

Review on Investigate the Growth Responses of Rooting and Shooting Performance of *Populus alba* Cuttings Grown in Hydroponic and Soil Cultures Environments

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Review Article

Received date: 09/03/2021

Accepted date: 26/04/2021

Published date: 05/05/2021

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Keywords: Amendments; Hydroponic Number of rooted Cuttings; Greenhouse; Media; Propagation, Soil Culture; plastic pots

ABSTRACT

Populus spp (poplars) are fast growing multipurpose trees. They have immense potential to benefit mankind and to protect the environment. In order to investigate the effects of biochar, Azolla and moss as components of growth media on root formation and shooting performance in mini cuttings of poplars, an experiment was conducted using plastic bottles in a greenhouse in hydroponic and soil media. The experiment was established on three different rooting media: sand, water and soil enriched with three concentrations (0gm, 10gm and 25gm per L of the amendments; (biochar Azolla and moss), with randomized block design, each block containing three replicates. The cutting length, cutting thickness and ages of cuttings were used to see root establishments. The data revealed the significant effect of different amendments and rooting media on the populus establishment. The rooted mini-cuttings were assessed for rooting percent; number of roots, root length, number of leaves and shoots height as well. In the study finding among all the amendments and rooting media, moss (25 gm L⁻¹) showed better results with water in terms of rooting percent (100%), number of roots (16.33), root length (19.83cm) and number of leaves (45.33) per rooted mini-cuttings. The formation of healthy plants after hardening under outdoor conditions showed that *populus alba* could be successfully propagated by mini-cutting techniques.

INTRODUCTION

Poplar is one of the most important economical tree species in temperate regions of the world due to its desirable attributes in adaptability, growth rate, wood biomass, and versatility of its wood for industry. ^[1] *Populus* is highly valued for a variety of purposes, due to its fast growth, broad range of genetic variation and coppice production potential.

It has suitable potential for the production of cellulose derived biofuels, electricity, and agroforestry, sources of valuable raw material for fiber, lumber, and plywood. It is also used for remediation in phytotechnologies. ^[2] Typically, it was thought that the poplar wood could also be used as a promising resource of renewable carbon for bioenergy and biofuel. ^[1] The conventional and molecular breeding packages have produced a considerable number of the best poplar genotypes. Reproduction of the best genotypes is extremely required, and clearly the clonal propagation method can make new accomplishment in genetic improvement accessible in a very short time, and allows a rapid return on investment. The physiological condition of stock plants is pivotal for the initiation of the rooting process of cuttings. Seasonal variation in the level of carbohydrates and hormones in the stock materials could affect the regeneration of the cuttings. ^[3] They reported that an adequate chilling period of physiological dormancy is highly required for production of the adventitious root of hardwood cuttings while suggested that summer is the best period for the rooting of poplar softwood cuttings when the donor plants are in optimal physiological condition for vegetative propagation. ^[4,5] Shoot position and cutting length also play important roles in the rooting process of poplar hardwood cuttings because the regeneration of cuttings requires energy. The Carbohydrate and hormonal content were found to be related to the size of the cutting (length and diameter) and to the original location of the cutting on the stock plant. Cuttings from the base of the shoot produced longer and heavier roots than that from tops of shoots, while the diameter of the cutting also strongly affects poplar rooting ability. ^[6] Commonly, the age of the stock plant could also influence the rooting ability of cuttings. ^[7] Cuttings from older plants may have lower potential to form adventitious roots than younger plants, because the production of rooting inhibitors increases with plant lignification. Cutting materials would better choose a younger tree and the position should be the base of the shoot. Poplars enrich the surface soil in a short rotation of 6–12 years by adding leaf litter and high productivity (25–50 m³-per hectare per year). ^[8] Studying the rooting ability may provide information for the development of improved cultural technologies and growth enhancement. ^[9] Such practices

may enhance the potential for success of commercial plantation deployment because rooting is the first biological prerequisite to stand establishment. This study was conducted in the following objectives. (1) To examine the effects of cutting length, thickness and age on the rooting and shooting characteristics of *Populus alba* cultures. (2) To evaluate the role of additives (Biochar, Moss and Azola) in hydroponic culture on the growth of *Populus alba*. (3) To study the effects of temperature variation and salinity on rooting and shooting growths of *Populus alba*. (4) To compare suitability of the sand and red soil, as basal media, for the growth of *Populus alba* with combination of biochar at uniform 20 cm cutting length.

