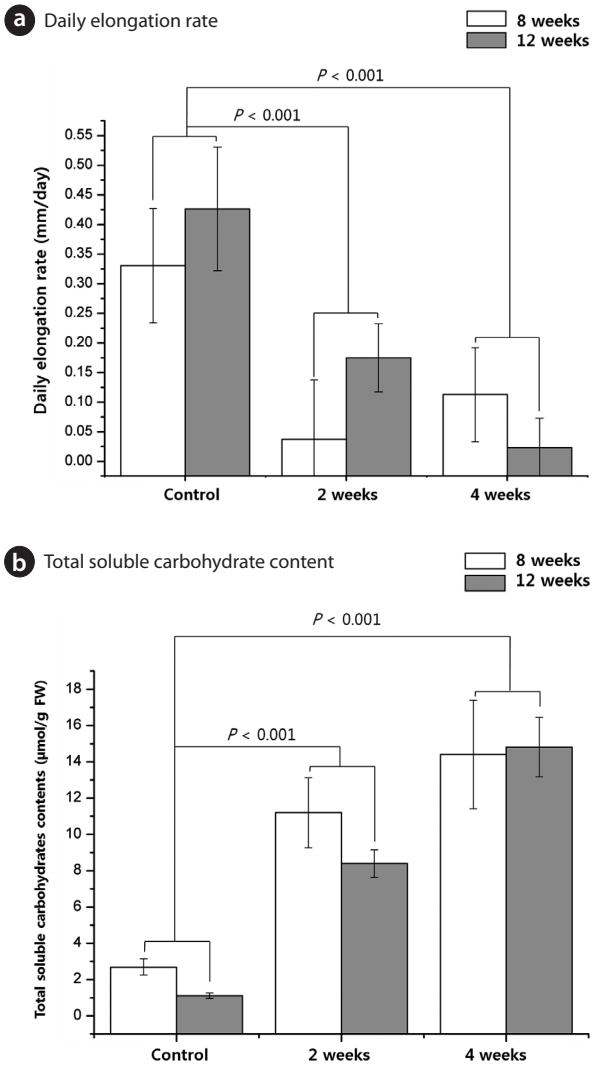

Fig. 3. (a) Daily elongation rate and (b) Total soluble carbohydrate content of *Tamarix chinensis* in response to the duration of growth and cold treatment. Error bars show mean ± standard error values.

that of 8-week-old seedlings ($P < 0.001$) (Fig. 2). There were no significant differences between the daily elongation rates in the 2 age groups (G8 vs. G12, $P = 0.46$), but seedlings that received cold treatment for 2 weeks and 4 weeks were smaller than those maintained at optimal temperature conditions (C0 vs. C2, $P < 0.05$; C0 vs. C4, $P < 0.001$) (Fig. 3).

Shoot, root, and total biomass decreased significantly with an increase in the duration of cold treatment and age ($P < 0.001$ for all C and G groups) (Table 3). During the treatment and under both control and stress conditions, the root:shoot ratio of the seedlings did not change significantly (Table 3). The shoot and total biomass content differed significantly in the treatment duration and seedling age groups (all $P < 0.01$), but root biomass did not show any significant intergroup differences ($P > 0.05$) (Table 3).

Photosynthetic pigment content

Cold treatment decreased the plant chlorophyll content in *T. chinensis* seedlings. The mean chlorophyll a and b content and total chlorophyll content decreased to almost less than half the initial value with cold treatment (C0 vs. C2; C0 vs. C4; $P < 0.001$), but did not vary between the treatment duration and seedling age groups (G × C; $P > 0.05$) (Table 4). With regard to the chlorophyll a content and total chlorophyll content, 12-week-old seedlings had slightly higher chlorophyll content than 8-week-old seedlings (G8 vs. G12, $P < 0.05$). However, chlorophyll b content showed no significant difference ($P = 0.05261$) (Table 4). The chlorophyll a:b ratio of the seedlings decreased after cold treatment ($P < 0.001$). Seedling age and interaction between cold-treatment duration and seedling age did not significantly affect the chlorophyll a:b ratio ($P = 0.05465$ and $P > 0.05$, respectively) (Table 4).

