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Effect of cutting position and indole butyric acid (auxin) concentration on rooting response of *Araucaria heterophylla*

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The effects of cutting position (tip, middle and basal) and concentration of indole butyric acid (IBA) (0, 5, 7.5 and 11 g/L) on root and shoot growth of *Araucaria heterophylla* were evaluated to develop a method for vegetative propagation for this tree species. Leaf number, number of adventitious roots, root length and survival rates were measured. These parameters were significantly influenced by the interactive effect of cutting position and hormonal concentration. Tip cuttings with the11 g/L IBA treatment showed higher root number, leaf number and root length whereas the other treatment combinations showed no root or shoot growth. Further, plant death was observed for the 5 and 7.5 g/L IBA treatments.

Key words: *Araucaria heterophylla,* cutting position, hormonal concentration, vegetative propagation.

INTRODUCTION

Araucaria heterophylla is a coniferous tree species with economic, social and environmental importance (Hazrat et al., 2006) in Africa. According to Bengoa (2000) and Azocar et al., (2005), *A. heterophylla* forests are a primary source of firewood, livestock shelter, construction materials and income for the Mapuche Pewenche community of southern Chile. *A. heterophylla* is also an important landscape tree species and is a dominant ornamental plant in urban areas of Ethiopia.

Despite these benefits, limited numbers of *A. heterophylla* seedlings are available in the market for gardeners with seedlings ranging in price from $43-195 USD as a result of few mature trees available and low seed production. A potential solution is the development of a vegetative propagation method (Pijut et al., 2011). Vegetative propagation can also be used to conserve superior genotypes, maintain valuable traits, reduce the high risk period when the tree is small and fragile, as well as reduce juvenile period (Hartmann et al., 2011; Gehlot et al., 2014). A successful system of vegetative production will allow producers to propagate plants throughout the year (Assis et al., 2004; Xavier et al., 2009).

An effective system of vegetative propagation is lacking for *A. heterophylla*. Propagules rooting is also influenced by endogenous and exogenous factors, such as

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Table 1. Comparison among cutting position (tip, middle and basal parts).

Dependent variable	Cutting position	Mean ± Std. Error
Root length	Tip	2.000±0.300 [a]
	Middle	2.220E-16±0.300 [b]
	Bottom	2.220E-16±0.300 [b]
Root number	Tip	1.333±0.173 [a]
	Middle	-4.626E-17 ±0.173[b]
	Bottom	-1.943E-16±0.173 [b]
Leaf number	Tip	1.167±0.127 [b]
	Middle	6.476E-17±0.127 [b]
	Bottom	-8.327E-17±0.127 [b]

Means with the same letter are not statistically different (P ≤ 0.05). The values represent mean ± S.E.

ontogenetic and physiological state, cutting position, humidity, temperature, light incidence, substrate, nutrition, hormonal balance and genetics (Li et al., 2009; Pijut et al., 2011). Furthermore, according to Ibironke (2017), rooting and shooting performance of cuttings are directly influenced by the types of growing media, thus, the selection and preparation of the medium is extremely important in terms of plant growth and quality because rooting performance depends on the type of medium used in propagation. One of the most effective and widely used auxins is indole-3-butyric acid (IBA), which has low toxicity, low mobility and high chemical stability (Hartmann et al., 2011). Thus, this research investigated the appropriate cutting position and auxin concentration (IBA) for rooting response of *A. heterophylla.* Thus, the research attempted to fill the gap by investigating the appropriate cutting position and auxin concentration (IBA) for rooting response of *A. heterophylla.*

MATERIALS AND METHODS

Study area

The experimental evaluation was conducted at the Teaching Nursery of Wondo Genet College of Forestry and Natural Resource (WGCF-NR), Ethiopia. The research site is located 263 km south of Addis Ababa and 13 km Southwest of Shashemene town. The campus is located on the eastern escarpment of the Ethiopian Rift Valley in the Southern Nation Nationalities and Peoples Regional State at 7° 6' N latitude and 38° 7' E longitudes with an altitude of 1700 m above sea level (Belaynesh, 2002). This region of Ethiopia is characterized by bimodal rainfall distribution with 1247 mm annual precipitation. The short rainy season ranges from March to May and the long rainy season lasts for five months from June to October. The mean monthly temperature is 19.5°C, with mean monthly maximum temperature of 26.3°C and mean monthly minimum temperature of 12.4°C (Amare et al., 2014).

