International Journal of Botany

ISSN: 1811-9700

International Journal of Botany 9 (1): 30-36, 2013
ISSN 1811-9700 / DOI: 10.3923/ijb.2013.30.36

Vegetative Propagation of Juvenile Leafy Stem Cuttings of *Prunus africana* (Hook.f.) Kalkm and *Syzygium guineense* (Willd.) DC

[1]Mamo Kebede, [2]Håkan Hultén and [3]Girma Balcha
[1]Wondo Genet College of Forestry and Natural Resource, P.O. Box 128, Shashamene, Ethiopia
[2]Department of Forest Management and Products, Swedish University of Agricultural Sciences, P.O. Box 7060, Uppsala, Sweden
[3]Institute of Biodiversity Conservation, P.O. Box 30726 Addis Ababa, Ethiopia

Abstract: *Prunus africana* (Hook.f.) Kalkm and *Syzygiym guineense* (Willd.) DC subsp. *guineense* are multipurpose indigenous trees to Ethiopia. Reforestation with these species is hindered due to their recalcitrant seeds and a higher seed predation. This study tested the effects of Indole-3-butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) applied at a concentration of 0.0, 0.2 and 0.4% on juvenile leafy stem cuttings of *P. africana* and *S. guineense* using non-mist polypropagator. Two leaves with an area of 30-35 cm^2 were left on each cutting. The bases of cuttings were dipped in the hormones for 3 seconds and placed into the rooting medium. All the rooted cuttings of the *S. guineense* and *P. africana* were potted in polythene bags containing a mixture of sand, forest soil and well decomposed manure. Auxin treatment had no significant effect on the final rooting percentage of both species. The effect of auxin treatment on root number, root and shoot length of the rooted cuttings of *P. africana* and *S. guineense* was significant ($p<0.01$). The establishment test on the rooted cuttings indicated that mortality accounted for only 0-2%. Prospects for vegetative propagation with the juvenile leafy stem cuttings of the above species are good as cuttings were easily rooted. The low technology non-mist polypropagator designed for tropical trees could serve as a tool for propagation of these species for conservation and capture of genetic variation. Further study on donor plant management is required for maintenance of juvenility to harvest more cuttings for plantation purposes.

Key words: Vegetative propagation, *P. africana*, *S. guineense*, polypropagator, growth hormone

INTRODUCTION

Prunus africana and *Syzygium guineense* subsp. *guineense* are important multipurpose tree species found in the remaining natural forests of Ethiopia and other African countries (Friis, 1992). The medicinal property of *P. africana* bark for the treatment of disorders of the prostate gland was recognized more than 20 years ago, when commercial harvesting of the species from natural populations in Cameroon and Madagascar began (Dawson, 1997). The edible fruit of *S. guineense* was reported to have high contents of Ca, Mg, Fe, P, K, *Syzygium guineense* (2247 $\mu g\ g^{-1}$) (Saka and Msonthi, 1994) and serve as an important tree species in the rural community subsidizing the rural food security. Both *P. africana* and *S .guineense* has timber, soil conservation, ornamental and bee floral values (Bekele-Tesemma *et al*., 1993).

Nevertheless, reforestation with these valuable multipurpose tree species had been hindered due mainly to their short seed viability and a high incidence of seed predation (Were and Munjuga, 1998; Hong *et al*., 1996). *Prunus africana* is classified as vulnerable species by the International Union for Conservation of Nature (IUCN) (Vivero *et al*., 2006). An alternative means of propagating these species, other than with their seed, would be the vegetative approach. Several studies have reported the possibility of vegetative propagation of tropical tree species (Nketiah *et al*., 1998; Shiembo *et al*., 1996; Ofori *et al*., 1996; Mesen *et al*., 1997). Vegetative propagation techniques has several advantages such as shortening rotations, increasing quality and allowing some of the biological problems hindering reforestation with hardwood species to be circumvented (Leakey, 1987). The recent advances in the development of a low-technology propagation system have enabled the successful propagation of a wide range of timber and multipurpose tree species from both tropical moist forest and semi-arid areas by leafy stem cuttings. Although leafy stem cuttings can propagate many species easily, there is still difficulty in some species. In a situation where full-scale study is inappropriate, considering a limited

Corresponding Author: Mamo Kebede, Wondo Genet College of Forestry and Natural Resources, P.O.Box 128, Shashamene, Ethiopia

number of key factors may be important for the propagation of an undomesticated species (Leakey and Storeton-West, 1992).

