



Macro-propagation of *Pouteria adolfi-friedericii* in a non-mist poly-propagator

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Report summary

Pouteria adolfi-friedericii is an important native timber species in Ethiopia. A vegetative propagation experiment was conducted aiming at developing a vegetative propagation technique for *Pouteria adolfi-freiderici*. A non-mist poly-propagator was constructed that consisted of a wooden frame enclosed in clear thick grade polythene and filled with drainage materials of different sizes. Four treatment combinations (hormone and without hormone and two sources of cuttings) were arranged in a complete randomized design in four replications. Then ten cuttings from each explant type was assigned to each box systematically. The cuttings from each explant source were dipped in the IBA-ethanol solution (0.4%) in separate beakers for about 24 hours before planting. Thus, a total of 40 hormone treated cuttings from the adult and the seedling cuttings each and another 40 non-treated ones were planted. Of the total 160 cuttings planted in the poly-propagator, only 22 (13.8%) responded (produced leaf and/or root) to the treatments, and most of the cuttings died out slowly. The adult cuttings/explants couldn't propagate vegetatively without hormone, but the seedling explants did. Generally, the rooting and leafing response of the species to the hormone was low and slow. Future experiments should consider different concentrations of IBA and perhaps also other type of rooting hormones.

Introduction

The industrial scale timber and pulpwood production is faced with several challenges, and critical gaps in the sub-sector including lack of proper propagation methods for some indigenous species. Hence vegetative propagation method was devised to scale up the use of industrial tree species in plantations.

It is widely realized that vegetative propagation and clonal selection offer a means to greatly enhance the yield and quality of forest products from commercial plantings in the tropics (Leakey, 1987). Macro-propagation is a simple technology that will enhance tree production through rapid multiplication and timely delivery of enough high-quality seedlings. The use of macro-propagation will enable production of forest trees with possible uniformity in growth, size and quality. One of the macro-propagation techniques is growing cuttings (explants) in a non-mist poly-propagator, which is a low technology propagator constructed from wooden frame enclosed in clear thick grade polythene and filled with drainage materials of different sizes (Kebede et al., 2013). Thus, using non-mist poly-propagators can help produce viable propagules with cheaper cost compared to other methods such as tissue culture.

Pouteria adolfi-friedericii (Engl.) A. Meeuse (Sapotaceae) is a tall tree attaining a height of 50 m with a clear straight bole with a small dense crown (Bekele-Tesemma, 2007). The genus, *Pouteria*, comprises approximately 320 species, and the species, *Pouteria adolfi-friedericii*, is subdivided into 5 subspecies (Lemmens, 2007). The species is widely distributed in upland forests in Ethiopia, Uganda, Kenya, DR Congo, Burundi, Rwanda, Tanzania, Zambia, Malawi, Zimbabwe (Anonymous, 2019). In East Africa the wood is valued for furniture. It is also suitable for light construction, light flooring, interior trim, joinery, cabinet work, boats, vehicle bodies, boxes and crates, veneer and plywood, and pulpwood. It is used as firewood and for charcoal production as well (Lemmens, 2007). The seeds of the species are sensitive to desiccation (Albrecht, 1993) and not storable for long but have high germination rate. However, collecting the seeds from these often very tall trees coupled with the short viability pose problems in growing the trees from seeds, and thus, vegetative propagation could give an alternative option to deal with the problem. Therefore, the objective of the study was to develop vegetative propagation technique for *Pouteria adolfi-friedericii* with the aim of using the technology for plantation, improvement and conservation of the species.

Materials and methods

Poly-propagator design and construction

A non-mist poly-propagator was constructed following the design of (Leakey et al., 1990). The poly-propagator consisted of a wooden frame enclosed in clear thick grade polythene and filled with drainage materials of different sizes. The inner polythene base of the propagator was covered with a thin layer of sand to prevent the polythene from being punctured by large stones (6-10 cm) which were placed on it to a depth of 4cm. These stones were then covered by successive layers of small stones (3-6cm) to a depth of 4cm and gravel (1cm) to a depth of 4cm adding up to a total of 12cm. All these drainage materials were washed thoroughly to remove all debris. Fine sand was thoroughly washed and sterilized by electric soil sterilizer and placed on the gravel on depth of 5cm. The propagator had eight boxes that are arranged in two rows. It was placed under a 60% shade-net to keep the temperature below 30 °C.

