Vegetative propagation of *Chrysophyllum albidum* G. Don by leafy stem cuttings

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Abstract

Chrysophyllum albidum is a tree species that is commonly intercropped with cocoa and is valued for its edible fruits. Since the provision of clonally propagated superior genotypes of this species would be beneficial to farmers, investigations were conducted to optimize its vegetative propagation. In the study, the first experiment examining the effect of leaf area on propagation success involved cuttings with leaf areas of 0 (leafless), 20, 40, 80, 160 and 200 cm^2. The results showed that cuttings with a leaf area of 40 cm^2 gave the highest rooting percentage (77.8% after 10 weeks), which was significantly higher than the other treatments ($P < 0.001$). The second experiment demonstrated that the use of different IBA concentrations (25, 50, 100, 200 and 300 $\mu g\ l^{-1}$ of water) did not give a higher rooting success than the control. The third experiment looked at the effect of four rooting media (fine sand, topsoil, sawdust and 1:1 mixture of topsoil and sawdust), and found that callusing was much lower for the latter treatment. It was concluded that *C. albidum* can be successfully propagated using stem cuttings with a leaf area of 40 cm^2 in a propagating medium of sawdust or a 1:1 mixture of topsoil and sawdust.

Original scientific paper. Received 12 Sep 13; revised 22 Oct 13

Introduction

Chrysophyllum albidum G. Don is a tree species that belongs to the family Sapotaceae and grows to a height of 40 m (Burkill, 1985). Its common name is white star apple and it is naturally distributed from Sierra Leone to Cameroon (Irvine, 1961; Burkill, 1985). Its fruit, which is edible, is globose in shape, yellow-orange in colour when ripe and sold in local markets (Taylor, 1960).

C. albidum is found in semi-deciduous tropical forests and also planted in plantations or on small holdings as shade trees with food crops or cocoa, for example, in the Eastern Region of Ghana. In southern Benin, it has been reported as a multipurpose tree that is used for food, medicine, firewood and timber (Houessou *et al*., 2012). Farmers who plant *C. albidum* need propagules from superior trees to plant to maximise profit. However, there is little information on the vegetative propagation of the tree, which would be of much value to farmers and extension agents.

A number of tropical tree species have been successfully vegetatively propagated through the rooting of stem cuttings (Shiembo, Newton & Lankey, 1996). There are a

number of key factors that influence such rooting. One of these is the leaf area of the cutting since the leaves provide carbohydrates and auxins for the cuttings thereby enhancing rooting (Leakey, Newton & Dick, 1994; Newton *et al.*, 1992). The size of the leaf has been found to be important for rooting as a large leaf area could lead to higher water loss through transpiration, and a small area may not be able to provide enough auxins and carbohydrates to support rooting as found in earlier studies in *Triplochiton scleroxylon* (Leakey & Coutts, 1989) and *Irvingia gabonensis* (Shiembo, Newton & Leakey, 1996). Different concentrations of exogenous application of auxins (IBA) can have varying effects on rooting percentage of stem cuttings of some tree species (Leakey, Chapman & Longman, 1982; Leakey, 1990). The type of rooting medium can also have an effect on percentage rooting and number of roots of some tree species (Leakey *et al*., 1990; Ofori *et al.*, 1996). Rooting is enhanced in a medium that has good drainage, quite high water retention capacity and porosity for aeration (Hartmann *et al*., 2002). Leakey (2004) has noted various other factors that can affect rooting of stem cuttings. These include the propagation chamber environment in terms of temperature, light and water status, stock plant physiology, management practices including phytosanitory conditions and the hardiness of cuttings.

In the study the non-mist propagation system (Leakey, 1990) was used. The effect of leaf area, auxin (IBA) concentration and rooting medium on rooting of stem cuttings of *C. albidum* were studied to determine an optimum treatment for clonal production using stem cuttings.