The main objective of this study was to analyse the potential of adventitious root formation in juvenile leafy stem cuttings of *Prunus africana* and *Syzygium guineense*. We set out to test the hypothesis that plant growth hormone Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) will promote adventitious root formation and that IBA will be more efficient than NAA.

MATERIALS AND METHODS

Management of stockplants: Seeds of *Prunus africana* and *Syzygium guineense* subsp. *guineense* were collected from Muneesa Shashamene area, near Wondo Genet College of Forestry and Natural Resource (7°5'30" N to 7°7'40" N latitude and 38°36'55" E to 38°39'00" E) and were sown in the College nursery. After germination, the seedlings were transplanted to polythene tubes 10 cm lay-flat diameter and 12 cm high filled with nursery soil comprising of a mixture of forest and ordinary soil, in a ratio of 1:3, respectively. Thatch grasses shaded the seedlings. Other than regular watering, the seedlings were grown with the application of neither pesticides nor fertilizers.

Environment of propagation: A low technology non-mist poly-propagator was constructed following the design of Leakey (1990). The polypropagator 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 8 cm. These stones were then covered by successive layers of small stones (3-6 cm) to a depth of 8 cm and gravel (0.5-1.0 cm) to a depth of 4 cm, adding up to a total depth of 20 cm. All these drainage/filling materials were washed thoroughly to remove all debris and then sterilized by a soil steriliser (Electric Soil Steriliser Complex, plantcare, England). Water was added to the drainage materials to field capacity. Fine sand (2 mm or less) was obtained by sieving river sand and placed on the gravel, separated by a perforated plastic tray to a depth of 5 cm after being thoroughly washed and sterilised. Six hollow plastic pipes with a diameter of 0.5 cm and a height of 30 cm were inserted vertically down into the drainage/filling materials in the centre and at each corner to control water levels and to allow water to be added when needed. A minimum and maximum temperature thermometer and a relative humidity meter were placed within the polypropagator. A wooden frame 2.5 m high was constructed over the whole propagation area, including the study area in order to provide shade. Light interception of about 80%, estimated by using a Spherical Densiometer (Lemmon, 1957) was achieved from palm fronds. A wire net stretched over the shading area, uniformly supported the shading material. Side shade was also provided with palm fronds hanging vertically and fixed to the ground to avoid sun radiation hitting the cuttings at an angle.

Preparation of cuttings: Seedlings grown at Wondo Genet College of Forestry and Natural Resource nursery for eight months from seed were transported to Addis Ababa and cuttings were harvested from these seedlings at the Forestry Research Centre, Addis Ababa, between 5 and 6 PM. A population of seedlings in a good physiological state were selected and placed under shade while they were still in pots. The top 3-4 cm was cut away and discarded. From each seedling two cuttings, each 10 cm long were taken. The top two leaves were left on the cuttings, the rest being taken away at their leaf node. Cuttings were placed in a beaker with distilled water as soon as they were excised from the stock plant. The total area of the two leaves left on the cuttings was 30 cm^2 and 35 cm^2 for *Prunus africana* and *Syzygium guineense*, respectively with minor variations within individuals. A leaf area meter (AM100 256 scanner, Analytical Development Co. Ltd, Hoddesdon, England) was used to measure the leaf area on a representative sample of cuttings. Forty cuttings, to receive the same treatment, were placed in each beaker and kept together with a rubber band.

