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Effects of cutting source and IBA concentration on shooting and rooting ability of *Pouteria adolfi-friederici* stem cuttings at polypropagator

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Abstract. *Bahru T, Derero A. 2023. Effects of cutting source and IBA concentration on shooting and rooting ability of* Pouteria adolfi-friederici *stem cuttings at polypropagator. Cell Biol Dev 7: 56-66. Pouteria adolfi-friederici* (Engl.) Baehni is an indigenous tree species extensively exploited for high-quality timber. Limited availability of seeds, intensive seed predation and recalcitrant seeds are bottlenecks for growing the tree species. Therefore, the present study aimed to evaluate the effect of cutting source and Indole-3-Butyric Acid (IBA) concentration on vegetative propagation of the species at a non-mist polypropagator. A total of 160 stem cuttings comprising four treatments (seedling cuttings + 0.2% IBA dose, seedling cuttings + 0.4% IBA dose, branch cuttings + 0.2% IBA dose and branch cuttings + 0.4% IBA dose) established using a completely randomized design for 180 days. The survival rate, rooting response, number of leaves/or buds, root number, and root length per rooted cuttings of seedling cuttings treated with 0.2% were significantly ($p<0.01$) the highest. Consequently, 70% of shooting and rooting success and higher mean root length (1.97+1.92 cm) were induced by seedling cuttings treated with 0.2% IBA concentration. In conclusion, seedling cuttings treated with 0.2% IBA concentration are more promising for vegetative propagation at a non-mist polypropagator. Hence, establishing mother stocks in nurseries and their proper management to serve as sources of juvenile stems will be essential for the successful macro-propagation of the species besides growing the species with seeds.

Keywords: IBA, *Kerero,* polypropagator, propagation, rooting

INTRODUCTION

The *Pouteria adolfi-friederici* (Engl.) Baehni is an indigenous multipurpose tree species belonging to the family Sapotaceae (Friis 2003). It is a very tall tree with a height of up to 45-50 m tall (Friis 2003) with an apparent straight bole nearly 16 m long (Maundu and Tengnäs 2005; Bekele 2007). The tree has a breast height diameter of up to 4 m (Fichtl and Adi 1994). According to Friis et al. (2011), it is one of the predominant characteristic species of Moist Evergreen Afromontane-Forest (MAF) in the vegetation types of Ethiopia. Therefore, the species is widely distributed in Shewa, Arsi, Welega, Illubabor, Keffa, Gamo Gofa, Sidamo and Bale floristic regions, Ethiopia (Friis 2003; Bekele 2007) within an altitudinal range of 1,350-2,450 m above sea level (Friis 2003). However, *P. adolfi-friederici* is extensively overexploited for its high-quality timber production in Ethiopia. Of course, the species is listed as Least Concern by the IUCN Red List of Threatened Species (IUCN 2020).

On the other hand, the limited availability of seeds and seedlings is a major practical problem for this species propagation. In addition, collecting, packing, and loading processed seeds or fruits, transportation, handling, and processing are additional practical problems to obtain fresh and mature seeds for further propagation and genetic conservation through seed storage. This is because the temperature and the moisture content affect the seed viability and germination potential. Consequently, fallen seeds or fruits are collected under mother trees, which are not fresh, well-matured, or easily attacked by insects or infected by disease. Fallen seeds or fruits, in turn, are eaten by seed predators (e.g., birds, rodents, smaller animals), decayed or rapidly germinated due to higher moisture availability. Seedlings and saplings are further easily free-grazed by herbivores, and there is little probability of being a mature tree. At the same time, there is a shift of highly suitable habitats for *P. adolfi-friederici* from the northern and central parts to the southern parts of Ethiopia (Tadesse et al. 2022).

Thus, to address the practical problems faced by *P. adolfi-friederici* species, vegetative propagation using stem cuttings might be a promising option to be investigated to reverse the current trend of species depletion and subsequent extinction. This is because vegetative propagation is an effective solution to a wide range of tropical tree species for mass propagation and plantation establishment within a tree improvement program (Negash 2010). This can be achieved within a short rotation period, especially for slow-growing plant species, for large-scale plantation expansion and harvesting of the intended end products. At the same time, due to its great economic contribution and the possibility of growing the species on-farm, it has been suggested as a potential candidate species for tree domestication under the agroforestry system or around homesteads. Similarly, an intraspecific morphological variation was identified using three main morphometric parameters (stem height, BDH and bole length) among the five populations in different natural forests of southwest Ethiopia (Seid and Mengesha, 2022). Such future research direction further helps conserve the species, establish seed orchards, improve its genetic and breeding base, improve productivity (fast growth and high timber quality and yield) and plantation expansion and development. In this respect, successful vegetative propagation using a low-technology non-mist polypropagator is one of the recent advances in the development of plant propagation for a wide range of plant species using leafy stem cuttings.

Nevertheless, various key factors contributed to the method's inappropriate application and limited practicality. Various sources reported that the shooting and rooting response of stem cuttings are influenced by sources of stem cuttings and the application of plant growth hormones for several plant species (Hartmann and Kester 1983; Negash 2010), particularly *P. adolfi-friederici* species (Derero et al. 2019). Of course, an earlier study conducted by Derero et al. (2019) confirmed that an encouraging result was obtained for the application of Indole-3-Butyric Acid (IBA) hormone on cutting sources (seedling and branch cuttings) in promoting shoot and root induction compared to the control treatment (without the application of IBA hormone). However, far earlier studies on various tree species reported that stem cuttings responded differently to shooting or rooting response or root number or root length due to different concentrations of IBA hormone (Leakey et al. 1982; Tchoundjeu and Leakey 2000, 2001; Tchoundjeu et al. 2002; Negash 2003; Tiwari and Das 2010; Asl et al. 2012; Kebede et al. 2013; Sevik and Guney 2013; de Souza et al. 2014; Elhaak et al. 2015; Junior et al. 2017; Phuyal et al. 2018; Pigatto et al. 2018; Tilahun et al. 2019; Vallejos-Torres et al. 2020; Olaniyi et al. 2021; Vallejos-Torres et al. 2021a, b; Khandaker et al. 2022; Zamora et al. 2022). Despite this, stem cuttings treated with different concentrations (doses) of IBA hormone have not yet been investigated. Further research is needed to select and apply the optimum IBA dose to improve the shooting and rooting success of *P. adolfi-friederici* stem cuttings. With this understanding, the present study aimed at investigating suitable cutting sources with the application of optimum IBA dose for the successful shooting and rooting ability of *P. adolfi-friederici* leafy stem cuttings at non-mist polypropagator.