Materials and methods

Study area and husbandry experimental practices

Stumps of 12 *C. albidum* trees were cut to a height of 30 cm at the Bunso Arboretum in the Eastern Region of Ghana (N 06° 16′, W 00° 27′, Altitude 257 m) in October 2008 to produce coppiced shoots. At the same time, seedlings were raised from seeds of *C. albidum* at a nursery in the Bunso Arboretum. The seedlings were watered until they were 5 months old after which they only received natural rainfall. The coppiced trees received inorganic fertilizer (N.P.K. 15:15:15) at a rate of 145 g per plant on two occasions when they were 2 and 5 months old to encourage growth of the shoots. They were sprayed with the fungicide Bendazim (Carbendazim) at 15 g/15 litres of water against *Phytophthora* spp. and the insecticide Cydim Super (a combination of Cypermethrin and Dimethoate), at a rate of 35 ml/15 litres of water to control leaf hoppers and mites, every 2 months.

Nine non-mist propagators were constructed following the design described by Leakey *et al.* (1990). The propagators consisted of wooden frames covered with a transparent polythene sheet. Each propagator measured 100 cm in length, 90 cm in width, 70 cm for lower height and 90 cm for the upper height. The bottom of each propagator box had 20 holes measuring 1 cm in diameter drilled at intervals to allow drainage of water. The floor of the box was covered with a transparent polythene sheet which had been punched with 40 holes of diameter of 3 mm. The propagators were placed in a greenhouse covered with a 50 per cent black shade cloth. The floor was then filled with sterilised fine sand to a depth

of 4 cm.

Plastic pots (top diameter 6 cm, bottom diameter 4 cm, height 6 cm) were used. The base of each container had a circular opening diameter of 2 cm. To prevent particles of the rooting medium from falling through, a 4-cm circular plastic mesh (mesh size 1 mm^2) was placed at the bottom of the container.

Three experiments were conducted simultaneously on the rooting of leafy stem cuttings of *C. albidum*. The first experiment examined the effect of leaf area, the second experiment examined the effect of Indole-3-butyric acid (IBA), and the third experiment considered the effect of rooting media.

The effect of leaf area on rooting of stem cuttings of C. albidum

The stumps from the 12 trees that were cut in October 2008 produced coppiced shoots ranging from 30 to 150 cm. On each stump, 10 or more shoots were allowed to grow. On 11 September 2009 the coppiced shoots were harvested from 6.00 a.m. to 6.30 a.m. in order to obtain turgid shoots. These were placed in polythene bags and sprayed with water and transported to the laboratory. For each stem cutting, the woody portion nearest the tree stump and the 3-cm terminal end were cut off as these portions are known to contain little carbohydrate (Hartmann *et al.* 2002). Cuttings were then made from the rest of the shoots. A single stem cutting consisted of one node, and a leaf at the next node up and as many stem cuttings as could be made from shoots were produced. A leaf on a stem cutting was trimmed to the appropriate leaf area for a given treatment using a paper template measured with a leaf area meter (LI-3100C Area Meter), (Li-cor Biosciences, Nebraska, U.S.A.).

Pots were arranged in a randomised complete block design with six replicates. The cuttings were randomly trimmed to the following areas: 0 (leaf removed), 20, 40, 80, 160 and 200 cm^2 using the paper templates, and these areas served as treatments. There were six cuttings for each treatment in each block. There were three propagators for the experiment and each propagator was divided into two blocks giving six blocks and 36 cuttings per treatment.

The base of each cutting was treated with 10 µ*l* of 20 µg l^{-1} of IBA concentration using a 10 – µ*l* micropipette (Thermo Fisher Scientific Co., Finland). The rooting medium was a 1:1 mixture of topsoil and sawdust put in plastic pots. The topsoil was obtained from the forest floor of the Bunso Arboretum. The sawdust which comprised a mixture of different tropical hard woods was from a sawmill at Bunso in Eastern Region of Ghana. The propagators were sprayed with the fungicide Bendazim (Carbendazim) (15 g/15 *l* water) against *Pythium* sp., *Fusarium* sp. and *Phytophthora* spp. and the insecticide Cydim Super (Cypermethrin and Dimethoate) (35 ml/15 *l* water) against aphids, mealy bugs and thrips 3 days prior to the experiment. The cuttings were sprayed with the same fungicide and insecticide prior to the insertion of the stem cuttings into the media.

