

Growth, Carbon and Nitrogen Status of Container Grown Black Pine (*Pinus thunbergii*) Seedlings at Various Levels of Foliar Fertilization

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Abstract : The growth, carbon and nitrogen status of container grown black pine (*Pinus thunbergii*) seedlings were examined at various levels of foliar fertilization (control, 0.1%, 0.2%, 0.3%). Root collar diameter, height and dry weight of black pine seedlings increased significantly with increasing levels of foliar fertilization ($P < 0.05$). Carbon concentration in needle of black pine seedlings was significantly higher in the foliar fertilization than in the control treatments ($P < 0.05$), while other seedling components such as stem and roots were not significantly different ($P > 0.05$) between the foliar fertilization and the control treatments. Nitrogen concentration and content were significantly greater in the foliar fertilization than in the control treatments ($P < 0.05$). Shoot/root ratio of black pine seedlings (needle+stem dry weight/root dry weight) was greater in the foliar fertilization (2.40-2.89) than in the control treatments (1.87). However, nitrogen use efficiency was significantly lower ($P < 0.05$) in the foliar fertilization (28-46) than in the control (111) treatments. The results indicate that morphological characteristics and nutritional status on container grown black pine seedlings were enhanced by various levels of foliar fertilization.

Key words : container seedling, liquid fertilization, *Pinus thunbergii*, seedling nutrition,

Introduction

Production of pine seedlings in containers has increased because of better performance of container seedlings over bareroot seedlings on outplanting sites (William and Stroupe, 2002; Ministry of Agriculture and Forestry, 2005; Korea Forest Research Institute, 2007). In addition, containerized seedlings are more resistant for poor handling practices in the field with less root disturbance and/or transplant shock (Landis *et al.*, 1989; William and Stroupe, 2002; Korea Forest Research Institute, 2007), and showed the ability to extend the planting season and to survive highly on adverse sites such as forest fire areas (Dumroese, 2003; Korea Forest Research Institute, 2007). These properties are important for the survival and early growth of seedlings at plantation establishment. Containerized seedlings with larger root collar diameters survived better and grew more vigorously than seedlings with smaller root collar diameter after out-planting for reforestation purpose (Landis *et al.*, 1989; Dumroese,

2003; Jackson *et al.*, 2005). Therefore, nursery manager must apply sufficient nutrients to stimulate growth to obtain larger root collar diameter (Landis *et al.*, 1989; Jackson *et al.*, 2005). Sufficient supply of nutrient in container seedlings showed better survival and growth compared with less fertilized container seedlings (Landis *et al.*, 1989; Munson and Timmer, 1990; Dumroese, 2003; Olet *et al.*, 2004; Dumroese *et al.*, 2005). In addition, application of water soluble fertilizers is the most common practice in container seedling production because foliar fertilization can be used to recharge nutrient depleted seedlings in container (Landis *et al.*, 1989). However, little is known about growth and nutrient status of container seedlings following application of water soluble fertilization in Korea.

Black pine (*Pinus thunbergii*) is one of a dominant and important reforestation species in southern Korea. However, there is little information for the growth and nutrition of seedlings related to container cultivation of black pine seedling. The main objective of this study was to determine the effect of carbon concentration and nitrogen distribution in container black pine seedlings receiving various levels of foliar fertilization. This objec-

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tive was met by determining responses (i.e., dry weight, carbon or nitrogen allocation among seedling components, and nitrogen use efficiency) of black pine seedlings in nutrient availability associated with different levels of foliar fertilization.

Materials and Methods

The experiment was conducted in a greenhouse at the Jinju National University. Black pine seeds were obtained from a local seed source and sown on 13 March, 2008 into PVC containers having a volume of 250 cm³ in each cavity (40 cavities, each cavity size : Ø47 mm×Ø30 mm ×H16 mm). Black pine seedlings were grown on benches of greenhouses in containers filled with 1:1:1 (V:V:V) peat moss:vermiculite:perlite medium mixed at the nursery. The seedlings were irrigated as needed to prevent plant water stress. Container black pine seedlings were treated with 4 different levels (Control, 0.1%, 0.2%, 0.3%) of foliar fertilizer (Planta Products 20N:20P₂O₅:20K₂O plus micro-elements) applied during the growing season. The foliar fertilization was applied (25 mL/seedling) with top-dressing over 5 applications (16 June, 30 June, 15 July, 28 July, 12 August, 2008) during the growing season. Six black pine seedlings were lifted randomly from foliar fertilization treatments of each container on 31 October, 2008. All seedlings were dried in a forced-air oven at 65°C for 48 hours, separated into needle, stem, and roots, and weighed. Seedling components from each treatment plot were combined and ground in a mortar. Carbon and total nitrogen from ground materials were determined on an elemental analyzer (CE Instruments EA1110 Elemental Analyzer, ThermoQuest Italia S.P.A. ITALY). Total nitrogen content of seedling components was calculated by multiplying dry weight by nitrogen concentration. Nitrogen use efficiency was calculated as seedling dry weight (mg)/seedling nitrogen content (mg) (Munson and Timmer 1990). Data were subjected to analysis of variance by using the general linear model procedure in SAS (SAS Institute Inc. 1989). Where appropriate, treatment means were compared using Tukey's test and

a significance level of $P=0.05$ was chosen.