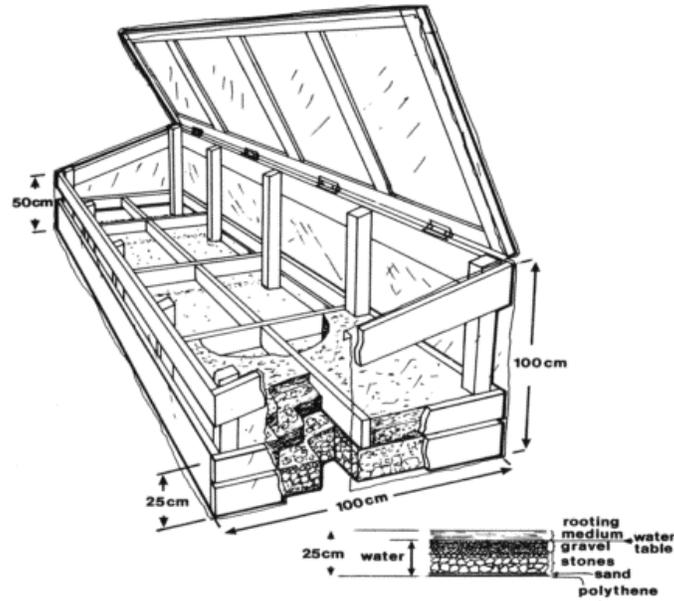


Figure 1 Schematic representation of a typical non-mist poly-propagator

Preparation of explants

Leafy stem cuttings with at least four nodes were taken from seedlings and adult tree. Two years old seedlings grown in FRC nursery were used. The nursery was located at altitude of 2380 m a.s.l. and at 9⁰¹' N and 38⁰⁴⁹' in Addis Ababa. The shoot tip of about 3-5 cm was removed both from the seedling and adult cuttings (from young branches) because the tip part can be too immature to bud. The lower leaves were removed at their nodes by leaving only 2 upper leaves after removal of the top part. Leaves were clipped to reduce leaf areas. All cuttings were kept in water to avoid drying out. The final size of the cuttings when planting was about 23 cm.



Figure 2 Tree climbing for collecting cuttings from an adult tree (a), preparation of cuttings to proper size (b), and dipping of cuttings in 0.4% hormone treated solution of the cuttings from the adult tree (c) and the cuttings from seedlings (d)

Preparation of solution

The commonly used rooting hormone, indolebutyric acid (IBA), was used in the experiment. A 0.4% IBA solution was prepared by dissolving the required amount of the powder in 96% ethanol.

Experimental design and treatments

Four treatment combinations (hormone and without hormone and two sources of cuttings) were used for the experiment. Separate boxes were used to arrange hormone and non-hormone treatments to avoid contamination. The hormone treatments were assigned to the different boxes in a complete randomized design in four replications. Then ten cuttings from each explant type was assigned to each box systematically. The cuttings from each explant source were dipped in the IBA-ethanol solution (0.4%) in separate beakers for about 24 hours before planting. Thus, a total of 40 hormone treated cuttings from

the adult and the seedling cuttings each and another 40 non-treated ones, and thus a total of 160 cuttings were prepared for planting.



Figure 3 The non-mist poly-propagator (a) is placed under a 60% shade-net and people were planting (b), and condition of the cuttings during planting (c) and a week after (d)

Planting and post planting management

The cuttings were planted on the sand-topped substrate making sure that two bottom nodes were buried. Watering of the plant was carried out 3-4 times a day to make sure that high humidity was created within the propagator.

Monitoring of growth environment within the poly-propagator

Temperature and relative humidity data were collected every 2 hours during the day beginning from 8:00 o'clock in the morning.