Preparation of the solution: Two plant growth regulators (auxins), Indole-3-butyric Acid (IBA) and Naphthalene Acetic Acid (NAA), were used to examine their effects on the rooting of juvenile leafy stem cuttings. Both chemicals were obtained in the powder form, from Sigma Chemical Company (St. Louis, USA). (Both hormones were stored at 4°C). Stock solutions of 0.2 and 0.4% of each auxin were prepared by dissolving the required amount of the chemical and dissolved in 96% ethanol and then the volume was adjusted by adding ethanol which was diluted to 50% by distilled water. Each stock solution was used immediately but only once.

Treatment, planting and assessment of the cuttings: There were ten treatment combinations (two growth hormones each with two concentration levels, one control and two species) having two replicates for each treatment with a total of 40 cuttings (20 cuttings in each replicate). The bases (3 cm long) of a bundle set of 20 cuttings were dipped in the hormones for 3 seconds. A current of cold air was directed on to the base of the cuttings to evaporate off the alcohol while the rubber band was loosened. The cuttings were then put into polythene tubes and the leaves sprayed with water using a handheld sprayer. Treatments were allocated randomly to plots. The basal 3 cm of the cuttings were inserted into the rooting medium. The polypropagators was opened once in the morning between 8 A.M and 9 A.M; and late in the afternoon between 4 P.M and 5 P.M for a brief period and given a fine foliar spray. At the end of the first week, cuttings were sprayed with the fungicide, Benomyl at a concentration of 10 g/10 L of water. The cuttings were also sprayed with the fungicide Mancozeb (Dithane M45, Rohm and Hass, France S.A) in the fourth week, at a concentration of 25 g/10 L of water. The cuttings were also sprayed with insecticide (Cymbush 10 EC, Imperial Chemical Industries Plc, Haslemere, UK) at a concentration of 5.8 mL 10^{-1} of water.

Assessment for rooting was begun after the observation on a few extra cuttings had confirmed the beginning of rooting at the end of the second week. Each week data was taken on the proportion of cuttings that rooted in the study. Cuttings were judged rooted when at least one adventitious root of a length 0.5 cm and shoot formed when a shoot growth of 0.5 cm was formed. In the eighth week the root number, root length and shoot length of each cutting was recorded.

Establishment of cuttings: Through decreasing foliar spray and keeping the polypropagator open during the night for three consecutive days and then left open until the late morning for the next two days and then left open the whole day for another two days the cuttings were adapted to a lower relative humidity. All the rooted cuttings of the *S. guineense* and *P. africana* were potted in polythene bags containing a mixture of sand, forest soil and well decomposed manure in the ratio of 1:3:1 after being sieved. The bottom of the pot was sealed, but perforated to drain out excess moisture. The rooted cuttings were gently lifted and transplanted into pots. To grow the cuttings under ordinary nursery conditions, the potted cuttings were taken out of the propagation shade area after 15 days to ordinary nursery bed shade provided by grass thatching. After about one week, the shade material was removed. Observations on the establishment and growth of cuttings were made three months after transplanting into the pots.

Data analysis: The data gathered on the effects of the growth hormones on root number, root length and shoot length were analysed by SPSS statistical package of computer software.

RESULTS

Rooting environment: The mean relative humidity was 85% with a range from 75 to 95%. The mean maximum and the mean minimum temperature in the polypropagator were 20 and 10°C , respectively. The mean maximum and minimum temperature in the rooting medium were 22.5 and 7°C, respectively during the rooting period.

Rooting response of cuttings: Adventitious rooting was developed in all treatments. More than half of the cuttings had rooted in the third week. However, the situation before the second week was not recorded. But it is not unlikely that some of the cuttings might have already started rooting before the second week. The proportion of cuttings that rooted in auxin treated cuttings was larger than those in the control during the third week. However, in the fifth and sixth week the control cuttings had also attained the same rooting percentage. By the sixth week all the treatments attained 100% rooting (Fig. 1a, b).