MATERIALS AND METHODS

Descriptions of Study Area

The study was conducted in Bahir Dar town, which is the capital city of Amhara Regional State (ANRS). It is located in North West Ethiopia approximately 565 km far from Addis Ababa, at altitude and longitude of 11°36'N and 37°23'E. The city has an elevation of 1840 meters above sea level with mean annual temperature of 18.42 to 27.15°C. Mean annual rainfall ranges from 895 to 2036 mm.^[10]

Plant Material Source and Preparation

Source of cutting materials were a three month seedlings and mature (1-2 years) donor *Populus alba* plant. Cuttings were collected from Selam Campus Bahir Dar University. Proper physiological conditions that promote ready adventitious rooting and freedom from insects and disease were considered for selection of both juvenile and mature donor plants. Seedlings and cuttings of mature donor plant enclosed in bags were transported to the propagation site at Zenzelma campus nursery site at the University of Bahir Dar and soaked in water until experiment, since soaking in water before experiment initiates rooting and greatly enhances the survival and growth of *Populus* during establishment.^[11]

Experimental Design

An experimental study was conducted from December, 2018 to May, 2019 to check the rooting and shooting (growth performance) of *Populus* cuttings in hydroponic and soil cultures. Effects of amendments (biochar, Azolla, moss) and salinity were tested in hydroponic culture. Complete randomized block experimental design was used whereby the experiment had five factors: cutting size and age related, water related, Amendment, Temperature and substrate related. Major treatment factor was the age of the donor plant which had two levels (juvenile and mature); sub minor treatment factor was the cutting length which had three levels (short (10 cm), medium size (20 cm) and long (30 cm)). Each treatment factor was replicated three times. With the three replications a total of 108 cuttings were used for the whole experiment in each treatment. Air temperature in the greenhouse was maintained at 23.4-42°C, with 10-60% relative humidity, and air temperature outside the greenhouse were 20.4-32°C with 10-45% relative humidity. The cuttings were regularly watered and monitored daily to avoid desiccation damage. In the present investigation, an attempt was also made to study the rooting ability in mini-cuttings by the influence of biochar, moss, azolla and their different concentration in hydroponic rooting media.

Experimental Procedures for Vegetative Propagation

Amendments were collected from different sources like biochar bought from market and grind in mortar, moss were collected around Saint Mary church and Azolla also collected from marshy place at Bahir Dar kebele eight. Then collected Moss and Azolla were dried and squished to be powder to mix with water. Next, powders of all biochar, moss and azolla were mixed with water by measuring in grams at 0 g, 10 g and 25 g per liters of water. Finally cutting lengths were measured in centimeters and planted in plastic pots for different treatment factors. For each treatment 36 cuttings were used.

Factor one: cutting related treatments; Cuttings taken from Salam campuses and soaked in water to avoid desiccation damage until experiment. In this treatment 36 cuttings to each treatment at cutting length 10, 20 and 30 cm cuttings with 3 replications, a total of 108 cuttings were used.

Cutting thickness; thin, medium and thick 36 cuttings were used for each and cutting age of donor plant from 3 month donor plant 36 cuttings and, 36 cuttings from 1-2 years donor *Populus* plants was planted at the nursery site. Generally, 288 cuttings were used in this cutting related treatment. For cutting thickness and cutting age 20 cm cuttings were used.

Factor two: water and salt effect on the growth; Cuttings for both juvenile and mature donor plants were divided into three cutting positions from their shoots namely apical, middle and basal position of stem cuttings. Then, 20 cm length Cuttings were planted and placed under saline solution for 21 days to check the degree of effects of salt stress on *Populus* growth related to cutting position. Treatments were at 0 gm, 10 gm and 25 g salts per liters of pure water. Then all cuttings from all treatments were rated on scale of 0-5 for rooting and quality, with 5 being high quality well-rooted cuttings and 0 being dead. In this treatment 12 cuttings with three replications in total 36 cuttings in pure water as a control and 12 cuttings with three replications in total of 36 cuttings of *Populus* were planted in saline solution which were prepared at 10 g salt per liter of water and 36 cuttings at 25 g salt per liter of water, i.e. 36 cuttings at 10 g salt per liters of saline solution and 25 g salt per liters of saline solution.

Factor three effects of amendments on growth: the following amendments were added to each treatment: biochar, moss and Azolla. 108 cuttings were planted in pure water + biochar at 0, 10 and 25 gm biochar per liters of pure water. 72 cuttings was

planted in pure water + moss at 0, 10 and 25 gm moss per liters of pure water. 72 cuttings were planted in pure water + Azola at 0, 10 and 25 gm per liter of pure water.

Factor four: Effects of temperature on growth: In these treatments 12 cuttings with three replications total of 36 cuttings at uniform 20 cm cutting length of populus alba in side greenhouse and 36 cuttings outside greenhouse total of 72 cuttings were tested to evaluate the effects of temperature on cutting propagation in soil media.

Factor five: Effects substrate related treatments on the growth; In this treatment red soil and sand soil were used with combinations of biochar as substrate or growth media to evaluate variation on propagations of populus alba. The biochar was added on both red soil and sand soil as follows: - 108 cuttings was planted in sand soil +biochar at 0, 10, 25 and 50 gm biochar per kg of sandy soil, and 108 cuttings was planted in red soil +biochar at 0, 10, 25 and 50 gm per kg of red soil. Data were collected in terms of rooting and shooting parameters like; - rooting percentage, number of roots per cutting, root Length per cutting, number of leaves per cutting, shoot height per cutting, branch number per cutting, root diameter per cutting shoot diameter per cutting leaf area and fresh weight and dry weight of root and shoot were correlated. Evaluation of cuttings was made after 30- 45 days from the day of planting and cuttings were considered rooted if it has at least one root. For root measurements, the sand and soil were removed from the rooted cuttings; their roots were washed and separated from the cuttings before subjecting to the root number and root length.