Data collection

After a close follow up on responses of the cuttings for weeks, the first count of new leaf buds and leaves was carried out on the 43rd day after establishment of the experiment and the second on the 90th day after establishment. The first assessment for rooting was conducted 195 days after planting on those shoots with well-developed and new leaf (leaves). Those suspected as rooted but didn't were replanted back in the poly-propagator. Subsequent monitoring of leafing and rooting was carried out on 297, 322, 368, 438 and 524 day after observing the leafing conditions. The final assessments and closing of the experiment were done 580 days after the onset of the experiment on 31 October 2019. Vegetative seedlings with roots and new leaves were immediately transferred to polybags and grown in a greenhouse after every count.

Statistical analysis

Descriptive statistics and analysis of variance in general liner model were conducted in SPSS 20 on the budding, leafing and rooting data. The LSD test was used for mean separation in the cases of significant differences.

Results and discussion

Growth environment within the poly-propagator

The highest mean diurnal temperature (25.3 ± 4.2 °C) was recorded at 2:00 PM, while the lowest mean diurnal temperature (16.6 ± 1.3 °C) was recorded at 8:00 AM. In terms of relative humidity (RH%), the highest value (88 ± 3.5) was recorded at 6:00 PM while the lowest value (79.3 ± 6.4) was recorded at 2:00 PM (Figure 4).

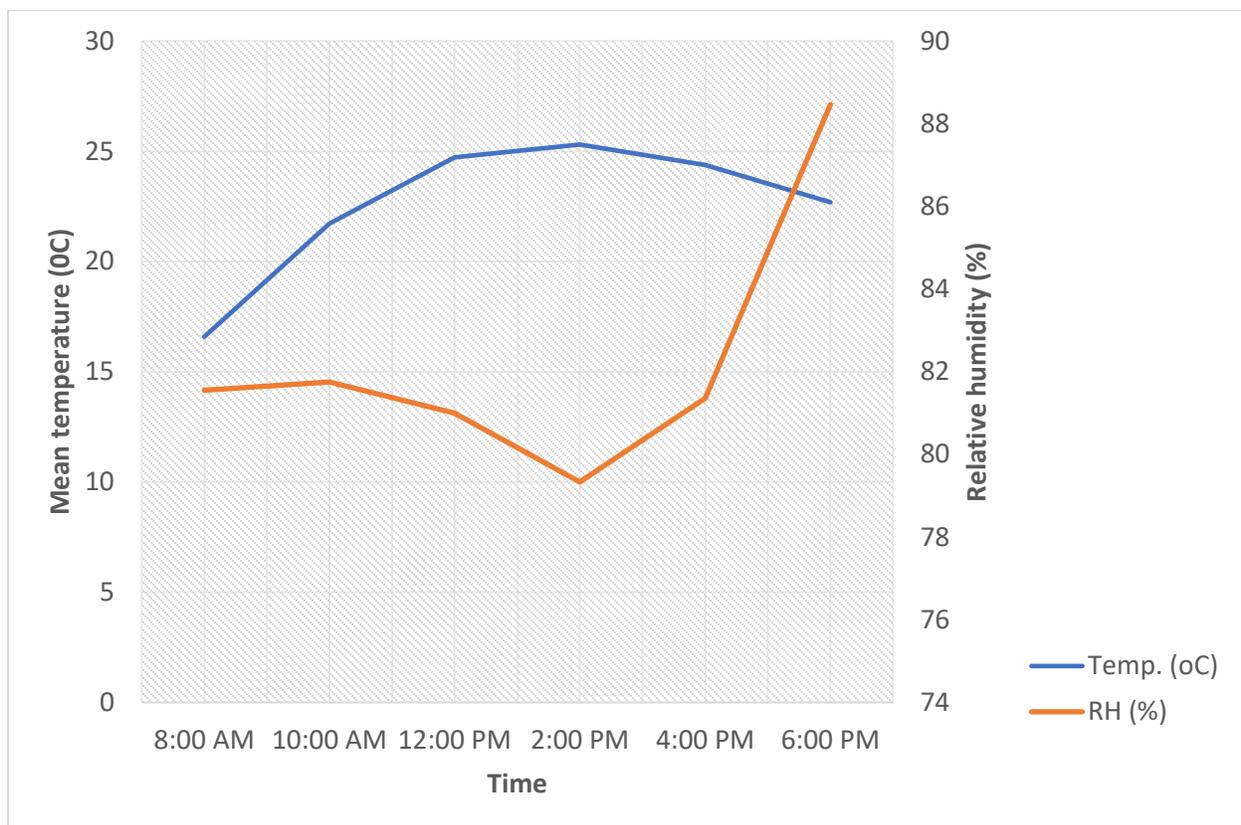


Figure 4 Mean diurnal temperature and relative humidity within the propagator.