Results

1. Morphological characteristics of seedlings

Root collar diameter and height of container black pine seedlings increased with increasing levels of foliar fertilization (Table 1). Root collar diameter and height of the seedlings were significantly higher in the foliar fertilization than in the control treatments ($P<0.05$). In addition, the height was significantly different among foliar fertilization treatments. Dry weight of seedling components (needle, stem, root) increased significantly ($P<0.05$) with increasing levels of foliar fertilization (Table 1). The greatest weight of the seedling was observed in the 0.3% level of foliar fertilization. The needle weight among seedling components was higher than the stem or root weight of the seedlings.

2. Carbon and nitrogen status of seedlings

Carbon concentration in needle of black pine seedlings was significantly higher in the foliar fertilization than in the control treatments ($P<0.05$), while other seedling components such as stem and roots were not significantly different ($P>0.05$) between the foliar fertilization and the control treatments (Table 2). Carbon concentration among the seedling components was generally higher in

Table 2. Carbon concentration of black pine seedlings grown at various levels of foliar fertilization in containers (n=6).

Treatment	Carbon concentration (%)		
	Needle	Stem	Root
Control	46.3±0.42b	47.4±0.36a	45.6±0.58a
0.1%	47.7±0.15a	47.4±0.32a	45.7±0.41a
0.2%	47.7±0.17a	47.6±0.34a	45.7±0.16a
0.3%	48.3±0.16a	44.4±1.96a	45.8±0.15a
<i>P</i> -value	<0.0001	0.1081	0.9753

Mean±SE. Numbers in each column followed by the same letter are not significantly different according to Tukey's test at $P=0.05$.

Table 1. Morphological characteristics of black pine seedlings grown at various levels of foliar fertilization in containers (n=6).

Treatment	Root collar diameter (mm)	Height (cm)	Dry weight (g)			
			Needle	Stem	Root	Total
Control	1.27±0.06b	5.67±0.21c	0.07±0.01c	0.03±0.01b	0.06±0.01b	0.15±0.02c
0.1%	2.24±0.14a	10.83±1.33b	0.46±0.03b	0.21±0.03a	0.29±0.04a	0.97±0.07b
0.2%	2.32±0.10a	12.00±1.41b	0.64±0.07ab	0.22±0.02a	0.33±0.04a	1.19±0.12ab
0.3%	2.52±0.09a	14.67±1.50a	0.76±0.07a	0.24±0.01a	0.36±0.04a	1.33±0.10a
<i>P</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Mean±SE. Numbers in each column followed by the same letter are not significantly different according to Tukey's test at $P=0.05$.

Table 3. Nitrogen concentration of black pine seedlings grown at various levels of foliar fertilization in containers (n=6).

Treatment	Nitrogen concentration (%)		
	Needle	Stem	Root
Control	0.46±0.01c	0.25±0.03b	0.52±0.01c
0.1%	1.05±0.03b	0.37±0.05ab	0.75±0.01b
0.2%	1.09±0.02b	0.53±0.08a	0.82±0.03ab
0.3%	1.32±0.004a	0.38±0.009ab	0.91±0.03a
P-value	<0.0001	<0.0001	<0.0001

Mean±SE. Numbers in each column followed by the same letter are not significantly different according to Tukey's test at $P=0.05$.

Table 4. Nitrogen content of black pine seedlings grown at various levels of foliar fertilization in containers (n=6).

Treatment	Nitrogen content (mg)			
	Needle	Stem	Root	Total
Control	1.0±0.2c	0.08±0.02b	0.3±0.07b	1.4±0.2c
0.1%	18.5±1.9b	0.8±0.2a	2.2±0.4a	21.6±2.1b
0.2%	24.9±2.1b	1.1±0.1a	2.7±0.3a	28.7±2.3b
0.3%	43.6±3.7a	0.9±0.04a	3.3±0.4a	47.8±3.9a
P-value	<0.0001	<0.0001	<0.0001	<0.0001

Mean±SE. Numbers in each column followed by the same letter are not significantly different according to Tukey's test at $P=0.05$.

Table 5. Shoot and root ratio (S/R) and nitrogen use efficiency (NUE) of black pine seedlings grown at various levels of foliar fertilization in containers (n=6).

Treatment	S/R	NUE
Control	1.87±0.27b	110±4.96a
0.1%	2.40±0.20ab	46±2.74b
0.2%	2.63±0.19ab	41±1.33b
0.3%	2.89±0.23a	28±1.05c
P-value	0.0299	<0.0001

Mean±SE. Numbers in each column followed by the same letter are not significantly different according to Tukey's test at $P=0.05$.

needle or stem than in roots. Nitrogen concentrations in the seedlings were greatest in needle, followed by roots and stem (Table 3). Nitrogen concentration of needle and roots was significantly greater in the foliar fertilization than in the control treatments ($P<0.05$).

