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The effect of soil heterogeneity and container length on the growth of *Populus euramericana* in a greenhouse study

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Abstract

Soil characteristics along with various container lengths have an important role in the early survival rate and growth of seedlings by influencing the seedling quality. This experiment was conducted to investigate the effect of container length and different soil mixtures on the growth of poplar in a greenhouse. Two types of soil, homogeneous vs. heterogeneous, were used along with two container lengths (30 vs. 60 cm). The heterogeneous soil was made by dividing 50% vermiculite from a mixture of 25% vermicompost and 25% nursery soil in volume. For the homogeneous soil, the above three soil types were mixed together. *Populus euramericana* clone cuttings were planted in late April, and then, the growth height, root collar diameter (RCD) and biomass were measured in August. The height of the poplar was not significantly affected by container length and soil type, but the RCD was significantly affected by soil type. Leaf and root biomass was higher at the long container than at the short container for both soil treatments, but stem biomass was lower at the heterogeneous soil than at the homogeneous soil treatment. Root to shoot biomass ratio was higher at the heterogeneous soil treatment than at the homogeneous soil treatment by 12%. In conclusion, heterogeneous soil along with a long container is suitable to increase the carbon allocation into the root.

Keywords : biomass, homogeneous, heterogeneous, root biomass, vermicompost

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Introduction

Forest restoration is a long term challenging process that needs effective planning, quality seedling and monitoring. Seedling quality plays an important role as it determine the initial survival of seedling after transplanting in the field. However, seedling quality requirement will vary with depending on the soil, climate and environment of the site. For example, seedlings could be under water stress because of poor contact of roots with soil, and low rooting system that is not able to uptake sufficient water from the soil in water-limited soil environments (Kozlowski and

Davis, 1975; Burdett, 1983). To overcome water stress, larger root system with greater water holding capacity is consider as an advantage for the initial survival of seedling especially for dry condition (Hobbs et al., 1982; Sands, 1984). In addition, seedling height and root collar diameter ratio is an important characteristics for seedling quality (Jacobs et al., 2012).

To achieve these characteristics in seedling, the seedling should be grown in suitable container with appropriate soil medium. Soil characteristics along with various container lengths play an important role in the initial growth of seedling which significantly influences on the initial survival rate and growth during restoration and regeneration process. Dominguez-Lerena et al. (2006) reported that the root volume and survival rate of *Pinus pinea* seedlings increase as the container height and diameter increases in both nursery and field study. They also suggested that the ratio of container depth and diameter should be four to get the best quality seedling. In addition of container shape, optimal growth of root depends on favorable soil or media for water, nutrient, and environment (Leskovar et al., 1990; Kim and Choi, 2016), because plants possess some important mechanisms like root proliferation and changes in nutrient uptakes depending on soil nutrient availability (Drew and Saker, 1978).

Soil heterogeneity is an important factor among plants for competition (Chapin, 1980) but there are very few studies for seedling growth in the forestry even though nutrient heterogeneity is common in natural habitats (Jackson and Caldwell, 1993; Gross et al., 1995; Ryel et al., 1996; Cain et al., 1999). Because nutrients are distributed in patchy manners in the soil, plant roots response to nutrient patches (Hodge, 2004). Plants can enter roots towards the high nutrient concentrated zone (Hutchings and de Kroon, 1994; Robinson, 1994; Robinson and Van Vuuren, 1998), especially fine roots proliferate in localized nutrient patches (Drew and Saker, 1978; Granato and Raper, 1989; Jackson and Caldwell, 1993; Pande et al., 2017). Fransen et al. (2001) reported that the both perennial grasses *Festuca rubra* and *Anthoxanthum odoratum* showed increasing root volume in response to localized nutrient enrichment, especially for N.

Container length also has a great influence on seedling growth. In the meta-analysis of 65 studies, the double container size will increase the biomass by 43% in average (Poorter et al., 2012a). Reduction in container size decreases the water and nutrient availability and also suppress the root growth (Jackson et al., 1996). Not only root but also the shoot of plants was affected by container size, Smaller containers decrease the photosynthesis because of lower light availability by higher density of plants (Robbins and Pharr, 1988; Climent et al., 2011), lower nutrient availability (Sinclair and Horie, 1989; NeSmith and Duval, 1998), and increase root mass fraction (Poorter et al., 2012b).