Early responses of the explants

Bud initiation and leafing

The first count on bud initiation and leafing on the 43rd day revealed that only cuttings that were hormone treated started budding and leafing. On the 90th day, all the hormone treated and untreated had leaf buds, but leaves were available only on the hormone treated explants (Table 1). At the age of 90 days, the highest mean number of leaves was recorded for the treatment “seedling + hormone”.

Table 1. Mean number of buds and leaves at 43 and 90 days after establishment of experiment

Treatment	Label*	Bud 43 days		Leaf 43 days		Bud 90 days		Leaf 90 days	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adult + Hormone	E1H1	0.03	0.16	0.05	0.32	0.72	1.66	0.05	0.32
Seedling + Hormone	E2H1	0.3	0.72	0.02	0.16	0.45	0.85	0.55	1.36
Adult + No hormone	E1H0	0	0	0	0	0.37	1.31	0	0
Seedling + No hormone	E2H0	0	0	0	0	0.03	0.16	0	0

* Hereafter only the labels are used in the Tables and Figures to refer to the treatments applied in the experiment

ANOVA revealed significant differences ($p < 0.05$) on leafing on the 90th day between the treatments. No significant difference was revealed for both budding and leafing among the treatments on the 43rd day, and there was no significant difference on budding on the 90th day among the treatments (Table 2).

Table 2. Analysis of variance among the four treatments on budding and leafing at 43 and 90 days after establishment of the experiment

Variable	df	Mean Square	F	P-value
No. of bud at 43 days	3	.856	3.416	.066
No. of leaf at 43 days	3	.023	.673	.590
No. of bud at 90 days	3	3.323	.676	.588
No. of leaf at 90 days	3	2.867	4.300	.038

Rooting

Four of the cuttings in one of the treatments (seedling + hormone) developed roots by the 195 day after planting. A picture of one of the rooted cuttings is given in Figure 5a, and the four transplanted seedlings in Figure 5b.



Figure 4 Rooting (A) and four transplanted seedlings (B) from cuttings (obtained from seedlings) treated with 0.4% IBA

Overall responses of the cuttings to the applied treatments

Figure 5 shows the cumulative response of the cuttings to the treatments. Of the total 160 cuttings planted in the poly-propagator, only 22 (13.8%) responded (produced leaf and/or root) to the treatments, and most of the cuttings died out slowly. The usual trend was to leaf first and then to produce the roots, but a

few (4 out of the 22) did root even before producing leaves, and this was observed and recorded while closing the experiment on day 580.