Effects of auxins on the root and shoot: The effect of IBA and NAA treatment on root number, root length and shoot length of the cutting of the species *Syzygium guineense* and *prunus africana* was significant ($p<0.01$).

The species responded differently to the auxins and to their concentration levels. In *Syzygium guineense*, the mean root number of rooted cuttings was 8.1 for the treatments applied at 0.0% (control) and IBA 0.2% compared to the other treatments. The corresponding mean root number for IBA 0.4%, NAA 0.2% and NAA 0.4% was 6.98, 6.73 and 6.53, respectively (Fig. 2a, b). The mean root number per rooted cutting was 17.18 and 14.13 at NAA 0.4% and IBA 0.4%, respectively. The corresponding mean root number per rooted cuttings treated with NAA 0.2% and IBA 0.2% was 13.68 and 6.73, respectively. The Mean root number was 7.39 for the control treatment (Fig. 2a, b).

In *Syzygium guineense* the mean root length per cutting was 3.85 cm in the cuttings treated with NAA 0.2% and the corresponding mean root length for the IBA 0.4% and the control was 3.18 cm and 3.14 cm, respectively. NAA 0.4 and IBA 0.2% treated cuttings produced 2.8 and 2.38 cm mean root length per cutting, respectively. In *Prunus africana* the maximum achieved

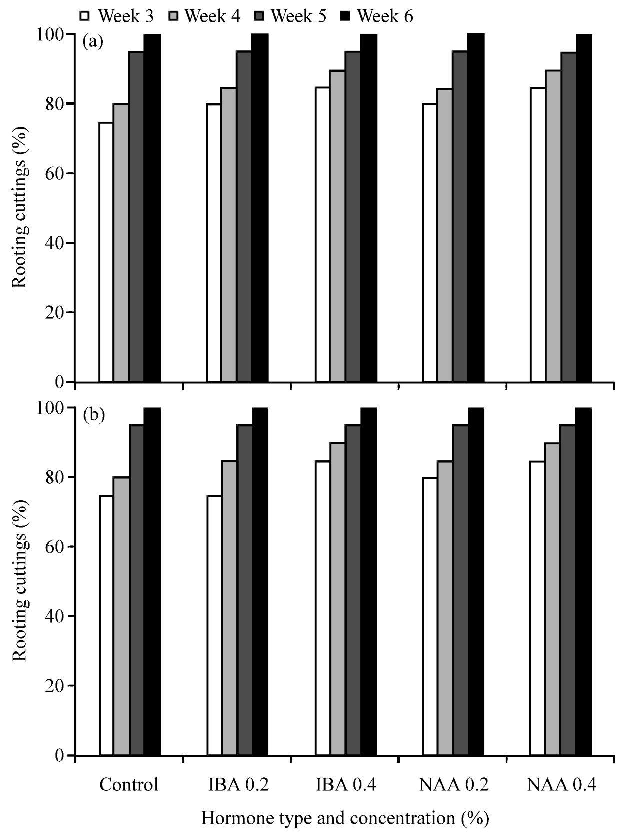

Fig. 1(a-b): Effect of hormone concentration on the rate of rooting of (a) *P. africana* and (b) *S. guineense* (n = 40 for each treatment and species)

