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EFFECT OF PHYTOHORMONE AND PHENOLOGY ON DOMESTICATION OF *Pentaclethra macrophylla* Benth. BY MARCOTTING IN DERIVED SAVANNA ZONE OF SOUTHEAST NIGERIA

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ABSTRACT
Marcotting is a method of vegetative propagation which still finds its relevance in the present day domestication and routine plant multiplication efforts of indigenous fruit trees including, *Pentaclethra macrophylla (Fabaceae* family). There is dearth of information regarding propagation of *P. macrophylla* by marcotting. Hence effects of phytohormone application and tree phenology on *P.macrophylla* propagation by marcotting were investigated in Ishiagu located in the derived savanna agro-ecological zone of South- east Nigeria. Marcotting of *P. macrophylla*, effect of Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) phytohormones application (range 0.1% – 0.3% and 0.05% – 0.1% concentrations, respectively) and different marcotting seasons were studied in field experiments. The plots were laid out in a randomized complete block design and replicated three times. Three branches of each tree replicate were marcotted at two-month interval (starting from January to November) across the year. Percent callus and adventitious roots formation, number and length of adventitious roots were assessed. Data were analysed using descriptive statistics and ANOVA.Results of combined effects of IBA and NAA on rooting of marcotted branches showed that both auxin treatments had generally unimpressive adventitious root production effect on *P. macrophylla* at eight weeks after marcotting (WAM). The branches however, produced callus tissues freely around the girdled portions of branches, which is a definite indication of ensuing rooting process that might have been delayed. Only treatment of 0.2% IBA+0NAA gave significantly highest mean with 100% callusing at 8 WAM; while least mean (50%) callusing was produced with 0.1% IBA+0 NAA and 0.2% IBA+0.05% NAA. July marcotted branches produced significantly highest percent callus (100%), adventitious roots (75%), number of roots (2.2±0.8) and root length (34.2±8.7cm) among the different marcotting seasons.

Keywords: Pentaclethra macrophylla, indigenous tree species, marcotting, and auxin

Introduction

The *Pentaclethra macrophylla* Benth., also known as African oil bean, is an indigenous economic leguminous tree (of mimosoid clade in the Caesalpinioideae sub-family); naturally distributed across west and central sub-Sahara African humid forest vegetation zone (Oboh, 2007; LPWG, 2017). The tree is often protected as volunteer stand or occasionally planted on the fringe of compound farms mainly for its edible seeds and its constituent edible oil which can be extracted for various domestic uses, although it is still largely under-exploited by the industries. Organized planting in grove or plantation of the tree is rare. It is also a major component of traditional home garden system in southern Nigeria, especially the southeast sub-region where the species seeds (locally known as 'ugba') are extensively harvested by farmers (Okeke *et al*., 2009; Ogbu and Awodoyin, 2017).

Asexual propagation (also known as regenerative or vegetative method) describes the regeneration and multiplication of plant kinds from vegetative parts, including buds, leaves, single cells/tissues as well as cuttings of roots and stems (Hartmann *et al.*, 2007). It offers a wide array of applications in Indigenous Fruit Tree (IFT) domestication efforts and general Plant Genetic Resources (PGR) conservation programmes.

With these applications, propagation experts are able to select desired characters available in the wild tree population by fixating the genetic variation of such trees in the natural stands. Ultimately, the objective is to multiply large quantity of improved seedlings for interested farmers and other users (Tchoundjeu *et al.*, 1997: Verheij, 2004; Awodoyin *et al*., 2015).

Marcotting, also known as air layering, is an age long established method of vegetative propagation which still finds relevance in present day domestication, PGR conservation and routine plant multiplication efforts. Unlike in stem cuttings, the induction of adventitious roots on a stem is made possible while the stem remain attached to the parentplant. The successful marcot stem is thereafter cut off to be an independent plant growing from its own roots. The need for a suitable/adaptable rootstock does not arise in marcotting, which gave it an edge over grafting. Since stem cuttings of full-grown tree experience hard to root tendency, marcotting therefore stands out as a preferred option for vegetative propagation plan in domestication of IFT. Moreover, marcots are known to come into fruiting earlier than slower-growing stem cuttings or grafted trees, but possess a shallow root systems (Jaenicke and Beniest, 2002; Asaah *et al*., 2012).