MATERIALS AND METHODS

Description of the study site

This experimental study was conducted at Central Ethiopia Forestry Development Center (CEFDC), Ethiopian Forestry Development (EFD), Addis Ababa, Ethiopia. It is situated at Gurd Shola, Bole Sub-District. The experimental site (Nursery) is located in the Highland (*Dega*) Agro-ecology at 2,368 masl, between 37°04'E Longitude and 09°96'N Latitude. Addis Ababa has a mean annual rainfall of 1,000 mm and a mean monthly temperature of 20°C.

Description of the overall research

This research was conducted at a non-mist polypropagator in the nursery site with two factorial experiments. These were sources of stem cuttings and application of different hormone concentrations, each factor with two levels or treatments. Leafy young stem cuttings (hereafter referred to as stem cuttings) were derived from about a 1-year-old raised seedlings (hereafter referred to as seedling cuttings) and young branches of matured mother trees (hereafter referred to as branch cuttings). On the other hand, Indole-3-Butyric Acid (IBA) hormone with two concentrations (doses), i.e., 0.2 and 0.4%, was applied for better initiation of shoots and roots on stem cuttings. This hormone is selected since IBA is one of the most effective and widely used auxins, with low toxicity, mobility, and high chemical stability (Hartmann and Kester 1983). At the same time, between 0.2 and 0.4% IBA doses were considered since far more previous studies (Hartmann and Kester 1983; Negash 2010; Derero et al. 2019; Zamora et al. 2022) found out that these doses had successful results in vegetative propagation of a wide range of plant species. Thus, these doses were selected and applied to be more efficient in time, energy, and resources. After that, prepared stem cuttings were established at a non-mist polypropagator, and the research was conducted for 180 days. Finally, survived stem cuttings with developed buds, leaves and roots were transplanted to polyethylene pots at the nursery, and the survival count was supervised further for 120 days.

Polypropagator design and construction

A low-technology non-mist polypropagator used for the vegetative propagation of *P. adolfi-friederici* stem cuttings in this study was constructed from wooden frames (see Figure 1.D), following the construction design of Leakey et al. (1990), which was applied for tropical trees. An earlier study on *P. adolfi-friederici* stem cuttings conducted by Derero et al. (2019) also applied the same propagator construction design. Likewise, vegetative propagation on *Khaya ivorensis* A.Chev. (Tchoundjeu and Leakey 2000), *Lovoa trichilioides* Harms (Tchoundjeu and Leakey 2001), *Prunus africana* (Hook.fil.) Kalkman (Tchoundjeu et al. 2002), *Podocarpus falcatus* A.Cunn. ex Parl., 1868 (Negash 2003), *Juniperus procera* Hochst. ex Endl., *P. falcatus* and *Olea europaea* L. (Negash 2010), *P. africana* and *Syzygium guineense* (Willd.) DC. (Kebede et al. 2013) and *Picralima nitida* (Stapf) T.Durand & H.Durand (Olaniyi et al. 2021) were carried out using this design.

Preparation of stem cuttings

In this experiment, two sources of *P. adolfi-friederici* stem cuttings were applied for the macro-propagation experiment in the non-mist polypropagator. These were seedling cuttings and branch cuttings. About 1-year-old seedling cuttings were collected from naturally regenerated seedlings around and adjacent to mother trees. By contrast, branch cuttings were taken from young branches of mother trees. The size of stem cuttings ranged from 0.2-0.6 mm in diameter, with at least four nodes considered. Matured mother trees in good physical condition and physically normal appearance were considered during sample collections (Figure 1.A). Following this, the shoot tip of about 3-5 cm was removed from stem cuttings to get matured stem cuttings. After that, the top 2-3 leaves were maintained on the stem cuttings, and the remaining were removed. In this regard, leaf area also considerably affects the rooting response of stem cuttings (Tchoundjeu et al. 2002). The leaf area considerably affects photosynthesis through light interception and transpiration rate for optimizing water loss. Thus, following the recommendation from various sources, leaves were trimmed to 50 cm^2 to reduce leaf areas and hence the transpiration rate (Leakey et al. 1982; Tchoundjeu and Leakey 2000, 2001; Tchoundjeu et al. 2002). Following this, all stem cuttings were prepared in such a way and safely put in the icebox and brought from the field to the experimental site (Figure 1.B). During transportation, stem cuttings were checked to maintain leaves on stems and ensure moisture did not dry out. During establishing stem cuttings in the non-mist polypropagator, each stem cutting (with 2-3 trimmed leaves per stem cutting) was planted in the rooting (sand) medium. Accordingly, 10 cm of the size of the stem cutting (two nodes) was maintained below the sand level, while about 20 cm was kept above the sand level.

Preparation of growth hormones

Various studies reported that IBA is better for rooting stem cuttings than α-Naphthalene Acetic Acid (NAA) (Leakey et al. 1982; Hartmann and Kester 1983; Kassahun and Mekonnen 2012; Osman et al. 2013; Kebede et al. 2013; Phuyal et al. 2018; Sahoo et al. 2021). Indole-3-butyric acid is the most reliable in stimulating cuttings in rooting in many plant species and is non-toxic to plants over a wide concentration range (Hartmann and Kester 1983). Consequently, the IBA hormone is preferred and mostly applied in other propagation studies instead of other hormones (NAA, IAA). Therefore, IBA was selected in this particular study over other auxins to be successful in our result and to be more efficient in resource use. Furthermore, the previous preliminary finding by Derero et al. (2019) on *P. adolfi-friederici* stem cuttings showed that 0.4% IBA was effective for initiating roots and shoots compared to the control treatment (treatment without hormone application). At the same time, the same study on *P. adolfi-friederici* (Derero et al. 2019) further suggested that different concentrations of IBA should be considered to enhance shooting and rooting response. Hence, in this experiment, the levels of IBA concentrations (0.2% and 0.4%) were evaluated without the control treatment. However, 0.4% dose was considered the control from the two levels. A wide range of previous studies also reported that hormone-treated stem cuttings had higher rooting ability than the control treatment, i.e., treatment without application of hormone (Leakey et al. 1982; Hartmann and Kester 1983; Leakey et al. 1990; Kebede et al. 2013). With this understanding and experience, IBA was selected and applied at two concentrations (0.2 and 0.4%) since these concentrations are the most effective for shooting and rooting of various tropical tree species (Hartmann and Kester 1983; Negash 2010; Kebede et al. 2013).