The stem cuttings were watered each day at 7.00 – 7.30 a.m. and 5.00 – 5.30 p.m. and also whenever the propagators were opened, with a 2.5-litre hand-pumped pressure sprayer to provide humid environment. Stem cuttings were assessed weekly starting from Week 2 to Week 10 and the following data were recorded: presence or absence of

callus, presence of roots (2 mm or longer), number of roots (2 mm or longer), length of the longest root, formation of a shoot, shedding of leaf and cutting mortality.

Effect of indole-3-butyric acid (IBA) concentration on rooting of stem cuttings of C. albidum

Stem cuttings from 1 year old seedlings of *C. albidum* were used for the experiment. A single stem cutting consisted of one node and a leaf at the next node up. As many stem cuttings as could be made from shoots were produced leaving out the terminal and lower parts. A leaf on a stem cutting was trimmed to 40 cm^2 using a paper template measured with a Leaf Area Meter (LI-3100C Area Meter), (Li-cor Biosciences, Nebraska, USA). The cuttings were randomly assigned to one of six different IBA solutions: 0, 25, 50, 100, 200 and 300 µg l^{-1} of water. In each treatment, 10 µ l^{-1} of the IBA solution was applied to the base of the stem cutting.

As described in Experiment 1, the rooting medium used was a 1:1 mixture of topsoil and sawdust put in plastic containers of lined small pots. The propagation unit and the rooting medium were sprayed with fungicide and insecticide 3 days prior to the experiment. The cuttings and the plastic were sprayed with the same fungicide and insecticide before they were inserted into the rooting medium as described in Experiment 1.

The randomised complete block design with six replicates was used. There were six blocks in three non-mist propagation units as in Experiment 1 (each propagator was divided into two blocks). There were six treatments in each block and each treatment had six cuttings giving 36 cuttings per treatment.

The experiment was sprayed with the same fungicide used as in Experiment 1 every 2 weeks. The stem cuttings were assessed weekly starting from Week 2 for the same parameters as in Experiment 1.

The effect of propagation media on rooting of stem cuttings of C. albidum

Coppiced shoots were obtained and stem cuttings prepared as described in Experiment 1. Randomised complete block design with three replicates was used. Three non-mist propagators were constructed for the experiment and each propagator was used as one block. Each block was divided into four sections, and each section contained 20 stem cuttings representing one treatment. The four rooting media examined were: fine sand (< 2 mm), top soil, sawdust and 1:1 mixture of topsoil and sawdust. Topsoil was obtained from the forest floor of the Bunso Arboretum. Sawdust which was a mixture of different tropical woods was collected from a sawmill in Bunso. Sand was collected from a site in Old Tafo-Akim. The four treatments were randomised in each of the three blocks. There were, therefore, a total of 60 plants per treatment. Each cutting was treated with 10 µl of 20 µg l^{-1} of IBA concentration as described in Experiment 1. The propagation units were treated 3 days prior to the experiment with a fungicide and insecticide as in Experiments 1 and 2. Subsequently at the end of every 2 weeks, the cuttings, the media and the propagators were sprayed with the same fungicide and insecticide. The stem cuttings were assessed at the end of every week starting from Week 2 as in Experiments 1 and 2.

Data analysis

Data collected were analysed using the

analysis of variance (ANOVA) test. At the end of Week 12 all the stem cuttings used in each of the three experiments were examined and scored as living and rooted, living but not rooted (dormant) or dead. The χ^2 test using contingency tables was used on the condition of cuttings at the end of Week 12. All statistical analyses were performed using the Genstat 10th edn statistical package.

