

Vegetative Propagation of African Walnut (*Plukenetia conophora* (Müll Arg) as Affected by Propagator, Plant Parts and Rooting Media

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Abstract

Plukenetia conophora is a seasonal fruiting liana with recalcitrant seeds that are highly competed for by man and rodents. This reduces seedling production from seeds, hence the need for alternative methods of production such as vegetative propagation. One thousand, one hundred and twenty (1,120) double node cuttings of *P. conophora* were set up in a factorial arrangement using a completely randomized design with four rooting media, two propagator chambers, and two plant parts, with seven replications. At the end of the 12th week, data collected on rooted cuttings (%), shoot length (cm), number of leaves, number of roots, and root length (cm) were subjected to analysis of variance and significant means were separated using Duncan multiple range test at 5% probability level. The results showed that the basal part of cuttings in sterilized river sand, placed inside the mist propagator produced the best result (87%), while the least rooting capacity (20%) was obtained for terminal cuttings rooted in unsterilized topsoil. The use of propagator and type of rooting media had significant effects on the number of roots produced by *P. conophora* cuttings. Cuttings placed in the propagator chamber had the highest number of leaves (7.31 ± 3.11), while plant part had a significant effect on the shoot length, with terminal cuttings producing the highest shoot length (3.92 ± 5.96 cm). *Plukenetia conophora* could be propagated vegetatively with due considerations to the parts of plant, media and the rooting environment.

Keywords: *Plukenetia conophora*, Mist propagator, Rooting media, Plant parts, Macro propagation.

Introduction

The propagation of most lianas is not given adequate attention due to their climbing nature. This makes establishing them in orchard very difficult and research on their performance challenging (Amadi *et al.*, 2014). However, these neglected plants are essential life forms that are underutilized in the rain forest ecosystem. One of such important lianas is the African walnut (*Plukenetia conophora* (Müll Arg) which provides immense benefits to man, animal and industries (Awodoyin *et al.*, 2000).

Tropical plant species are mostly propagated through seeds, which have some limitations (Bowes, 1999; Adebisi *et al.*, 2011). These include scarcity, storage and variability problems, competition for the seeds that have multiple uses and dormancy problems (Akinyele, 2010; Alaje *et al.*, 2019). These limitations hamper the production of large quantities of seedlings for plantation establishment. There is, therefore, the need for alternative means of propagation other than the use of seeds. Vegetative propagation is a viable option for mass production of quality planting materials (Tchoundjeu *et al.*, 2004).

Many studies have reported the advantages of producing plant vegetatively through stem cuttings. Anegbeh *et al.* (2006) and Atangana *et al.* (2006) highlighted that vegetative propagation of *Allanblackia floribunda* would ensure the regeneration of the plant in the absence of seeds. Koukou *et al.* (2016) reported a high success in rooting of *Garcinia kola*, a dormant seed which takes longer years to reach maturity when propagated through seeds. Plants produced through stem cuttings produce plantlets that have the same genetic materials as the parent tree (Tchoundjeu *et al.*, 2006; Leakey and Akinnifesi, 2008). These plants mature early by by-passing the seedling phase which leads to reduction in maturity and fruiting age. They have high uniformity in yield and quality, and meet up with the market demands (Tchoundjeu *et al.*, 2002; 2004; 2006; Leakey and Akinnifesi, 2008).

Stem cuttings are used in vegetative propagation to achieve improvements in yield and traits of plants (Leakey, 2005; Miller and Gross, 2011).

Nevertheless, stem cuttings propagation is affected by many factors among which are plant growth regulators, age and part of the plant, growth media, size of the cuttings, and the environment in which the plant is propagated (Hartmann *et al.*, 2002; Husen and Pal, 2006; Paparozzi, 2008; Yeboah *et al.*, 2009; 2011).