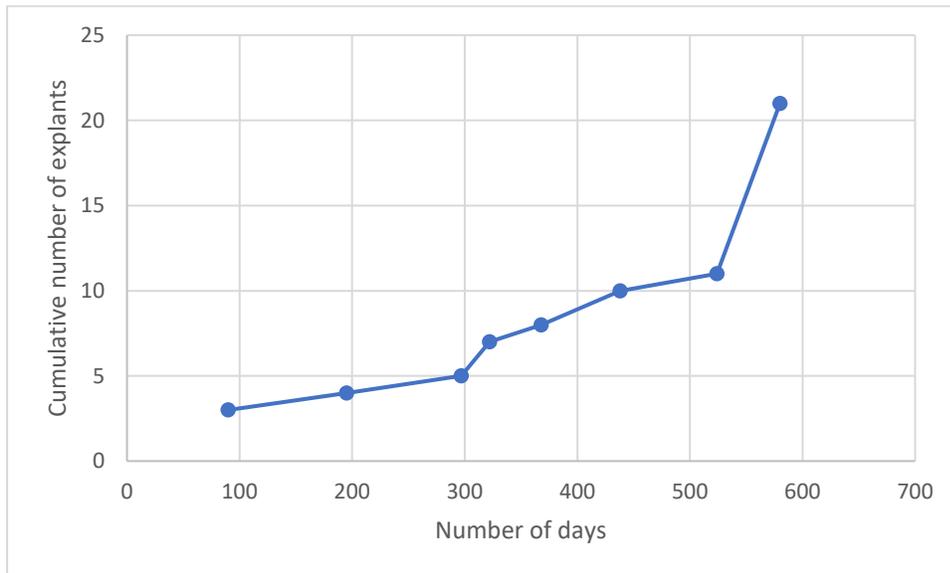


Figure 5 Cumulative response of the cuttings to the applied treatments at different ages of the experiment

The response was the strongest for the seedling than the adult explants (Figure 6). Results showed that while only 7.5% of the adult explants responded, on the contrary 20% of the seedling explants responded. The relatively higher success on seedling cuttings is perhaps related to juvenility as this enhances rooting potential (Palanisamy and Subramanian, 2001). However, when compared to the 100% rooting response of seedling explants of *Prunus africana* and *Syzygium guineense* in just six weeks in Addis Ababa (Kebede et al., 2013), *Pouteria* happens to be a very slow and poorly responding species to propagation by cuttings.

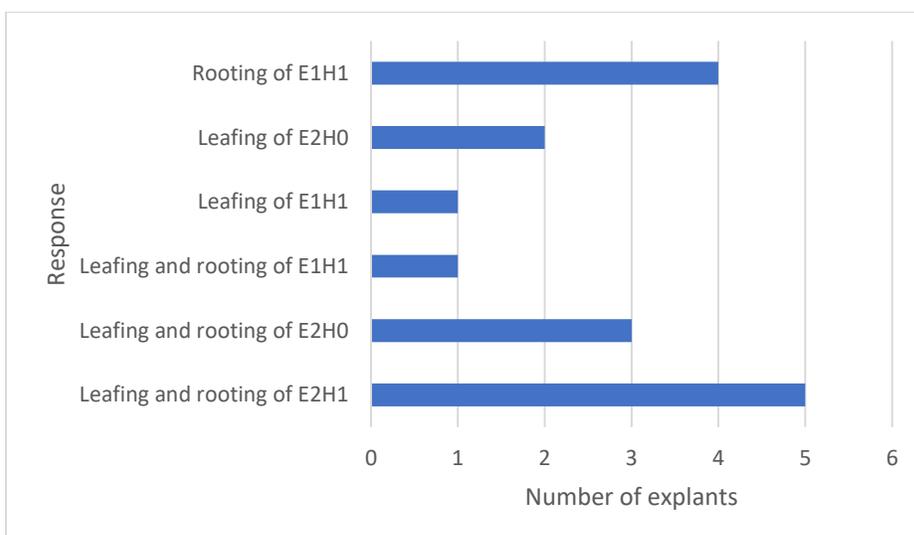


Figure 6. Number of explants that produced roots and/or leaves with and without the rooting hormone application

The IBA application had significant effects on rooting of the explants, all the hormone untreated adult explants failed to root and/or shoot (Figure 7). Whereas 21.3% of the hormone treated explants responded, only 6.3% of the untreated explants responded. Some other works reported high rooting success (over 80%) for IBA treated leafy cuttings of *Pausinystalia johimbe* and *Prunus africana* in Cameroon with a very significant performance when compared to untreated ones (Tchoundjeu et al., 2002; Tchoundjeu et al., 2004). Some species such as *Milicia excelsa* show high rooting performance (about 80%) even without IBA owing to endogenous auxins available in the species (Ofori et al., 1996).