Approach

The experiment included 12 treatment combinations. Three concentrations of IBA (5, 7.5 and 11 g/L) were evaluated for root induction with no auxin treatment used as a control, along with three cutting positions upper, middle and lower segments of *A. heterophylla* stems. Each treatment combination has three cuttings totaling 36 cuttings. Cuttings were harvested early in the morning from 6-year old *A. heterophylla* trees form (WGCF-NR). Cuttings were 30 cm long and each cutting had 38 leaves.

Cuttings were maintained under moist condition to prevent desiccation before treatment. Hormonal treatments were conducted by placing the 4 to 5 cm distal portion of each cutting in one of three IBA solutions for five minutes. Cuttings were placed in water for the control treatment and all cuttings were planted at the same time and date in 25 cm × 30 cm polyethylene tunnel. Polyethylene tunnels were placed under the lat-house in the nursery site of WGCF-NR to reduce direct sunlight. Treatments were arranged in a completely randomized design with three replications. Cuttings were watered regularly to maintain the humid environment needed for rooting. Growing medium consisted of a mixture of three soil types (forest, sand and clay soils) using a 3:2:1 ratio based on volume.

Newly developed number of leaves**,** number of roots, root length and visual quality of auxiliary shoots were recorded for each explant 50 days after planting. Data were analyzed using two factorial ANOVA test at 5% level of significance. The statistical analysis was done using SPSS version 16.0.

RESULTS

Effect of cutting position on root and shoot performance

The number of roots, root length and leave number varied for stem cutting positions (Table 1). The tip cuttings appeared green in color as compared to cuttings from the middle and basal part of the stem (Figure 1).

Effect of different IBA treatments on root and shoot performance

Cuttings treated with 11 g/L IBA showed significantly greater root lengths (Table 2). Control cuttings with no

Figure 1. Effect of cutting position on rooting and shoot performance of *A. heterophylla:* (A) Basal cuttings position treated with 11g/l IBA. (B) Tip cuttings position with no IBA treated.

Table 2. Comparison among different concentration of IBA.

Dependent variable	IBA concentration (g/L)	Mean±Std.Error
Root length	Control	1.975E-16±0.347[b]
	5	2.715E-16[b]±0.347[b]
	7.5	-9.861E-17[b]±0.347[b]
	11	2.667[a]±0.347[b]
Root Number	Control	-1.563E-16±0.200[b]
	5	4.048E-18±0.200[b]
	7.5	-7.406E-17±0.200[b]
	11	1.778±0.200[b]
Leaf Number	Control	7.615E-17±0.147[b]
	5	-1.637E-17±0.147[b]
	7.5	-1.849E-16±0.147[b]
	11	1.556±0.147[a]

Means with the same letter are not statistically different ($P \leq 0.05$). The values represent mean ± S.E

IBA treatment were alive and remained green without the production of root systems. Cuttings treated with 5 and 7.5 g/L IBA wilted and perished (Figure 2). The highest numbers of dead and wilted cuttings were recorded for the 7.5 g/L IBA treatment. The highest root number was recorded for 11 g/L treatment, whereas root systems failed to develop for the other treatments. Additionally, the 11 g/L showed a significantly higher leaf number.

Comparative effect of cutting position and different IBA concentration

The comparisons between cutting position and IBA treatment are presented in Table 3. Root development was only observed for the tip cuttings with the 11 g/L IBA treatment, which resulted in significantly higher mean root number, root length, and leaf number for this treatment

Figure 2. Effect different of IBA treatments on rooting and shoot performance of *A. heterophylla*. (A) Tip cuttings position with 11 g/l IBA treated. (B) Tip cuttings position with no IBA treated. (C and D) Middle and lower segments position treated with 11 g/L.

compared to other treatments.