Adequate knowledge on the requirements for vegetative propagation of liana species is important for mass regeneration and conservation. It is necessary to develop the right regimes for macro propagation of *P. conophora* using stem cuttings in order to limit the dependence on seeds. In particular, the seeds are consumed by man and rodents with little left for regeneration purposes. Furthermore, the seeds lose viability with time and have a recalcitrant nature (Amadi *et al.*, 2014). Seed production is also seasonal and does not usually tally with seedling production time. Hence, the urgent need for the development of alternative methods of propagation of this multipurpose plant.

This research examined the vegetative propagation of *P. conophora* through stem cuttings. It determined the effects of the use of mist propagator, different plant parts and rooting media on rooting of *P. conophora*.

Materials and Methods

Experimental Site

This study was conducted at the Tree Breeding and Physiology nursery of Forestry Research Institute of Nigeria (FRIN) Headquarters, Jericho, Ibadan, Nigeria.

Source of Cuttings and Rooting Media

The stem cuttings were collected from mature climbers at the arboretum of The Forestry Research Institute of Nigeria Ibadan. Cuttings from two nodes (approximately 7 cm) were prepared from the terminal and basal regions of shoots. The lengths of the cuttings were chosen based on the number of leaves present and the nodal arrangement of double nodes, with full internodes underneath. A high humidity mist propagator was constructed, following the method described by Leakey *et al.* (1990). This was placed under a screen house roofed with transparent plastic sheets with 20 % light intensity. The topsoil was collected from the FRIN arboretum, while river sand was sourced from the flowing river at FRIN. Sterilization of the media was done with the use of autoclave sterilizer for three hours.

Experimental Procedure

The factorial experiment was laid in a completely randomized design with seven replicates. There were three factors; rooting media: topsoil, sterilized topsoil, river sand and sterilized river sand; planting environment: inside the humid propagator and under nursery shed, without the use of propagator; Parts of plant used: terminal (cuttings collected from the apical part) and basal (cuttings collected from the base).

A total of 1,120 double node cuttings of *P. conophora* were collected from basal and terminal parts of the plant. The temperature and relative humidity inside and outside the propagator were monitored. The cuttings were set in propagating sieves of diameter 10 cm with each containing ten cuttings. The cuttings were treated with fungicides, watered daily with a hand sprayer and monitored for 12 weeks.

Data Collection and Analysis

The percentage rooted cuttings (%), shoot length (cm), number of new leaves, number of roots, and root length (cm) were assessed. Data collected were analyzed

using Analysis of Variance (ANOVA) and descriptive statistics. Data analysis was performed using SAS (SAS 2000) and significantly different means were separated using the Duncan Multiple Range Test (DMRT) at 5% level of probability.

Results

The cuttings taken from the base, set in sterilized river sand and placed inside the mist propagator produced the highest rooting (87%), followed by the terminal cuttings set in sterilized river sand and placed inside the propagator (78%) (Table 1). The lowest rooting (20%) was obtained from terminal cuttings set in unsterilized topsoil and placed outside the propagator. The planting environment, part of plant used and their interaction had significant effects on number of leaves produced by the cuttings of *P. conophora* (Table 2).

The cuttings set inside the propagator chamber had higher number of leaves (7.31 ± 3.11) when compared with those outside the propagator chamber (6.55 ± 1.25). The basal cuttings had a higher number of leaves (7.48 ± 1.12) than the terminal cuttings (6.29 ± 2.05). In the two-way interactions between propagator and plant parts, the basal cuttings set inside the propagator had higher number of leaves when

compared with terminal cuttings set inside or outside the propagator chamber. Hence, sterilized river sand and basal cuttings produced higher number of leaves when compared with other rooting media and terminal cuttings. In the three-way interactions between the propagator, plant parts and rooting media, basal cuttings set in sterilized river sand and placed inside the propagator had the highest number of leaves (9.14 ± 0.21), though they were not statistically different from others.

