EFFECT OF DIFFERENT CONCENTRATIONS OF INDOLE-3-BUTYRIC ACID ON THE ROOTING OF LEAFY STEM CUTTINGS OF *IRVINGIA WOMBOLU* (VERMOESEN)

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Abstract

The effects of different concentrations of applied auxin (IBA) on the rooting potentials of leafy stem cuttings of Irvingia wombolu obtained from coppiced shoots and inserted in a low technology non mist propagation system was also assessed in an experiment conducted at the Teaching and Research Farm of the Delta State University, Asaba Campus, Nigeria. Five treatments namely 0, 25, 50, 100 and 200 ppm IBA dissolved in 50% industrial alcohol applied by dipping the base of the cuttings for 5 seconds. The result of the experiment displayed no pronounced effect of IBA concentration on rooting percentage with 25 ppm recording (51.1%), 50 ppm and 200 ppm each recording 50.3%, 100 ppm, (43.4) and the control, (49.6%). The mean number of roots, leaf abscission, cutting mortality and the number of cuttings forming new shoots were unaffected by IBA concentration. Root length was however influenced by IBA concentration with 200 ppm higher (P = 0.01) than the rest treatments. The results suggest that the application of IBA for mass clonal propagation of I. wombolu may not be necessary.

Key words: *Irvingia wombolu*, vegetative propagation, softwood cuttings, IBA

INTRODUCTION

Irvingia gabonensis and *I. wombolu* commonly called bush mango have enormous potential, both in economic terms and as species for sustainable production (Angie and Brown, 2001). They are the source of "ogbono" the *Irvingia* kernel which is popularly used as soup thickener in most West and Central African countries. The popularity of the kernels in the local and International markets has highlighted its potentials for a true commercial crop thus resulting in more intensive collection in the forests (Ladipo, 2000). ICRAF (1975), cited by Ladipo (1999) reported that the market for kernel products was worth in the region of US $50 million. This market which extends to local, regional and International levels is growing. The products are traded within Nigeria and between countries in West and Central Africa. They are also transported to Europe, United States, and Japan and to other areas where African migrants abound in large numbers (Ladipo and Boland, 1994), cited by Ladipo (1999).

The high value of the species to farmers (Jeanicke et al., 1995) long gestation period of seed sown trees, (Moss, 1995; Ladipo et al., 1996), poor germination capacity (Nya et al., 2000), variability of fruits, kernel characteristics and tree size (Ladipo et al., 1996: Schreckenberg et al., 2002) and limited knowledge base (Tchoundjeu et al., 2002) indicates the need for improvement of the species for domestication. Vegetative propagation may greatly facilitate the process of domestication of Irvingia and other fruit trees by enabling the rapid multiplication of selected genotypes and the production of superior planting stock (Leakey and Newton, 1994; Leakey et al., 1994; Mesen et al., 2001), and shortening fruiting time for farmers (Moss, 1995). A wide variety of factors may influence the rooting ability of a number of tropical trees (Leakey et al., 1990; Mesen et al., 2001; Leakey 2004; Dick et al., 2004; Ludwig-Müller et al., 2005; Ehiagbonare and Onyibe 2008).

This experiment investigated the vegetative propagation by leafy stem cuttings of *I. wombolu* as influenced by different concentration of indole-3-butyric acid (IBA) using a non-mist propagation system.

MATERIALS AND METHODS

The experiment was carried out at the Delta State University, Asaba Campus (06º14´N and 06º49´E) in Oshimili South local Government Area of Delta State, Nigeria. Asaba lies in the tropical rainforest zone with annual rainfall range of 1500 mm to 1849.3 mm. Mean temperature are 23.3ºC with a maximum of 37.3ºC. Mean monthly soil temperature at 100 cm depth and sunshine is 28.3ºCelsius and 4.8 hours respectively (Asaba Meteorological Office, 2003).