Data Analysis

These data were subjected to descriptive statistics, tests of analysis of variance, correlation and independent sample t-test to analyze two independent samples inside greenhouse and outside greenhouse and effects of cutting age variation on rooting and growth performance. SPSS-Statistical packages for social science version 21 were used to do statistical analysis techniques for different treatments. The analysis of variance (ANOVA) procedures were used to test for significant effect of treatments, and for comparisons of different means of different treatments. Pearson correlation coefficients were used to correlate fresh and dry weight of roots and shoots in substrate related treatments.

RESULTS

Effects of Cutting Length and Cutting Thickness

Cutting length significantly affected shoot height, leaf area and branch number at $P \leq 0.05$. But cutting thickness had a significant effect on parameters like; (root percentage, shoot height, root diameter and shoot diameter at $p < 0.05$ as indicated in **(Table 1)**. Even though significant variations were not seen for all shooting and rooting performance due to cutting length effect, there were morphological variations which were seen especially in shooting performance as it is indicated in which relatively 10 cm cutting length showed better results. The effects of cutting length did not show a significant difference in percentages of rooting of populus. Since the rooting percentage (75%), (88.8%) and (77.7%) at cutting length of 10 cm, 20 cm and 30 cm respectively different impacts: thin cuttings and medium thickness cuttings showed best results compared to thick cuttings. From the total of 108 cuttings (36cuttings to each treatment) thin, medium and thick cuttings rooting percentage were 91.6%, 86.1 %and 55.5% respectively. **(Figure 1)**

Effect of Stock Plant Maturation (Cutting Age)

Rooting responses of cuttings varied significantly with stock plant types or donor plant maturation (25%) and produced more roots per rooted cutting. The cutting age significantly affected only the percentage of rooted Cuttings taken from 3months stock plant rooted significantly better (50%) than cuttings from 1-2 years' trees cuttings, root length and leaf number were not significantly affected by stock plant maturation at ($p < 0.05$) according to T test, as indicated below in figure by the same colors. **(Figure 2)**

As the result indicated in **(Figure 3)** only rooting percentage of 3 month cutting showed better results at $p < 0.05$ compared to 1-2 years' cuttings due to cutting age effect on populus rooting in the hydroponic media.

Effects of Biochar on Growth Performance of Populus Alba

In the present study, in hydroponic, populus root and shoot growth increased with increased rate of biochar but it was statistically non-significant until the fourth week. Additions of biochar at 10 gm /liter, 25 gm /per liter and 50 gm /liter as compared to control (0 gm of biochar amendment per liter) is shown **(Table 2)**. There were significantly higher growth performances at each treatment ($p < 0.05$). The rooting percentage is shown by **(Figure 3)**.

As the result indicated in **(Figure 4)** only rooting percentage of 3 month cutting showed better results at $p < 0.05$ compared to 1-2 years cuttings due to cutting age effect on populus rooting in the hydroponic media.

Effects of Moss on Growth of Populus in Hydroponic Media

The hydroponic media containing moss (25g/l) and 10g/l demonstrated greater percentage of rooting (100%) and (77.8%) respectively in comparison with 0g/l moss (control) and other media combinations as shown in **(Figure 5)**.

Similarly, applications of 10g/l and 25g/l moss showed significance difference in terms of number of roots per cutting and leaf number per cutting, but not significant difference was observed in terms of root length per cutting and shoot height per cutting at ($p < 0.05$) as indicated in **(Table 3)** below.

Table 1. Effects of cutting length and cutting thickness on root number per cutting, root length per cutting, leaf number per cutting and shoot height per cutting in soil.

Cutting related Factors		Root number Per cutting	Root length per cutting(cm)	Leaf number precutting	Shoot height per cutting(cm)
Cutting length	10 cm	8.33 ± 4.09a	15.83 ± 2.20a	28.00 ± 3.05a	29.60 ± 0.20a
	20 cm	6.33 ± 1.33a	17.83 ± 1.92a	34.67 ± 4.71a	38.60 ± 3.08ab
	30 cm	10.3 ± 6.83a	18.00 ± 0.57a	46.67 ± 4.91b	45.50 ± 2.11b
F		0.18	0.49	5.68	13.57
P value		0.84	0.63	0.04	0.006
Cutting thickness	Thin	7.33 ± 0.88a	14.83 ± 1.45a	17.67 ± 4.17a	26.76 ± 1.63a
	M thick	5.00 ± 0.00a	14.33 ± 0.28a	22.33 ± 3.75a	25.83 ± 1.92ab
	Thick	3.67 ± 1.20a	16.43 ± 2.79a	36.00 ± 9.53a	33.10 ± 1.51b
F		4.65	0.34		5.42
P value		0.06	0.73		0.045

Note: Mean ± SE followed by the same letters are not significantly different (p<0.05).