The use of propagator, type of rooting media and their interactions significantly affected the number of roots produced by *P. conophora* cuttings. Similarly, the three-way interaction between propagator, plant parts and rooting media had significant effect on the number of roots produced. Cuttings inside the propagator had higher number of roots (1.91 ± 0.63) than those outside the propagator. Cuttings from the terminal points set in sterilized river sand produced the highest number of roots (2.93 ± 0.43). Basal cuttings set in the propagator had the highest number of roots (2.24 ± 0.22), closely followed by basal cuttings set outside the propagator.

In the three-way treatment combinations, terminal cuttings set in sterilized river sand placed outside the propagator had the highest number of roots (3.00 ± 0.31), followed by terminal cuttings in unsterilized river sand placed outside the propagator.

Table 1: Effect of propagator, plant parts and rooting media on the rooting ability of *Plukenetia conophora* cuttings

Propagator	Plant part	Rooting media	Percentage rooted (%)
Inside propagator	Terminal	Sterilized topsoil	62
Inside propagator	Terminal	Unsterilized topsoil	38
Inside propagator	Terminal	Sterilized river sand	78
Inside propagator	Terminal	Unsterilized river sand	42
Inside propagator	Basal	Sterilized topsoil	65
Inside propagator	Basal	Unsterilized topsoil	33
Inside propagator	Basal	Sterilized river sand	87
Inside propagator	Basal	Unsterilized river sand	34
Outside propagator	Terminal	Sterilized topsoil	50
Outside propagator	Terminal	Unsterilized topsoil	20
Outside propagator	Terminal	Sterilized river sand	58
Outside propagator	Terminal	Unsterilized river sand	22
Outside propagator	Basal	Sterilized topsoil	55
Outside propagator	Basal	Unsterilized topsoil	30
Outside propagator	Basal	Sterilized river sand	60
Outside propagator	Basal	Unsterilized river sand	34

Table 2: Effect of propagator, plant parts and rooting media on number of leaves, number of roots, root length and shoot length of rooted *Plukenetia conophora* cuttings

Treatment Effects			Number of leaves	Number of roots	Root length (cm)	Shoot length (cm)
Propagator						
Outside			6.55±1.25b	1.91±0.63a	3.45±1.12b	8.42±3.14b
Inside			7.31±3.11a	1.88±0.12b	10.19±3.10a	10.19±4.66a
Plant parts						
Terminal			6.29±2.05b	1.86±0.37a	8.52±0.38b	3.92±5.96a
Basal			7.48±1.12a	1.93±0.37a	9.77±0.53a	3.26±1.56b
Rooting media						
Sterilized topsoil			6.69±1.57a	1.71±0.29ab	2.97±0.47c	8.48±2.47 a
Unsterilized topsoil			6.70±1.57a	1.79±0.29ab	2.26±0.42d	8.94±2.05a
Sterilized river sand			7.88±3.57a	2.57±0.68a	4.25±0.54a	11.23±1.31a
Unsterilized river sand			5.86±1.22a	1.430±0.26b	3.98±0.43b	7.36±2.22a
Propagator	Rooting media					
Inside	Sterilized topsoil		6.46 ±1.04a	1.79±0.02a	3.28±0.36 b	8.63±2.03 a
	Unsterilized topsoil		5.84±1.16a	1.93±0.43a	3.64±0.34b	6.94±0.21 a
	Sterilized river sand		8.04±1.20a	3.00±0.20a	4.61±0.54 a	10.73±2.03 a
	Unsterilized river sand		5.86±0.63a	1.430.20a	2.26±0.56 c	7.36±0.86 a
Outside	Sterilized topsoil		6.93±0.86a	1.64±0.20a	3.89±0.14c	8.32±2.22 a
	Unsterilized topsoil		7.56±1.31a	1.64±0.20a	2.34±0.43c	10.94±1.53a
	Sterilized river sand		7.57±1.49a	2.36±0.34a	4.31±0.46 a	12.43± a
	Unsterilized river sand		5.96±1.23a	1.98±0.08a	4.21±1.26a	8.29±2.0 a
Plant parts	Rooting media					
Terminal	Sterilized topsoil		6.30±1.58a	1.00±0.34b	3.23±0.38b	8.24±1.98a
	Unsterilized topsoil		5.57±1.04a	1.79±0.55ab	3.05b	6.26±2.09a
	Sterilized river sand		7.25±1.16a	2.93±0.43a	5.24±0.56a	11.62±1.95a
	Unsterilized river sand		5.80±1.2a	1.29±0.42b	1.63±0.55c	7.44±2.03a
Basal	Sterilized topsoil		7.09±1.35a	1.79±0.55ab	2.80±0.26c	9.51±1.76a
	Unsterilized topsoil		7.83±1.16a	1.86±0.43ab	3.64±0.56b	9.87±1.95 a
	Sterilized river sand		9.14±0.63a	2.14±0.20a	4.32±0.38ab	12.01±0.53a
	Unsterilized river sand		5.91±0.97a	1.86±0.71ab	2.88±0.21c	6.48±2.19a
Propagator	Plant parts	Rooting media				
Inside	Terminal	Sterilized topsoil	4.74±1.04a	1.00±0.00b	5.63 ±2.09a	3.57±0.207b
		Unsterilized topsoil	3.06±0.53a	1.43±0.02b	3.06±0.53a	4.07±0.38b
		Sterilized river sand	8.93±0.97a	2.86±0.71ab	11.59±2.19a	5.66±0.21 a
		Unsterilized river sand	5.80±1.31a	1.00±0.00b	8.24±1.53a	1.63±0.43d
	Basal	Sterilized topsoil	8.19±0.16a	2.57±0.43ab	11.63±1.95a	3.75b
		Unsterilized topsoil	8.08±1.20a	2.43±0.43ab	10.82±2.03a	3.21±0.55bc

