EFFECT OF SOME SEED EXTRACTS AND IBA ON THE ROOTING OF LEAFY STEM CUTTINGS OF *IRVINGIA WOMBOLU* (VERMOESEN)

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Abstract

The effects of some post severance treatments on the rooting potentials of juvenile stem cuttings of Irvingia wombolu was assessed in an experiment conducted at the Teaching and Research Farm of the Delta State University, Asaba Campus, Nigeria. 400 single node softwood cuttings of I. wombolu were randomly assigned to the 5 treatments namely orange seed extract, pawpaw seed extract, African pear seed extract, 250 ppm indole-3-butyric acid (IBA) dissolved in industrial alcohol and a control (50% alcohol) applied by dipping the base of the cuttings for 5 seconds The results displayed a pronounced effect of seed extract and IBA on the rooting percentage with IBA (34.4%) and pawpaw seed extract (26.7%) not different from each other but higher ($P < 0.001$) than the control (14.8%), orange (11.9%) and African pear (10.8%). The mean numbers of roots ranged between 0.71 and 2.01 in African pear and IBA respectively. A similar result was obtained for root length. Seed extracts and IBA did not significantly ($P > 0.05$) influence leaf abscission, cutting mortality and shoot formation. The results suggest the pawpaw seed extract can replace synthetic IBA in inducing rooting of cuttings of I. wombolu for mass clonal propagation.

Key words: *Irvingia wombolu*, dika nut, ogbono, vegetative propagation, seed extracts, pawpaw, orange, African pear, IBA, auxin, domestication, non-mist propagation system

INTRODUCTION

Irvingia gabonensis and *Irvingia wombolu* are two trees species that produces the most economically viable non-timber forest products (NTFPs) from the rain forest zone of West Africa (Ladipo, 2000; Tchounjeu et al., 2002). Both *Irvingia* species are found growing wild in the humid lowland forests of tropical Africa in Angola, Cameroon, Central African Republic, Congo, Equatorial Guinea, Gabon and Zaire (Harris, 1996) with *I. wombolu* extending to Senegal (Ndoye et al., 1997, cited by Ainge and Brown, 2001).

Various authors have described the enormous potentials of *Irvingia* both in economic terms and as a species for sustainable production). According to Ladipo (2000) the consideration of *Irvingia* as a strategic crop and one that has immense value for food security cannot be overemphasized considering its nutritional values. The attractiveness of *Irvingia* is derived from the highly valuable by-product of the fruit – the kernels which are ground into a paste or cake called 'dika bread' and used as soups, stews or sauce additive for flavouring and thickening (Agbor, 1994; Leakey and Newton, 1994; Leakey et al., 2003). These products are traded within Nigeria and between countries in West and Central Africa (Ndoye et al., 1997). These products 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).

Bush mango and other high value indigenous fruit trees are becoming increasingly difficult to collect due to deforestation and old tree age (Okafor, 1980; Ladipo, 2000). There is therefore an urgent need to domesticate these fruit trees and integrate them fully into farmlands or as enrichment planting inside the forest. Constraints to domestication of the species include the long gestation period of seed sown *Irvingia* (Moss, 1995), poor germination capacity (Nya et al., 2000) variability of fruits and kernel characteristics, variability in tree size (Ladipo et al., 1996; Schreckenberg et al., 2001) and limited knowledge base (Tchoundjeu et al., 2002).

Bush mango has poor seed germination potential which less than 50% germination capacity when freshly collected and sown (Nya et al., 2006). Furthermore, bush mango trees grown from seed may take over 10 years to start producing fruits and although cultivation can be improved more easily through seeds, farmers would like to see financial returns sooner (Moss, 1995; Ladipo et al., 1996). Faster propagation techniques are therefore needed to produce trees of selected desirable phenotypes based on improvement objective identified by farmers for planting (Angie and Brown, 2001). Vegetative propagation techniques are indispensable for the capture and multiplication of the phenotypic variation expressed (Leakey and Simon, 2000) and offers the means to achieve greater improvement in the shortest possible time (Mesen et al., 2001; Leakey et al., 2003). Mass clonal propagation involves the production of adventitious roots which are facilitated by the treatments of cuttings with synthetic hormones (auxins) which are very expensive and often difficult to get. Many natural occurring compounds that exert auxin-like effects have been revealed (Kogl et al., 1934, cited by Hartmann et al., 1997; Ludwig-Müller, 2000; Bartel et al*.,* 2001).