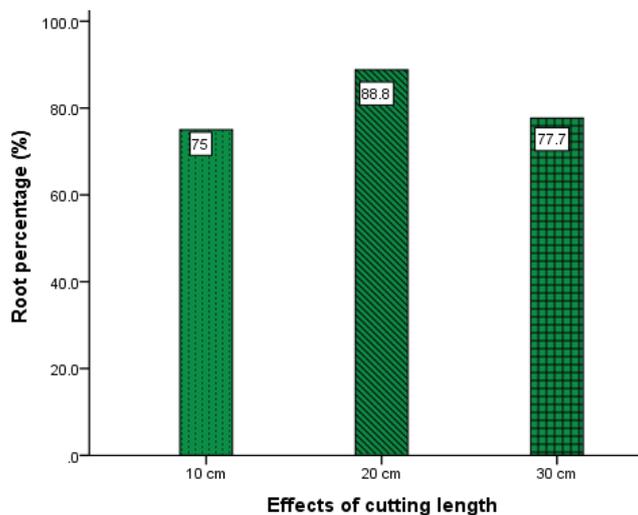


Figure 1. Effects of cutting length on rooting percentages of populus in soil.

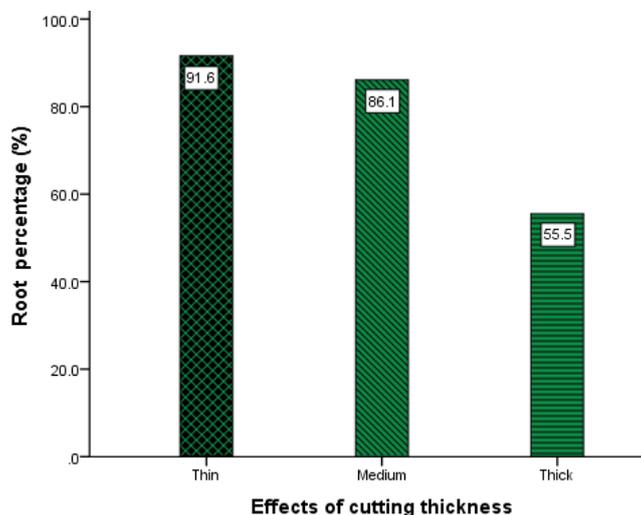


Figure 2. Effects of cutting thickness on rooting percentages of populus in soil.

As the result indicated in (Figure 5) applications of moss at 10 g/L and 25 g /L of water showed significant effect on rooting of populus in hydroponic growth media compared to 0 g moss /L water (control), applications of 25 g moss/L water showed best result in rooting (100%) followed by applications of 10 g moss/L water (77.8%) and lastly 0 g moss/L (control) were (16.7%) in rooting percentage from the total 36 cuttings for each treatment. i.e. 108 cuttings were.

Effects of Azolla on Rooting and Shooting Performance of Populus

Application of Azolla has been found to significantly improve the physical and chemical properties of the soil especially nitrogen, organic matter and other cations such as Magnesium, Calcium and Sodium released into the soil which are important nutrients for plant growth. [12] Combined incorporation of nitrogen fixing green manures such as Sesbania and Azolla shows significant enhancement in the activity of soil enzymes such as dehydrogenase, phosphatase, cellulose and amylase. [13] Similarly, in current

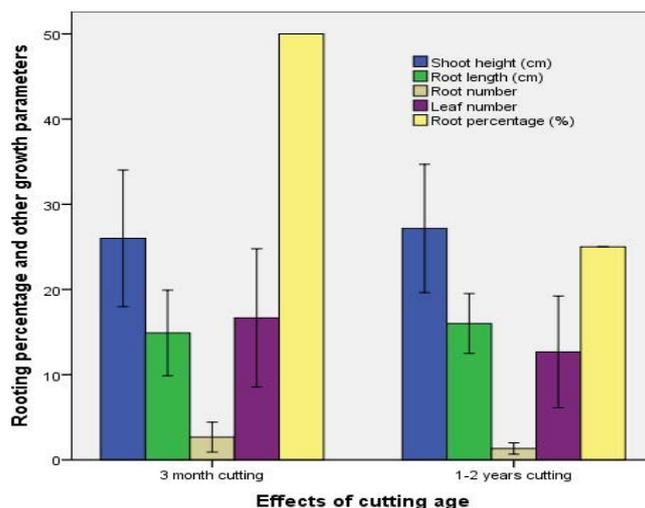


Figure 3. Effects of cutting age on rooting and shootings of populus in hydroponic media.

Table 2. Effects of biochar on root number, root length, leaf number and shoot height per cuttings of populus in hydroponic media.

Biochar	Root number per cutting	Root length per cutting(cm)	Leaf number per cutting	Shoot height per cutting(cm)
0 gm/L	1.00 ± 0.00a	10.50 ± 2.08a	7.33 ± 1.45b	13.66 ± 1.92a
10 gm/L	7.67 ± 0.88b	14.33 ± 1.85ac	20.00 ± 1.00c	28.60 ± 0.78b
25 gm/L	8.33 ± 0.88b	19.83 ± 1.76c	21.67 ± 1.20c	26.66 ± 1.01b
F	31.71	6.064	40.46	37.11
P value	0.001	0.036	0.000	0.000

Note: Mean ±SE (standard error) followed by the same letters are not significantly different at (p<0.05).

