



Article The Use of Deep Container and Heterogeneous Substrate as Potentially Effective Nursery Practice to Produce Good Quality Nodal Seedlings of Populus sibirica Tausch

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Abstract: Nursery practices are considered major factors influencing seedling quality, which are likely to be maintained in the early establishment phase in the field. Here, we investigated the effects of container depth and substrate heterogeneity on the growth of Populus sibirica nodal seedlings to suggest an effective nursery practice for producing quality seedlings appropriate for forest establishment in a dry environment. We used two substrate heterogeneities (homogeneous and heterogeneous) and two container depth treatments (30 and 60 cm). Variations in root collar diameter (RCD) growth, height growth, stem and root biomass, root to stem ratio, and root mass in the first 15 cm depth from the soil surface across the treatments were computed. Results revealed that both substrate heterogeneity and container depth had no significant effects on the RCD and height growth of P. sibirica seedlings but significantly improved their root and stem biomass. Seedlings in the 60 cm containers generally accumulated higher root biomass than those in the 30 cm containers. There was an interaction effect of container depth and substrate heterogeneity treatments on root and total dry mass, such that seedlings grown in the 60 cm container using heterogeneous substrate resulted in the highest root and total biomass. Analyses of proportional root growth in the upper 15 cm of the containers compared to the total indicated that both the main effects of deeper containers (60 cm) and heterogeneous substrate have fewer roots at this depth, indicating a greater root density in the bottom of the deeper containers. Therefore, deeper containers and heterogeneous substrate may be used as an effective nursery practice to produce seedlings with root traits potentially suitable for harsh conditions, such as arid and semi-arid environments. However, further studies using other seedling morphological traits in conjunction with field-trial tests are needed for a definitive assessment of the effectiveness of deeper containers and heterogeneous substrate in producing good quality seedlings potentially suitable in a dry environment.

Keywords: afforestation; arid; container size; nursery practice; root to stem ratio; seedling root traits; semi-arid



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1. Introduction

In forest-poor arid and semi-arid zones, forests play a crucial role in combating desertification [1]. In Mongolia, for instance, forests are of vital importance for the protection of the fragile landscape and the provision of many environmental services for the country's growing population, making afforestation and forest restoration programs very important. However, tree cultivation on barren landscapes and restoration of degraded forests are much more difficult in arid zones, characterized by slow growth and a high mortality rate of newly planted seedlings [2–4]. Arid regions are also known to have a high rate of seed dormancy, which generally increases with aridity [5,6]. Moreover, the success of a forest establishment effort depends on the quality or traits of the seedlings used and the ability to survive under an anticipated range of environmental conditions, particularly upon outplanting [7]. Thus, an increased understanding of seedling quality during the nursery phase is deemed necessary to ensure that the newly planted seedlings will exhibit the desired level of growth and survival under harsh conditions in arid and semi-arid environments.

Studies have shown that nursery practices are considered to have a major influence on seedling quality during the nursery or hardening phase of the seedlings, and these qualities are likely to be maintained in the early establishment phase in the field [8,9]. One of the reported determinants of seedling quality is the container characteristics, which affect the morpho-physiological characteristics of the roots [9–11]. Specifically, container depth influences water holding capacity, humidity, temperature, and aeration, and is thereby considered an important factor determining root architecture and phenology [12–14]. Field and nursery studies indicated that the root volume and survival rate of *Pinus pinea* L. seedlings increased with container height and diameter [15]. A meta-analysis of 65 studies concluded also that, on average, doubling the size of the containers increased the biomass growth by 43% [16]. Additionally, a smaller container size can inadvertently decrease the soil nutrients available for plants over time, thereby affecting above-ground growth via indirect effects on photosynthesis and other essential physiological processes [17,18]. Consequently, an investigation on the effect of container size on seedling growth is important for seedling survival, especially in arid and semi-arid environments in which plant-soil interactions may have a major impact on seedlings upon outplanting. Furthermore, there is no consensus on the target seedling traits determining the survival and proliferation of seedlings after outplanting in water-limited environments to date [19].