This study was undertaken to provide more insight on the domestication of the species by vegetative means and to assess the effect of different seed extracts and synthetic IBA on the rooting of the juvenile stem cuttings of the species.

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 1 500 mm to 1 849.3 mm. Mean temperature are 23.3ºC with a maximum of 37.3ºC. Mean monthly soil temperature at 100 cm depth and sunshine is 28.3ºC and 4.8 bars respectively (Asaba Meteorological Centre, 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 prior to severance.

A propagation unit was established in the Department of Forestry and Wildlife for the experiments. The low-tech non-mist propagator, measuring 3.05 m × 6.10 m × 2.14 m is a modified design described Leakey et al. (1990). The propagation unit was cited in a shade-house providing irradiance inside the propagator of sporo-ximately 15–30 percent of that received outside the unit.

Mature fruits of *Carica papaya* (pawpaw), *Citrus spp* (orange) and *Dacryodes edulis* (African pear) were collected from fruit trees in the campus. The seeds were extracted from the fruits and washed thoroughly in clean water and sun dried for 2 weeks after which they were ground separately in a sterile mortar to obtain 100 g each of the dry seed powder. Ethanol extracts of the seeds were obtained by adding 100 g each of the seed powder to 100 ml of ethanol in a beaker to obtain 100 g (wt/v) of the extracts, and left to settle for 5 hours at room temperature before the extracts were filtered using cheesecloth. Sterilization of the extracts was done by applying 125 mg of streptopenicillin (a mixture of 62.5 mg of streptomycin & 62.5 mg of penicillin (Gupta and Banerjee, 1990). 400 SNSWC, four from each shoot were harvested from the stock plants and randomly assigned to the 5 treatments namely orange seed extract, pawpaw seed extract African pear seed extract, 250 ppm IBA dissolved in industrial alcohol and a control (50% alcohol). The base of the cuttings were dipped in the extracts, evaporated in a gentle air before setting in composted sawdust in the propagator in a Randomized Complete Block Design with 4 replicates. 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

Treatment effect on rooting percentage was highly significant ($P < 0.001$) at the commencement of rooting at Week 3 with IBA different from the rest treatments which were yet to root. Results at Week 4 were similar to Week 3. At Week 5, treatment effect on rooting percentage was highly significant ($P < 0.001$) with IBA recording higher rooting than pawpaw, which in turn was not different from the rest treatments. At Week 6, a highly significant ($P < 0.001$) treatment effect was obtained with IBA, different from pawpaw, which in turn was different from the rest treatments, which were not different from each other. At the final assessment at Week 7, IBA and pawpaw display significantly ($P < 0.001$) higher rooting percentage than the rest treatments, which were not different from each other. At this time, rooting percentage ranged between 0% to 34.4% in African pear and IBA respectively (Figure 1).

Treatment effect on number of roots was highly significant at Week 3 ($P < 0.009$) and Week 4 ($P < 0.001$) with IBA, different from the rest treatments, which were yet to root. At Week 5, IBA recorded significantly higher ($P < 0.02$) number of roots than the rest treatments, which were not different from each other. At Week 6 a highly significant ($P < 0.001$) effect of treatment was obtained with IBA, different from the pawpaw, which was not different orange and the control which in turn was not different from African pear. At the final assessment at Week 7, the overall effect of treatment on number of roots was highly significant ($P < 0.001$) with IBA, higher than pawpaw, which in turn was higher than the rest treatments, which were not different from each other (Figure 2).