One hundred and fifty fruits of *I. wombolu* were procured from collectors in Ossissa, Delta State. The fruits were depulped and sun dried for three days and sown afterwards in 0.20 litter polythene pots filled with top soil. Two weeks after germination the seedlings were sown directly in the field with a spacing of 20 cm × 20 cm and raised under shade. The vigorous seedlings were cut back to maintain a supply of coppice shoots and used as stock plants. Plants were watered daily to field capacity. Stock plants were sprayed with a systemic fungicides and insecticides (Imidacloprid 10% + Metalaxyl 10% + Carbendazim 10% WS) prior to severance.

A propagation unit was established in the Teaching and Research Farm, Delta State University, Asaba Campus, Asaba for the experiments. The propagator consisted of a metal frame measuring 3.05 m × 6.10 m × 2.14 m and enclosed in a clear polyethylene with a water-tight block work base. The base of the propagator is covered by a thin layer of sand (10 cm depth) and then successive layers of small and medium size granite (0.5–5 cm, to a depth of 25 cm) and filled with water. The access to the propagator is a hinge fitted metal gate which is also clad in polyethylene. This (low-technology) non-mist propagator is a modified design described by Leakey et al. (1990), was used for the study. The propagation unit was cited in a shade-house providing irradiance inside the propagator of approximately 15–30 percent of that received outside the unit.

Four hundred single node softwood cuttings, four from each shoot were harvested from stock plants described above. 80 cuttings were randomly allocated to each of five treatments namely 0, 25, 50, 100 and 200 ppm IBA dissolved in 50% industrial alcohol applied by dipping the base of the cuttings for 5 seconds with the control dipped in alcohol only. Thereafter, the alcohol was evaporated in a gentle air prior to insertion in the propagator. The cuttings were inserted in composted sawdust in a randomized complete block design and replicated 4 times. Cuttings were assessed weekly for the presence and number of roots (≥ 2 mm in length), rooting percentage, root length leaf abscission, cutting mortality and shoot formation. Data collected were subjected to analysis of variance (ANOVA) and significant means were separated by Fisher's Least Significant Difference (LSD) at 5% level of probability, using Genstat 3 Discovery edition (Genstat 2007). Prior to ANOVA, all percentage data were arcsine transformed, root length data was log transformed while number of roots, leaf abscission, cutting mortality and shoot formation data were square root transformed, and (Gomez and Gomez, 1984).

RESULTS

Rooting percentage was not affected by IBA concentration at Week 3 when rooting commenced ($P > 0.05$). At Week 4, the control and 200 ppm recorded significantly ($P = 0.05$) higher rooting percentage than 100 ppm. However no significant difference was observed between 50 ppm and 100 ppm though 100 ppm was not different from 25 ppm. Treatment effect on rooting percentage tended to diminish thereafter. At Week 5 the following values were recorded; control –33.1%, 200 ppm – 31.4%,

Figure 1: Effect of IBA concentration on the rooting percentage of leafy stem cuttings of *Irvingia wombolu*