Results

Effect of leaf area on C. albidum *stem cuttings*

Stem cuttings started rooting at Week 5. The onset of rooting was not significantly affected by the leaf area treatments (Fig. 1a). From Week 6 (range of 5.6 – 41.9%) (P = 0.005) to Week 10 (range of 2.8 – 77.8%) (P < 0.001), there were significant differences between the treatments (Fig. 1a) with the 40 cm^2 treatment showing the highest percentage of cuttings rooted. Treatment 0 cm^2 (leafless stem cuttings), had the lowest proportion of rooted cuttings during the entire 10-week period. By Week 9 and 10, apart from treatment 40 cm^2 which showed an optimal performance and treatment 0 cm^2 (leafless stem cuttings) where rooting was almost 0 per cent, there was a pattern in which treatments of progressively higher leaf areas showed progressively higher percentage rooting.

Stem cuttings with a leaf area of 40 cm^2 had a significantly higher mean number of roots (P < 0.001) than the other treatments. This was followed by stem cuttings with a leaf area of 160 cm^2, then 200 cm^2, 80 cm^2 and 20 cm^2. The 0 cm^2 (leafless stem cuttings) had the lowest figure which was close to 0 (Fig. 1b).

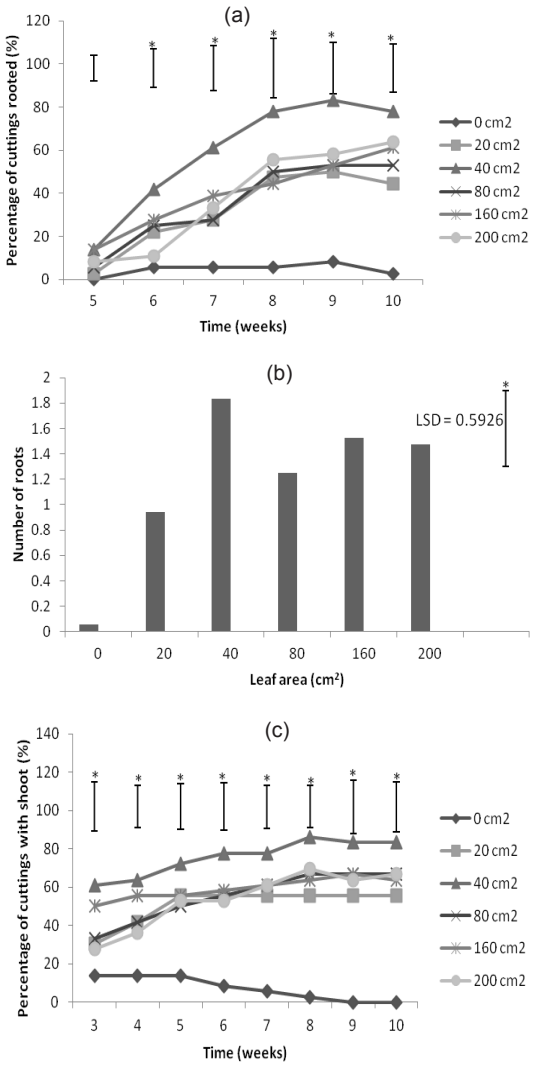

Fig. 1. The effect of leaf area on (a) stem cutting rooting percentage, (b) number of roots at week 10 and (c) shoot formation on cuttings of C. albidum. Each point represents the mean of 6 replicates. The bars show value of LSD (0.05) and * on LSD bar indicates significant difference between the treatments for that week.

There was no callus formation in Weeks 2 and 3 but callus was observed on two out of 216 cuttings from Week 4 to 9. By Week

10, callus was observed on 5 out of 216 stem cuttings (2.3%). There was no effect of leaf area on callus formation.

Formation of shoots by the stem cuttings started in Week 3 for all the treatments. With the exception of the 0 cm^2 treatment (leafless stem cuttings), all other leaf area treatments exhibited greater than 20 per cent shoot formation (Fig. 1c) at this stage. The highest percentage of shoot formation was recorded for the 40 cm^2 leaf area treatment from Week 3 until the end of the experiment. There were significant differences between the treatment means for each week of the study (Week 3, P = 0.012; Week 4, P = 0.002; Week 5, P = 0.001; Week 6-10, $P < 0.001$) (Fig. 1c).