The purpose of this study was to investigate the effect of soil heterogeneity and container length on the growth of *Populus euramericana* cuttings in a greenhouse. We hypothesized that the long container length with soil heterogeneity could increase root biomass as well as seedling quality. As root will proliferate to more nutrient rich zone, it will grow deeper in heterogeneous condition. This study will give us knowledge about the effect of soil and container length variation for seedling production in forestry area.

Materials and Method

Study site and species

The experiment was conducted in the greenhouse located in the Chungnam National University Experimental Forest at Daejeon, South Korea (36°22'16"N and 127°21'08"E). The mean air temperature was 23.6℃ and humidity was 70% in the greenhouse from May to August 2016.

We used branches in 1 cm diameter of clone of *Populus euramericana* for cuttings, which were provided from National Institute of Forest Science in Korea.

Experimental design

This experiment was designed for 2 × 2 factorial experiment with soil type (homogeneous vs. heterogeneous) and container length (30 vs. 60 cm) with five replications in a completely randomized block design.

Plastic containers were made by PVC lay-plat hose (15 cm in diameter). For making containers, PVC lay-plat hose was cut into 70 cm for 60 cm container length treatment and 40 cm for 30 cm container length treatment. The bottom of each PVC lay-plat hose was sealed with stapler and holes (5 mm in diameter) were made by punching on both side of the container with two lines with 3 cm interval between holes for ensuring aeration and water drainage.

The homogeneous soil treatment was mixed by 25% soil in the nursery, 50% vermiculite, and 25% vermicompost (VERMIFARM, Republic of Korea) in volume. These mixed soil was filled at both container lengths for homogeneous soil treatment (Fig. 1).

Fifty percent vermiculite was separated from the mixed soil with 25% soil and 25% vermicompost for

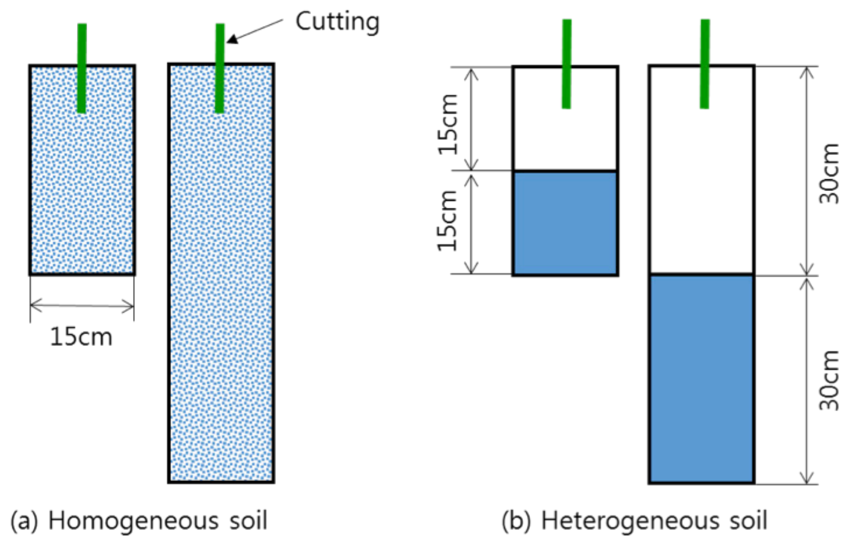

Fig. 1. 2 × 2 factorial experimental design with container length and soil type. 30 cm and 60 cm container length with homogenous soil (left) and heterogeneous soil (right).

the heterogeneous soil treatment. Vermiculite was filled of half upper part of the both container lengths, whereas the mixed soil was putted on half lower part of the both container lengths (Fig. 1).

One cutting with 12 cm in height and 1 cm in diameter was planted at each container at late April, 2016. We used a rooting accelerator (Luton, Farm Hannong, Republic of Korea) for inducing roots and a plant liniment (Lac Balsam, Frunol Delicia, Germany) for reducing water evaporation from the cut. Wooden rack was used to keep containers vertically.

Water was irrigated for 15 minutes at everyday by hand spray. Pruning was done at early May to keep one branch per cutting.