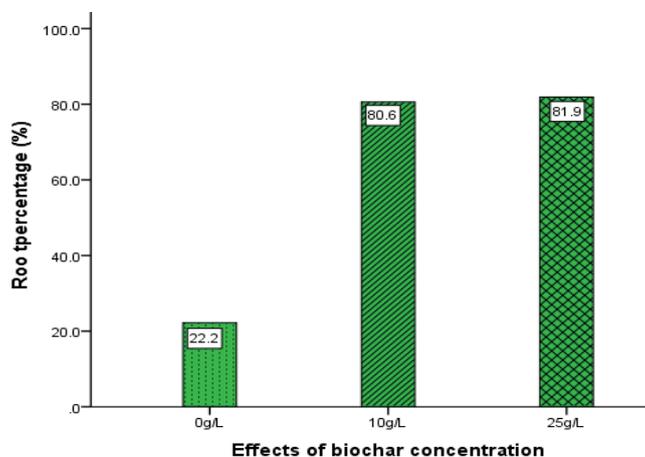


Figure 4. Effects of biochar amendment on rooting percentages of populus in hydroponic growth media.

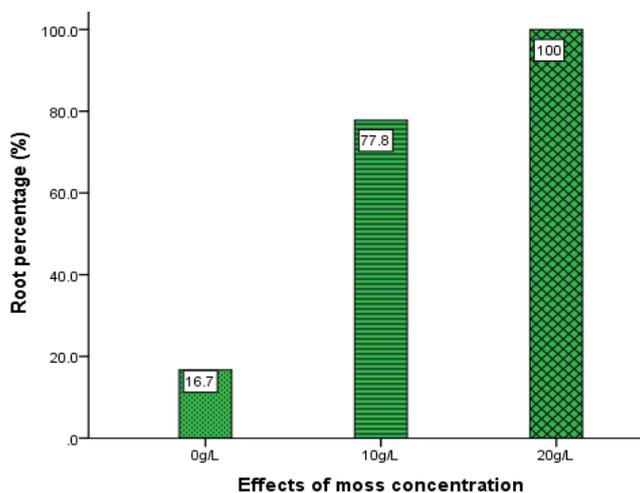


Figure 5. Effects of moss on rooting of populus in hydroponic growth media.

Table 3. Effects of moss on root (%), root number per cutting, root length per cutting, leaf numbers per cutting and shoot height per cutting of populus alba in hydroponic media. n=36 cuttings for each treatment.

Moss+moss	Root number per cutting	Root length per cutting(cm)	Leaf number per cutting	Shoot height per cutting(cm)
0 gm/L	3.00 ± 1.15a	14.33 ± 4.10a	11.67 ± 0.88d	27.00 ± 3.12b
10 g/L	13.33 ± 1.76b	9.33 ± 1.36a	18.67 ± 0.88bc	24.16 ± 3.17b
25g/L	16.33 ± 1.45b	7.16 ± 0.44a	19.67 ± 2.33bc	22.83 ± 2.25b
F	22.39	2.14	8.14	1.64
P value	0.002	0.20	0.02	0.27

Note: Mean ±SE followed by the same letters are not significantly different at (p<0.05).

Table 4. Effects of Azolla on root length, root number per cutting, leaf number per cutting and shoot height per cutting in hydroponic media.

Azolla+water	Root length per cutting(cm)	Root number per cutting	Leaf number per cutting	Shoot height per cutting(cm)
0 gm azola/L	10.83 ± 1.69a	6.00 ± 1.52a	12.67 ± 3.18a	26.66 ± 0.60ab
10 gm/L	9.00 ± 2.29a	12.00 ± 0.77b	35.00 ± 5.68b	28.00 ± 3.51ab
25 gm L	6.33 ± 0.44a	16.33 ± 1.33c	45.33 ± 2.60b	32.66 ± 1.16ab
F	1.85	18.175	16.991	2.12
P value	0.23	0.003	0.003	0.20

Note: Mean±SE followed by the same letters are not significantly different at (p<0.05).

study applications of 10g/l and 25g/l of Azolla showed significance difference in terms of number of roots per cutting and leaf number per cutting compared to without Azolla amendment (control), but not significant difference was observed in terms of root length per cutting and shoot height per cutting at p<0.05 as indicated in **Table 4**.

Effects of Temperature

Effect of temperature on the rooting process increased rooting performance may be due to increasing enzymatic activity for forming calluses, root primordia and adventitious roots of populus cuttings under controlled environment compared to outdoor uncontrolled treatments. When we see rooting periods of the populus inside the greenhouse and outside the greenhouse, the inside greenhouse rooted three days before than the populus cuttings which were planted outside the greenhouse in which other factors were maintained constant. Therefore, root% per cutting, root number per cuttings, leaf number per cuttings and shoot height per cutting showed significant differences at p<0.05. All below in the figure listed rooting and shooting parameters showed better results in inside the greenhouse as it was tested by independent sample t-test. As indicated in **(Figure 6)** effects of temperature inside the greenhouse showed better results on root percentage, root number, root length, shoot height, leaf number compared to effects outside the greenhouse in all rooting and shooting parameters as shown above by different colors. Variations in all parameters were significant at (p<0.05).