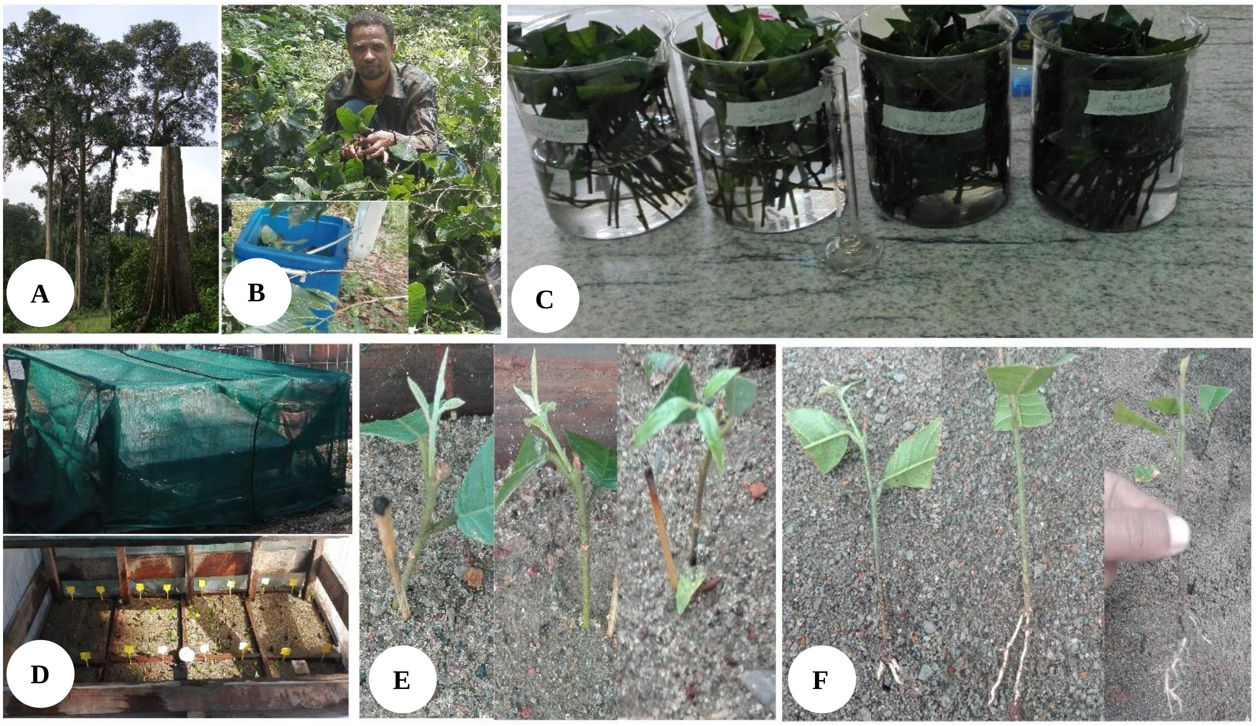

Figure 1. Vegetative propagation process of *Pouteria adolfi-friederici* stem cuttings at non-mist polypropagator: A. Selected mother tree for collection of stem cuttings; B. Preparation of enough stem cuttings with 2-3 leaves placed in the icebox; C. Stem cuttings dipped at 0.2% and 0.4% IBA concentrations for 24 hours; D. Established & tagged stem cuttings on sand medium at non-mist polypropagator and covered with shade-net; E. Shooting success of stem cuttings on sand medium; and F. Rooting success of stem cuttings on sand medium

Hence, the levels were dissolved and prepared with 96% ethanol, following the method on *S. guineense* and *P. africana* tree species by Kebede et al. (2013). Accordingly, 100 mg IBA will be diluted in 100 mL distilled water to prepare an IBA stock solution, and 1 mL 95% ethanol alcohol will be added to dissolve the hormone (Smith 2000; Trigiano and Gray 2000). After that, from the IBA stock solution, 20 mg 100 mL^{-1} and 40 mg 100 mL^{-1} solution were prepared to make 0.2% and 0.4% IBA solution, respectively (Trigiano and Gray 2000). Eventually, each cutting will be dipped into the respective IBA solution (either 0.2% or 0.4%) for 24 hours (Figure 1.C) and planted in the rooting medium (Figure 1.E), following the method by Hartmann and Kester (1983) and Derero et al. (2019).

Experimental design and treatment combinations

In this experiment, four treatment combinations (2 sources of stem cuttings and 2 IBA hormone concentrations at 0.2% and 0.4%) having 4 replicates for each treatment were applied. According to Hartmann and Kester (1983) and Hartmann et al. (2014) measurements, 0.2% IBA hormone equals 2,000 ppm or 2 g L^{-1} or 2,000 mg L^{-1} IBA concentration. However, this experiment did not include the control treatment (treatment without applying hormones). This is because stem cuttings had a better rooting response than the control treatment in the previous experiment application of IBA at 0.4% concentration (Derero et al. 2019). With this, the experiment was planned to evaluate the effect of different IBA concentrations (doses) on the shooting and rooting success of *P. adolfi-friederici* stem cuttings. Using Completely Randomized Design (CRD), 10 stem cuttings (repetitions) were prepared from each treatment to give a total of 160 (n=160) stem cuttings (10 stem cuttings x 2 stem cutting sources x 2 hormone concentrations x 4 replicates). All stem cuttings were dipped in the respective IBA solution, i.e., 0.2 or 0.4%, for 24 hours and established in a well-prepared non-mist polypropagator. The polypropagator was divided into 8 boxes (sections), and each box was again divided into two sub-sections. As a result, seedling cuttings and branch cuttings treated with the same IBA hormone (0.2 or 0.4%) were placed within the same box under sub-sections. With this, treated seedling cuttings and branch cuttings were tagged separately and planted in each sub-section.

On the contrary, stem cuttings treated with different concentrations of IBA hormone (0.2 or 0.4%) were established with different boxes separately to avoid contamination. Finally, stem cuttings were established on sand as a rooting medium at non-mist polypropagator (Figure 1.E). After that, watering every day early in the morning and late in the afternoon was conducted throughout the experimental period (180 days). Regular supervision was conducted to manage the relative humidity, daily temperature, and overall experimental conditions. Daily supervision was carried out since the establishment of stem cuttings in the non-mist polypropagator. Side by side, data on survivability, shooting and rooting ability, number of buds and leaves per stem cutting, number of roots per stem cutting and root length per stem cutting were collected. Following this, newly-rooted stem cuttings (Figure 1.F) having at least one root longer than 1 cm (Tchoundjeu and Leakey 2001) were uprooted, counted, marked to avoid double counting, and maintained in the rooting medium. The remaining unrooted or rooted stem cuttings of less than 1 cm were still maintained in the rooting medium. At the end of 180 days, all the survived stem cuttings were uprooted separately, and the root length and the total number of roots from each stem cutting were recorded. Eventually, all stem cuttings with developed shoots and roots were transplanted to pots for further survival study. Accordingly, seedling polyethylene pots (20 cm height and 16 cm diameter size) were pre-prepared with the required 2:1:1:1 soil mix ratio (2 forest soil: 1 local soil: 1 manure: 1 sand), and the pots were kept at nursery under shade. The stem cuttings were further supervised for an additional 120 days after being transplanted into nursery pots, and the cuttings' survival rates were recorded. Finally, statistical analyses on observed parameters, including survival rate, shooting response, rooting response, number of leaves and buds per cutting, number of roots per cutting and root length per cutting, were further performed using both descriptive (percentage, table, and graph) and quantitative statistics using SPSS version 27 software.