Another nursery practice that may have a major impact on seedling morpho-functional traits is spatial heterogeneity in soil nutrient availability [19–21]. Many studies reported that plants respond to resource heterogeneity through redirecting the nutrient-absorbing organs to nutrient-rich areas, resulting in increased growth performance [22–24]. A study by Cahill and Casper [25], for instance, reported that the above-ground biomass of *Phy*tolacca americana L. and Ambrosia artemisiiifolia L. was greater in a localized placement of nutrients than in the homogeneous one. Similarly, heterogeneous soil significantly increased the above-ground biomass of ten clonal plants in the recent study of Gao et al. [23], who attributed the result to the enhanced resource foraging response of the species in heterogeneous soil conditions. Heterogeneous resource distribution plays a key role in the function, structure, and vegetation patterns in arid and semi-arid ecosystems [26]. The "islands of fertility" phenomenon is also common in arid and semi-arid ecosystems, which can change vegetation growth characteristics and soil nutrient distribution [27]. Therefore, understanding how seedlings respond to soil nutrient heterogeneity will help us understand the appropriate seedling functional traits for such a phenomenon in arid and semi-arid environments.

Plants generally perform better in heterogeneous environments due to root proliferation and enhanced foraging of soil resources, which are all soil-volume dependent. Thus, we investigated the effect of container depth and substrate heterogeneity on the growth of *Populus sibirica* Tausch nodal seedlings to suggest an effective nursery practice for producing quality seedlings potentially suitable for forest establishment in a dry environment. Here, we hypothesized that the growth of *P. sibirica* seedlings is better in a deeper container with heterogeneous soil compared with shallower ones with homogenous soil. This study will provide us preliminary insights into considering the container size and substrate heterogeneity in nursery seedling production for afforestation and restoration projects in dry environments.

2. Materials and Methods

2.1. Experimental Site and Species

In April 2016, the experiment was conducted in the greenhouse in the Mine Reclamation Corporation (MIRECO) nursery, Ulaanbaatar, Mongolia (36°22'16" N and 127°21'08" E) with an elevation of 1130 m. The area was in a semi-arid region with a mean air temperature and relative humidity of 13.6 °C and 49.8%, respectively [2]. The average annual precipitation was approximately 190 mm and the summer precipitation usually occurred from June to August [2]. The mean annual evapotranspiration was approximately 750 mm. The growing season started in May or June, usually with the onset of summer rains, and ended in September.

Clonal seedlings (hereinafter "seedlings") of *P. sibirica*, from the population native to Mongolia, were used in our study. Here, we selected the fast-growing *P. sibirica* as the test species based on the reports that most of the species in the genus of *Populus* have a high potential for vegetative propagation, can tolerate a certain degree of drought, and play important economic and environmental roles in many countries [1,28–30]. It is also one of the commonly used tree species to solve desertification in Mongolia [31]. The stem cuttings (c.a. 1 cm in diameter and 12 cm in height) used in this experiment were collected from an approximately 10-year-old plantation in the experimental site.

2.2. Experimental Design and Management

The experiment used a two-factorial design of substrate heterogeneity and container size treatments. Seedlings were subjected to two substrate heterogeneity treatments (homogeneous and heterogeneous) and two container depth treatments (30 and 60 cm in depth). There were ten container replicates for each of the four treatments and thus 40 containers in total (50 cm distance between containers) in a completely randomized design. Each experimental container, which was made of PVC lay-flat hose, was 15.24 cm in diameter and 70 and 40 cm in length for 60 (10.6 L in volume) and 30 cm (5.3 L in volume) treatments, respectively. The bottom of each container was sealed and perforated for aeration. The homogeneous substrate treatment was made of a mixture of 25% nursery soil, 25% vermicompost, and 50% peatmoss in volume (Table 1), and was put to both container depths (Figure 1). The heterogeneous substrate treatment was placed in the bottom part of the container, and 50% peatmoss, which was placed in the upper part of the container (Figure 1).