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Outside	Terminal	Sterilized river sand	9.14±0.21a	1.86±0.34ab	9.87±2.22a	2.80±0.14c
		Unsterilized river sand	5.91±1.58a	1.86±0.34ab	6.48±1.98a	2.88±0.38c
		Sterilized topsoil	7.86±1.49a	1.57±0.2b	9.24±2.59a	2.89±0.46c
		Unsterilized topsoil	7.55±1.35a	2.14±0.55ab	9.45±1.79a	4.57 ±0.26a
	Basal	Sterilized river sand	7.57±2.49a	3.00±0.31a	12.43±3.57a	2.34±0.09c
		Unsterilized river sand	5.88±0.00a	2.98±0.01a	10.99±0.71a	1.44±0.21d
		Sterilized topsoil	6.00a	1.71b	7.40a	4.89a
		Unsterilized topsoil	7.57a	1.14b	12.43a	4.06ab
		Sterilized river sand	9.12a	2.33ab	9.97a	2.75c
		Unsterilized river sand	7.27a	2.64ab	7.22a	2.99bc

The use of the mist propagator had a significant effect on the root length of *P. conophora* cuttings. The cuttings inside the propagator had higher root length (10.19±3.1 cm) when compared with those outside the propagator (Table 2). The part of plant used and rooting media influenced rooting with root length of terminal cuttings being higher (3.92±5.96 cm). Sterilized river sand had the highest root length (4.25±0.54 cm) followed by unsterilized river sand (3.98±0.43 cm), while the least was observed in unsterilized top soil. Hence, interactions among the propagator, rooting media, and plant parts significantly affected root length.

Similarly, the use of propagator significantly affected shoot length, with cuttings set inside the propagator producing the longest shoot (10.19±4.66 cm) (Table 2). The plant part used also had a significant effect on shoot length with basal cuttings having the highest shoot length (9.77±0.53 cm) when compared with the terminal cuttings (8.52±0.38 cm). The two-way interaction between propagator and plant parts produced a significant effect as basal cuttings set inside the propagator had the longest root (10.37±2.63 cm).