After Week 3 some of the leafless cuttings started shoot formation although the overall proportion was low (14%). This was maintained up to Week 5 but decreased gradually until none had shoots by Week 9 and 10. This treatment showed significantly lower percentage shoots than the other treatments throughout the experimental period.

At the end of Week 12 when an assessment was made on the status of the stem cuttings, the 40 cm^2 leaf area treatment had the highest number of rooted stem cuttings (83%) compared to the other treatments. This was followed by treatments 160 cm^2 (67% living and rooted) and 200 cm^2 (61% rooted), and these were in turn higher than treatments 80 cm^2 (61% rooted) and 20 cm^2 (53% rooted). The leafless stem cuttings (0 cm^2) showed the highest cutting mortality of 94 per cent with 6 per cent dormant cuttings and none rooted. There

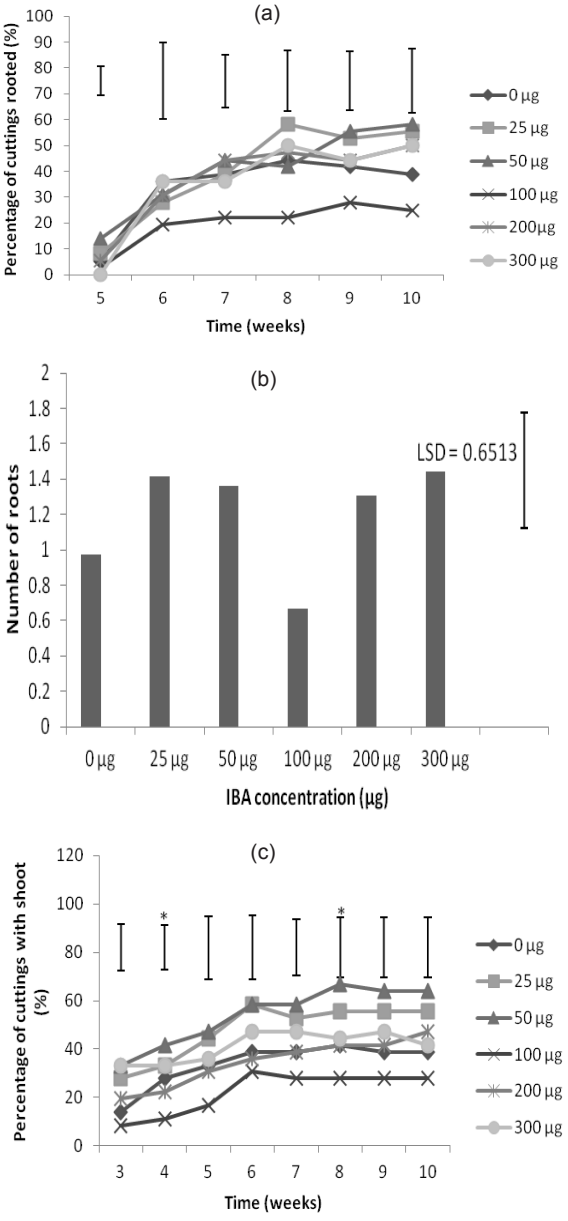

Fig. 2. The effect of IBA concentration on (a) stem cutting rooting percentage, (b) number of roots at week 10 and (c) shoot formation on cuttings of C. albidum. Each point represents the mean value of 6 replicates. The bars with different lengths represent the value of LSD at 5 per cent and * on LSD bars show significant differences between treatment means for the particular week.

were significant differences between the treatments ($P < 0.001$).