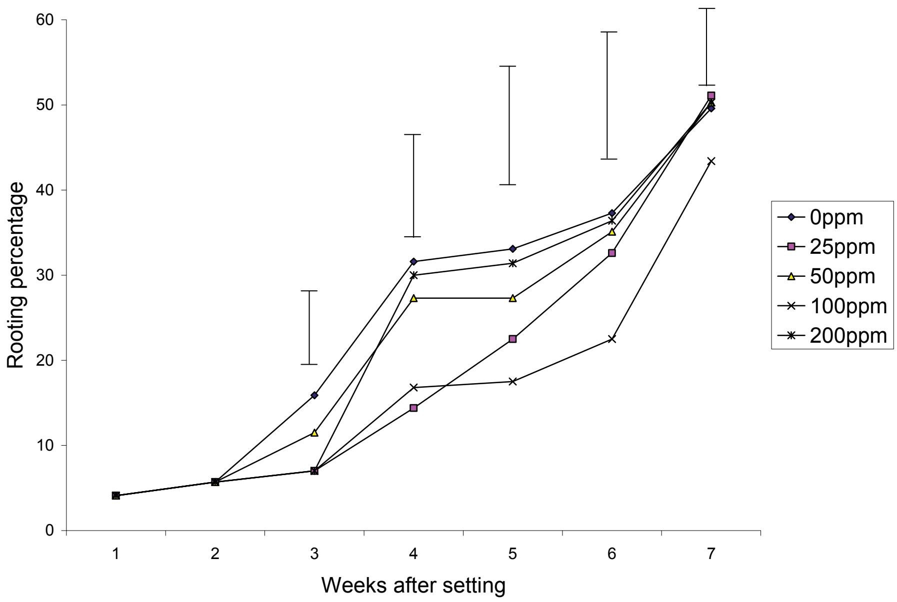
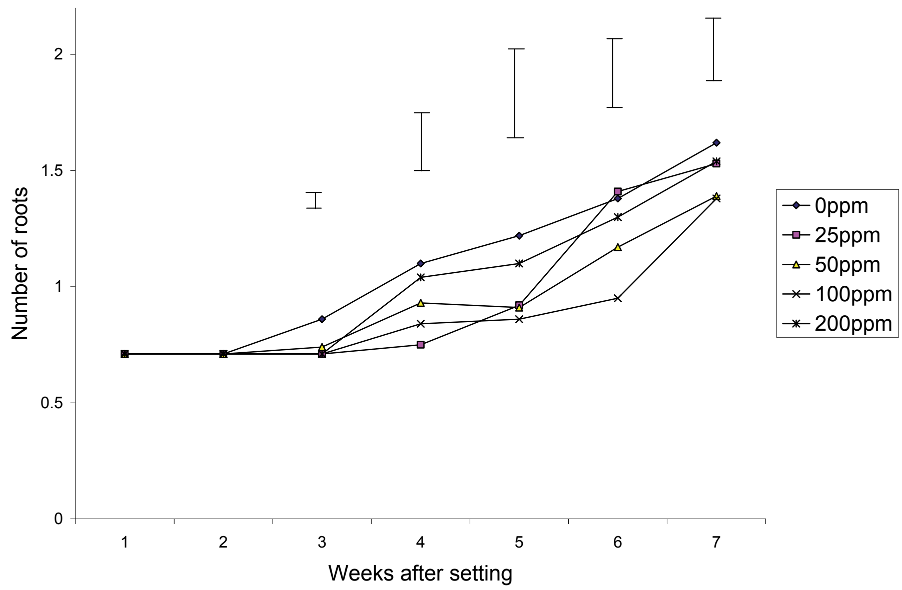


Figure 2: Effect of IBA concentration on the number of roots of leafy stem cuttings of *Irvingia wombolu*



50 ppm – 27.3%, 25 ppm 22.5% and 100 ppm – 17.5%. At the final assessment at Week 7, rooting percentage ranged from 43.4 to 51% in 100 ppm and 25 ppm respectively (Figure 1).

At Week 3, the number of roots was significantly higher (P = 0.01) in the control than the other treatments, which were not different from each other. Treatment effect on number of roots was negligible (P > 0.05) at Week 4 and Week 5. At Week 6, the control, 25 ppm and 200 ppm, were not different from 50 ppm but significantly higher (P = 0.05) than 100 ppm. Treatment effect was not significant (P > 0.05) at Week 7. The number of rooted cuttings ranged from 1.38 to 1.63 in 100 ppm and 0 ppm respectively (Figure 2).

The effect of IBA concentration on root length was negligible at Week 5. At Week 6 treatment effect on root length was significant (P = 0.03). 25 ppm recorded higher root length, though not different from 200 ppm and 50 ppm which in turn were not different from the control. However, 50 ppm, the control and 100 ppm were not different from each other. At Week 7, treatment effect was highly significant (P = 0.01). 200 ppm exhibited a higher root length though not different from 25 ppm which in turn was not different from 50 ppm which was not different from 100 ppm and the control (Figure 3).