Total N content of seedlings was closely related to seedling weight at various levels of foliar fertilization (0.3% > 0.2-0.1% > control) treatments (Table 4). In addition, there was a significant foliar fertilization effect among the nitrogen content of seedling components ($P<0.05$). Nitrogen content of seedlings was highest in needle followed by roots and stem. Shoot/root ratio of seedlings (Table 5) was greater in the foliar fertilization

than in the control treatments (control, 1.87; foliar fertilization, 2.40-2.89). In contrast, nitrogen use efficiency was significantly lower ($P<0.05$) in the foliar fertilization than in the control treatments (Table 5).

Discussion

Various levels of foliar fertilization have a strong influence on growth of container black pine seedlings. Seedling height ranged from 5.67 cm for the control to 14.67 cm for the 0.3% level, while root collar diameter ranged from 1.27 mm for the control to 2.52 mm for the 0.3% level. Total seedling biomass ranged 0.15 g for the control to 1.33 g for the 0.3% level. Because nutrient availability is generally considered the major environmental factor limiting growth in many container seedlings (Landis *et al.*, 1989; William and Stroupe, 2002; Dumroese *et al.*, 2005), growth and nitrogen uptake of black pine seedlings were expected to be greatest in the treatment with the greatest levels of foliar fertilization, i.e., in the 0.3% level treatment. In addition, black pine seedlings growing in the foliar fertilization treatments would have greater dry weight, nitrogen concentration, and nitrogen content than those growing in the control treatment. In this study, the seedlings in foliar fertilization had up to about 2-3 times the root collar diameter and height growth of the seedling of the control treatments. Needle weight of the foliar fertilization seedlings was also much greater than that of the control seedlings. Needle nitrogen concentrations of black pine seedlings was related to differences in application of available nitrogen because there was a significant difference in various levels of foliar fertilization. The significantly greater needle nitrogen concentrations of black pine seedlings in the foliar fertilization (1.05-1.32%) than in the control treatments (<0.46%) may be due to the increased nitrogen availability after fertilization. However, needle nitrogen concentration found in the control treatment is quite low, which may indicate a nitrogen deficiency in case of no fertilization in container black pine seedlings. The lowest needle nitrogen concentrations found in the control would limit black pine seedling growth. Therefore, seedlings in the control treatment showed the lowest weight of seedling components compared with other foliar fertilization treatments. Although seedlings given the highest level of foliar fertilizer (0.3% level) have a foliar nitrogen concentration of 1.32%, all levels of foliar fertilization in this study yield needle nitrogen concentrations below optimum range (1.5% to 2.5%) of coniferous seedlings suggested by Landis *et al.* (1989). This result suggest that the application levels of foliar fertilization in container black pine seedlings of this study were insufficient to maintain the optimum range of nitrogen

concentrations. In contrast to nitrogen concentration, carbon concentration in black pine seedlings was not significantly different at various levels of foliar fertilization except for needle carbon concentration. Needle carbon concentration was lower in the control than in the foliar fertilization treatments because low nutrient availability could induce a high carbon consumption (Cheng *et al.*, 2004). However, carbon concentration in stem and roots was a poor indicator of response to foliar fertilization and was not related to black pine seedling growth response at various levels of foliar fertilization.

Shoot/root ratio of black pine seedlings was greater in the foliar fertilization than in the control treatments. These greater shoot/root ratio in foliar fertilizer treatments may reflect increase in nutrient availability following foliar fertilization because of less carbon allocation to roots of seedling in the fertilizer treatments compared with the control treatments. In contrast, low shoot/root ratio of black pine seedlings in the control treatment could be due to a phenotypic response such as a decrease in needle growth and an increase in root growth (Table 1) in response to low nitrogen availability.

Nitrogen use efficiency, usually defined as seedling weight per unit seedling nitrogen content, has frequently been used to compare the response of plant species in nitrogen availability (Aerts, 1990). Seedlings growing under conditions of low nitrogen availability have high nitrogen use efficiency, which may indicate internal adjustments to tolerate nitrogen stress (Munson and Timmer, 1990; Elliott and White, 1994). High nitrogen use efficiency with the control and the low level of foliar fertilizer treatments may result from decreases in nitrogen in storage compounds.

Conclusions

The growth, carbon and nitrogen status of black pine seedlings grown in containers were influenced by various levels of foliar fertilization. Root collar diameter, height, nitrogen concentration, and nitrogen content of black pine seedlings increased with increasing levels of foliar fertilization, while black pine seedlings in the control treatment showed the lowest biomass, nitrogen concentration and content, indicating insufficient nitrogen availability and probably nutrient deficiency. The results indicate that application levels of foliar fertilization are a major environmental factor limiting growth in container black pine seedlings.

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