DISCUSSION

Vegetative propagation has been an excellent method to support genetic improvement of forest species, allowing the reproduction of genetically superior individuals and providing greater uniformity of the plants (Sutton, 2002). The main aim of the study was to know whether growth regulators (IBA) would have any better response than the untreated control on root and shoot initiation of *A. heterophylla* tree species. This tree species were successfully propagated using tip cuttings with 11 g/L IBA (Tworkoski and Takeda, 2007), whereas the other cuttings did not respond to root even though they were treated with IBA treatments. Plant deaths were recorded during data collection from cuttings treated with auxin. Effect of auxin treatments on initiation and promotion of roots and shoots were found inconsistent in the cuttings. Different cutting positions had different response for root and shoot initiation. The rooting percentages observed in this study were low when compared to other ornamental species with established vegetative propagation protocols (Almeida et al., 2007; Negishi et al., 2014). However, performance of hormonal concentration has a positive correlation in promoting root and shoots initiation of the cuttings (Kala et al., 2017; Singh, 2017; Rambabu et al., 2017). In contrast to Eganathan et al., (2002) finding, no significant variation was observed in plant height and leave number per plant among the different treatments which might be attributed to the slow growth rate of the plant.

Conclusion and recommendation

This study is the first description of vegetative propagation in *A. heterophylla* using cutting positions and auxin concentrations. The study clearly indicated the feasibility of developing an *in vivo* propagation protocol for the plant from tip cutting as explants. The present established

Table 3. Comparison of the interaction effect between cutting position and different IBA concentration.

Dependent variable	Cutting position	Auxin concentration (g/L)	Mean±Std.Error
Root length	Tip	0 (Control)	1.483E-16±0.601[b]
		5	3.704E-16±0.601[b]
		7.5	-1.184E-15±0.601[b]
		11	8.000±0.601[a]
	Middle	0	2.961E-16±0.601[b]
		5	7.401E-17±0.601[b]
		7.5	2.961E-16±0.601[b]
		11	2.220E-16±0.601[b]
	Bottom	0 (Control)	1.480E-16±0.601[b]
		5	3.701E-16±0.601[b]
		7.5	5.921E-16±0.601[b]
		11	-2.220E-16±0.601[b]
Root number	Tip	0 (Control)	-1.726E-16±0.347[b]
		5	8.630E-17±0.347[b]
		7.5	-1.232E-16±0.347[b]
		11	5.333a±0.347[b]
	Middle	0 (Control)	-1.234E-16±0.347[b]
		5	-1.604E-16±0.347[b]
		7.5	2.465E-17±0.347[b]
		11	7.404E-17±0.347[b]
	Bottom	0 (Control)	-1.727E-16±0.347[b]
		5	8.635E-17±0.347[b]
		7.5	-1.234E-16±0.347[b]
		11	-5.674E-16±0.347[b]
Leaf number	Tip	0 (Control)	7.980E-17±0.255[b]
		5	2.429E-17±0.255[b]
		7.5	-7.282E-16±0.255[b]
		11	4.667±0.255[a]
	Middle	0 (Control)	6.785E-17±0.255[b]
		5	-9.869E-1±0.255[b]
		7.5	1.236E-17±0.255[b]
		11	2.775E-16±0.255[b]
	Bottom	0 (Control)	8.018E-17±0.255[b]
		5	2.467E-17±0.255[b]
		7.5	1.604E-16±0.255[b]
		11	-5.983E-16±0.255[b]

Means with the same letter are not statistically different ($P \leq 0.05$). The values represent mean ± S.E

vegetative propagation protocol for *A. heterophylla* has a considerable practical significance and the process has to be successfully exploited for large scale production of cloned plants for sustainable utilization and supply of this valuable ornamental plant. Shoot tip stem cuttings showed better performance and survival rate than other stem cutting. Therefore, vegetative propagation of *A. heterophylla* using other types of auxin, either alone or in combination, should be studied so as to identify the most suitable auxin type and/or combination for successful *in vivo* propagation of the plant. Further, the experiment should be tested on different soil mixtures to determine the best soil mixture for better root ability. The experiment should be further studied for extended period to detect the effects of the different auxin concentrations on the different stem cuttings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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