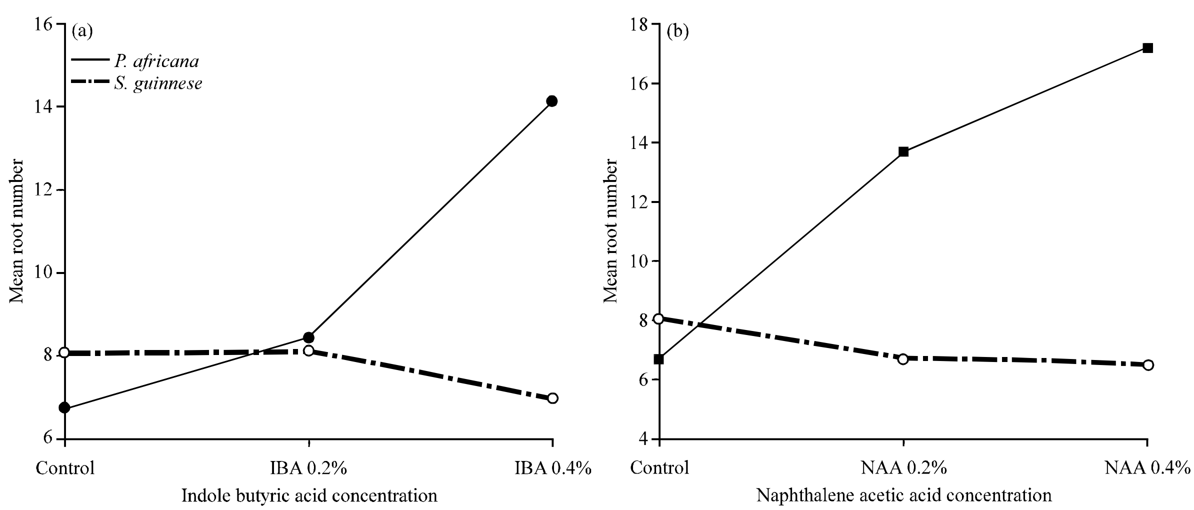

Fig. 2(a-b): Effect of concentration of (a) IBA and (b) NAA, on the mean root number of the rooted cuttings of *P. africana* and *S. guineense* (n = 40 for each treatment and species)

mean root length was 4.65 and 4.38 cm at 0.2% IBA and 0.0%, respectively. The corresponding mean root length attained for NAA 0.2%, IBA 0.4% and NAA 0.4% was 37.8, 3.3 and 24.5 per cutting, respectively (Fig. 3a, b).

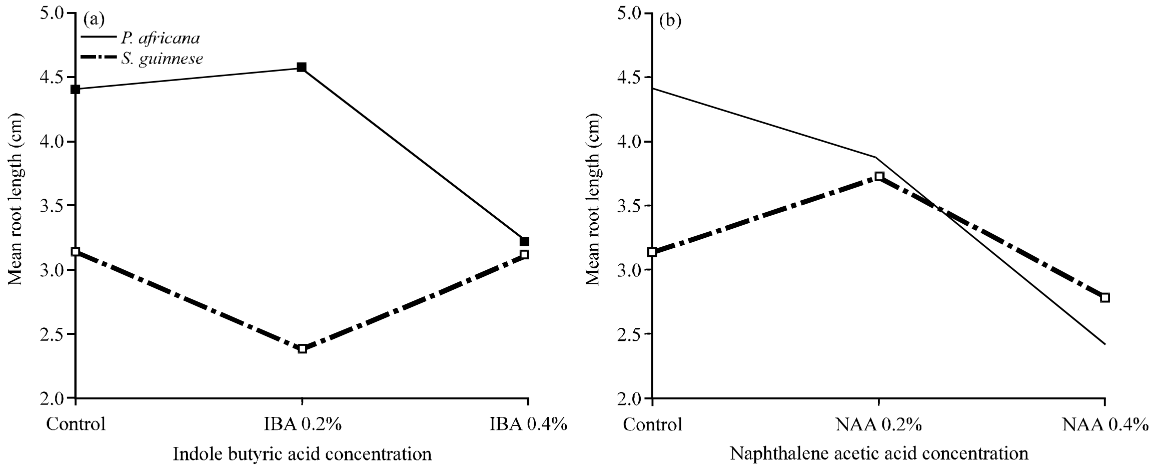

Fig. 3(a-b): Effect of concentration of (a) IBA and (b) NAA, on the mean root length of the rooted cuttings of *P. africana* and *S. guineense* (n = 40 for each treatment and species)