Effect of IBA on stem cuttings of C. albidum

Stem cuttings began to root in Week 5, and the time to the beginning of rooting was independent of the treatments imposed (Fig. 2a). Rooting percentage progressively increased over the experimental period. The treatment with 50 µg IBA showed the highest rooting of the treatments at Week 10 (58.3%), followed by the treatment with 25 µg IBA which reached a maximum at Week 8 but diminished gradually from Week 9 to Week 10. The treatments with 300 µg and 200 µg IBA showed a lower percentage of rooted cuttings. However, the differences between the treatments were not significant over the experimental period (Fig. 2a).

With the exception of the treatment with 100 µg IBA, stem cuttings treated with IBA showed a higher mean number of roots per cutting compared to the 0 µg which is the control, although the differences were not significant ($P = 0.123$) (Fig. 2b).

There was no callus formation on the stem cuttings treated with different concentrations of IBA during the 10-week experimental period. Shoot formation started in all IBA treatments in Week 3 (Fig. 2c) and continued to increase up to Week 6. Shoot formation was significantly higher under the 50 and 25 µg IBA treatments compared to 200 and 300 µg IBA treatments and the untreated control in weeks 3 ($P = 0.018$), 4 ($P = 0.022$), and 8 (0.054). The treatment

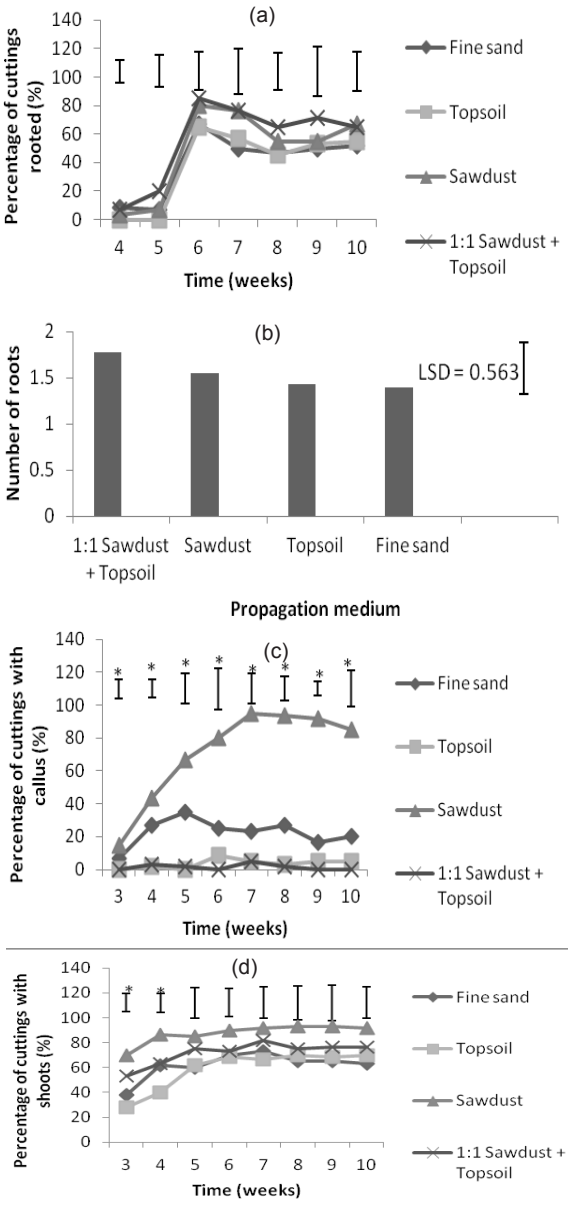

Figure 3. Effect of propagation media concentration on (a) stem cutting rooting percentage, (b) number of roots at week 10, (c) proportion of cuttings with callus and (d) shoot formation on cuttings of C. albidum. Each point represents the mean value of 3 replicates. The bars with different lengths represent the value of LSD at 5 per cent and * on LSD bars show significant differences between treatment means for the particular week.

with 100 µg IBA had the lowest percentage shoot formation.