RESULTS AND DISCUSSION

The propagator environment

In this experiment, the test result confirmed that both temperature and relative humidity recorded in the propagator significantly ($p<0.001$) varied across months and daily duration. However, temperature and relative humidity significantly ($p<0.01$) but negatively correlated for the vegetative propagation of stem cuttings. A mean temperature and mean relative humidity of 22.4+4.75ºc and 75.5+6.50%, respectively, with 12 hours natural light and 12 hours dark was recorded within 180 days at non-mist polypropagator kept under 60% shade-net (Figures 2.A and 2.B). The mean minimum and maximum daily temperatures were recorded at 16.5+2.76ºc at 08:30 a.m. and 25.1+2.72ºc at 02:30 p.m., respectively. In the same way, the mean maximum (24.6+3.32ºc) temperature was recorded in May, while the mean minimum temperature (20.7+5.21ºc) was observed in December. By contrast, the highest mean daily relative humidity (78.8+2.81%) was recorded at 08:30 a.m., whereas the lowest mean daily relative humidity (72.5+8.11%) was recorded at 02:30 p.m. The highest (78.2+3.76%) and the lowest (72.2+4.73%) mean monthly relative humidity was recorded in April and May, respectively.

Survival rate of stem cuttings

In our finding, the mean survival rate differed significantly between cutting sources ($F=59.91$; $p<0.001$) and IBA doses ($F=4.15$; $p=0.043$), as indicated in Table 1. In the same way, a significant ($F=8.13$; $p<0.01$) interaction effect on survival rate was also observed between cutting sources and IBA doses. A 2-tailed Pearson correlation analysis further confirmed that the survivability was significant (p<0.01) and positively associated with other recorded parameters (Table 2). Out of 160 stem cuttings planted in the non-mist polypropagator, 50 (31.3%) stem cuttings survived at the end of 180 days (Figure 3). However, 40% survived at the end of 30 days and gradually declined. During this time, 1-6 leaves and 1-5 buds in each stem cutting were developed.

Nevertheless, some developed leaves and buds on stem cuttings were gradually wilted and died out during the experimental period. The developed buds also grew to the leaves or wilted and eventually died. Some other stem cuttings survived for a few months or the entire experimental period without initiation of buds and leaves or even adventitious roots. A few stem cuttings also developed adventitious roots without initiation of buds and leaves, or stem cuttings developed buds and leaves without roots. Figure 4 shows the developed adventitious roots on some stem cuttings.

By the age of 180 days, 30 (60%) stem cuttings survived by applying 0.2% IBA concentration, while 0.4% IBA level contributed to a 40% survival rate. However, stem cuttings treated with both IBA doses (0.2 and 0.4%) showed a similar declining trend (except seedling cuttings treated with 0.2% IBA dose) in survival rate across the entire experimental period (30 to 180 days). On the other hand, stem cuttings treated with 0.2% IBA level contributed to the survival of 28 (56%) seedling cuttings compared to seedling cuttings treated with 0.4% IBA dose, which accounted for 32% (16 seedling cuttings) at the age of 180 days. By contrast, branch cuttings treated with 0.4% IBA dose had a better survival count (8% or 4 branch cuttings) than 0.2% IBA application (4%) in the same period.

In conclusion, the present result indicates that seedling cuttings treated with 0.2% IBA concentration considerably increased the survival rate compared to those treated with 0.4% IBA or branch cuttings treated with 0.2% or 0.4% IBA dose.

Shooting response of stem cuttings

In this study, the analyses of variance further showed that sources of stem cuttings significantly (F=34.25; p<0.001) affected the shooting success of *P. adolfi-friederici* stem cuttings at the non-mist polypropagator. Application of IBA concentrations and the interaction between both factors (cutting sources and IBA doses), however, did not affect (p<0.05) the shooting response of stem cuttings. However, the shooting response was significant (p<0.01) and positively correlated with other recorded parameters (Table 2). In this regard, *P. adolfi-friederici* stem cuttings responded differently to applying IBA concentrations at the non-mist polypropagator. The rate of shooting response (initiation and development of buds and leaves) was not observed in the first week of planting at the non-mist polypropagator. However, the first initiation of buds was observed during the second week of planting on 6 stem cuttings, which accounted for 9.8%. This was followed by the third and fourth weeks after establishing 8 stem cuttings (13.1%) and 6 stem cuttings (9.8%). Of these, 83.3, 75 and 83.3% of seedling cuttings treated with 0.2% IBA application were initiated buds during the 2^{nd}, thi^{rd}, and fourth weeks, respectively. Overall, 16 stem cuttings (28.6%) out of the total survived cuttings showed shooting response at 120 days. This was followed by 28.1, 25.9, and 12.7% shooting success at the end of 30, 90, and 150 days after planting. Of these, stem cuttings treated with 0.2% IBA dose induced 27.5% of the shooting response compared to 0.4% IBA application (17.5% shooting ability) at the age of 30 days (Figure 4). Application of 0.2% IBA concentration on stem cuttings further induced a 22.5% shooting response in seedling cuttings, while 0.4% IBA dose induced 12.5% seedling cuttings in a given period. Similarly, branch cuttings treated with 0.2% or 0.4% IBA concentrations had lower shooting percentages (5% each) than seedling cuttings. Conversely, out of the total seedling cuttings (i.e., 40 samples) treated with 0.2% IBA dose, 70% showed rooting success throughout the period (Figure 5). By contrast, applying 0.4% IBA dose on seedling cuttings induced a 47.5% shooting response, while branch cuttings treated with 0.2% and 0.4% IBA concentrations contributed 32.5% in the same period and samples. Almost in all the treatments, the shooting response was steeply reduced across the entire vegetative propagation period at the non-mist polypropagator. In conclusion, our finding confirmed that seedling cuttings treated with 0.2% IBA concentration substantially improved shooting response as opposed to seedling cuttings treated with 0.4% IBA dose or branch cuttings treated with 0.2% or 0.4% IBA dose.

Number of leaves and buds on stem cuttings

Overall, the mean total number of leaves and buds developed across the entire duration significantly varied between cutting sources (F=37.17; p<0.001), IBA doses (F=4.01; p<0.05) and their interaction effect between them (F=7.29; p<0.01) as indicated in Table 1. Despite this fact, a correlation analysis affirmed that the total number of leaves and buds was significant (p<0.01) and positively related to other dependent variables parameters (Table 2). A similar increasing trend was observed among all the treatments despite the variation in the total number of leaves and buds developed between 30 to 180 days after establishment at non-mist polypropagator (Figure 6). The highest number of leaves and buds was recorded by seedling cuttings treated with 0.2% IBA concentration, which accounted for 18-111 total numbers. This was followed by seedling cuttings treated by 0.4% IBA level with 9-59 total numbers of leaves and or buds. In contrast, branch cuttings treated with 0.2% or 0.4% IBA concentrations had relatively lower (2-18) total numbers of leaves and buds. In conclusion, our finding approved that seedling cuttings treated with 0.2% IBA concentration significantly enhanced the total numbers of leaves and buds as opposed to seedling cuttings treated with 0.4% IBA dose or branch cuttings treated with 0.2% or 0.4% IBA dose.