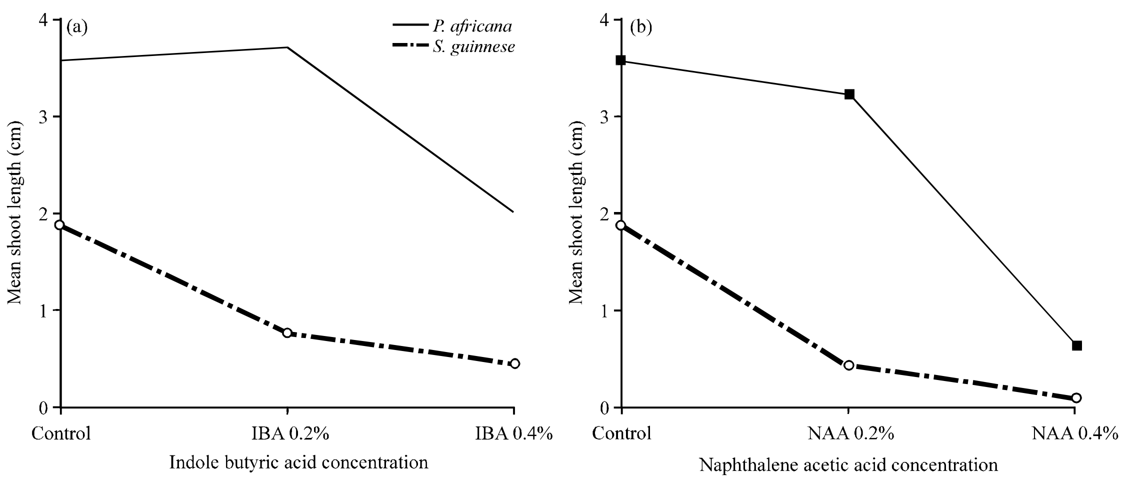

Fig. 4(a-b): Effect of concentration of (a) IBA and (b) NAA, on the mean shoot length of the rooted cuttings of *P. africana* and *S. guineense* (n = 40 for each treatment

Shoot length was not promoted with hormone application. Only IBA 0.2% slightly promoted shoot elongation. The mean shoot length was 3.75 and 3.65 cm at IBA 0.2 and 0.0%, respectively for *Prunus africana*. In this species NAA 0.2%, NAA 0.4% and IBA 0.4% attained mean shoot lengths of 3.3, 2.03 and 0.65 cm, respectively (Fig. 4, b).

With *Syzygium guineense* the maximum mean shoot length attained was 1.9 cm in the cuttings treated with 0.0%. Auxin treatment of the cuttings produced negligible mean shoot length by the end of the study. At the higher concentration adventitious shoot formation was inhibited (Fig. 4).

From the observation made after three months, almost all of the rooted cuttings of *P. africana* and *S. guineense* had established. Field planting was carried out at the beginning of August. Future observation will be made as to their final field survival.

DISCUSSION

The low-technology non-misted polypropagator especially designed for tropical conditions has enabled the propagation of these species. Regardless of high ambient temperature outside, a relative humidity of more than 85% was achieved. This might have contributed to the survival and subsequent rooting of the species under this study. The rooting of *Prunus africana* and *Syzygium guineense* was 100% in all treatments. However, a greater proportion of cuttings treated with auxins rooted earlier than the control. The exogenously supplied auxins might have triggered the rooting processes earlier.