At the end of Week 12, stem cuttings treated with 50 µg IBA showed the best performance with 72 per cent rooted cuttings. This treatment also had three per cent living but unrooted (dormant) cuttings and 25 per cent cutting mortality. Cuttings treated with 25 µg IBA gave 58 per cent rooted cuttings, three per cent dormant cuttings and 39 per cent cutting mortality. Treatments with 300 µg IBA, 200 µg IBA and 0 µg IBA gave 58, 50 and 44 per cent rooted cuttings, respectively, which was higher than 28 per cent living and rooted cuttings when treated with 100 µg IBA. There were significant differences between treatments ($P < 001$).

Effect of different rooting media on rooting of stem cuttings of C. albidum

Rooting of cuttings began between Week 4 and 5 for all treatments and reached a maximum at Week 6 (Fig. 3a). A higher rooting percentage was observed in the mixture of sawdust and topsoil treatment and the sawdust from Week 5 to 10, although there were no significant differences between the treatment means (Fig. 3a). By the end of Week 10, there was no significant effect of growing medium on the number of roots (Fig. 3b).

The highest number of cuttings observed with callus formation was recorded in those rooted in the sawdust medium (Fig. 3c). Callus formation started in Week 3 in all the different propagation media with 6.7 and 15 per cent in the fine sand and sawdust medium, respectively. From Week 5 the differences in callus formation between the different treatments began to widen. Maximum callus formation was recorded in the sawdust medium in Week 7 (95%) (Fig. 3c). Both the topsoil medium treatment and the 1:1 mixture of sawdust and topsoil exhibited low levels of callus formation throughout the experiment. There were significant differences between the different propagating media for all the weeks assessed (Week 3, $P = 0.054$; Week 4-10, $P < 0.001$) (Fig. 3c).

From Week 3 to Week 10, the highest percentage of shoots formed on stem cuttings was observed for the sawdust medium (93.3% in Week 8 and 9). This was followed by the 1:1 mixture of topsoil and sawdust (Fig. 3d). The fine sand and topsoil treatments had a similar percentage of cuttings with shoots from Week 5 onwards. It was only in Week 3 ($P = 0.002$) and Week 4 ($P = 0.002$) that there were significant differences between the treatment means (Fig. 3d).

At the end of Week 12, the percentage of living and rooted cuttings were higher in the sawdust medium (78%) and 1:1 mixture of sawdust and topsoil medium (75%) than topsoil (60%) and fine sand (58%) treatments. There was a significant difference between the treatments ($P < 0.001$).

Discussion

Whilst leaves on cuttings are known to provide carbohydrates for plant growth through photosynthesis, water is also lost through transpiration which can adversely affect stem cuttings with large leaves. Therefore, reducing leaf area by leaf trimming on stem cuttings has been used to enhance rooting of cuttings (Leakey & Coutts, 1989).

In the experiment, reducing the leaf area of *C. albidum* cuttings to 40 cm^2 significantly increased the percentage rooting compared to treatments in which more or

less leaf area was removed. This also had a consequent effect on shoot formation. Thus, maximum percentage rooting at the end of the experiment of cuttings with a leaf area of 40 cm^2 was 77.8 per cent. The fact that the maximum percentage rooting was observed with cuttings with a leaf area of 40 cm^2 was probably a compromise between assimilate gain through photosynthesis and water loss through transpiration. This is comparable to other studies in which an optimum rooting of cuttings was achieved with trimming leaf areas to particular sizes that were neither too small nor too big; for example *T. scleroxylon* (50 cm^2) (Leakey *et al*., 1982), *Cleistopholis glauca* (50 cm^2) (Leakey, 1985) and *Khaya ivorensis* (10-30 cm^2) (Tchoundjeu, 1989). The number of cuttings living, rooted and with shoots at the end of the experiment was also highest for the 40 cm^2 treatment and lowest for treatment 0 cm^2 (leafless) (0% survivorship). This indicates that the presence of leaves on cuttings and their size influence rooting of stem cuttings of *C. albidum.*