Vegetative propagation applied in the conservation and domestication of IFT, essentially, offers viable opportunities for research, sustainable development and use of plant resources. The aims of vegetative propagation options are enumerated as follows: mitigating challenges of poor seed germination and storage behaviour, reducing gestation period of fruit bearing species, maintenance of superior genotypes, uniformity of orchards/plantations, fixing desired attributes of two or more plant genotypes into one plant stand and controlling phases of tree development, among others (Leakey, 2000). Among the indigenous fruit species of sub-Saharan regions that have benefited in marked reduction of their gestation periods and subsequent early fruiting, through enhanced use of vegetative propagation include: *Dacryodis edulis* (5 - 2 years), *Adansonia digitata* (10 - 4 years), *Irvingia gabonensis* and *I. wombolu* (7 - 3 years each), and *Vitellaria paradoxa* (20 to below 5 years), following the studies of Jaenicke and Beniest, (2002) and Jamnadass *et al.*, (2011). There is however, limited information regarding vegetative propagation of *Pentaclethra macrophylla* by marcotting which leads to marked reduction in gestation period, early fruiting and dwarfing effect. Hence effects of phytohormone application and tree phenology on *P.macrophylla* vegetative propagation by marcotting were investigated in Ishiagu located in the derived savanna Agro-ecological zone of Southeastern Nigeria.

Materials and Methods

The experiments involving marcotting technique were conducted on selected matured tree stands of *Pentaclethra macrophylla* established in 2002 and located at the mixed Tree Crops Plantation site of Federal College of Agriculture (FCA) Ishiagu, Ebonyi State Nigeria.

Experiment 1: **Response of *P. macrophylla* marcots to treatment with varying concentrations of NAA and IBA growth hormones**

This experiment followed a 3 x 4 factorial experimental layout. Thus, there were three concentrations of Naphthalene Acetic Acid (NAA); 0, 0.05, 0.1% and four concentrations of Indol-3 Butyric Acid (IBA), 0, 0.1, 0.2, and 0.3%. This gave twelve treatment combinations replicated three times and laid out in factorial randomised complete block design. In this manner, each parent tree used constituted a block/replicate. Thus, there were three (3) tree stands representing the three blocks/replicates. Branches that were 1.0 ± 0.2 cm in diameter were selected for the study. Each branch was girdled at 40 cm length from its shoot tip bud by removing ring of bark (5 cm wide) to expose the cambial layer (Hartmann *et al*., 2007; Sthapit *et al*., 2016). Appropriate diluted solutions of the various concentrations of the plant growth hormones were applied to the girdled portion of the respective stems (1.0 ± 0.2 cm diameter) that were selected. Thereafter, the girdled portions were covered with moist growth medium held in polythene sheet and tied firmly with twine at its two ends. Mixture of river sand/ saw dust (1:1 v/v) served as growth medium. Parameters assessed include: per cent callus formation at 8 WAM, number of roots in successful marcots at 8 WAM; and root length (cm) in successful marcots at 8 WAM.

Experiment 2: Evaluation of different seasons on rooting ability of *P. macrophylla* marcots in Ishiagu, Southeast Nigeria

This study was set to span the vegetative and reproductive phases of the plant's phenology in a year (Awodoyin and Olaniyan, 2000; Tchoundjeu *et al*., 2010). The plot was laid out in a randomised complete block design and replicated three times (number of trees). Three branches of each tree replicate were marcotted at two-month interval (starting from January to November) across the year. Branches (1.0 ± 0.2 cm in diameter) were girdled by cutting a 5 cm wide ring of bark at the proximal end. Moistened mixture of river sand and saw dust (1:1; w/w) was tied around the girdled part using polythene sheet. The growth medium held in the polythene sheet around the girdled portion was kept moist by injecting 100 ml of distilled water into each marcot twice a week using hypodermic syringe. Administration of water was increased to four times per week during dry season. At eight weeks after each marcotting operation, the branches were detached from the parent tree, untied and assessed for callus formation and adventitious root production, in terms of percentage of callus, number of roots per marcot and root length (cm) per marcot. Callus formation was assessed by visual observation of number of girdled branches that formed callus.