| Table 1. Chemical properties of the nursery soil, vermicompost, and peatmoss used | l in t | the stud | зłу |
|---|--------|----------|-----|
|---|--------|----------|-----|

| Chemical Properties | Nurse | Nursery Soil | | Vermicompost | | moss |
|--|-------|--------------|-------|--------------|------|--------|
| pН | 8.5 | (0.1) | 6.5 | (0.0) | 5.9 | (0.1) |
| Organic matter (%) | 12.6 | (2.0) | 27.4 | (0.3) | 56.0 | (1.4) |
| Total N (%) | 0.35 | (0.02) | 0.57 | (0.15) | 0.31 | (0.02) |
| Available P (mg kg $^{-1}$) | 53 | (12) | 2872 | (101) | 123 | (5) |
| Exchangeable K^+ (cmol _c kg ⁻¹) | 22.9 | (0.5) | 37.6 | (1.2) | 23.6 | (0.6) |
| Exchangeable Ca^{2+} (cmol _c kg ⁻¹) | 0.20 | (0.01) | 10.76 | (1.24) | 2.90 | (0.12) |
| Exchangeable Mg^{2+} (cmol _c kg ⁻¹) | 5.5 | (0.1) | 10.1 | (0.4) | 2.6 | (0.1) |
| Exchangeable Na^+ (cmol _c kg ⁻¹) | 1.27 | (0.11) | 12.50 | (0.15) | 0.49 | (0.01) |
| CEC (cmol _c kg ⁻¹) | 22.8 | (0.4) | 29.7 | (1.2) | 15.4 | (0.1) |

Available P and CEC represent H_2PO_4 and cation exchange capacity, respectively. Total N is the sum of organic N and inorganic N. Values in parentheses are standard errors (n = 3).

During the course of the experiment, sturdy wooden racks were used to keep the orientation of the containers straight and vertical. Five hundred milliliters of water per day was supplied to each container via drip irrigation. Pruning was also done in early May to allow only one branch per seedling.



Figure 1. Layout of the experimental design showing the different container depth and substrate heterogeneity treatments: 30 and 60 cm container lengths with (**a**) homogenous and (**b**) heterogeneous substrates.

2.3. Soil Analysis

Three soil samples, 500 g each, were randomly collected at the nursery for soil chemical analysis, following the procedures in Park et al. [32]. The collected soil samples were first stored at 4 °C until further analysis. Soil organic matter (OM) content was determined using the Tyurin method. Soil pH was measured using a 1:5 (w/v) soil: distilled water suspension. Total nitrogen (TN) was measured in 1 g soil using the micro-Kjeldahl method. Available phosphorus (P₂O₅; AP) was determined using the Lancaster method. The cation exchange capacity (CEC) was determined in 1N HN₄OAc and CH₃COOH extracts using the Brown method. Exchangeable cations K⁺, Ca²⁺, Mg²⁺, and Na⁺ in the 1N NH₄OAc extract were determined using an atomic absorption spectrometer (AA280FS; Agilent Technologies, Santa Clara, CA, USA). Three 500 g vermicompost and peatmoss samples were also collected in three different bags for chemical analysis following the same procedures mentioned above.

2.4. Growth Measurements

The height and root collar diameter (RCD) of the seedlings were measured in September after they had grown for five months. The height was measured from the branch emerging point to the highest apical meristem and the RCD was measured at the branch emerging point. At the end of the experiment, the seedlings were harvested and divided into the stem and root components. Leaf dry mass was not measured because of sudden leaf drop caused by abrupt cold temperatures. For harvesting roots, the container was cut into every 15 cm in length from top to the bottom, then the roots were carefully sieved with a 2 mm mesh and washed with tap water to remove the soil particles and other extraneous materials. All plant components were oven-dried at 65 °C for 48 h down to a constant mass. The dry mass of roots from the first 15 cm depth was also determined.

2.5. Statistical Analysis

Two-way analysis of variance (ANOVA) with Duncan's multiple comparison tests was run to test the effects of container depth and substrate heterogeneity treatments on the changes in height, RCD, and dry mass. All statistical analyses were performed using SAS 9.5 and all probabilities were tested at a $\alpha = 0.05$ significance level.