Total soluble carbohydrate content

Total soluble carbohydrate (TSCH) content of control seedlings and seedlings that received cold treatment for 2 weeks and 4 weeks were 2.69 μmol/g FW, 11.41 μmol/g FW, and 14.40 μmol/g FW, respectively, in 8-week-old seedlings, and 1.11 μmol/g FW, 8.39 μmol/g FW, and 14.47 μmol/g FW, respectively, in 12-week-old seedlings, respectively. The TSCH content increased significantly with cold treatment ($P < 0.001$ for all C0 vs. C2, C0 vs. C4) (Fig. 3a). Both seedling age and interaction of cold-treatment duration and seedling age significantly affected the TSCH content in *T. chinensis* seedlings ($P > 0.05$) (Fig. 3a).

DISCUSSION

The freezing tolerance of many plants increases on exposure to low, non-freezing temperatures (Thomashow 1999). Studies on deciduous trees have shown that bud dormancy and absence of visible growth are frequent morphological changes in deciduous plants (Rohde and Bhalerao 2007). Mature *T. chinensis* also make dormancy buds when they overwintered, but *T. chinensis* seedlings aged less than 1 year did not undergo bud dormancy, rather the entire plant overwintered (Fig. 1). Therefore, cold acclimation responses of *T. chinensis* may help to know about seedling recruitment trait in Korea.

We investigated some growth factors and physiological factors. The key factor in the induction of acclimation appeared to be growth cessation (Weiser 1970). In many cases, the first step towards establishing dormancy is growth cessation by environmental factors such as cold (Rohde and Bhalerao 2007). Growth cessation is considered as a prerequisite to cold acclimation. Moreover the shoot apex of an overwintering perennial ceases its morphogenetic activity at the end of the growing season to develop freezing tolerance (Rinne et al. 2001). In this study, cold-treated seedlings showed almost no growth. The results clearly show acclimation responses of *T. chinensis* seedlings, such as growth cessation (Fig. 3a), which could help determine how *T. chinensis* seedlings cope with cold stress after a short period of cold treatment.

Color changes in the stem and apical buds of seedlings, which had turned red, were visible after 2 weeks of cold treatment (personal observation). This color change may caused by cold treatment may be partly caused by degradation of chlorophylls (Table 4) and accumulation of ca-

Table 3. Shoot, root, total biomass, and root:shoot ratio in *Tamarix chinensis* seedlings

		Biomass							
		Shoot (mg)		Root (mg)		Total biomass (mg)		Root:Shoot ratio	
Treatment		G8	G12	G8	G12	G8	G12	G8	G12
C0 (control)		75.6 ± 15.1^{a}	216.1 ± 24.9^{a}	12.3 ± 2.4^{a}	29.1 ± 5.8^{a}	87.9 ± 17.3^{a}	226.1 ± 30.4^{a}	0.18 ± 0.03^{a}	0.16 ± 0.03^{c}
C2		21.6 ± 5.7^{b}	66.2 ± 13.7^{b}	2.4 ± 0.72^{c}	15.7 ± 3.5^{b}	25.6 ± 5.7^{b}	92.0 ± 8.9^{b}	0.12 ± 0.02^{c}	0.22 ± 0.03^{a}
C4		25.3 ± 3.4^{b}	72.7 ± 9.6^{b}	3.9 ± 1.1^{b}	13.0 ± 2.3^{b}	26.1 ± 4.4^{b}	78.3 ± 12.6^{b}	0.16 ± 0.03^{b}	0.18 ± 0.01^{b}
ANOVA result	G	***		***		***		NS	
	C	***		***		***		NS	
	G × C	**		NS		**		*	

*P < 0.05; **P < 0.01; ***P < 0.001; NS: P > 0.05; Mean ± SE values are given.
Means with different letters within a column are significantly different at P < 0.05 using Tukey's honestly significant difference (HSD) test (N = 7).
G, seedling age (week); C, cold treatment (week); G × C, seedling age and cold treatment period; NS, not significant; SE, standard error.