Effects of Substrate on Growth of Populus Cuttings

In the present study, in sand with biochar amendment, populus root length increased with increased rate of biochar

The increased growth in biochar treated plants as compared to control plants could be associated with the porosity of the biochar that enhances infiltration of water in pots. In the red soil with biochar combination, the numbers of roots per cutting and root number per cutting were higher compared to the sand with biochar treatments; this might be due to high nutrient availability in red soil compared to sand which is poor in nutrients relatively compared to red soil. In cases, red soil and sand, the addition of biochar has a significant effect compared to control (without biochar amendment) but higher in the red soil. Normally, biochar has charges and increases the cation exchange capacity of the growth media, supplementing nutrients to the plants.

As shown in **(figure 7)** the shoot height and root number per cuttings were significantly higher in red soil compared to sandy soil whereas root length showed better result significantly in sandy soil compared to red soil, but leaf number did not show any significant difference at p< 0.05.

Effects of Salt Stress on Propagations of Populus Cuttings

As observed from the result, effects of salt were pronounced compared to non-saline (control), from the experiment, for each treatment 36 cuttings which were planted at 10 gm salt per liter, 25 gm per liter and 0 gm per liter as control. After one week cuttings were started shooting slightly but, at the second week started drying and all cuttings were dried from second to third weeks. But the control group showed good rooting and shooting, and persisted to time of data collection and recording for analysis.

Correlation between Fresh and Dry Weight of Root and Shoot

There was significant positive correlation between fresh weights and dry weights of the populus with respect to root dry weight and shoot dry weight in red soil and sand substrate at p<0.05 **(Table 5,6)**.

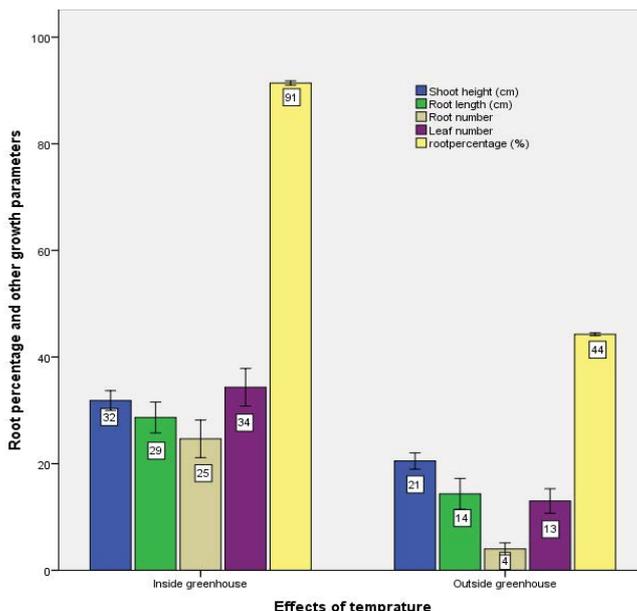


Figure 6. Effects of temperature on root percentage, roots number per cutting, root length per cutting, leaf number per cutting and shoot.

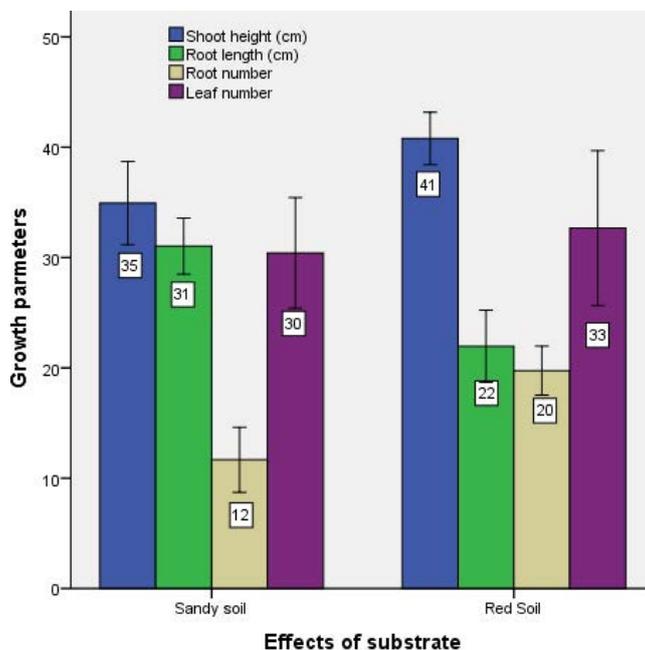


Figure 7. Effects of sand versus red soil substrate on the growth of populus cuttings.

Table 5. Correlation between fresh and dry weight of shoot and root grown in red soil.