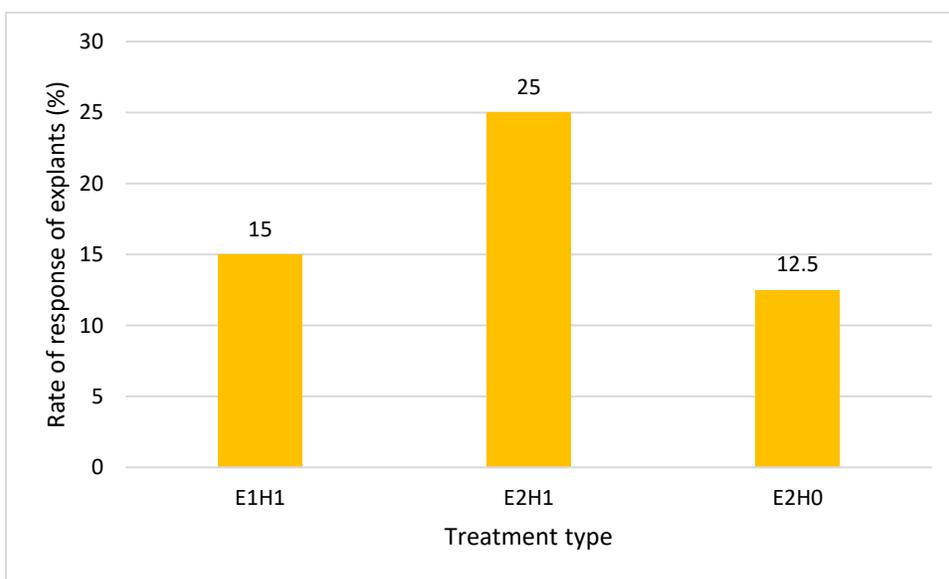


Figure 7. Rate of responses of the explants to the treatments applied

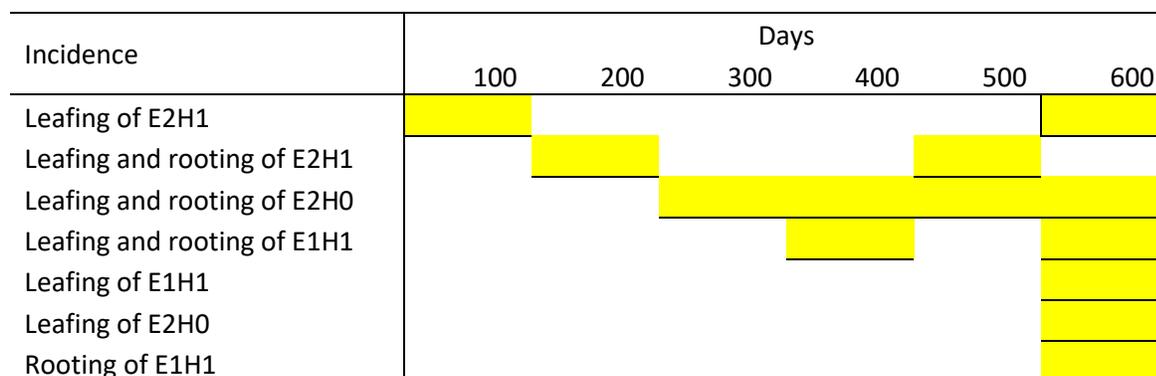


Figure 8. Modes of leafing and rooting responses across the experimental period

The overall root number and root length of the cuttings that responded to the treatments was 4.4 (± 2.4) and 8.6 (± 5) cm, respectively. The highest rooting number and rooting length was recorded on adult explants treated with hormone (Figure 9). The adult explants had significantly higher number of roots than

the seedling explant roots, but there was no significant difference among the treatments in their effect on rooting length.