Figure 3: Effect of IBA concentration on the root length of leafy stem cuttings of *Irvingia wombolu*

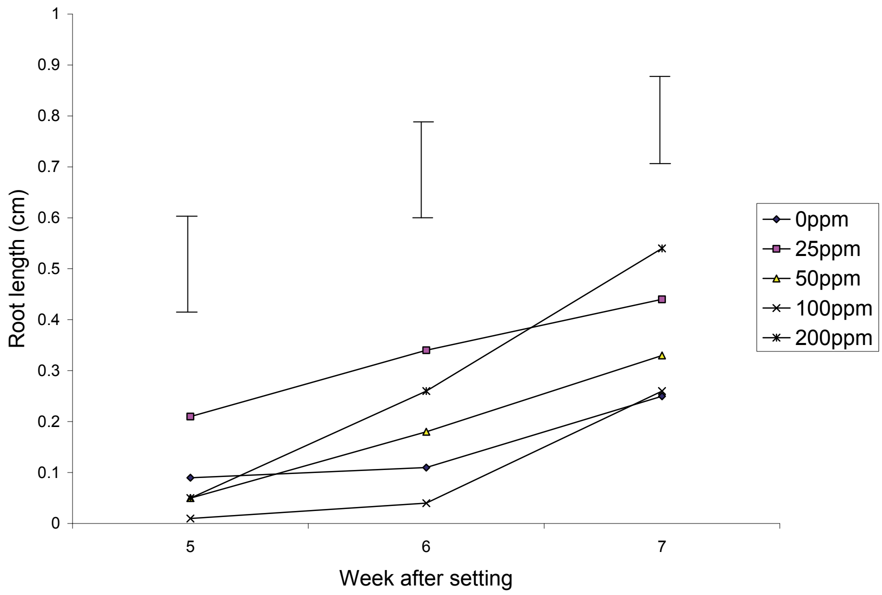
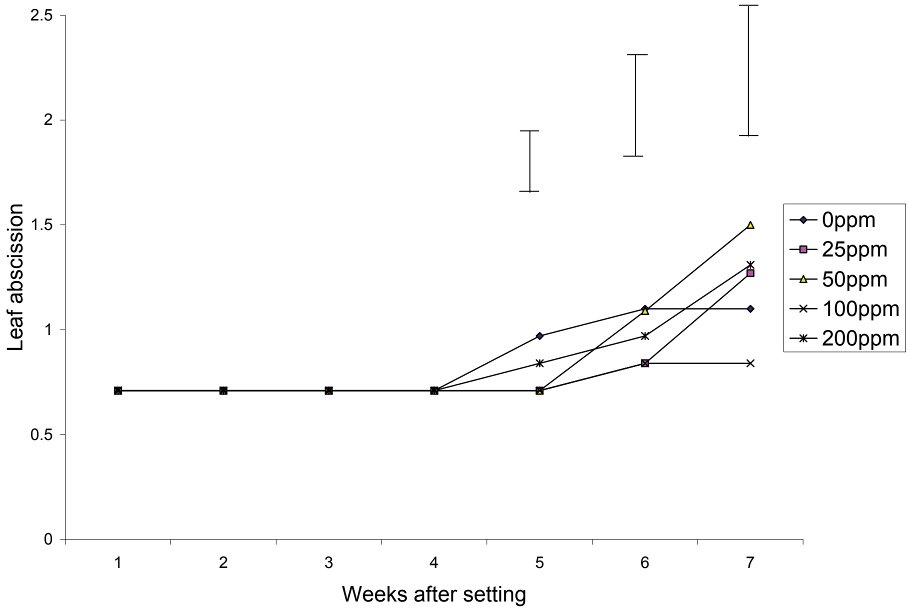


Figure 4: Effect of IBA concentration on leaf abscission of leafy stem cuttings of *Irvingia wombolu*



Leaf abscission was unaffected by treatment (P > 0.05). Leaf abscission was not observed until Week 5, with 1.25 percent and 2.5 percent leaf abscission recorded in 200 ppm & the control respectively. At Week 7 leaf abscission ranged between 1.2 to 10% in 100 ppm and 50 ppm respectively (Figure 4).

Cutting mortality was unaffected by treatment (P > 0.05). No cutting death was recorded until Week 5 in 0 ppm, 100 ppm and 200 ppm. At Week 7, cutting mortality ranged between 2.5% in 25 ppm and 1 200 ppm to 3.75% in the control (Figure 5).

Similarly, shoot formation was unaffected by IBA concentration (P > 0.05). The cuttings treated with 200 ppm IBA recorded a consistent higher number of new shoots produced throughout the trial. At the final assessment at Week 7, the proportion of cuttings forming new shoots range from 7.1% to 19.6% in 100 ppm and 200 ppm respectively (Figure 6).