Red Soil+biochar	SHFW	RFW	SHDW	RDW
SHFW	1			
RFW	0.58*	1		
SHDW	0.99**	0.55	1	
RDW	0.53	0.98**	0.52	1

Table 6. Correlation between fresh and dry weight of shoot and root grown in sand.

*Correlation is significant at 0.05 levels (2-tailed);
**Correlation is significant at 0.01 levels (2-tailed).

Sand+biochar	SHFW	RFW	SHDW	RDW
SHFW	1			
RFW	0.60*	1		
SHDW	0.96**	0.64*	1	
RDW	0.47	0.90**	0.56	1

DISCUSSION

The study showed that the mini-cutting propagation techniques can be utilized for mass and rapid production of the populus alba. The rooting capacity found in the study indicated that the mini-cutting management and induction treatments imposed significantly higher rooting ability of populus alba. The high root percentage (100%) was observed with 25 gm/L moss application, which shows that populus alba can be successfully mass propagated under greenhouse conditions and might show better survival in the field conditions after one year. Mini-cuttings planted in field conditions were demonstrating the efficiency of our hardening protocol. It is important to consider that growth amendments not only influence the percentage of rooting, it also may accelerate the onset of the rooting process and increase the number and quality of roots. Positive relationships between rooting and cutting length have been reported for a number of tropical tree species, including Triplochiton scleroxylon and Eucalyptus spp such relationships between cutting length and rooting ability are thought to reflect the importance of carbohydrate reserves stored in the stem, which support adventitious root development. ^[14,15]

In contrast, there was a lack of any pronounced relationship between cutting length and rooting ability in the much larger cuttings used in the present study, since stored food in small cuttings are sufficient. Only the leaf numbers per cutting, shoot height per cutting and branch number per cutting were significantly affected by cutting length at ($p < 0.05$), but rest of rooting and shooting parameters were not significantly affected by cutting length, as shown above in **(Table 1)**.

In the cutting thickness, there were rooting ability variations among three mini -cuttings namely thin, medium thickness and thick cuttings. The thin cuttings showed better performance in root percentage per treatment compared to medium and thick cuttings. The variation is significant at ($p < 0.05$) according to Tukey's multiple comparison test as it indicated in (Table 1). Root diameter and shoot diameter also showed significant variation at ($p < 0.05$).

The most critical factor affecting vegetative propagation of populus alba by stem cuttings was found to be the age of the stock plant. Cuttings taken from 3 month-old seedlings rooted well and produced more roots than cuttings obtained from older 1-2 years trees and show significant difference at ($p < 0.05$). Cuttings from 1-2year old stock plants did not root very well. Even if the variation is not significant for all growth parameters, cuttings taken from 3-month stock plants showed good performance in rooting and shooting parameters. The variation might be due to active cell division at the shoot meristematic tissue and root apices. Stem cuttings of populus spp taken from juvenile donor plants rooted better significantly than cuttings taken from mature donor plants indicating that age of donor plants is crucial for rooting. This study is line up with the studies, researchers conducted with other species have shown that the ability to form adventitious roots on stem cuttings decreases with increasing age of donor plant . Stem cuttings taken from juvenile donor plants are generally considered easy to propagate by cuttings due to their young ontogenetic, physiological and chronological age and possibly to its low production of secondary metabolites. Decrease in rooting potential of stem cuttings due to increased age of donor plants may be due to a decrease in the content of endogenous auxins or accumulation of inhibitory substances which inhibit rooting. ^[16,17]

In the Azolla application, great variation was observed among the concentration forms of application compared to un treated with Azolla or control group. Applications of 25 gm of azolla /L of water showed better results in root number per cutting and number of leaf per cuttings. Azolla is important to supply and fix atmospheric nitrogen to assist the growth of the plant as well as the rooting. Other studies in Cuttack in line with present study, demonstrated that use of Azolla enhanced crop yield and crop N uptake significantly as compared to treatments without Azolla. ^[18]

Addition of biochar significantly increased rooting percentage, root number, root length and shoot height of populus alba compared to treatments without biochar application control. Therefore, all parameters showed beneficial effects of biochar application over control. The significant effect of biochar on leaf number at the later stage of growth showed that the biochar may influence growth in the long-term. Similarly, it has been reported that when biochar is used as a soil amendment, it stimulates soil fertility and improves soil quality by increasing soil pH, increasing the ability to retain moisture, attracting more useful fungi and other microbes, improving the ability of cation exchange, and preserving the nutrients in the soil. ^[19] The biochar increased germination rate in all soils tested, enhanced plant height and green biomass of populus in peaty and clay soils. ^[20]

Biochar can increase the value of non-harvested agricultural products and promote the plant growth. ^[21] In the present study, in sand, water and red soil with biochar amendment, populus growth increased with increased rate of biochar was statistically significant at the fourth week at ($p < 0.05$).