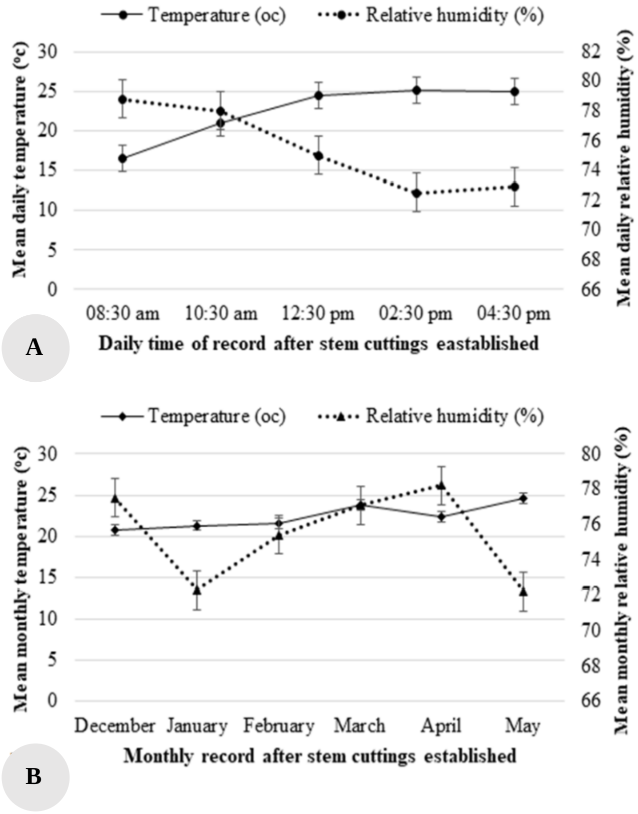

Figure 2. Mean daily (A) and mean monthly (B) temperature and relative humidity record of *Pouteria adolfi-friederici* stem cuttings after established at the non-mist polypropagator

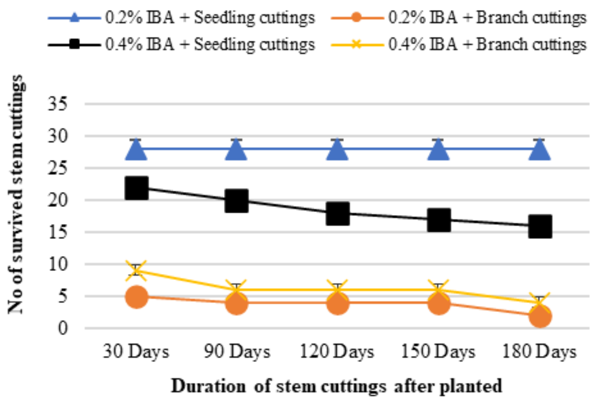

Figure 3. Survival ability of *P. adolfi-friederici* stem cuttings at non-mist polypropagator during the entire vegetative propagation period. Seedling cuttings were 1-year-old raised seedlings at the nursery, while branch cuttings were young branches of matured mother trees collected from mother trees at the field

A B C D E

Figure 4. Rooting ability of *P. adolfi-friederici* stem cuttings at non-mist polypropagator. Rooting success of stem cuttings in A, B, and C indicated stem cuttings treated with 0.2% IBA concentrations for 24 hours, while stem cuttings in D & E showed stem cuttings dipped in 0.4% IBA levels for 24 hours

Table 1. Effects of cutting sources, IBA concentrations and their interaction on vegetative propagation of *Pouteria adolfi-friederici* stem cuttings at non-mist polypropagator

Source of variation	Survival rate	Shooting response	Rooting response	No of leaves &/or buds/cutting	No roots/cutting	Root length/cutting
Cutting sources	59.91***	34.25***	55.91***	37.17***	43.34***	49.88***
IBA concentration	4.15*	1.20^{ns}	3.31^{ns}	4.01*	16.24***	7.42**
Cutting sources*IBA concentration	8.13**	3.35^{ns}	6.90**	7.29**	17.75***	9.48**
R^2	0.316	0.199	0.298	0.237	0.331	0.300

Note: $F^{p\text{-value}}$; significance levels were *** -p<0.001; ** -P<0.01; * -P<0.05; ^{ns} -non significant

Table 2. Pearson correlation analysis of recorded parameters on vegetative propagation of *Pouteria adolfi-friederici* stem cuttings at the non-mist polypropagator

	Survival rate	Shooting response	Rooting response	No of leaves & buds/cutting	No roots/cutting	Root length/cutting
Survival rate						
Shooting response	0.834**					
Rooting response	0.956**	0.820**				
No of leaves & buds/cutting	0.774**	0.813**	0.740**			
No roots/cutting	0.661**	0.506**	0.671**	0.532**		
Root length/cutting	0.771**	0.625**	0.783**	0.609**	0.927**	

Note: **correlation is significant at the 0.01 level (2-tailed)

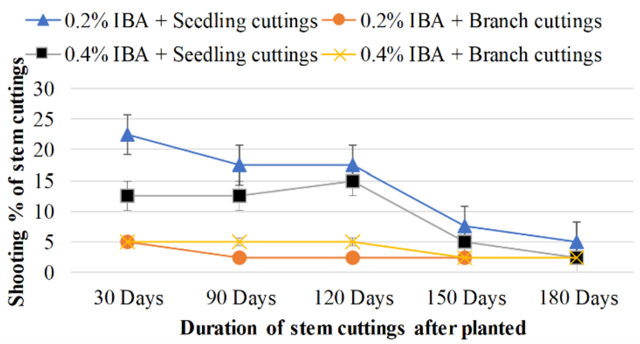

Figure 5. Shooting response of *Pouteria adolfi-friederici* stem cuttings at non-mist polypropagator during the entire vegetative propagation period

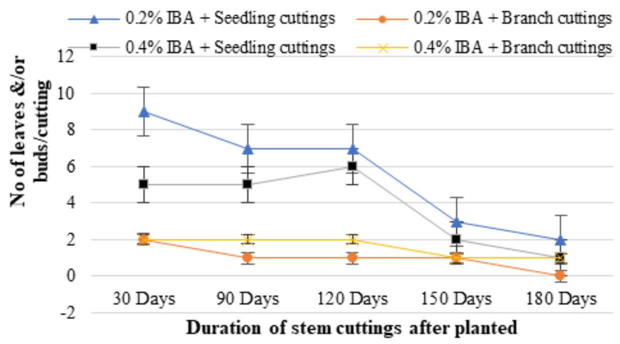

Figure 6. Number of leaves and buds on *Pouteria adolfi-friederici* stem cuttings at non-mist polypropagator during the entire vegetative propagation period