DISCUSSION

The results from this study revealed that *Irvingia wombolu* can be successfully propagated by leafy stem cuttings in a non mist propagation system. This suggests that vegetative multiplication of this species is feasible.

Figure 5: Effect of IBA concentration on cutting mortality of leafy stem cuttings of *Irvingia wombolu*

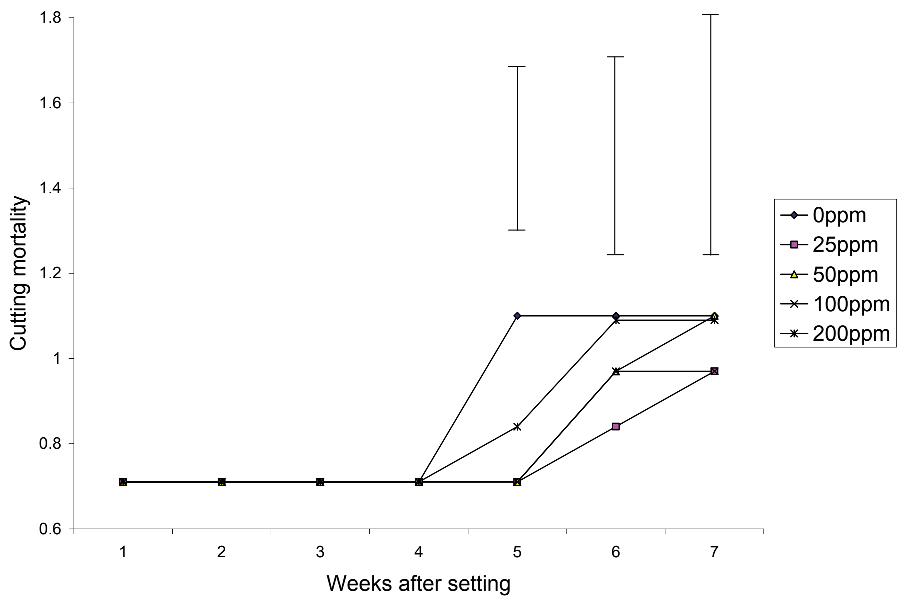
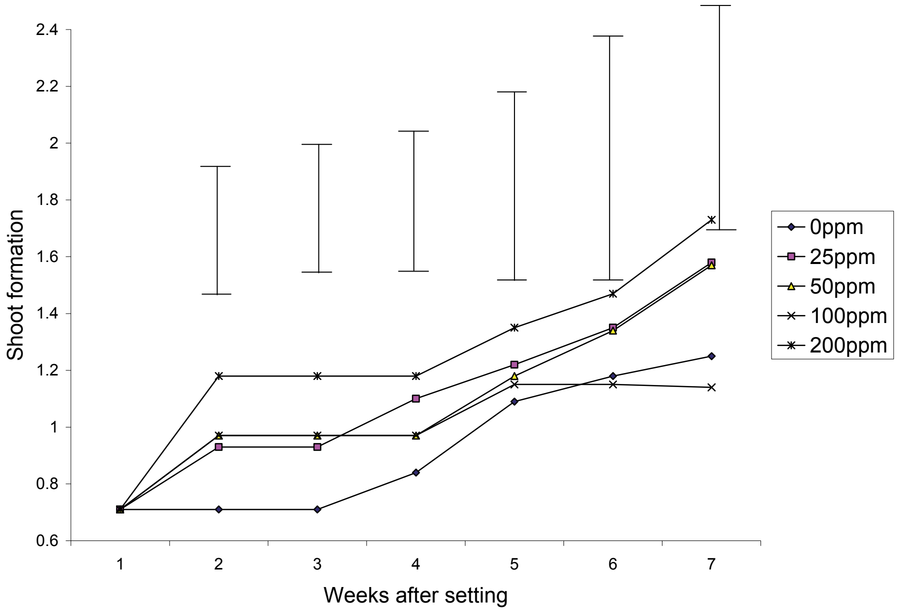


Figure 6: Effect of IBA concentration on shoot formation of leafy stem cuttings of *Irvingia wombolu*