Root length was significantly ($P < 0.02$) influenced by treatment at Week 3 with IBA, higher than the rest treatments. At Week 4, treatment effect was significant ($P < 0.01$) with IBA higher than the rest treatments which

Figure 1: Effect of seed extracts and IBA on rooting percentage of leafy stem cuttings of *Irvingia wombolu*

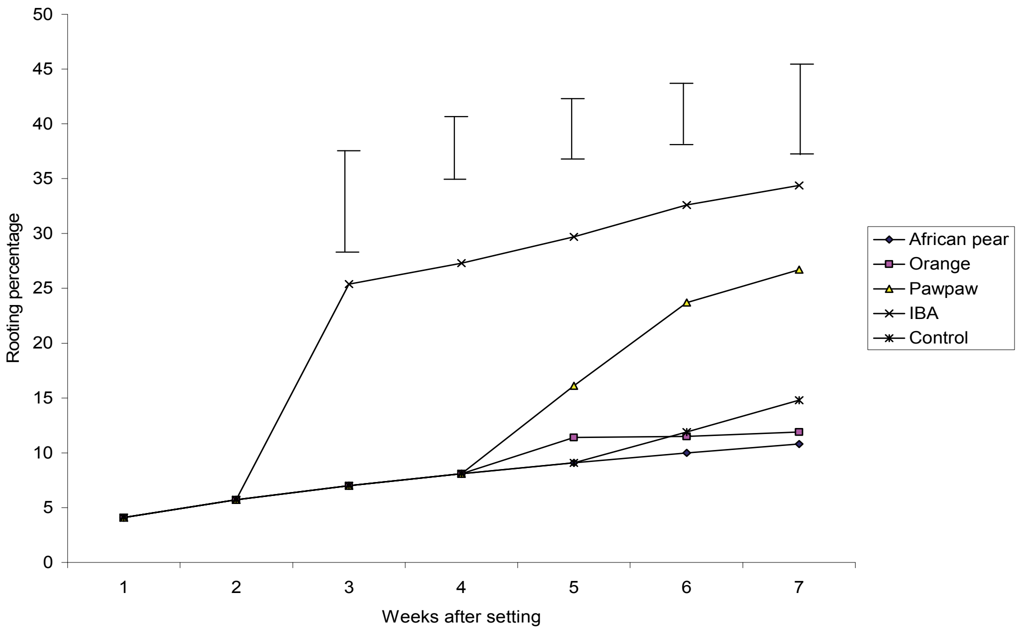
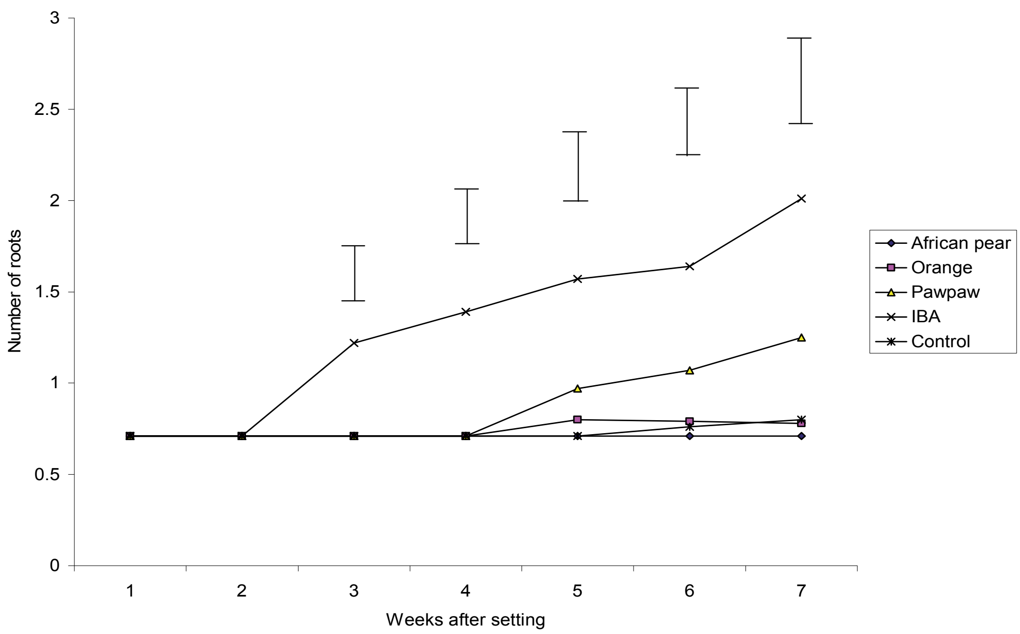