Results and Discussion

Experiment 1: Response of *P. macrophylla* marcots to treatment with varying concentrations of NAA and IBA growth hormones

Simple effect of hormone treatment concentrations on percent callus formation

At the end of this study, none of the branches air-layered produced noticeable roots, but most of them callused freely, which is a definite sign of rooting although somewhat delayed. Results obtained from two seasons trials (Table 1) showed that the application of IBA and NAA growth hormones significantly ($p<0.05$) impacted on callus formation, although there were not visible root productions at 8 WAM. At the various hormone treatment combinations applied, most of the girdled branches formed callus tissues ranging from 33.3% - 100% in both trials. The treatment 0.2% IBA+0 NAA gave the highest (100%) callus formation which was significantly different ($p<0.05$) from the second highest percent callus formation (66.7%) in the two trials. At 0.1% of the growth hormones concentration, NAA showed more impact than IBA on callus formation in the two trials.

Main effect of IBA and NAA growth hormones on percent callus formation

It is apparent from Table 1 also, that the *P. macrophylla* treated branches responded directly to increased concentrations of IBA and NAA growth hormones as reflected in the percent callus formation. However, impact of increased concentrations of NAA did not elicit significant response from the two trials; unlike IBA increased concentrations that indicated significant effect ($p<0.05$) on percent callus formation among similar treated branches only at the second trial. Combination of the two hormones had highly significant interaction effect on the branches callusing ability.

Mean percent callus formation from two trials

Table 1 also showed that although visible roots were not formed from the hormone treated branches of *P. macrophylla* for air-layering at various concentrations, callus tissue was mostly produced which is a particular evidence of belated rooting. Only treatment 0.2% IBA+0NAA was able to give mean 100% callusing at 8 WAM, the least mean (50%) callusing was produced by 0.1% IBA+0 NAA and 0.2% IBA+0.05% NAA. There is also clear evidence of higher percent callusing with increase in the hormones concentrations except in 0.3% IBA application which indicated drop in mean number of callused branches.

Table 1: Effect of varying concentrations of IBA and NAA phytohormones on callus formation (%) of *P. macrophylla* marcots at 8 WAM in 2015

Treatments (%)	First trial	Second trial	Average
0 IBA+0 NAA	66.7±11.8	100.0±0.0	83.4
0 IBA+0.05 NAA	66.7±9.4	66.7±2.3	66.7
0 IBA+0.1 NAA	66.7±23.6	100±0.0	83.4
0.1 IBA+0 NAA	66.7±4.7	33.3±11.7	50.0
0.1 IBA+0.05 NAA	100.0±0.0	66.7±9.4	83.4
0.1 IBA+0.1 NAA	66.7±2.4	66.7±16.5	66.7
0.2 IBA+0 NAA	100.0±0.0	100.0±0.0	100.0
0.2 IBA+0.05 NAA	33.3±11.8	66.7±2.4	50.0
0.2 IBA+0.1 NAA	66.7±11.7	66.7±4.8	66.7
0.3 IBA+0 NAA	66.7±2.3	100.0±0.0	83.4
0.3 IBA+0.05 NAA	66.7±9.4	100.0±0.0	83.4
0.3 IBA+0.1 NAA	100.0±0.0	66.7±11.8	83.4
LSD(0.05)	16.88	13.19	
Factor IBA:			
0 IBA	77.8±15.7	77.8±19.2	77.8
0.1 IBA	66.7±0.0	77.8±15.7	72.3
0.2 IBA	77.8±19.2	100.0±0.0	88.9
0.3 IBA	66.7±27.2	55.6±15.7	61.2
Factor NAA:			
0 NAA	66.7±0.0	75.0±27.6	70.9
0.05 NAA	75.0±27.6	75.0±14.4	75.0
0.1 NAA	75.0±14.4	83.5±16.7	79.3
LSD(0.05)	ns	9.33	
Interaction: IBA x NAA	**	**	
CV (%)	29.39	28.81	

Values are mean ± SD (n = 9)

Experiment 2: Evaluation of different seasons on rooting ability of *P. macrophylla* marcots in Ishiagu, Southeast Nigeria: Period of the year and percent callus formation

The period of the year showed remarkable effects on the performance of air layering as a means of vegetative propagation of *P. macrophylla*, especially with reference to percent callus formation of the branches. From Table 2, it is evident that percent callus formation increased from 25% in January to 100% in July and September, and thereafter dropped in November, as the season progressed from dry to wet weather, as the tree switched from flower bloom (reproductive) phase to end of fruit set (vegetative) phase. In other words, 100% of the branches formed callus and some proceeded to form adventitious root by eight weeks after marcotting.