The very low rooting percentage shown by the complete removal of leaves from stem cuttings was probably due to depletion of stored carbohydrates and auxins necessary for root initiation and development (Haissig, 1986; Hartmann *et al.*, 2002). Similar results have also been observed in *Irvingia gabonensis* (Shiembo, Newton & Leakey, 1996), *T. scleroxylon* (Leakey *et al*., 1982) and *Terminalia spinosa* (Newton *et al.*, 1992). Allowing higher leaf areas to remain on the cutting gave intermediate percentages of rooting and number of roots. The results showed that from Week 3 some of the leafless cuttings (0 cm^2) did produce shoots from leaf buds, but these could not be sustained because there was insufficient carbohydrate reserves to support new shoots, and so the number of live cuttings decreased until Week 9 when all the cuttings had died.

There were no significant differences between the different concentrations of IBA used, although a trend was observed of low levels of IBA improving rooting percentages. Comparable observations showing little effect of IBA on rooting have also been observed in *Shorea macrophylla* (Lo, 1985), *Milicia exselsa* (Ofori *et al*., 1996), *Nauclea diderrichii* (Leakey, 1990), *I. gabonensis* (Shiembo *et al.,* 1996) and *Allanbrackia floribunda* (Atangana *et al*., 2006).

Propagation medium is critical for rooting of cuttings. The porosity of the medium to facilitate aeration, and the ability of the particles to absorb moisture is important for the rooting of stem cuttings. In the study, percentage rooting for all the treatments peaked at Week 6 although there were no significant differences between the rooting media. This shows that all the treatments had good characteristics to enhance rooting. Sawdust is reported to have good water absorbing capacity and aeration to enhance rooting of cuttings (Ofori *et al.*, 1996). The 1:1 mixture of sawdust and topsoil also has similar attributes, which would have contributed to the higher number of rooted cuttings, and number of roots per cutting than fine sand and topsoil media. Fine sand has high porosity and little absorptive capacity while topsoil has high water holding capacity and reduced porosity.

The number of roots and length of roots of cuttings in sawdust medium and the equal parts mixture of sawdust and topsoil medium maintained higher values than fine sand and topsoil media. This could be attributed

to the porous nature and quite high water holding capacity of the propagating media with higher values which enhanced rooting.

The percentage of callus formation was higher in the sawdust and fine sand media than in topsoil and 1:1 mixture of sawdust and topsoil media. The 1:1 mixture of sawdust and topsoil recorded 0 per cent for most of the weeks. This was the medium used for the other two experiments and it is, therefore, not surprising that callus formation was not found in IBA-treated cuttings and was very low in cuttings with different leaf area treatments. Callusing among cuttings is normally found in media with high temperatures and low water holding capacity (Hartmann *et al*., 2002). The temperature in the sawdust medium could easily rise due to the low water absorptive capacity of the wood particles and could favour callusing. Fine sand also has low water retentive capacity. On the other hand, topsoil medium and 1:1 mixture of sawdust and topsoil medium have high water retentive capacity which would be inhibitory to callusing.

Generally, the number of roots per cutting in the study was low compared to tree species like *T. scleroxylon* and *I. gabonensis*, and is likely to be a reflection of the nature of the tree species. Some species are difficult to root and such species take a longer time to produce a high number of roots. This could also be due to lack of cell sensitivity to respond to root initiation hormones (auxins) (Hartmann *et al.*, 2002).

Conclusion

The study has shown that propagation of *C. albidum* by stem cuttings is possible. A leafy stem cutting with 40 cm^2 of leaf area in propagating medium of either sawdust or 1:1 mixture of sawdust and topsoil in a non-mist propagation chamber will enhance propagating *C. albidum* stem cuttings. The use of IBA would not be essential although application at a rate of 20 – 50 µg l^{-1} may increase the success rate. The results of the study could be used to help farmers involved in the cultivation of *C. albidum* fruit trees to obtain propagated stock from superior trees.

Acknowledgement

The financial support provided by GET-FUND for the study is gratefully acknowledged. The author is grateful to Prof. P. Hadley and Dr A. J. Daymond for their useful suggestions and comments.

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