Figure 2: Effect of seed extracts and IBA on number of roots of leafy stem cuttings of *Irvingia wombolu*



were not different from each other. A highly significant ($P < 0.01$) effect of treatment on root length was recorded with IBA, higher than pawpaw, orange which in turn was higher than the rest treatments, which were not different from each other at Week 5. At the final assessment at Week 7, treatment effect on root length was highly significant overall ($P < 0.001$) with IBA recording higher value than pawpaw which in turn was higher than the rest treatments, which were not different from each other (Figure 3).

Leaf abscission was unaffected by treatment ($P > 0.05$). IBA recorded the least value consistently from Week 1 to Week 7. At the final assessment at Week 7, the proportion of leaf death ranged between 3.5% to 10.7% in IBA and the control respectively (Figure 4).

Cutting mortality was unaffected by treatment ($P > 0.05$) from week 1 to week 3. No cutting death was recorded in Week 1, in Week 2, African pear, orange and pawpaw recorded any cutting mortality. At Week 4, treatment ef-

Figure 3: Effect of seed extracts and IBA on root length of leafy stem cuttings of *Irvingia wombolu*

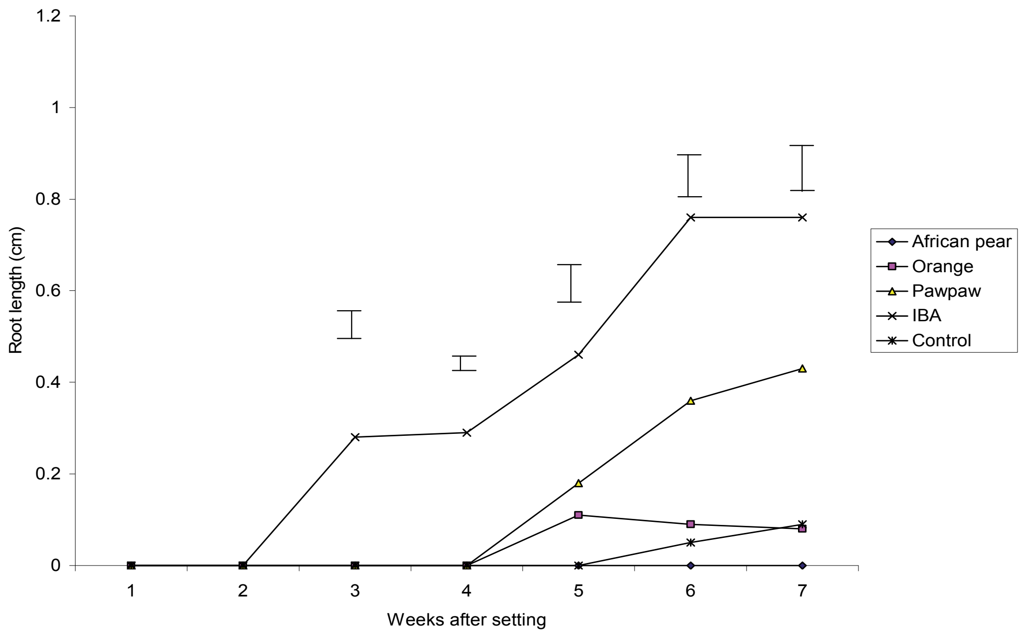