Table 2: Mean basic weather data, percent callus and adventitious root formation of *P. macrophylla* marcots across different months in Ishiagu, Southeast Nigeria†.

	Local weather data		Callus formation (%)		Adventitious root formation (%)	
Months of marcotting	**Rainfall (mm)**	**Max. temp.(^{0}C)**	**2013/2014**	**2014/2015**	**2013/2014**	**2014/2015**
January	48.3	34	41.7±11.8	25.0±4.1	0.0±0.0	0.0±0.0
March	45.1	35	100.0±0.0	83.3±4.7	33.3±11.7	8.3±2.4
May	250.3	32	100.0±0.0	91.7±11.8	16.7±4.7	8.3±2.4
July	304.9	29	100.0±0.0	100.0±0.0	75.0±20.4	66.7±15.5
September	293.6	30	100.0±0.0	100.0±0.0	16.7±4.7	33.3±4.7
November	42.5	32	100.0±0.0	83.3±9.4	0.0±0.0	33.3±9.4
$LSD_{(0.05)}$			32.71	25.37	23.20	15.22
CV (%)			30.75	35.71	26.81	23.09

†Main flowering period of *P. macrophylla* is March – April, with smaller flushes in November (Keay, 1989). Callus and root formation (%) taken at 8 weeks after marcotting (WAM). Values are mean ± SD (n = 9)

The result also revealed that callus formation and subsequent root growth were minimal (gave as low as 25% and 41.7% for 2014/2015 and 2013/2014 trials respectively) during the dry season months which spanned from November to March in the study location.

Period of the year and percent adventitious roots formation

None of the girdled branches produced adventitious roots at January operation period during which the local weather was quite dry (with mean of 48.3 mm rainfall) and hot with mean atmospheric maximum temperature of 34°C (Table 2). Percent root formation significantly rose to the peak in July (75% and 66.7% for 2013/2014 and 2014/2015 trials respectively) which apparently had the highest mean monthly rainfall of 304.9 mm and least mean day temperature of 29°C. Just like percent callus formation (which may be regarded as early stage of emerging root system), the adventitious root production responded positively and significantly ($p<0.05$) to an increased moisture content in the atmosphere and a reduced day temperature. However, not all callused branches successfully stroke roots depending on season.

Period of the year and number of adventitious roots per successful marcot

The numbers of roots produced at eight weeks after girdling were only marginally different across the wet and dry season weathers, and were non-significant for both trials (Table 3). However, July and September girdled branches still showed more number of root formation per successful marcot branch than other periods, as both had 2.1 and 1.5 roots respectively.

Table 3: Effect of period of the year and root production of *P. macrophylla* marcot seedlings at 8 WAM in Ishiagu southeast Nigeria†

Months of marcotting	**Mean number of roots**		**Mean root length (mm)**	
	2013/2014	**2014/2015**	**2013/2014**	**2014/2015**
January	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
March	1.0±0.4	0.2±0.02	7.8±1.6	2.3±0.62
May	1.5±1.4	0.3±0.1	25.0±4.1	5.3±1.24
July	2.2±0.8	2.1±0.82	34.2±8.7	10.8±1.34
September	1.5±1.4	1.5±0.41	20.3±4.1	30.3±6.24
November	0.0±0.0	1.0±0.12	0.0±0.0	12.8±3.0
$LSD_{(0.05)}$	ns	ns	10.42	6.60
CV (%)	27.79	37.43	25.07	20.9

WAM = Week after marcotting; † As in table 4.15 above; ns = non-significant. Values are mean ± SD (n = 9)

Period of the year and length of adventitious roots per successful marcot

Root length per successful marcot branch showed significant response ($p<0.05$) to season (Table 3). July and September marcotted branches had relatively more profuse and extended roots ranging from 10.8 to 34.2 mm and 20.3 to 30.3 mm respectively. *Pentaclethra macrophylla* marcot seedlings that produced least adventitious root lengths (0.0 to 2.3 mm) came from those branches girdled during dry season (November - March) in the study area which also coincided with major flower blooming phase of the test plant.