The lack of significant effect of IBA concentration on rooting percentage overall contrasted with the result of the study by Nya et al. (2007) who obtained a higher rooting percentage with 500 ppm compared to the control. IBA treatment usually increase rooting dramatically (Leakey, 2004) and it is reasonable to assume that root formation is related to the level of free auxin. Shiembo et al. (1996) obtained a relatively slight effect of IBA concentration on rooting and asserted that *I. gabonesis* is relatively well supplied with endogenous auxins. Other tropical tree species such as *Nauclea diderrichii* (Leakey, 1990) and *Vochysia hundurensis* (Leakey et al., 1990) are also relatively insensitive to IBA concentration. However, the lack of pronounced effect of IBA concentration on rooting percentage may not be confusing. The lack of increased rooting with IBA concentration may be explained by Wiesman et al. (1989) who reported that IBA applied to the base of mung beans cuttings was transported to the upper part of the cutting as IBA conjugates which serves as an auxin source during later stages of rooting.

The lack of pronounced effect of IBA concentration on the mean number of roots seems unusual, although it agreed with the finding of Shiembo et al. (1996). A positive trend was recorded between IBA concentration and number of roots in pea cuttings (Nordstrom et al., 1991), *Albizia guachepele* and *Cordia alliodora* (Mesen, 1993), *Nauclea diderrichii* (Leakey, 1990), *Cinatum africanum* (Shiembo et al., 1996) and *I. gabonensis* (Nya et al., 2007). Nordstrom et al. (1991) pointed out that auxin content was apparently not the factor limiting root formation as a 100-fold increase in the internal IAA level that occurred during the first few days after application did not increase the rooting pattern in pea cuttings. They stated further that for root stimulation to occur obviously requires that the internal auxin level remains high during day 3 and 4. Thus the number of roots formed should therefore be regarded as a function of both concentration and time. This was confirmed by Ludwig-Müller et al. (2005).

The pronounced effect of IBA concentration on root length, specifically with 200 ppm recording the highest value in terms of mean root length tended to suggest that increasing IBA concentration will result in increased root length produced. However, the mean length obtained in 25 ppm being higher than 50 ppm and 100 ppm is therefore unusual.

The lack of treatment effect on leaf abscission is unusual. All the other treatments except 100 ppm recorded higher values than the control. No reason could be adduced for this seemingly lack of a clear trend in the values observed. The slightly higher percentage of leaf abscission in some of the treatments over the control appears incidental as leaf losses in all the treatments were recorded from the 5th week. This may be due to auxin induced leaf abscission as reported by Middleton et al. (1980), cited by Shiembo et al. (1996).

The lack of treatment effect and the seemingly lack of clear trend observed in cutting mortality, with 25 ppm and 100 ppm recording lower values than the control, with 200 ppm recording the highest value cannot be ascribed to auxin toxicity: deleterious effect of supraoptimal IBA concentration as indicated by Middleton et al. (1980) and Haissig (1986), cited by Shiembo et al. (1996). No relationship was observed between cutting mortality and leaf abscission, although Shiembo et al. (1996), linked cutting death with leaf abscission.

The lack of treatment effect on shoot formation is not unusual. This may be due to the propagation unit being saturated with watar making the unit highly humid and thereby reducing transpiration. Aloni et al. (2005) reported the absence of lateral buds of plant grown under condition of almost no transpiration. Cytokinins have been implicated in the regulation, promotion and development of shoot growth (Rowland and Ogden, 1992; Rahayu et al., 2005). This was supported by Fajimi et al. (2007) who observed shoot development in *I. gabonensis in-vitro* only when excised embryo was transferred to a kinetin supplemented media. Aloni et al. (2004, 2005) confirmed specifically that the root cap cells as the main site for cytokinin synthesis and is transported by transpiration stream mainly to developing shoot organs with high transpiration rates.

CONCLUSION

The study has revealed a lack of pronounced effect of IBA concentration on rooting of leafy stem cuttings of the species. This implies that *I. wombolu* is endowed with endogenous auxins hence may not require the application of synthetic auxin to induce rooting of the leafy stem cuttings for mass clonal propagation and it should therefore not be referred to as a difficult-to-root species. This is an indication that farmers can vegetative propagate this species by stem cuttings with or without IBA treatment.