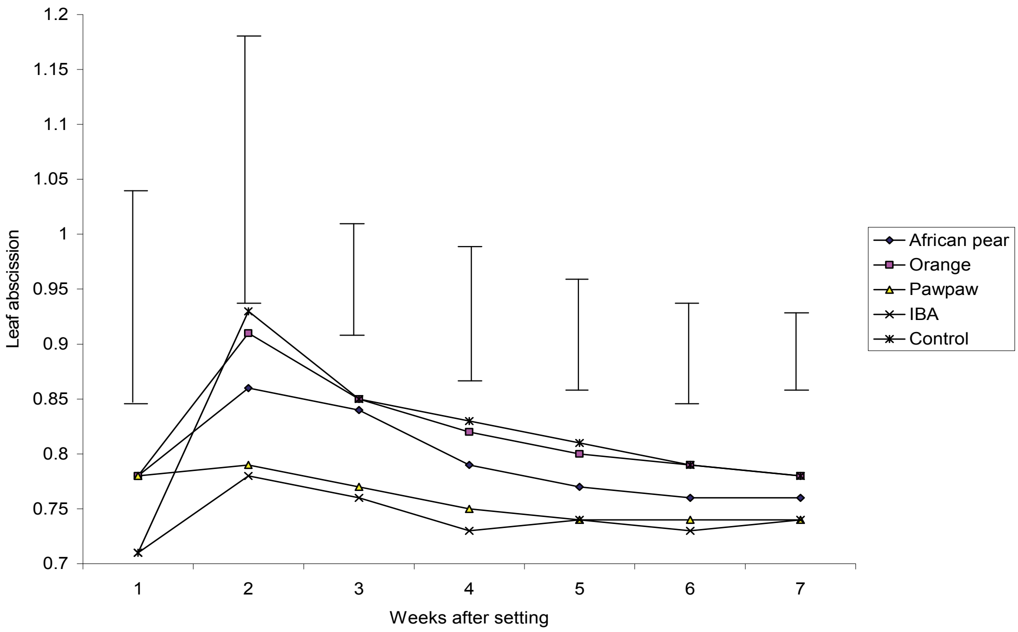


Figure 4: Effect of seed extracts and IBA on leaf abscission of leafy stem cuttings of *Irvingia wombolu*



fect was highly significant ($P < 0.03$) with IBA, recording higher value than the rest treatments, which were not different from each other. Treatment effect tended to diminish thereafter. At Week 7, there was no treatment effect overall ($P > 0.05$) with proportion of cutting death ranging from 0% to 21.3% in pawpaw and the control respectively (Figure 5).

By Week 2, shoot formation was unaffected by treatment ($P > 0.05$) with orange and African pear recording higher values than pawpaw, while no shoot was formed yet in IBA and the control. Week 3 and Week 4 displayed a highly significant ($P < 0.001$) treatment effect with orange African Pear recording higher values than the rest treatments, which were not different from each other. At Week 5, treatment effect was highly significant ($P < 0.002$) with IBA recording lowest mean, although not different from pawpaw and the control which in turn were not different from African pear, which was not different from orange.

Figure 5: Effect of seed extracts and IBA on cutting mortality of leafy stem cuttings of *Irvingia wombolu*