3. Results

3.1. Effects of Different Container Depths and Substrate Heterogeniety on the RCD and Height Growth

ANOVA results yielded no container depth and substrate interactions for either height or RCD growth parameters. The differences in height and RCD growth were statistically similar between the 30 and 60 cm container depth treatments (Figure 2, Table 2). Similarly, there was no significant difference in height and RCD growth between homogeneous and heterogeneous substrates (Figure 2, Table 2).



Figure 2. (**a**,**c**) Height and root collar diameter growth of *Populus sibirica* seedlings in 30 and 60 cm containers and in (**b**,**d**) homogenous and heterogeneous substrates. Vertical bars show standard errors.

| | | Probability (Pr > F) | | | | | | | |
|-------------------------|---|----------------------|------|----------|--------|--------|------------|-----------------|--|
| Source of Variable D | | TTL | RCD | Dry Mass | | | Root to | Root in 0–15 cm | |
| vallable | | Ht | KCD | Stem | Root | Total | Stem Ratio | of Total Root | |
| Substrate | 1 | 0.24 | 0.27 | < 0.01 | < 0.01 | < 0.01 | 0.11 | < 0.01 | |
| Depth | 1 | 0.97 | 0.26 | 0.05 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | |
| Substrate × Depth | 1 | 0.70 | 0.20 | 0.15 | < 0.01 | < 0.01 | 0.13 | 0.82 | |

Table 2. The p-values of two-way ANOVA ($\alpha = 0.05$) for growth parameters of *Populus sibirica* in response to substrate and container treatments.

3.2. Effects of Different Container Depths and Substrate Heterogeneity Treatments on Stem and Root Biomass Growth

The stem and root biomass growth of *P. sibirica* seedlings varied significantly between 30 and 60 cm container depths and between homogeneous and heterogeneous substrates (Figures 3 and 4; Table 2). A significant container depth \times substrate heterogeneity interaction effect on root biomass was also found (Table 2). Specifically, the combination of heterogeneous substrate and a 60 cm container increased both root and total biomass compared with the shallower container or homogeneous substrate (Figure 4; Table 2).



Figure 3. (a) Stem dry mass of *Populus sibirica* seedlings in 30 and 60 cm containers and in (b) homogenous and heterogeneous substrates. Different lowercase letters indicate significant differences between substrate heterogeneity treatments or container depth treatments. Vertical bars represent standard errors.



Figure 4. Root dry mass of *Populus sibirica* seedlings in 30 and 60 cm containers and in homogenous and heterogeneous substrates. Different lowercase letters indicate significant differences between substrate heterogeneity treatments \times container depth treatments.

3.3. Root to Stem Ratio and Root Mass in First 15 cm Soil Depth

There were no significant container depth and substrate interactions for either root to stem ratio or root mass in the first upper 15 cm depth of the container (Table 2). The dry mass ratio of root to stem did not vary significantly between homogeneous and heterogeneous substrates, but it did between 30 and 60 cm container depth treatments (Figure 5; Table 2). Here, we found that the 60 cm containers resulted in a significantly larger root to stem ratio than the 30 cm containers regardless of substrate type.

We observed significant effects of container depth and substrate heterogeneity treatments on the root mass in the first 0–15 cm depth from the surface of the soil (Figure 5; Table 2). The 30 cm treatment showed a higher percentage of the root biomass in the first 15 cm depth, i.e., 63% for homogeneous and 50% for heterogeneous substrate, compared with that of the 60 cm treatment, i.e., 44% and <30% for homogeneous and heterogeneous substrates, respectively (Figure 5).



Figure 5. (**a**,**c**) Root to stem ratio and root dry mass in the first 0–15 cm depth of total root of *Populus sibirica* seedlings in 30 and 60 cm containers and in (**b**,**d**) homogenous and heterogeneous substrates. Different lowercase letters indicate significant differences between substrate treatments or container depth treatments. Vertical bars show standard errors.