The influence of auxins in promoting adventitious root formation through their ability to promote the initiation of lateral root and enhancing the transport of carbohydrates to the cutting base, is well documented in several studies (Davis, 1988; Leaky *et al*., 1982; Hartmann *et al*., 1997). However, contrasting effects of the addition of auxins recorded with different tree species ranged from no effect to significant effect. The present result also showed that *Prunus africana* and *Syzygium guineense* were successfully rooted without hormone application in the control treatment. The optimum IBA concentration for *Triplochiton scleroxylon* was found to be 0.4% (Leaky *et al*., 1982), *Cordia alliodora* 1.6% but very low rooting occurred when no auxin was supplied (Mesen *et al*., 1997). Successful rooting without applied auxin was reported in a number of tropical tree species, such as *Shorea macrophylla* (Lo, 1985), *Nauclea didderrichii* (Leakey, 1990), *Vochysia hondurensis* (Leakey, 1990), *Milicia excellsa* (Ofori *et al*., 1996) and *Irvinga gabonensis* (Shiembo *et al*., 1996). References state that such contrasting results may reflect a variation in endogenous auxin content at the time of severance (Leakey, 1990; Hartmann *et al*., 1997).

In *Prunus africana* a positive trend was observed between IBA and NAA concentration and root number. Earlier studies produced similar results in *Gnetum africanum* (Shiembo *et al*., 1996), *Triplochiton scleroxylon* (Leaky *et al*., 1982), *Nauclea didderrichii* (Leakey, 1990), *Milicia excelsa* (Ofori *et al*., 1996) *Cordia alliodora* and *Albizia guachepele* (Mesen *et al*., 1997). This may be attributed to the effect of the auxin on the mobilization of carbohydrates and their transfer to the rooting zone.

With *Syzygium guineense* different concentration of IBA and NAA had varying effects on the root number. There was lack of response at lower concentration and even a negative (inhibitory) response at higher concentrations with both auxins. Shiembo *et al*. (1996) has observed this unusual relationship in *Irvingia gabonensis*, a West African fruit tree. In this study it was observed that root length was slightly promoted by the lower concentration of both hormones. The mean root length per cutting was reduced at the higher concentration for all the species tested. However, due to lack of instrument the root dry weight was not measured to make further comparison.

Shoot growth in *Prunus africana* and *Syzygium guineense* had a negative relation. At higher concentrations of both IBA and NAA the inhibitory effect is pronounced. The inhibition of shoot production by higher concentrations of IBA has been recorded in other tree species, such as *Milicia excelsa* (Ofori *et al*., 1996) and *Cordia alliodora* (Mesen *et al*., 1997). This may arise as a result of IBA-induced basipetal transport of assimilates; with sink strength successively enhanced by increase in IBA (0.4% concentration) (Ofori *et al*., 1996). IBA 0.4% and NAA 0.4% produced a higher mean number of roots per cuttings of *Prunus africana*, produced almost negligible proportions of cuttings that produced shoots when compared to the lower concentration and the control.

CONCLUSION

Prospects for vegetative propagation with *Prunus africana* and *Syzygium guineense* under low-technology non-mist polypropagator are good as cuttings were easily rooted. The rooting ability of these species without hormone treatment is found to be encouraging when the cost and the availability of the hormones are considered.

It can be concluded that the problem of short viability of *P. Africana* and *S.guineense* can be mitigated by rooting cuttings. The nursery level establishment of the rooted cuttings is encouraging. Planting programs can make use of this propagation tool to overcome their vulnerability to being threatened in the wild. Studies on establishment of the rooted cuttings on the planting sites should be conducted. Further research on mass propagation through establishment of stockplant (donor plant), is required as juvenile cuttings are easily rooted. Other factors controlling the rooting ability of these species are deemed necessary.

ACKNOWLEDGMENTS

We would like to thank the financial assistance of the Swedish International Development Authority (SIDA) and Wondo Genet College of Forestry and Natural Resources for the MSc study opportunity for the first author and coverage of cost for his thesis. We are grateful to the anonymous reviewer (s) for their many useful comments and suggestions on improving our manuscript.

REFERENCES

Bekele-Tesemma, A., A. Brinie and B. Tegnas, 1993. Useful Trees and Shrubs for Ethiopia: Identification, Propagation and Management for Agricultural and Pastoral Communities. Regional Soil Conservation Unit (RSCU), Swedish International Development Authority (SIDA), Nairobi, Kenya, Pages: 474.