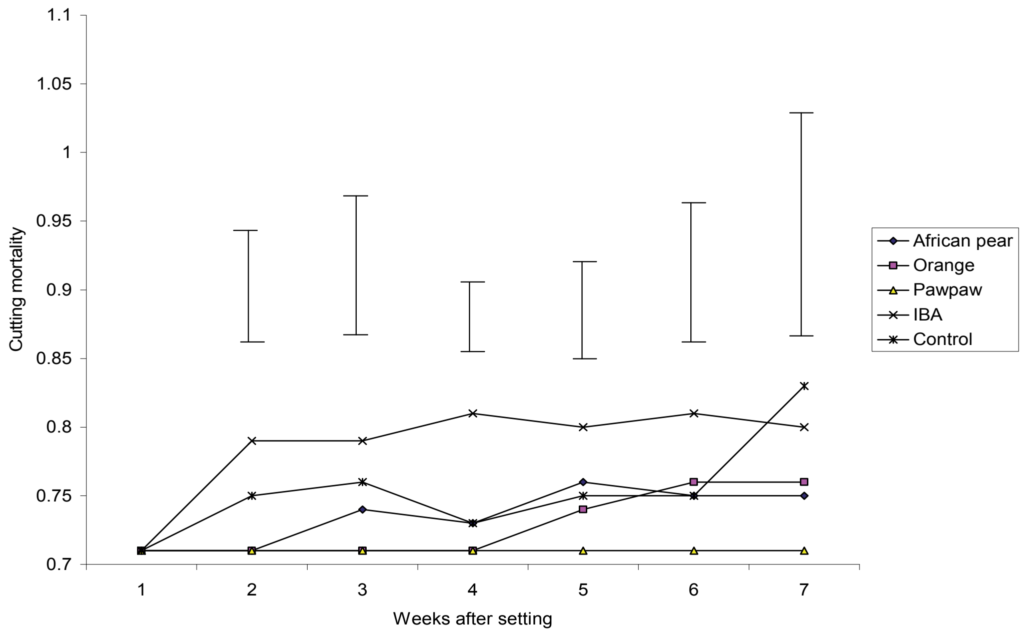
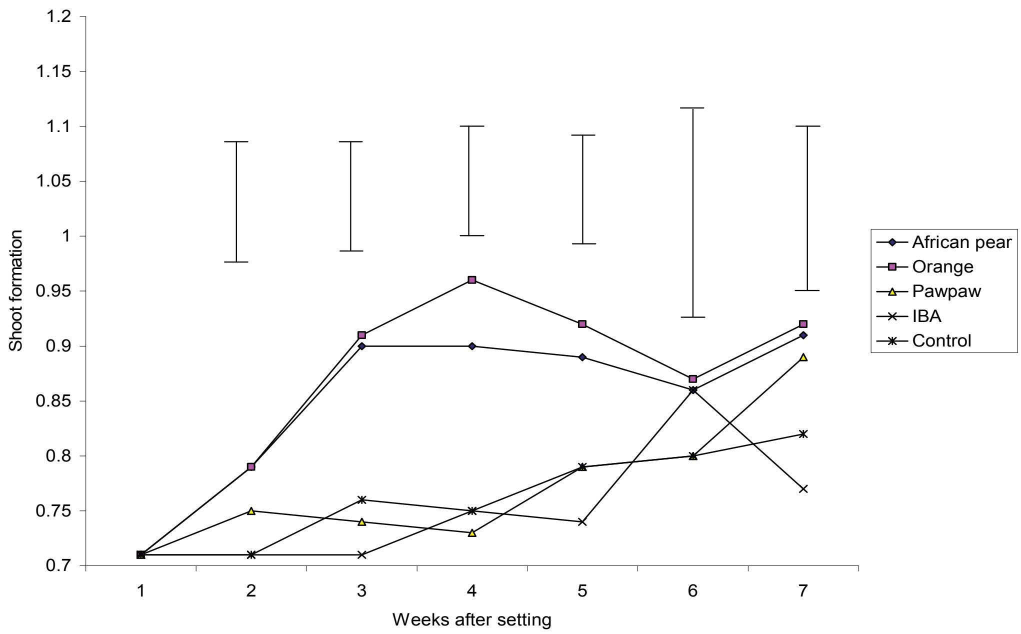


Figure 6: Effect of seed extracts and IBA on shoot formation of leafy stem cuttings of *Irvingia wombolu*



There was no treatment effect on cutting mortality at Week 6. At Week 7, the proportion of cuttings producing new shoot ($P > 0.05$) ranged from 8.9% to 35.7% in IBA and orange respectively (Figure 6).