Rooting response of stem cuttings

The present finding revealed that the rooting percentage of *P. adolfi-friederici* stem cuttings strongly varied by cutting sources (F=55.91; p<0.001), although the application of different concentrations of IBA was insignificant (F=3.31; p<0.05). On the other hand, the interaction effect also showed a significant difference (F=6.90; p<0.01) between cutting sources and IBA concentrations. Adventitious roots sprouted and developed on stem cuttings despite its too-late response (120 days after planting) compared to the shooting response. Of the total seedling cuttings (i.e., 40 samples), 70% and 40% rooting success were induced by 0.2% and 0.4% IBA concentrations across the entire propagation period (Figure 7). On the contrary, only a 15% rooting response in branch cuttings was observed by 0.2% and 0.4% IBA concentrations in a given period and samples. Conversely, 35.7% of the rooting response was observed out of the survived cuttings at the end of 120 days after establishment. This was followed by a 36.4% rooting response of *P. adolfi-friederici* stem cuttings at 150 days of planting. However, 47 stem cuttings (94%) out of the survived cuttings showed rooting response in the entire propagation period. Furthermore, a 2-talied Pearson correlation analysis confirmed a significant (p<0.01) and positive association between rooting response and other recorded variables (Table 2). In conclusion, this result affirmed that seedling cuttings treated with 0.2% IBA concentration significantly promoted rooting success compared to seedling cuttings treated with 0.4% IBA dose or branch cuttings treated with 0.2% or 0.4% IBA dose.

Mean root number per rooted cutting

The mean root number per rooted cutting was strongly influenced by the sources of stem cuttings (F=43.34; p<0.001), application of IBA doses (F=16.24; p<0.001) as well as their interaction effect between them (F=17.75; p<0.001) as shown in Table 1. The highest mean root number per rooted cutting (7.45+8.19 cm) was recorded in seedling cuttings treated with 0.2% IBA dose (Figure 8). The corresponding mean root number of rooted cuttings applied at 0.4% IBA dose for seedling cuttings and branch cuttings was (1.80+2.78 cm) and (0.18+0.59 cm), respectively. On the contrary, several roots per rooted cuttings were counted for branch cuttings (0.05+0.22 mean root number) treated with 0.2% IBA concentration. The test result also reported that strong significant differences (p<0.001) were observed for cutting sources, IBA concentrations and the interaction effect between both factors on recorded mean root number per rooted cutting. At the same time, a significant (p<0.01) and positive correlation between the mean root number and other variables was observed (refer to Table 2). The present finding supported that those treated with 0.2% IBA concentration considerably increased mean root number per rooted cutting than seedling cuttings treated with 0.4% IBA or branch cuttings treated with 0.2% or 0.4% IBA doses. Furthermore, the multiple comparisons using the Post Hoc test showed that seedling cuttings treated with 0.2% IBA dose significantly varied (p<0.001) in the mean root number per rooted cutting from seedling cuttings treated with 0.4% IBA dose, branch cuttings treated with 0.2% and 0.4% IBA dose.

Mean root length per rooted cutting

The test statistics in this analysis confirmed that root length per cutting was significantly affected by cutting sources (F=49.88; p<0.001), IBA concentrations (7.42; p<0.01) and the interaction between the two factors (9.48; p<0.01) on rooted stem cuttings. In our finding, the longest mean root length (1.97+1.92 cm) was measured for seedling cuttings treated with 0.2% IBA concentration compared to branch cuttings (0.04+0.24 cm) treated with the same dose (Figure 9). The longest and the shortest root length was also 2.34 and 1.59 cm, respectively, for seedling cuttings treated with 0.2% IBA dose. The corresponding mean root length attained was 1.24 and 0.49 cm for seedling cuttings treated with 0.4% IBA dose. By contrast, the shortest mean root length per rooted cutting was recorded from branch cuttings (0.04+0.24 cm) treated with 0.2% IBA concentration, followed by branch cuttings (0.11+0.40 cm) treated with 0.4% IBA doses. In conclusion, our result confirmed that seedling cuttings treated with 0.2% IBA concentration significantly promoted mean root length per rooted cutting as opposed to seedling cuttings treated with 0.4% IBA dose or branch cuttings treated with 0.2% or 0.4% IBA dose. In the same manner, the Post Hoc test also showed that except for the comparison between seedling cuttings treated with 0.4% IBA dose and branch cuttings treated with 0.4% IBA dose, all treatments significantly differed (p<0.05) in the mean root length per rooted cutting.

Survival rate of stem cuttings at nursery

At the end of 120 days, the survivability of *P. adolfi-friederici* stem cuttings transplanted on polyethylene pots at the nursery was recorded. Out of the total transplanted stem cuttings, a 48.9% survival rate of stem cuttings was recorded at the age of 120 days of nursery lifespan. Of these, 69.6% of seedling cuttings, compared to branch cuttings, successfully showed higher survival potential. During this stage, the researcher's regular observation of stem cuttings indicated that seedling cuttings had good morphological characteristics (number of buds and leaves, size of leaves, number of branches, number of roots and fibrous roots and size of roots) over a few branch cuttings. Seedling cuttings were grown uniformly and showed limited morphological variation during the entire nursery's lifespan. In contrast, most of the branch cuttings showed stunted growth or wilted and failed to survive and eventually died, while only a few cuttings were able to survive well during this period.