Table 4. Chlorophyll a, b, and total chlorophyll content and chlorophyll a:b ratio in *Tamarix chinensis* seedlings

		Chlorophyll content (μg/g FW)							
		Chlorophyll a		Chlorophyll b		Total chlorophyll		Chlorophyll a:b ratio	
Treatment		G8	G12	G8	G12	G8	G12	G8	G12
C0 (control)		8.3 ± 1.6^{a}	11.5 ± 0.6^{a}	3.5 ± 0.6^{a}	4.5 ± 0.2^{a}	13.3 ± 1.9^{a}	15.9 ± 0.8^{a}	2.4 ± 0.1^{a}	2.5 ± 0.0^{a}
C2		5.0 ± 0.7^{b}	5.4 ± 0.2^{b}	2.4 ± 0.2^{b}	2.6 ± 0.1^{b}	7.4 ± 0.9^{b}	7.9 ± 0.3^{b}	2.1 ± 0.1^{b}	2.1 ± 0.1^{b}
C4		3.4 ± 0.5^{c}	4.9 ± 0.5^{b}	1.7 ± 0.19^{c}	2.2 ± 0.1^{b}	5.1 ± 0.7^{c}	7.1 ± 0.6^{b}	1.9 ± 0.1^{b}	2.2 ± 0.1^{b}
ANOVA result	G	*		NS		*		NS	
	C	***		***		***		***	
	G × C	NS		NS		NS		NS	

*P < 0.05; **P < 0.01; ***P < 0.001; NS: P > 0.05, Mean ± SE values.
Means with different letters within a column are significantly different at P < 0.05 using Tukey's honestly significant difference (HSD) test (N = 7).
G, seedling age (week); C, cold treatment (week); G × C, seedling age and cold treatment period; NS, not significant; SE, standard error.

rotenoids and anthocyanins (Lichtenthaler 1987, Chalker-Scott 1999). After cold treatment for 2 and 4 weeks, the chlorophyll a:b ratio decreased (Table 4). With a decrease in the chlorophyll a:b ratio, the decrease in the activity of photosystem I should be greater than that in the activity of photosystem II. Decrease in the chlorophyll a:b ratio may result in a decrease in photosynthesis because of an imbalance in photosystems I and II (Watts and Eley 1981, Renaut et al. 2005). Therefore cold treatment accelerated the breakdown of chlorophyll a, thereby decreasing the overall photosynthesis rate.

Although the chlorophyll content was reduced, concentrations of total soluble carbohydrates in cold-treated seedlings were higher than those in the control seedlings (Fig. 3b). Carbohydrates accumulate under stress conditions. Circumstantial evidence points to the possibility that sugar may be the translocatable promoting factor. Sugars play diverse roles in cells, e.g., they can serve as energy sources for general metabolism and synthesis of stress-responsive materials (Liu et al. 2004, Renaut et al. 2005). Moreover, accumulation of compatible solutes, sugars, and certain proteins can protect cell structures during dehydration by binding water molecules (Kerepesi and Galiba 2000, Welling et al. 2002). Soluble carbohydrates protect the plasma membrane and proteins from freezing and dehydration (Steponkus 1984). Many studies supported soluble carbohydrates can be regarded as indicators of cold adaptation mechanism in plants. Therefore, our results show that *T. chinensis* seedlings aged less than 1 year show cold acclimation responses and a low degree of tolerance to cold treatment, which is not influenced by seedling age.

In conclusion, we suggest that *T. chinensis* seedlings have a potential ability to adapt to cold stress. Changes in biomass and growth, which are affected by low non-freezing temperatures, take place in the seedlings in response to unfavorable conditions. Since cold acclimation is a cumulative and multifactorial process, it is difficult to explain in detail how *T. chinensis* seedlings with age less than 1 year can withstand the harsh winter. However, we observed a trend in plant growth and material to adapt to cold stress by acclimation in seedlings aged less than 1 year in controlled environment.

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