Chemical Analysis

Three soil samples were collected to analyze the chemical properties of nursery soil. For chemical analyses, 500 g soil samples were randomly collected from the surface soil approximately 5 cm in depth from the nursery. The collected soil samples were stored at 4℃ until analysis. The soil organic matter content was determined using the Tyurin method (Walkley and Black, 1934; Nelson and Sommers, 1996). The soil pH and electrical conductivity were measured using a 1 : 5 (w/v) soil : distilled water suspension. Total nitrogen was measured in 1 g soil by using the micro-Kjeldahl method (Bremner et al., 1996) and available phosphate (P_2O_5) was measured with the Lancaster method (Kuo et al., 1996; Cox, 2001). The cation exchange capacity (CEC) was determined in 1N HN_4OAc and CH_3COOH extracts by using the Brown method (Sumner and Miller, 1996). Exchangeable cations K^+, Ca^{2+}, Mg^{2+}, and Na^+ in the 1N NH_4OAc extract were determined using an atomic absorption spectrometer (AA280FS; Agilent Technologies, Santa Clara, CA, U.S.A.).

Three 500 g vermicompost and vermiculite were collected at three different bags for chemical analysis. The analysis method was the same with above soil chemical analysis.

Growth Measurements

The height and root collar diameter of cuttings were measured on late August. The height was measured from the branch emerging point to apical meristem and the root collar diameter was measured at the branch emerging point. The cuttings were harvested in September and divided into leaf, stem and root. For root harvesting, the container was cut into upper and lower parts on 15 cm from the bottom for 30 cm container length and 30 cm from the bottom for 60 cm container length. The roots were sieved with 2 mm mesh and then were washed in tap water to remove soil from roots. All components were dried at 65°C for 48 hours in a dry oven.

Statistical analysis

Analysis of variance (ANOVA) with Duncan's multiple comparison tests was applied to test the effects of container length and soil treatment on height, root collar diameter and dry weight. All probabilities were tested at the significant level at 0.05.

Results and Discussion

Soil characteristics

The pH of soil and vermicompost was from 6.5 to 8.5, neutral or somewhat low basic (Table 1). Most of properties Organic matter was 21 - 110 times higher in vermicompost than vermiculite and nursery soil. Available P was higher in vermicompost with the range 14 - 33 times than in nursery soil and vermiculite. Total N, exchangeable K and Na, CEC, and EC in soil were higher in vermicompost than vermiculite and nursery soil. To survive in harsh conditions, seedlings with better root system are required so that plants can easily uptake nutrients and water from soil. Plant roots tend to grow towards nutrient rich patches in heterogeneous conditions, but was not in homogeneous soil. Nutrient was placed mostly in lower part in heterogeneous soil where as in homogeneous soil nutrient is equally distributed that will induce root proliferation more deep into the soil (Fransen et al., 2001). As a result, root growth was higher in heterogeneous soil treatment than in homogeneous soil treatment.

Growth characteristics

There were no interaction effects as well as main effects of soil type and container length on the growth of height (Table 2, Fig. 2(a)). The growth of root collar diameter was significantly higher at homogeneous soil treatment than at heterogeneous soil treatment ($p < 0.01$), but there were no differences between container length treatments (Table 2, Fig. 2(b)).

The total biomass including above- and belowground was similarly 66 g per plant (Fig. 3), but each tissue dry weight showed different trends. Leaf dry weight was significantly higher at 60 cm container

Table 1. Soil chemical analysis of each soil type of experiment.

Chemical properties	Nursery soil	Vermicompost	Vermiculite
pH	6.60 (0.1)	7.90 (0.0)	6.70 (0.1)
Organic matter (%)	0.15 (0.01)	16.49 (0.35)	0.77 (0.04)
Total N (%)	0.02 (0.00)	0.90 (0.02)	0.07 (0.01)
Available P (mg kg^{-1})	29.90 (0.6)	416.10 (2.5)	12.50 (11.3)
Exchangeable K^+ ($cmol_c$ kg^{-1})	3.80 (0.2)	32.4 (0.8)	26.6 (0.9)
Exchangeable Ca^{2+} ($cmol_c$ kg^{-1})	0.16 (0.07)	22.96 (0.57)	0.07 (0.00)
Exchangeable Mg^{2+} ($cmol_c$ kg^{-1})	3.20 (0.0)	18.70 (0.1)	0.00 (0.0)
Exchangeable Na^+ ($cmol_c$ kg^{-1})	0.27 (0.01)	7.25 (0.05)	0.33 (0.09)
CEC ($cmol_c$ kg^{-1})	0.08 (0.01)	6.40 (0.09)	0.42 (0.17)
EC (dS m^{-1})	0.11 (0.01)	26.68 (0.30)	0.02 (0.01)

Available P, CEC and EC represent $H_2PO_4^-$, cation exchange capacity, and electrical conductivity, respectively. Total N is the sum of organic N and inorganic N. Parenthesis is standard error (n = 3).