Davis, T.D., 1988. Photosynthesis During Adventitious Rooting. In: Adventitious Root Formation in Cuttings, Davis, T.D., B.E. Haissing and N. Sankhla (Eds.). Vol. 2, Disocorides Press, Portland, USA.

Dawson, I.K., 1997. *Prunus africana*: How agroforestry can help save an endangered medicinal tree. Agroforestry Today, 9: 15-17.

Friis, I., 1992. Forests and Forest Trees of Northeast Tropical Africa. Kew Bulletin Additional Series XV. Her Majesty's Stationery Office, London, UK.

Hartmann, H.T., D.E. Kester, D.F.T. Jnr and R.L. Geneve, 1997. Plant Propagation: Principles and Practices. 6th Edn., Prentice Hall of India, New Delhi, India, Pages: 770.

Hong, T.D., S. Linington and R.H. Ellis, 1996. Seed Storage Behaviour: A Compendium. Handbook for Genebanks: No. 4. International Plant Genetic Resources Instiute, Rome, Italy.

Leakey, R.R.B. and R. Storeton-West, 1992. The rooting ability of *Triplochiton scleroxylon* cuttings: The interactions between stockplant irradiance, light quality and nutrients. For. Ecol. Manage., 49: 133-150.

Leakey, R.R.B., 1987. Clonal forestry in the tropics: A review of developments, strategies and opportunities. Commons, For. Rev., 66: 61-75.

Leakey, R.R.B., 1990. Nauclea diderriochi: Rooting of stem cuttings, clonal variation in shoot dominance and branch plagiotropism. Trees, 4: 164-169.

Leaky, R.R.B., F.T. Last and K.A. Longman, 1982. Domestication of tropical trees: An approach in securing future productivity and diversity in managed ecosystem. Comm. Foresty Rev., 61: 33-42.

Lemmon, P.E., 1957. A new instrument for measuring forest overstorey density. J. Forest., 55: 667-668.

Lo, Y.N., 1985. Root initiation of *Shorea macrophylla* cuttings: Effect of node position, growth regulators and misting regime. For. Ecol. Manage., 12: 43-52.

Mesen, J.F., A.C. Newton and R.R.B. Leakey, 1997. Vegetative propagation of *Cordia alliodora* (Ruiz and Pavon) Oken: The effect of IBA concentration, propagation medium and cutting origin. For. Ecol. Manage., 92: 45-54.

Nketiah, T., A.C. Newton and R.R.B. Leaky, 1998. Vegetative propagation of *Triplochiton scleroxylon* K. Schum in Ghana. For. Ecol. Manage., 105: 99-105.

Ofori, D.A., A.C. Newton, R.R.B. Leakey and J. Grace, 1996. Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: Effect of auxin concentration, leaf area and rooting medium. For. Ecol. Manage., 84: 39-48.

Saka, J.D.K. and J.D. Msonthi, 1994. The Nutritional value of edible fruits of indigenous wild trees in Malawi. For. Ecol. Manage., 64: 245-248.

Shiembo, P.N., A.C. Newton and R.R.B. Leakey, 1996. Vegetative propagation of *Irvingia gabonensis*, a West African fruit tree. For. Ecol. Manage., 87: 185-192.

Vivero, J.L., E. Kelbessa and S. Demissew, 2006. Progress on the Red List of Plants of Ethiopia and Eritrea: Conservation and Biogeography of Endemic Flowering Taxa. In: Taxonomy and Ecology of African Plants, their Conservation and Sustainable Use, Ghazanfar, S.A. and H.J. Beentje (Eds.). Royal Botanic Gardens, Kew, London, UK pp: 761-778.

Were, J. and M. Munjuga, 1998. Preliminary findings on the storage behaviour of *Prunus Africana* and *Sclerocarya birrea* seed in Kenya. Proceedings of the Conference IUFRO Seed Symposium Recalcitrant Seeds, October 12-15, 1998, Kuala Lumpur, Malaysia, pp: 431-437.