DISCUSSION

The results from the experiment revealed that *I. wombolu* can be successfully propagated by leafy stem cuttings in a non mist propagation system, which suggests that vegetative multiplication of this species is feasible. The results clearly established the potentials of pawpaw seed extracts in enhancing the rooting of leafy stem cuttings of *I. wombolu.* The results also indicate that pawpaw seed extracts could replace synthetic IBA in enhancing the rooting of the stem cutting of the species for multiplication. Although IBA recorded a higher rooting percentage than pawpaw seed extract, they were not different from each other. The significantly higher rooting percentage in pawpaw seed extract compared to the control may be due to the ability of plants to store IAA in the form of conjugates and IBA in their seeds which can provide free IAA upon hydrolysis or β-oxidation respectively (Zolman et al., 2000). According to Ashanmugavalu (1985), extracts of cashew seeds when chemically assayed contained natural auxin IAA with Rf of 0.03. The poor performance of African pear (which produced no roots) and orange may not be confusing. According to Sztein et al. (1999), among divergent plants, endogenous auxin levels are quite variable. Furthermore, different plant species have distinct IAA conjugate profiles (Cohen and Bandurski, 1982; Slovin et al., 1999), cited by Woodward and Bartel (2005). According to Normanly et al. (1993) and Tam et al. (2000), cited by Woodward and Bartel (2005), in experiments using alkaline hydrolysis to release free IAA from conjugates revealed that *Arabidopsis* maintains approximately 90% of IAA in amide linkages, 10% as ester-linked conjugates and 1% as free IAA. Ljung et al. (2002) stated that most of the amide-linked conjugates in *Arabidopsis* seeds are solvent insoluble, pointing out that a certain IAA-peptide are present; the large size of this conjugate may contribute to the solvent insolubility of amide conjugates. This may lead one to suggest that even when auxins are present in high levels, it may not available to the cuttings. Another reason for the poor performance of African pear and orange seed extracts may be due to the oily nature of the extracts which may have created a firm around the base of the cuttings thereby cutting off access to much needed oxygen for respiration. The delayed rooting (5^{th} week) in the cut-tings treated with seed extracts when compared to those dipped in IBA (3^{rd} week) may not be unconnected with the slow release of free IAA in the cuttings treated with seed extracts.

Although the result revealed no pronounced effect of treatment on the mean number of roots, the means mimicked the trend obtained in rooting percentage, with IBA and pawpaw seed extracts recording higher values, though not significantly different from the control and the rest treatments. The reason for the lack of treatment effect is not clear. Future studies focusing on increasing the seed extract concentrations could reveal the potentials of pawpaw seed extract in enhancing the number of roots produced.

The profound influenced of root length by IBA and pawpaw seed extracts agrees with Middleton *et* al. (1980), who attributed it to the effect of auxin mobilization of carbohydrates and subsequent transfer to the root zone. This suggests that the application of pawpaw seed extracts to the base of the cuttings of the species could enhance the number of roots produced. The inability of the other seed extracts to influence the number of roots produced may not be unconnected with the reasons adduced earlier.

Although no pronounced effect of seed extracts and IBA on leaf abscission, cutting mortality and shoot formation was obtained, there is an indication from the trend in the results that the seed extracts may have the potentials to lower leaf abscission and cutting mortality, while enhancing the number of cuttings forming new shoots, inspite of the lack significant effect of treatments recorded.

In conclusion, the present study has demonstrated that pawpaw seed extract can enhance the rooting of the leafy stem cuttings of *I. wombolu* for mass clonal propagation and could therefore replace the expensive synthetic IBA in enhancing the rooting of the stem cuttings of the species.

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Received for publication on May 19, 2009
Accepted for publication on October 19, 2009

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