Table 2. ANOVA table for growth parameters.

Source of variation	Degree of freedom	Probability (Pr > F)							
		Height	Root collar diameter	Dry weight				Root to shoot ratio	Upper to lower root ratio
				Leaf	Stem	Root	Total		
Soil	1	0.24	0.01	0.43	0.02	0.80	0.08	0.04	0.18
Length	1	0.40	0.60	< 0.01	0.30	< 0.01	< 0.01	0.70	< 0.01
Soil × Length	1	0.86	0.59	< 0.01	0.39	0.13	0.08	0.75	0.59

length than at 30 cm container length, but there were no differences between homogeneous soil and heterogeneous soil treatments (Table 2, Fig. 3(a)). Stem dry weight was significantly higher at homogeneous soil than at heterogeneous soil by 20%, but there were no differences between container length treatments (Table 2, Fig. 3(b)). Root dry weight was significantly higher at 60 cm container length than at 30 cm container length by 21%, but there were no differences between soil treatments (Table 2, Fig. 3(c)).

The average of root to shoot ratio was 21% (Fig. 4(a)). Root to shoot ratio at heterogeneous soil was higher than at homogeneous soil. Generally, 7% more roots were located at above the half of container

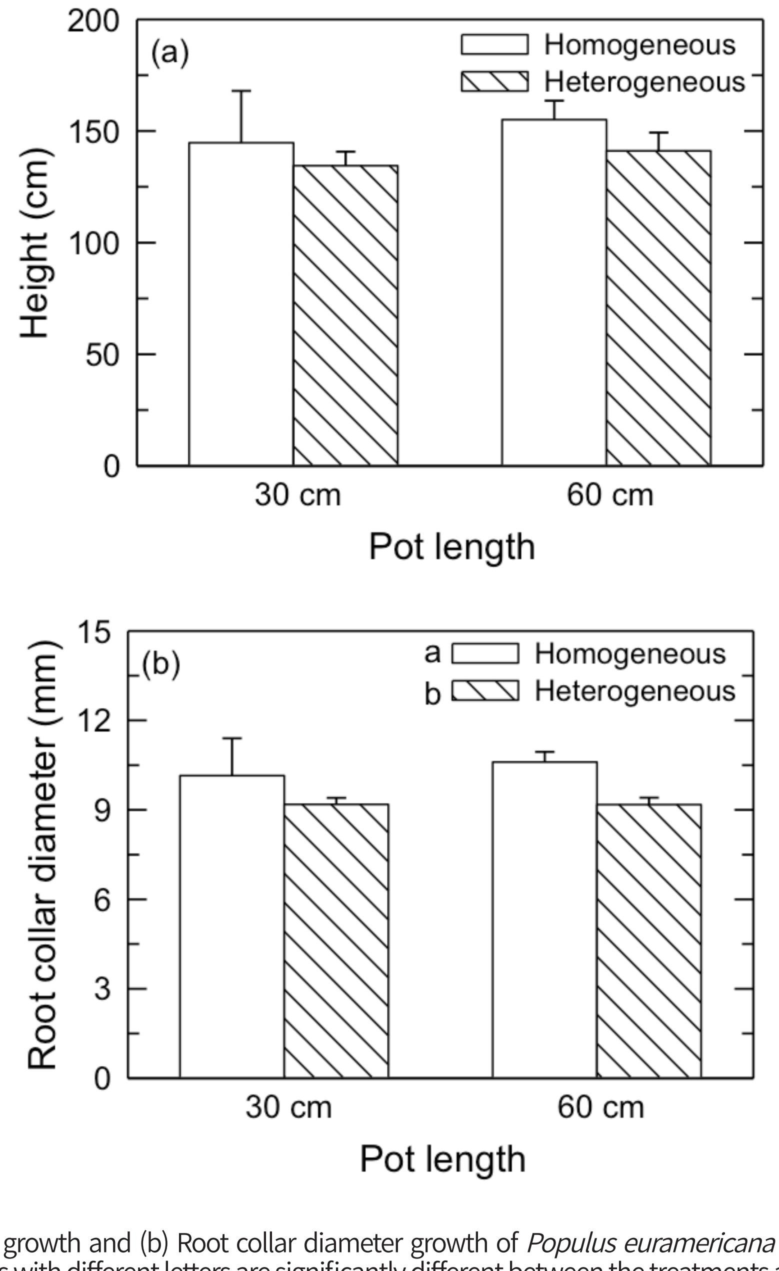

Fig. 2. (a) Height growth and (b) Root collar diameter growth of *Populus euramericana* cuttings between the treatments. Means with different letters are significantly different between the treatments at α=0.05. Vertical bars show standard errors (n = 5).