Results of the experiment on combined effects of IBA and NAA on rooting of air-layered branches showed that both auxin treatments had generally unimpressive root production effect on *P. macrophylla* at eight week after girdling, although the branches fairly callused, which is a definite indication of ensuing rooting process that might have been delayed. Interaction of the two hormones revealed synergism as evident by significant effect on the branches callusing ability, despite the absence of visible adventitious root production on the air-layered branches. Single effect of IBA treatment proved to have slightly suppressive influence on callus formation and invariably on root production with increasing concentrations from 0 – 0.3% IBA, unlike the NAA. This observation is in consonance with Tchoundjeu *et al*. (2010), Yeboah *et al.* (2010) and Akwatulira *et al.* (2011), although on different tree species. These authors equally reported the suppressive effect of high concentrations of IBA on percent root production of layered branches. In contrast, low IBA concentration is reported to enhance rooting of *Irvingia garbonensis* cuttings with optimal performance at 250 μg concentration (Schiembo *et al.*, 1996). Similar response had also been reported by Awodoyin and Olaniyan (2000), of the positive effect of low auxin concentrations on adventitious root production of air-layered guava (*Psidium guajava*) branches with optimal performance at 100 ppm (= 0.01%) IBA. Thus, the negative impact of both rooting hormones (especially IBA) observed in this study, may be due to the high levels of these auxin substances ranging from 0.1 – 0.3% (equivalent to 1000 to 3000 mg/L IBA) used, apart from the role of season which could be quite crucial as well.

With regard to the effect of season on rooting success of *P. macrophylla* in layering operation, the best rooting was obtained in July, followed by September. It is apparent from the result that adventitious root formation by marcotted branches of *P. macrophylla* required high humid condition which is naturally guaranteed during the peak of wet season weather, and vice versa. Obviously, this behaviour may be attributed to the prevailing high moisture and cool temperature associated with the period of peak rainfall. In other words, July was the coolest and wettest month of the year at the study station, followed by September. The poor root formation obtained in the month of May when rainfall was also fairly high could be an indication that roots formation and elongation in African oil bean air-layering is not determined only by weather condition, but also by interplay of intrinsic specific factors including the tree reproductive and vegetative growth cycle in the season. It has been long established that root formation is influenced by auxins produced by the plant itself (Awodoyin and Olaniyan, 2000; Jaenicke and Beniest, 2002; Hartmann *et al.*, 2007). The period between November and January coincides with the period of reproductive cycle of the Afrcan oil bean tree, with minor blooming on April; while the period from May to September coincides with the season of active vegetative growth (Keay, 1989; Oboh, 2007). The physiology of tree growth phases show that as plant undergoes active vegetative growth cycle, buds release endogenous auxin that moves basipetally to stimulate root production (Jaenicke and Beniest, 2002; Leakey, 2004). So by implication and in response to the deliberate wound caused by girdling in layering operation, branches of the species began formation of adventitious roots. This is an affirmation of similar reports by Awodoyin and Olaniyan (2000), Leakey (2004) and Hartmann *et al.* (2007) that infliction of wounds on stem brings about auxin-induced stimulation of vascular meristematic tissues to form adventitious roots in cuttings and layering techniques.

On the other hand, when plants undergo period of reproductive cycle, anti-auxin substances are released that make active vegetative growth to be suspended and the buds appear to enter state of dormancy. Therefore, shoots marcotted or collected for stem cuttings at such reproductive phase may largely be unresponsive to formation of adventitious roots due to inhibiting endogenous factors (Jaenicke and Beniest, 2002; Hartmann *et al.*, 2007; Chadha, 2009). This may explain the poor callus formation and root production observed among *P. macrophylla* stems air-layered during November, January and March.

Conclusion

Single and combined effects of IBA and NAA rooting hormones application (in the range 0.1% – 0.3% and 0.05% – 0.1%, respectively) on air layered branches of *Pentaclethra macrophylla* showed that both auxin treatments had little adventitious root production at eight weeks after girdling. However, the branches fairly callused which is a definite mark of ensuing rooting process. The marcotting done in July and September performed best in the formation of adventitious roots. These periods coincided with the two peaks of rainfall in the area studied. Also, phenology of *P. macrophylla* shows that the species undergo active vegetative phase during June –

September; while period between November and April coincides with the reproductive (flower set) cycle of the species.

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