Journal of Applied Horticulture, 20(3): 233-236, 2018

The response of cutting position and auxin concentration on rooting and shooting of *Araucaria heterophylla*

Abera Tilahun[1]*, Begashaw Manahlie[2], Getachew Abebe[1] and Genet Negash[1]

*[1]Department of Dryland Forestry, Wondo Genet College of Forestry and Natural Resource, Hawassa University, Hawassa, Ethiopia. P.O.Box 128, Shashemene, Ethiopia. [2]Hawassa University, College of Natural and Computational Science, P.O.Box 05, Ethiopia. *E-mail: abt2003@gmail.com*

Abstract

This study was carried out to determine the effects of cutting position (tip, middle and basal) and different concentrations of IBA (IBA) (0, 5, 7.5 and 11g/L) on the rooting and shooting performance of *Araucaria heterophylla*. Two factorial experiments were conducted. Number of leaf, number of adventitious root and root length produced by cutting materials and the survival rates were measured. These parameters were significantly influenced by the interactive effect of cutting position and hormonal concentration. The interaction between tip cuttings and 11 g/L IBA concentration had higher root number, leaf number and root length as compared other interactions had not rooting and shooting performance on the cutting materials. Vegetative propagation of *A. heterophylla* through cuttings can be appropriately achieved by treating cuttings with IBA.

Key words: *Araucaria heterophylla*, cutting position, hormonal concentration

Introduction

Araucaria heterophylla is a coniferous tree with great economic, social and environmental importance (Hazrat *et al.*, 2006*)*. According to Bengoa (2000) and Azocar *et al.* (2005), *A. heterophylla* forest stands are a primary source of firewood, livestock shelter, construction materials and income for the Mapuche Pewenche community. The species makes a very bold statement in the landscape; in youth it is almost prehistoric in appearance and at maturity, it lends an exotic other-worldly look to vistas. Currently, *A. heterophylla* is becoming dominant ornamental plant in urban areas of Ethiopia and has been receiving a good attention by gardeners as one of the best ornamental plants in different landscapes for environmental, social, economic and aesthetic benefits.

Despite its multiple benefits, supplied numbers of *A. heterophylla* seedlings by gardeners is limited and the current unit cost of each seedling ranges between 43-195 USD (1290-5850 in Ethiopian currency) depending on the length of the seedling (authors personal observation). This implies that the current cost of the seedlings is high and unaffordable. This is because of the species is effectively reproduced only through sexual method and also individual plant require more than 30 years to bear seed (Azocar *et al.*, 2005). Hence, to address the issue a the potential alternative was the development of large scale vegetative propagation methods (Pijut *et al.*, 2011). Such a system has added advantage of conserving superior genotypes, maintaining valuable traits, and reducing the high risk period when the tree is small and fragile, and increase the availability of seedling resources of species and reducing juvenile period (Cuevas-Perez *et al.*, 1992). The development of a successful vegetative production system will also allow producers to propagate plants with the same characteristics as the parent trees (Eldridge *et al.*, 1994) and potential to engage in propagation throughout the year (Assis *et al.*, 2004; Xavier *et al.*, 2009).

Despite these potential and the presence of possible propagation options, there is a lack of research that investigates and assists the development of effective systems of vegetative propagation for *A. heterophylla*. Furthermore, since any propagule rooting is influenced by endogenous and exogenous factors, such as ontogenetic and physiological state, propagule type, cutting position, humidity, temperature, light incidence, substrate, nutritional and hormonal balance, as well as certain genes and enzyme activity (Basak *et al.*, 2000; Li *et al.*, 2009; Pijut *et al.*, 2011). Nevertheless, the probability of successful rooting can be enhanced with growth regulators, such as auxins. 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 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 study was conducted in the teaching nursery of Wondo Genet College of Forestry and Natural Resource (WGCFNR). It is located 263 km South of Addis Ababa, and about 13 km south west of Shashemene town. The Campus is found on the eastern escarpment of the Ethiopian Rift Valley in the Southern Nation Nationalities and Peoples Regional (SNNPR) state. Geographically, it is located within 7^0 6' N latitude and 38^0 7' E longitudes, and has an altitude of 1700 m.a.s.l. (Belaynesh, 2002). This part of the country gets a bimodal rainfall distribution (1247 mm precipitation annually) with the short rainy season ranging from March to May and the long rainy season lasts for five months from June to October. The mean monthly temperature is 19.5 ^{0}C, with mean monthly maximum temperature of 26.3 ^{0}C and mean monthly minimum temperature of 12.4 ^{0}C, respectively (Teshale, 2003, unpublished data).

Methodology: Various concentrations of IBA (0, 5, 7.5 and 11 g/L) were evaluated for root induction and shoot production from upper, middle and lower segment of *A. heterophylla* stems. Twelve treatment combinations were replicated 3 times. Each treatment combination had three cuttings, and thus a total of 36 cuttings. Cuttings were harvested early in the morning from 6 years old of *A. heterophylla* trees.