4. Discussion

4.1. Deeper Container and Heterogeneous Substrate Improved the Root and Stem Biomass Growth of P. sibirica

In this study, substrate and container depth had no significant effects on the RCD and height growth of *P. sibirica*, but they significantly improved the root and total biomass growth of the seedlings. Results showed that the seedlings placed in the 60 cm container treatment generally accumulated higher root biomass than those placed in the 30 cm containers, suggesting that a larger container can promote root growth of P. sibirica. Here, root growth between heterogeneous soil and homogeneous soil was similar for seedlings grown in 30 cm containers but significantly varied in seedlings grown in bigger containers (i.e., heterogeneous > homogeneous). This implies that the root growth of *P. sibirica* can be soil-volume-dependent, which means reduced soil resource (e.g., water and nutrients) availability. A meta-analysis of 65 different studies reported that increasing the pot size increased total biomass production by 43% [16]. Economically and logistically, the use of shallow pots is more preferred by many tree growers for nursey seedling production over deep ones; however, studies have shown that a smaller pot hinders root growth due to reduced soil volume or rooting space, post-transplant performance, and resource availability, including water and nutrients [33–35]. The negative impacts of the use of smallsized containers on root health and stability were also documented in some studies [36,37].

We also found a significant interaction effect between substrate heterogeneity and container depth on root biomass. Evidently, seedlings planted in heterogeneous substrate using 60 cm containers resulted in the highest root dry mass, suggesting that the interactive effects of both treatments may further improve the root growth of the seedlings. Our findings are consistent with those of several studies, which reported that fast-growing species accumulated more biomass in heterogeneous than in homogeneous substrate treatments [38–40]. Our study on plant response to different fertilizer placement treatments also showed that the bottom placement resulted in the longest root and largest below-ground biomass in both fast- and slow-growing species [41]. The results of the present study can be attributed to the ability of *P. sibirica* to re-direct root growth towards localized placement or

availability of soil nutrients, thereby improving plant growth through enhanced resource acquisition. This can be supported by the observed significantly lower root dry mass (i.e., <30%) in the first 0–15 cm from the surface of the heterogeneous substrate in 60 cm containers, expressing phenotypic plasticity in root growth and proliferation of *P. sibirica* in a patchy distribution of soil nutrients. Here the vermicompost + nursery soil and peatmoss were mixed evenly in the homogeneous substrate, whereas those in the heterogeneous treatment were placed in the bottom and upper part of the container, respectively. Few studies have long demonstrated that the allocation of root biomass can be profoundly influenced by the pattern of the supply of soil-based resources, as roots often proliferate in nutrient-rich locations [42,43].

4.2. Implications for Effective Afforestation and Restoration of Arid and Semi-Arid Lands Using Seedlings with Better Rooting Characteristcis

The limited availability of viable seeds, the high mortality rate of newly planted seedlings, and drought are some of the major constraining factors relating to afforestation and restoration projects in arid ecosystems, such as Mongolia [2,4-6]. New practices or techniques to boost seedling quality and sustain the survival rate and growth of newly planted seedlings in a dry environment are therefore very important. Our results are relevant to producing superior quality seedlings using stem cuttings of fast-growing P. sibirica grown in 60 cm containers and heterogeneous substrate for the effective afforestation and restoration of degraded drylands in Mongolia and/or other xeric habitats. First, the use of stem cuttings will provide a rapid and cost-effective solution to the limited availability and high dormancy of seeds in arid ecosystems. An estimate of 85% of plant species in arid regions produce dormant seeds, and the prevalence of dormancy increases with aridity [5,6]. Therefore, the propagation of new seedlings from stem cuttings can avoid the difficulties in germinating dormant seeds due to low and short-lived rainfall events in arid and semi-arid lands, thereby meeting the production criteria of afforestation and restoration projects. Further, needing the use of artificial methods to facilitate seedling production for forest plantations in dryland is one of the major issues that the Food and Agriculture Organization (FAO) pointed out [44].