depth if we assumed root distributed evenly across container depth. More roots were located at above half of container length at 60 cm container length than at 30 cm container length.

Seedling quality is an important factor for successful regeneration. For seedling establishment and survival, physiological stress resistance under harsh environmental conditions at the time of

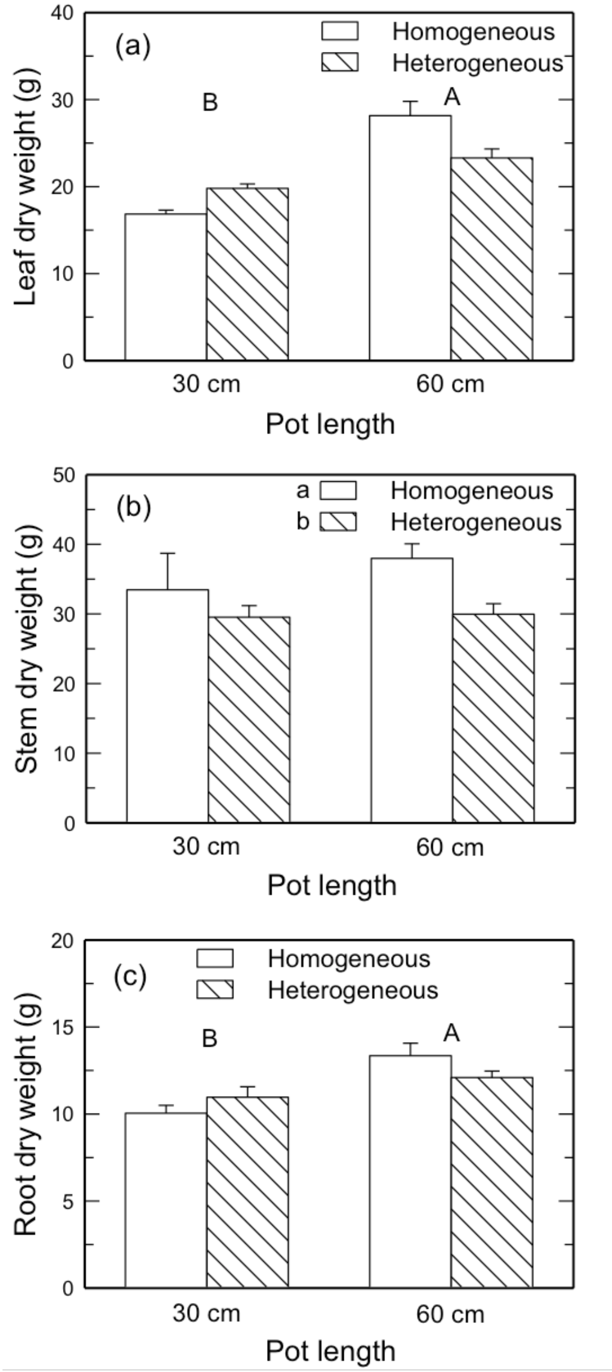

Fig. 3. (a) Leaf, (b) stem, and (c) root dry weight of *Populus euramericana* cuttings between the treatments. Means with different capital letters are significantly different between pot length treatments and means with different small letters are significantly different between soil treatments at α=0.05. Vertical bars show standard errors (n = 5).

transplanting is an essential characteristics for qualified seedlings (Timmis, 1980; Ritchie, 1984; McCreary and Duryea, 1985; Rietveld, 1989). Because water absorption capacity of seedlings depends on root volume (Carlson, 1986), we hypothesized that the long container and heterogeneous soil might improve the survival and initial growth of seedlings by increasing poor rooting system (Ritchie, 1984; Sands, 1984; Rietveld, 1989). In this study, 60 cm container length showed the higher growth for leaf, stem and root and homogenous soil showed higher stem growth than other treatment (Table 2; Fig. 3). Our findings indicate that the larger the container size the more the tree growth. This is because growth rates of shoots and roots in large size container are independent (Tonutti and Giulivo, 1990).