Discussion

The low-technology non-mist polypropagator is one of the most promising options to propagate *P. adolfi-friederici* stem cuttings using the vegetative propagation method. This system provides suitable propagation conditions (optimum temperature, relative humidity and suitable propagation substrate) in maintaining a favorable environment for successful survival rate, shooting and rooting ability, and the required number and length of roots on stem cuttings. Our analysis showed that the propagator's temperature and relative humidity significantly (p<0.001) varied across months and daily duration. In this regard, the shooting and rooting response of *P. adolfi-friederici* stem cuttings were observed in the entire propagation period (180 days), when the temperature in the propagator was maintained from 20 to 25°C. The influence of temperature in the propagator on the shooting and rooting ability of stem cuttings was consistent with other findings reported previously by Leakey et al. (1982) and Derero et al. (2019). Other studies further discussed and confirmed our findings (Leakey et al. 1982; Sevik and Guney 2013; Caplan et al. 2018; Derero et al. 2019). Even the higher temperature (28-30°c) reported by Shekhawat and Manokari (2016), 26-34°c by Zamora et al. (2022) and 28-35°c by Vallejos-Torres et al. (2020 and 2021a,b) was maintained for suitable shoot and root induction at a relative humidity of about 80-90% (Shekhawat and Manokari 2016) or above 80% (Zamora et al. 2022). This is because the higher temperature observed during the propagation period can be compensated by keeping a higher percentage of relative humidity. This, in turn, helps to compensate for the faster loss of water from stem cuttings through evapotranspiration and thereby maintains the process of photosynthesis as opposed to the slower absorption of water by the initially incipient roots. Similarly, relative humidity (75.5%) maintained in the propagator was also in line with other findings in earlier studies despite a slightly higher than 75% relative humidity (Vallejos-Torres et al. 2020 and 2021a, b), around 80% (Pigatto et al. 2018), 85% (Leakey et al. 1982; Kebede et al. 2013) and approximately 100% (Junior et al. 2017). In contrast to these and the present findings, the relative humidity was maintained at a wider range (60-95%) since the establishment of the cannabis stem cuttings up to the end of the experiment (Caplan et al. 2018).

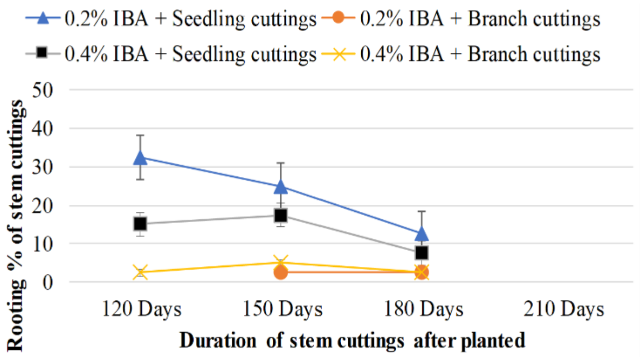

Figure 7. Rooting response of *Pouteria adolfi-friederici* stem cuttings at non-mist polypropagator during the entire vegetative propagation period

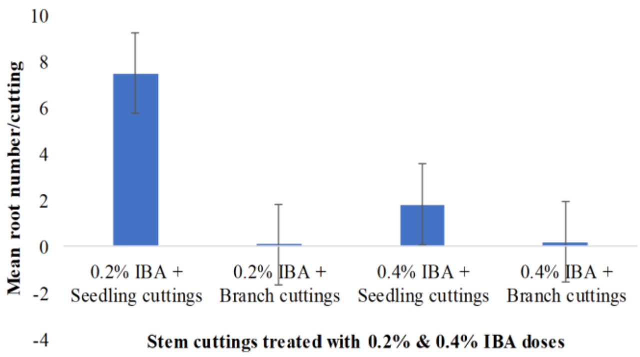

Figure 8. Mean root number per rooted cutting of *Pouteria adolfi-friederici* at non-mist polypropagator during the entire vegetative propagation period

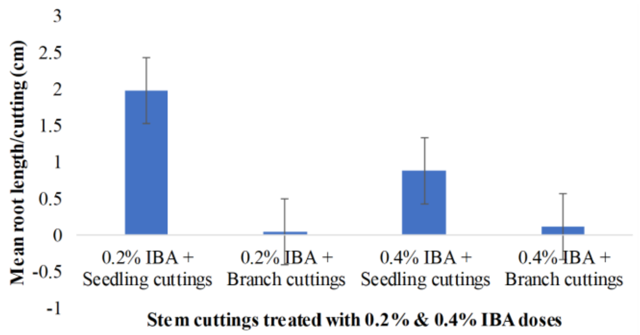

Figure 9. Mean root length per rooted cutting of *Pouteria adolfi-friederici* at non-mist polypropagator during the entire vegetative propagation period

On the other hand, seedling cuttings retained most of the leaves compared to branch cuttings that shed their leaves during the entire shooting period, which probably suggests higher survivability, bud initiation, shooting, and rooting response of seedling cuttings. This is because most of the leaves retained on seedling cuttings might contribute to a higher rate of photosynthesis instead of water stress by enhancing the rate of evapotranspiration. By contrast, branch cuttings reduced water stress by minimizing evapotranspiration rate by shedding most of their leaves despite reducing the photosynthesis rate on leaves. This is because the present experiment was conducted during the hot and dry months (December to May), so the higher temperature outside the non-mist polypropagator contributed to moisture stress and hence higher evapotranspiration inside the propagator. Nevertheless, such an effect does not seem to be influenced by the shedding of most leaves from branch cuttings compared to retained leaves on seedling cuttings. This is because much effort was taken to maintain the temperature and relative humidity to the required level at the non-mist polypropagator by installing a 60% shade net. At the same time, regular supervision and watering of stem cuttings early in the morning and late in the afternoon throughout the entire propagation period was carried out. In conclusion, the present finding proposes that the temperature and relative humidity are optimum for the propagation of the species at the non-mist polypropagator.