REFERENCES

AINGE L., BROWN N. (2001): *Irvingia gabonensis* and *Irvingia wombolu.* A State of the Knowledge Report. CARPE. Available at http://carpe.umd.edu/resources/Dorcuments/report-aingebrown2001pdf/wiew.20K

ALONI R., LANGHANS M., ALONI E., ULLRICH C.I. (2004): Role of cytokinin in the regulation of root gravitropism. Planta, 220: 177–182.

ALONI R., LANGHANS M. ALONI E., DREIEICHER E., ULLRICH C.I. (2005): Root-synthesized cytokinin in *Arabidopsis* is distributed in the shoot by the transpiration stream. Journal of Experimental Botany, 56: 1535–1544.

Asaba Meteorological Office (2003). National meteorological report. Meteorological Bulletin, Lagos, Nigeria.

BANGERTH F., LI C.J., GRUGER J. (2000): Mutual interaction of auxins and cytokinins in regulating correlative dominance. Plant Growth Regulators, 32 (2–3): 205–217.

DICK J.MCP., LEAKEY R., MCBEATH C., HARVEY F., SMITH R.I., WOODS C. (2004): Influence of nutrient application rate on the growth and rooting potential of West Africa hardwood Triplohition Scleroxylon. Tree Physiology, 24: 35–44.

EHIAGBONARE J.E., ONYIBE H.I. (2008): Regeneration studies on *Pentaclethra macrophylla* Bth. Scientific Research and Essay, 3 (11): 531–536.

FAJIMI O., SARUMI M.B., OLAYODE M.N., GAMRA E.O., SANUSI S.I. (2007): *In-vitro* propagation of *Irvingia gabonensis*. African Journal of Biotechnology, 6 (8): 976–978.

Genstat (2007). Genstat discovery edition 3. Lawes Agricultural Research Trust, Rothamsted Experimental Station.

GOMEZ K.A., GOMEZ A.A. (1984): Statistical procedures for agricultural research. 2nd ed. John Wiley and Sons, N.Y.

HAISSIG B.E., DAVIS T.D. (1994) (eds.): A historical evaluation of adventitious rooting research to 1993. In Biology of Adventitious root formation. Plenum Press, New York.

JAENICKE H., FRANZEL S., BOLAND D. (1995): Towards a method to set priorities amongst multipurpose trees for improvement activities: a case study from West Africa. Journal of Tropical Forest Science, 7: 490–506.

LADIPO D.O. (1999): The development of quality control standards for Ogbone *(Irvingia gabonensis* and *Irvingia wombolu*) kernels: Efforts towards encouraging organized and further international trade in NWFP in West and Central Africa. Non-Wood Forest Products of Central Africa, Current Research Issues and Prospects for Conservation and Development. Available at www.fao.org/docrep/x2161/x2161e.htm

LADIPO D.O. (2000): Harvesting of Irvingia gabonensis and Irvingia wombulu in Nigerian forests; potentials for the development of sustainable systems. Paper presented at the Seminar Harvesting of Non-Wood Forest Products, held at Menemen-Izmir, Turkey on 2–8 October 2000. Available at http://www.fao.org/docrep/005/y4496e/y4496e32.htm

LADIPO D.O., BOLAND D.J. (1994): Trade in *Irvingia wombolu* kernels. In: Ladipo D.O., Bolands D.J. (eds.): Proceedings of the International Workshop on *Irvingia* in West Africa. International Centre for Research in Agroforestry (ICRAF), Nairobi, Kenya.

LADIPO D.O., FONDOUN J.M., GANA N. (1996): Domestication of the bush mango (*Irvingia spp*): Some exploitable intra-specific variation in West and Central Africa. In: Leakey R.R.B., Temu A.B., Melnyk M., VANTOMME P. (eds.): Domestication and Commercialization of Non-Timber Forest Products for Agroforestry. Non-Timber Forest Products, Paper 9, FAO Rome, pp. 193–206.