In moss application, there was significant effect in increment of root number over the control in a special manner to other treatments. Additive values of moss were used as a source of oxygen to run metabolism fast in the propagation system, since microbial activity is assisted by oxygen. The highest root number per treatment was observed in this treatment, up to 100%, at 25 gm of moss per liters of pure water, there was complete rooting 36 cutting out of 36 cuttings initially planted. Other studies in India in lined with current study showed that regarding the effect of rooting media on the rooting percentage irrespective of PGRs used, the media containing sand and peat moss demonstrated greater percentage of rooting in comparison with other media. ^[22] Temperature affects the rooting and shooting performance of populus alba significantly. Since temperature is essential for growth of plants to optimize enzyme's activity. From a total of 36 cuttings planted inside greenhouse 33 cuttings were rooted after four weeks whereas outside of the green house in shade area only 16 cuttings per 36 planted cuttings rooted after four weeks. Shoot height was also similarly showed significantly better results inside the greenhouse as indicated in **(Figure 6)**.

In substrate related treatments, the current study was interested to compare suitability of the sand and red soil with combinations of biochar on rooting and shooting performance of populus alba. As the experimental results showed, rooting parameters in sand particularly root length showed relatively better results, while root number and shoot height shown good results with cuttings planted in red soil at $p < 0.05$. The reason that root length performs better in sand might be due to its porosity which enables roots to move easily to down ward and the highest root number and shoot height observed in red soil might be also due to availability of nutrients in soil which enables plants to generate more roots per plant compared to sandy soil. But the addition of the biochar amendment showed significant effect on the root length per cutting, root number per cuttings, leaf number per cuttings and shoot height of populus alba on both sand and red soil substrate media. (**Figure 8-11**)



Figure 8. EPhotos showing effects cutting length on the growth of populus. 10 cm 20 cm 30 cm.



Figure 9. Photos showing effects of cutting thickness on the growth of populus. Thin medium thick



Figure 10. Effects of temprature on the growth of populus.



Figure 11. Photos showin taking of during data collection.in lab.

Cuttings planted in salty media did not perform well as compared to the control. Thirty -six cuttings were planted at 10 gm salt per liter, 25 gm per liter and 0 gm per liter as control. After a week cuttings were started shooting slightly but, at the second week started drying and all cuttings were dried from second to third weeks. So, nothing was collected from dried cuttings, but the control group showed good rooting and shooting. From this experiment, it is possible to conclude that poplars are intolerant to salt stress. Other findings in line with current study showed that Poplars are intolerant to saline conditions and organic soils, then should be avoided, since they are generally waterlogged and very acidic.^[23] The salt-induced decrease in plant growth at high salinity might be due to energy losses through increased respiration and/salt pumping and biosynthesis of organic solutes for osmotic adjustment. Correlations between fresh and dry weights of root and shoot in substrate related treatments.^[24-26]

As observed in the results there was significant positive correlation in fresh weights and dry weights of root and shoot of Populus in substrate related treatments.

The positive correlation between fresh weight and dry weight shows that effectiveness of sand and red soil for growth of populus with combinations of biochar as amendment, since it shows positive Pearson coefficients of correlation(r) at ($p < 0.05$) and ($0 < r < 1$) for both sand and red soil substrate treatments as indicated in above (Table 6).

CONCLUSION

In plant propagation, multiplication at mass minimizes the cost of afforestation. A high survival rate with high percent of rooting is needed for effective seedling production. In the present study, vegetative propagation through mini cuttings of Populus was found to be very promising methods, which need to be communicated to farmers using Populus alba propagation. The size of cuttings was correlated with the number of roots and its paramount importance to teach foresters about it. Adding amendments to rooting media showed significant improvement. The formation of healthy roots and shoots with applications of biochar, Azolla and moss in hydroponic, and soil related media (red soil and sand) in the greenhouse showed that it is possible to propagate populus in hot climatic condition. Populus growth under outdoor conditions showed that as it could be successfully propagated by using mini cutting techniques.

Many poor farmers are interested in fast growing; multipurpose tree species for quick earning, therefore vegetative propagation may reduce the rotation time from seed germination to maturity for wood harvesting. Propagation through stem cuttings will be a rapid means for mass propagation of the species and will serve as an alternative to the use of seeds which have poor germination. It is also recommended that should be incorporated into agroforestry systems especially where soil is known to be degraded because of its ability to adapt to the environment easily and enable it to restore the degraded area sooner in reforestation.

Based on the findings of current study the following recommendations were forwarded:

Present study shows that techniques of culturing populus plants in hydroponic and soil media using cuttings enhance

the rate of seedling production. So, foresters can use this technique. Nowadays global warming is increasing dramatically due to deforestation and uncontrolled human activities, to restore the degraded ecosystem population should be planted, since it is fast growing and brings changes to the environment in short periods of time.

Populus plants should be planted at the sides of farmland and water bodies, since they are important to control soil erosion to sustain soil fertility and filter harmful chemicals by phytoremediation.

ACKNOWLEDGEMENTS

The authors' special word of thanks is to Ethiopian biodiversity institute, Harar biodiversity center and the studied area of local communities who were so kind and willing to supply us with information on Rooting and Shooting of Populus alba cuttings grown in Hydroponic and Soil Cultures.

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