The results of the present study suggested promising options for a simple, effective, and rapid vegetative propagation protocol for *P. adolfi-friederici* stem cuttings. This, in turn, is an important step towards conservation and genetic improvement of the species and promoting small- and large-scale plantation development under plantation forest or agroforestry systems. This is because, nowadays, the species is confronted with various major practical problems, including fresh and mature seed provision, poor seed viability and storage, and limited means of propagation. In line with this, there is a decline of suitable habitats associated with climate change and, thereby, a subsequent shift of highly suitable habitats of *P. adolfi-friederici* from the northern and central parts to the southern parts of Ethiopia (Tadesse et al. 2022). However, an intraspecific morphological variation among the different *P. adolfi-friederici* populations in different natural forests of southwest Ethiopia (Seid and Mengesha 2022) create a good opportunity for its genetic resource conservation and improvement options. Our finding showed that cutting source ($p<0.001$), application of IBA concentration ($p<0.05$ except shooting and rooting response) and the interaction between the source of stem cuttings and IBA concentration ($p<0.01$ except for shooting response) considerably influenced survival rate, shooting and rooting success, number of leaves and buds, root number and root length of stem cuttings throughout the propagation period, i.e., 180 days. Initially, higher survived stem cuttings (31.3%) in our study were recorded compared to 13.8% by Derero et al. (2019). In turn, the first initiation of buds was observed during the second and third weeks, followed by the rooting response at the age of 120 days in this study, while bud initiation after the sixth week and rooting response on 195 days were observed in the previous research (Derero et al. 2019). At the same time, 70% shooting and rooting success with 0.2% IBA dose in the present study was 5 times higher than 13.8% shooting and rooting response with 0.4% IBA dose in the former investigation (Derero et al. 2019). All these findings suggest that the application of optimum IBA concentration (0.2%) on selected suitable cutting source (seedling cuttings) in our investigation resulted in earlier, faster and higher survivability, bud initiation, shooting and rooting response, greater number of leaves and roots and longer roots on *P. adolfi-friederici* stem cuttings as opposed to seedling cuttings treated with 0.4%. Consistent with the present study, several investigators have also reported that optimal IBA concentration considerably contributed to the successful survival rate, shooting and rooting response of stem cuttings (Leakey et al. 1982; Kebede et al. 2013). Furthermore, our findings confirmed that *P. adolfi-friederici* stem cuttings considerably vary in response to survival rate, shooting, and rooting success due to various concentrations of IBA and cutting sources. During vegetative propagation of *P. adolfi-friederici* at polypropagator, stem cuttings responded differently to different levels of IBA hormone (0.2 and 0.4%). The experimental result in the present study indicated that seedling cuttings treated with 0.2% IBA concentration had far better survival rate, shooting, and rooting response than seedling cuttings treated with 0.4% IBA concentration. That probably suggests that less IBA concentration (0.2% IBA), instead of a higher concentration (0.4%), easily stimulates and triggers young plant parts, i.e., seedling cuttings; the response was inhibited at more IBA doses (0.4% IBA). Our results are also consistent with those of many other tree species, *P. falcatus* (Negash 2003), *Bougainvillea* sp. (Asl et al. 2012), *Melissa officinalis* L. (Sevik and Guney 2013), *Prosopis alba* Griseb. (de Souza et al. 2014), *Rosmarinus officinalis* L. (Elhaak et al. 2015), *Couroupita guianensis* Aubl. (Shekhawat and Manokari 2016), *Ficus benjamina* L. (Topacoglu et al. 2016), *Theobroma cacao* L. (Junior et al. 2017), *Stevia rebaudiana* (Bertoni) Bertoni (Pigatto et al. 2018), *Coffea arabica* L. (Vallejos-Torres et al. 2020), *P. nitida* (Olaniyi et al. 2021), *Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg. (Vallejos-Torres et al. 2021a), *Manilkara bidentata* (A.DC.) A.Chev. (Vallejos-Torres et al. 2021b), *Syzygium samarangense* (Blume) Merr. & L.M.Perry (Khandaker et al. 2022) and *T. cacao* (Zamora et al. 2022), in which stem cuttings treated with lower IBA concentration had higher root numbers, root length and rooting response than higher IBA concentration. Even applying other plant growth hormones (BAP or NAA) instead of IBA further triggered and enhanced better shooting and rooting ability and increased root length and numbers at lower concentrations compared to higher concentrations. Unlike the present study and the aforementioned earlier studies, successful rooting ability, root number and root length in *Embelia tsjeriam-cottam* (Roem. & Schult.) A.DC. (Tiwari and Das 2010), *Zanthoxylum armatum* DC. (Phuyal et al. 2018) and

Araucaria heterophylla (Salisb.) Franco (Tilahun et al. 2019) stem cuttings were observed as cuttings treated with higher IBA concentration as opposed to lower concentration. Likewise, seedling cuttings treated with 0.4% IBA concentration further triggered better shooting and rooting responses than branch cuttings treated with 0.2% and 0.4% IBA concentrations. This might be further associated with younger plant parts responding better than matured (older) parts to applying growth hormone (IBA hormone). Of course, this trend was reversed in branch cuttings treated with 0.4% IBA dose as opposed to branch cuttings treated with 0.2% IBA concentration. This probably suggests that matured stem cuttings (in this case, branch cuttings) required more doses (0.4% IBA) to induce better shooting and rooting response compared to fewer doses (0.2% IBA). Several other comparative studies (Leakey et al. 1982; Tchoundjeu and Leakey 2000, 2001; Tchoundjeu et al. 2002; Negash 2003; Kebede et al. 2013) on different concentrations of growth hormone have also demonstrated that optimum IBA concentration prompts rooting response, while supra-optimal (overdoses) IBA concentration inhibits the rooting ability. On the other hand, faster and higher survival rate, shooting and rooting response in seedling cuttings compared to branch cuttings on *P. adolfi-friederici* stem cuttings both in our finding and the previous study (Derero et al. 2019) further confirmed that the selection of suitable cutting source a great contribution to successful vegetative propagation. Consequently, higher survival rate and rooting response in seedling cuttings compared to branch cuttings in *P. adolfi-friederici* tree species, which is in line with the apical cuttings as opposed to middle or basal cuttings of *K. ivorensis* (Tchoundjeu and Leakey 2000), *L. trichilioides* (Tchoundjeu and Leakey 2001), *P. falcatus* (Negash 2003), *S. rebaudiana* (Kassahun and Mekonnen 2012), *Cannabis sativa* (Caplan et al. 2018), *A. heterophylla* (Tilahun et al. 2019), suggests young plant parts promote better propagation than mature plant parts.

In this study, vegetatively propagated stem cuttings of *P. adolfi-friederici* at a non-mist polypropagator were transplanted to pots after shooting and rooting success for further acclimatization process at nursery for 120 days. This is because successful stem-cutting establishment after transplantation at greenhouses and nurseries is a major bottleneck since seedlings are continuously exposed to a unique microenvironment, including lower relative humidity, high light intensity, scarce nutrient and water availability, disease and insect pest infestation, as well as other stressful conditions. In this stage, seedling cuttings, as opposed to branch cuttings, were healthy, greenish, and grew very well on pots during the 120-day nursery lifespan. More buds and leaves and roots and root systems were successfully developed. Thus, the satisfactory result on the transplantation and acclimatization process of *P. adolfi-friederici* seedling cuttings (69.6% survival rate) at the nursery is an indication of the efficiency of the developed protocol for its vegetative propagation despite a lower survival rate (58.9%). This further contributes to the successful survival rate, growth performance, and adaptation potential of stem cuttings in the field. Negash (2010) also reported the positive response of vegetative propagation of *Hagenia abyssinica* (Bruce) J.F.Gmel. and hence the successful transplantation of stem cuttings at nursery and field establishments. In conclusion, stem cuttings' survival potential, growth performance of stem cuttings at greenhouses and nurseries, and field establishment and adaptation potential are further suggested.

In conclusion, the low-technology non-mist polypropagator is one of the most promising options for propagating *P. adolfi-friederici* stem cuttings using the vegetative propagation method. In our experiment, 70% of shooting and rooting success and higher mean root length (1.97+1.92 cm) were induced by seedling cuttings treated with 0.2% IBA concentration with a mean temperature of 22.4+4.75ºc and mean relative humidity of 75.5+6.50% at non-mist polypropagator kept under 60% shade-net. Hence, the results confirmed that the species can be propagated vegetatively by seedling cuttings treated with 0.2% IBA concentration at non-mist polypropagator. Therefore, establishing mother stocks in nurseries and their proper management to serve as sources of juvenile stems will be essential for the successful macro-propagation of the species besides growing the species with seeds.

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