LEAKEY R.R.B. (1990): *Nauclea diderrichii:* rooting of stem cuttings, clonal variation in shoot dominance and branch plaglotropism. Trees, 4: 164–169.

LEAKEY R.R.B. (2004): Physiology of vegetative propagation: In: Burley J., Evans J., Youngquist J.A. (eds.): Encyclopedia of Forest Science. Academic Press, London, pp. 1655–1668.

LEAKEY R.R.B., MESSEN J.F., TCHOUNDJEU Z., LONGMAN K.A, DICK J.MCP., NEWTON A., MATIN A., GRACE J., MUNRO R.C., MUTHOKA P.N. (1990): Low-technology techniques for vegetative propagation of tropical trees. Commonwealth Forestry Review, 69: 237–257.

LEAKEY R.R.B., NEWTON A.C. (1994): Domestication of "Cinderella" species as an art of "woody plant revolution". In: Leakey R.R.B., Newton A.C. (eds.): Tropical Trees: the Potentials for Domestication and the Rebuilding of Forestry Resources. HMSO, London, pp. 3–6.

LUDWIG-MÜLLER J., VERTOCNIK A. TOWN C.D. (2005): Analysis of indole-3-butyric acid-induced adventitious root formation on *Arabidopsis* stem segments. Journal of Experimental Botany, 56 (418): 2095–2105.

MESSEN F., LEAKEY R.R.B., NEWTON A.C. (2001): The influence of stock plant environment on morphology, physiology and rooting of leafy stem cuttings of *Albizia guachapele.* New Forests, 22: 213–227.

MIDDLETON W. JAVIS B.C., BOOTH A. (1980): The role of leaves in auxin and boron dependent rooting of stem cuttings of *phasolus aureus.* New Phytologist, 84: 251–259.

MOSS R. (1995): Unexploited tree crops: components of productive and more sustainable farming systems. Journal for Farming Systems Research Extension, 5 (1): 107–117.

NORDSTROM A.C., JACOBS F.A., ELIASSON L. (1991): Effect of exogenous indole-3-acetic acid and indole-3-butyric acid on internal levels of the respective auxins and their conjugation with aspartic acid during adventitious root formation in pea cuttings. Plant physiology, 96: 856–861.

NYA P.I., OMOKARO D.N., NKANG A.E. (2006): Effect of pretreatments on seed viability during fruit development of two varieties of *Irvingia gabonensis*. Global Journal of Pure and Applied Sciences, 12 (2): 141–148.

NYA P.I., OMOKARO D.N., NKANG A.E. (2007): Effect of hormonal treatments on sprouting and rooting of stem cutting of *Irvingia gabonensis*. Nigerian Journal of Botany, 20 (1): 119–131.

RAHAYU Y.S., WALCH-LIU P., NEUMANN G., ROMHELD V., VON WIREN N., BANGERT F. (2005): Root-derived cytokinins as long-distance signals for NO_3-induced stimulation of leaf growth. Journal of Experimental Botany, 56: 1143–1152.

ROWLAND L.J., OGDEN E.L. (1992): Use of a cytokinin conjugate for efficient shoot regeneration from leaf sections of highbush blueberry. HortScience, 27: 1127–1129.

SCHRECKENBERG D.K., MBOSSO A., BABOULE C., ENYONG L., KANMEGNE L., NGONG C. (2002). The social and economic importance of *Dacryodes edulis* (G.Don) H.J.Lam is southern Cameroon. Forests, Trees and Livelihood, 12: 15–40.

SHIEMBO P.N., NEWTON A.C. , LEAKEY R.R.B. (1996): Vegetative propagation of *Irvingia gabonensis*, a West Africa fruit tree. Forest Ecology and Management, 87: 185–192.

TCHOUNDJEU Z., KENGUE J., LEAKEY R.R.B. (2002): Domestication 71 of *Darcryodes edulis*: state-of-the-art. Forests, Trees and Livelihoods, 12: 3–13.

WIESMAN Z., RIOV J., EPSTEIN E. (1989): Characterization and rooting ability of indole-3-butyric acid conjugates formed during rooting of mung bean cuttings. Plant Physiology, 91: 1080–1084.

Received for publication on May 19, 2009
Accepted for publication on June 6, 2010

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