Discussion

Vegetative propagation is a technique used to capture and transfer the genetic potential present in the mother tree for certain traits to their offspring (Tchoundjeu *et al.*, 2006; Leakey and Akinnifesi, 2008). Hettasch *et al.* (2009) stated that even with low narrow-sense heritability, genetic gains can still be doubled by using vegetative propagation. Vegetative propagation also contributes to the acceleration of successful tree improvement programmes. It is a viable option in the conservation and multiplication of plant species (Tchoundjeu, *et al.*, 2001, 2002, 2004; Akinyele 2010;

Yakubu *et al.*, 2014, 2020). In this study, *Plukenetia conophora*, a seasonal fruiting liana with recalcitrant seeds that are in high demand, could be vegetatively propagated using stem cuttings. Basal stem cuttings in sterilized river sand placed under a propagator had the highest rooting (87%). Plant parts, rooting environment and growth media affected rooting success of stem cuttings of *P. conophora*.

The rooting environment and microclimatic factors such as humidity, temperature and water were essential conditions for rooting success under the propagator. The propagator helps to maintain and regulate the osmotic pressure in the cells and promote formation of root initials in the stem cuttings (Hartmann *et al.*, 2002). The high relative humidity in the propagator, ensures the cuttings are not subjected to water deficit due to transpiration. (Nketiah *et al.*, 1998; Leakey, 2004).

Temperature affects root initiation and elongation resulting in large numbers of cells dividing, differentiating, and elongating (James and Brain, 2007). Temperature is also important in various enzymatic reactions such as respiration and the dark reactions of photosynthesis which increase with rising temperatures. Temperature adversely affects respiration and photosynthesis in carbohydrate utilization and translocation as roots grow and develop (Hartman *et al.*, 2002). The adverse effects are however, modulated under the propagator chamber thus increasing the number of leaves and roots as well as root and shoot length.

Basal cuttings were more amenable to vegetative propagation, producing higher number of leaves and roots, than cuttings from the terminal parts of the plant. This could be due to higher concentration of carbohydrate content at the base (Leakey, 2014). Carbohydrate in the form of food reserves plays an important role in the growth of stem cuttings. Hoad and Leakey (1996)

observed that carbohydrate reserve was an important criterion for rooting of stem cuttings of *Eucalyptus grandis*. Similarly, Amri *et al.* (2010) and Swarts *et al.* (2018) observed that basal cuttings of *Dalbergia melanoxylon* and *Lobostemon fruticosus* achieved optimum rooting success due to food reserves.

However, this may not be true for all plant species. For example, according to Olaniyi *et al.* (2021), cutting position did not influence rooting, while stem cuttings obtained from the apical position had the greatest tendency to improve rooting capacity in *Picalima nitida*. Tchoundjeu and Leakey (2001) and Tate (2018) also reported higher rooting successes for apical cuttings compared to basal cuttings in *Lovoa trichilioides* and *Santalum austrocaledonicum*, respectively. This could be due to aging and maturation of tissues, reducing rooting success in physiologically older shoots (Wendling *et al.*, 2014).

The rooting success of cuttings is affected by the growth media (Swarts *et al.*, 2018). Ercis *et al.* (2002) and Swarts *et al.* (2018) opined that using the right rooting medium was a key factor to achieving success in rooting of cuttings. In this study, the rooting media affected the number of roots and root length. This is in line with the work of Yeboah *et al.* (2009) who reported that growth media affected the number of roots obtained in stem cuttings of Shea nut tree (*Vitellaria paradoxa*).

The results of this study indicate that soil type and sterilization influenced root development. When soils are sterilized, pathogens and microbes that could hinder root development are eliminated. River sand has large pores that allow for easy movement of air and water. This will aid the proliferation of fine roots with little or no obstruction.

Conclusion

The use of propagator chamber, basal stem cuttings, and sterilized river sand enhanced rooting success of *P. conophora*. The vegetative propagation of the species would reduce problems associated with non-availability of seeds during the off-season periods. This would also reduce competition for seeds among users.

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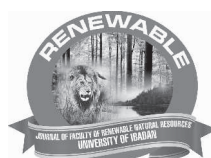
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