Cuttings used were 30 cm long and each cutting had 38 leaves. Cuttings were kept in moist condition so as to protect from desiccation before being dipped in the different hormonal concentrations. 4-5 cm distal portion of the cuttings basal were dipped for five minutes into different IBA concentrations except the control treatment one. After treating, all cuttings were planted at the same time and date in 25 x 30 cm area of polyethylene tunnel. Polyethylene tubes were placed under the latch-house in the nursery site of Wondo Genet College of Forestry and Natural Resource so as to reduce the direct sunlight from the cuttings. Complete random design was implemented. Cuttings were watered regularly to maintain the humid environment needed for rooting. The growing media were prepared from the mixture of three types of soil *i.e.* forest soil, sand soil and clay soil in the ratio of 3:2:1 were mixed, respectively. Mean number of leaves per explants, mean number of root per explants, mean length of roots per explants and visual quality of auxiliary shoots were recorded after nearly 50 days. For visual quality test, the proliferated shoots were ranked according to their colour and abnormality (yellowing of the leaves, formation of malformed leaves). Each experiment was carried out as a completely randomized design with 12 treatment combinations and were replicated 3 times. 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 rooting and shoot performance of *A. heterophylla*: The number of roots developed, root length and leaf number varied as influenced by stem cutting positions (Table 1). Furthermore, the cutting materials taken from the tip part appeared green in colour as compared to the cutting materials taken from the middle and basal part of the stem (Fig. 1).

Effect of different IBA treatments on rooting and shoot performance of *A. heterophylla:* Cuttings treated with 11 g/L have shown a high significance mean difference as comparared to other treatments in terms of root length. The cuttings treated with control (no IBA), 5 g/L and 7.5 g/L did not initiate root formation (Table 2). Cuttings treated with control (no IBA) were alive and appeared green without bearing a root system. Whereas, cuttings treated with 5 g/L and 7.5 g/L were wilted and dried (Table 2). The cuttings treated with 7.5 g/L recorded the highest number of dead and wilted cutting materials (Fig. 2). The highest root number was recorded in cuttings treated with 11 mg/L, whereas; the cuttings treated with other concentrations did not show initiation of root system (Table 2). The average mean values of leaf nubmer was higher in cuttings treated with 11 g/L IBA as compared to other treatments.

Table 1. Mean comparison among cutting position (Tip, Middle and Basal parts)

Dependent Variable	Cutting position	Mean
Root length	Tip	2.000 [a]
	Middle	2.220E-16 [b]
	Bottom	2.220E-16 [b]
Root Number	Tip	1.333 [a]
	Middle	-4.626E-17 [b]
	Bottom	-1.943E-16 [b]
Leaf Number	Tip	1.167 [b]
	Middle	6.476E-17 [b]
	Bottom	-8.327E-17 [b]

Means with the same letter are not statistically different ($P \leq 0.05$).

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

Comparative effect of cutting position and different IBA concentration: As shown in Table 3, significant variations in root length were found in the interaction between tip cutting positions with 11 g/L IBA concentration. However, there was no mean significant difference in the root length in the interaction with the middle and basal position of cuttings with all IBA concentration (*i.e.* no IBA, 5 g/L, 7.5 g/L and 11 g/L). In terms of root number, interaction of tip cuittings with 11 g/L IBA showed significant variation as compared to other treatments Furthermore, in terms of leaf number, it was found that tip cutting treated with 11 g/L IBA had a significant mean difference than other interactions.

Table 2. Mean comparison among different concentration of IBA

Parameters	IBA concentration (g/L)	Mean
Root length	Control	1.975E-16 b
	5	2.715E-16 b
	7.5	-9.861E-17 b
	11	2.667a
Root Number	Control	-1.563E-16 b
	5	4.048E-18b
	7.5	-7.406E-17 b
	11	1.778 a
Leaf Number	Control	7.615E-17 b
	5	-1.637E-17 b
	7.5	-1.849E-16 b
	11	1.556 a

Means with the same letter are not statistically different ($P \leq 0.05$).

Discussion

The results of the study showed that *A. heterophylla* tree species can propagated by cuttings. Interaction of the tip cuttings with the maximum IBA concentration (11g/L) showed significant results in terms of leaf number, root length and number. This finding is supported by the study of Tworkoski and Takeda (2007) conducted on the performance of cutting position with the same concentration of IBA. Thus, this finding indicated that

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

Table 3. Mean comparison of the interaction effect between Cutting position and different IBA concentration

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

Means with the same letter are not statistically different ($P \leq 0.05$).

cuttings from compact trees have less rooting performance than cuttings from young trees at the same hormonal concentration of 250 g/L. In addition, the research showed that when the IBA concentrations increased from 5 g/L to 11 g/L the rooting performance on the cutting materials increased. Accordingly, the highest number (70±3) of roots per shoot tip stem cutting was observed in 11 g/L auxin, the best auxin concentration for inducing a resilient root system. This result is also supported by Haissig (1970), Eliasson and Areblad (1984). Guerrero *et al.* (1999) studied effect of hormonal concentration on rooting performance and reported that higher endogenous auxin concentrations improved rooting. No significant variation was observed in plant height and leaf number per plant among the different treatments which might be accounted to the slow growth rate of the plant.

In conclusion, this study is the first description of vegetative propagation in *A. heterophylla* using different stem cuttings and auxin concentrations. The study clearly indicated the feasibility of developing an *in vivo* propagation protocol for the plant using shoot tip stem cutting as explants. The present established vegetative propagation protocol for *A. heterophylla* has a considerable practical significance and the process can 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 types (basal and middle) in all treatments. Vegetative propagation of *A. heterophylla* using other types of auxins, 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 rooting environment.

Acknowledgements

The authors are grateful to the Hawassa University, Wondo Genet College of Forestry and Natural Resource Research and Development office for providing both financial and technical assistance. We also sincerely thank all the Wondo Genet nursery site assistants for their valuable efforts.

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Received: November, 2017; Revised: November, 2017; Accepted: December, 2017