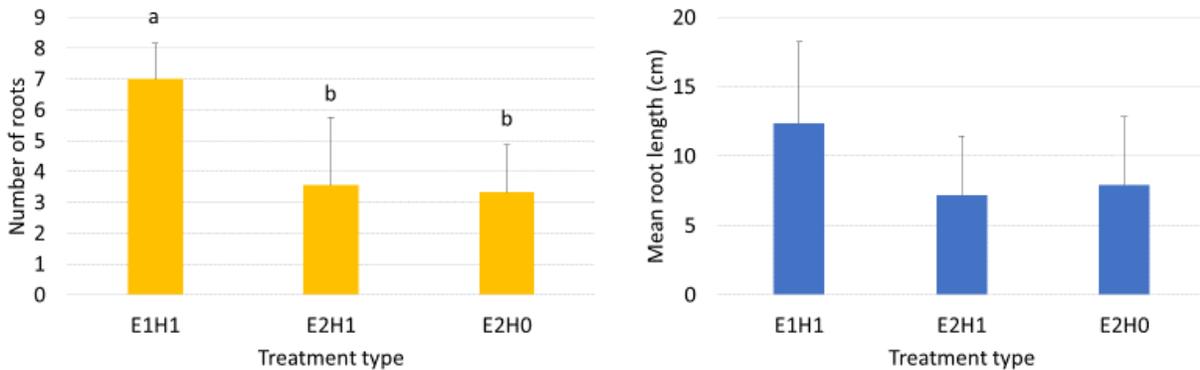


Figure 9 Mean number of roots and mean root length of explants under three different treatments

The mean root length of the seedling explants (7.2 cm) is higher than the mean root length reported for *Syzygium guineense* and *Prunus africana* seedling explants (Kebede et al., 2013).

Conclusions and implications

The study reports on the results of the vegetative propagation experiment carried out on one of the important timber species in Ethiopia, *Pouteria adolfi-friedericii*, in a non-mist poly-propagator with the use of a rooting hormone, IBA. Adult cuttings/explants of the species don't propagate vegetatively without hormone, but the seedling explants do. Generally, the rooting and leafing response of the species to the hormone is low. Future experiments should consider different concentrations of IBA and perhaps also other type of rooting hormones.

Acknowledgments

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References

Albrecht, J. (1993). Forest seed handling. *Tropical forestry handbook* 1, 381-462.

- Anonymous (2019). Tropical Plants Database, Ken Fern. tropical.theferns.info. 2019-12-25. <tropical.theferns.info/viewtropical.php?id=Pouteria+adolphi-friedericii>.
- Bekele-Tesemma, A. (2007). "Useful trees and shrubs of Ethiopia: identification, propagation, and management for 17 agroclimatic zones," RELMA in ICRAF Project, World Agroforestry Centre, Eastern Africa Region.
- Kebede, M., Hulten, H., and Balcha, G. (2013). Vegetative propagation of juvenile leafy stem cuttings of *Prunus africana* (Hook. f.) Kalkm and *Syzygium guineense* (Willd.) DC. *International Journal of Botany* **9**, 30-36.
- Leakey, R. (1987). Clonal forestry in the tropics—a review of developments, strategies and opportunities. *The Commonwealth Forestry Review*, 61-75.
- Leakey, R. R., Mesen, J., Tchoundjeu, Z., Longman, K., Dick, J. M., Newton, A., Matin, A., Grace, J., Munro, R., and Muthoka, P. (1990). Low-technology techniques for the vegetative propagation of tropical trees. *The Commonwealth Forestry Review*, 247-257.
- Lemmens, R. H. M. J. (2007). *Pouteria adolphi-friedericii* (Engl.) A. Meeuse. In: Louppe, D., Oteng-Amoako, A.A. & Brink, M. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l’Afrique tropicale), Wageningen, Netherlands. Accessed 24 October 2019.
- Ofori, D., Newton, A., Leakey, R., and Grace, J. (1996). Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentration, leaf area and rooting medium. *Forest Ecology and Management* **84**, 39-48.
- Palanisamy, K., and Subramanian, K. (2001). Vegetative propagation of mature teak trees (*Tectona grandis* L.). *Silvae Genetica* **50**, 188-190.
- Tchoundjeu, Z., Avana, M., Leakey, R., Simons, A., Assah, E., Duguma, B., and Bell, J. (2002). Vegetative propagation of *Prunus africana*: effects of rooting medium, auxin concentrations and leaf area. *Agroforestry systems* **54**, 183-192.
- Tchoundjeu, Z., Mpeck, M.-L. N., Asaah, E., and Amougou, A. (2004). The role of vegetative propagation in the domestication of *Pausinystalia johimbe* (K. Schum), a highly threatened medicinal species of West and Central Africa. *Forest Ecology and Management* **188**, 175-183.