Some of the important lessons from the tree planting initiative in Mongolia were that the high mortality rates of seedlings were due to the inhibition of taproot growth by the design of the nursery seedling container, harsh and dry climate, poor quality seedlings, and poor preparation and planting techniques [2,45,46]. Thus, better rooting characteristics (rate, volume, and stability) are important root traits that should be targeted to help ensure high survival rate and effective foraging for soil resources in harsh and dry environments. Our results suggest that the use of deep containers will improve the root to stem ratio and rooting deeper in the container relative to the top portion, and similar advantages can be obtained using heterogeneous substrate. Thus, we suggest that the root biomass growth can be used as a good seedling quality indicator for *P. sibirica* using either 60 cm containers or heterogeneous substrate and/or a combination of the treatments. Davis and Jacobs [7] stated that root characteristics may provide a more accurate indication of good seedling quality than above-ground morphology, particularly after transplanting. They further noted that large root volume showed a distinct correlation with improved field performance. Moreover, breeders emphasized that root morphological traits associated with sustaining survival rate under drought conditions included high root length density, especially at depths in soil with available water [47].

In this study, we found that the deeper containers resulted in a better root growth compared with the shallower containers. A review pointed out that the use of smaller containers for nursery seedling production is more practical and economical than the use of bigger/deeper containers in terms of space requirements, labor and transport costs, and maintenance requirements [16]. However, our study revealed that shallower containers resulted in poor root growth, which is obviously less feasible in a dryland compared with the deep-rooted seedlings produced using deeper containers. This is because plants need to effectively forage for water from deep ground in order to keep hydrated until the next

rain. The size of a plant's root system is a key trait of interest related to foraging for ground water as erratic rain is among the most constraining factors in arid lands [44,47,48]. Without human intervention, such as a high-throughput irrigation system, plants can only acquire water from the ground through roots as rain water may not be available in the subsurface of the soil in arid and semi-arid lands. Having deep-rooted seedlings upon the early phase of outplanting could potentially reduce the need for human intervention and, therefore, the overall costs. Several studies reported that multiple constraints for dryland plant establishment were ameliorated by strategic deep planting and deep soil mixing methods [49,50], and such reports seem to go hand in hand with deep-rooted seedlings produced through deep containers. Therefore, in the case of drylands, deeper containers may be favored over shallower ones for the sake of producing planting stocks better adapted to a water-limited environment. To our knowledge, no study has yet shown detailed pros and cons of using both shallow and deep containers for seedling production, especially in drylands. Hence, the insights we presented herein could give way to more advanced studies on the optimization of pot depth designed only for special environmental conditions in dryland.

However, a root to shoot ratio reflects the water-absorbing and transpiring area; thus, a higher root to stem ratio of *P. sibirica* in 60 cm containers and heterogeneous substrate may imply further lowering of groundwater in afforestation and restoration sites in xeric habitats. Increased extraction of groundwater has been already attributed to increased evapotranspiration in afforestation sites [51,52]. Therefore, some silvicultural treatments can be employed to modify stand transpiration, and further research is necessary to better understand the suitability of *P. sibirica* seedlings, with their high rooting rate, to afforestation projects in drylands.

5. Conclusions

The present study highlights the importance of science-based and new investigations in considering the container depth and substrate heterogeneity as effective nursery practices for seedling production for afforestation and restoration projects in arid and semi-arid lands. Our results revealed that deeper containers and heterogeneous substrate remarkably increased the growth of *P. sibirica* seedlings, particularly the higher root to stem ratio. Therefore, deeper containers and heterogeneous substrate may be used as a potentially effective nursery practice to produce clonal seedlings with root growth characteristics potentially helpful for ensuring high survivability of the species during the early phase of outplanting in drylands. The findings of the present study may provide preliminary insights into considering container depth and substrate type in producing planting stocks for dryland forest establishment.

Because there are still many other factors influencing seedling quality in the nursery phase, further study is needed using additional seedling morphological variables and a range of possible environmental conditions or treatments. Additionally, for a field application, an economical and automated deep container system should be explored and developed in future study.

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Data Availability Statement: The data used in the article is available from the primary author's repository upon request.

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