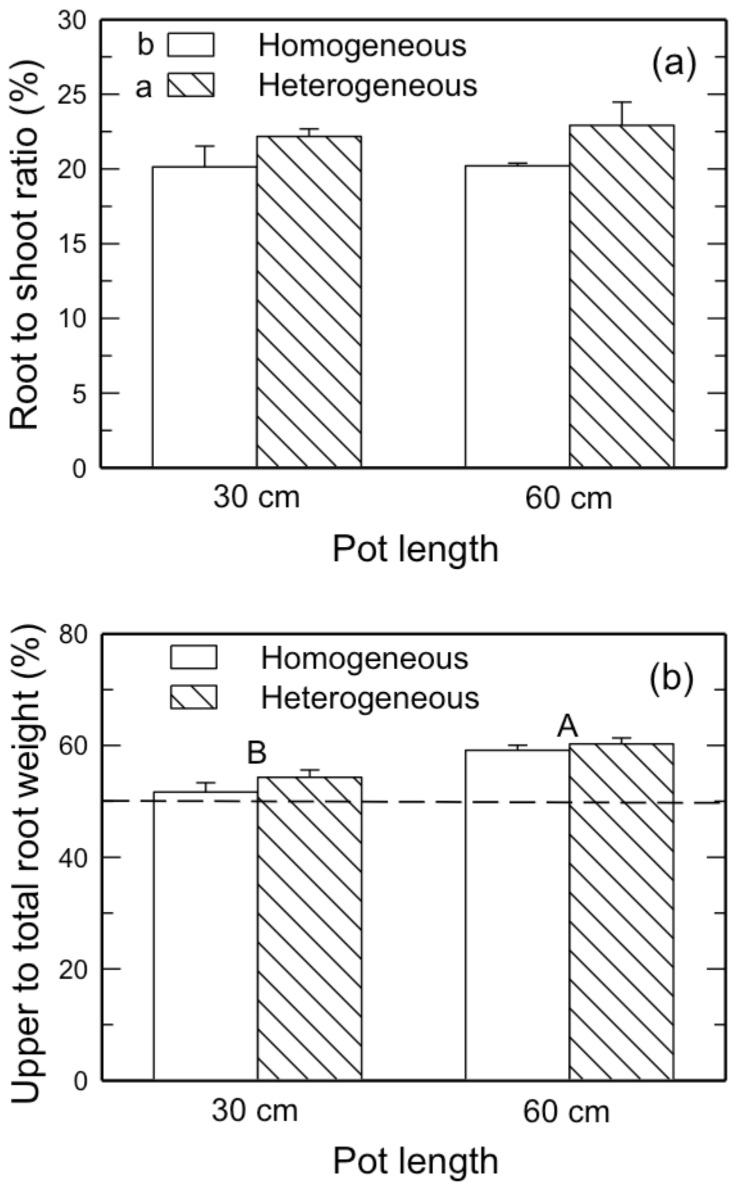

Fig. 4. (a) Root to shoot ratio and (b) upper root to total root weight of *Populus euramericana* cuttings between the treatments. Means with different capital letters are significantly different between pot length treatments and means with different small letters are significantly different between soil treatments at α=0.05. Vertical bars show standard errors (n = 5).

Conclusion

This research was conducted to investigate the effect of soil heterogeneity and container length on *Populus* growth. Soil heterogeneity and container length have great influence on seedling growth as well as biomass production. It was found that container length positively affects the growth of seedling like root growth, shoot height and biomass production and also for maintaining the balance between shoot and root growth. Because carbon allocation to root growth was higher in heterogeneous soil treatment than in homogeneous soil treatment, heterogeneous soil in containers could make seedlings more vigorous so that it can survive after transplanting. This study gives us knowledge that if seedlings produced on heterogeneous soil and larger container will have high root and shoot growth that will lead to increase the survival rate for successful restoration in harsh condition.

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