

Original Research Article

Conservation of a rare medicinal plant: A case study of *Griffonia simplicifolia* (Vahl ex DC)

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Abstract: This study aimed at carrying out the vegetative propagation of *Griffonia simplicifolia*. This was achieved by growing nodal and stem cuttings of *G. simplicifolia* using two types of soils under varying environmental conditions including open area and humidity propagator. This revealed that terminal nodal stem cuttings showed the best growth forms in terms of shoot development as well as root development when subjected to open area and topsoil. However, when subjected to the humid propagator and river sand, the distal stem cuttings gave the best growth forms thereby promoting shoot and root development. Hence, it is concluded that the terminal nodes are the best parts to be used for the propagation of *G. simplicifolia* and the best growth medium is river sand. Furthermore, this research demonstrates that *G. simplicifolia* can be reproduced through macropropagation using stem cuttings and it can be seen as a basis for conservation and other breeding research on *G. simplicifolia*.

Keyword: Conservation, *Griffonia simplicifolia*, macropropagation, stem cutting, terminal nodes

INTRODUCTION

Griffonia is a genus of flowering plant in the legume family Fabaceae. It belongs to the sub-family Caesalpinioideae. There are four members of the genus; *G. physocarpa* (Ball), *G. speciosa* (Benth.) Tanb, *G. tessmannii* (De wild) Compere, *G. simplicifolia* (Vahl ex DC) Baill (Kokou *et al.*, 2008; Wunderlin, 2010). *G. simplicifolia* is a woody climbing shrub native to West Africa and Central Africa (Carnevale *et al.*, 2010). Kokou *et al.*, (2008) stated further that *G. simplicifolia* occurs in thickets, usually associated with mounds of the termite *Macrotermes spp.*

on plains, in forests, in secondary vegetation and on old farms. It is evergreen, vigorous and has wide adaptability.

In West Africa, *G. simplicifolia* flowers from July till November. It occurs in grass savanna, in coastal plains on termite mounds, in scrub thickets, secondary and disturbed forest and along the margins of primary forest. It grows to about 3 m and bears greenish flowers followed by black pods(Beattie *et al.*, 2011). The plant produces fair quantities of highly palatable herbage of good nutritive value and fair qualities of large, viable seeds(Beattie *et al.*, 2011; Irvine, 1961). Pods ripen from August onwards. Leaves alternate, simple, glabrous; stipules triangular, 1mm long, petiole up to 1.5 m long, blade ovate, 6-12 m x 3-6 cm, based round to short, acuminate, 3(-5)-veined from the base, reticulate veins prominent on both sides. The inflorescence is an auxiliary, pyramidal raceme 5-20 cm long, bracts and bracteoles triangular, very small, and persistent, developing into an oblique-cylindrical fruit. Seedling is with epigeal germination(Aubreville, 1968; Carnevale *et al.*, 2010).

The plant has been reputed as an important African medicinal shrub with potent anti-depressant and healing properties. While every part of the plant is used in native healing systems of the region, western use has focused on the high content of a serotonin precursor and management of sickle cell anaemia(Mehta *et al.*, 2015). Other traditional uses of the plant include; healing of infected skin lesions (topical leaf and bark decoction), purgative for constipation (oral stem and leaf decoction), treatment of eye inflammation (topical drops from leaf sap) and as an aphrodisiac(Muszyńska *et al.*, 2015; Van Andel *et al.*, 2012). Carnevale *et al.*, (2010) and Meybeck, (2009)also stated that a decoction of the stems and leaves is also used to stop vomiting and to treat congestion of the pelvis.

The modern phytochemistry study found that 5-hydroxytryptophan is the main bioactive component of *G. simplicifolia* extract(Vigliante *et al.*, 2019). The 5-hydroxytryptophan (5-HTP), an amino acid that helps the body produce serotonin is a natural relaxant and has been widely reported for some pharmacological activities including anti-depression, inhibition of obesity, anti-oxidation, alleviation of insomnia and other biological effects(Beattie *et al.*, 2011). Serotonin is important in regulating brain chemistry especially in countering problems such as depression, schizophrenia, insomnia and eating disorders(Eggers, 2013; Meltzer *et al.*, 2003). *G. simplicifolia* seeds also regulate appetite through the increase in serotonin, leading to weight reduction in obese persons, while helping normalize the weight of people suffering from anorexia nervosa(Carnevale *et al.*, 2011; Lemaire and Adosraku, 2002). Theseed of the plant is being used in treating fibromyalgia, chronic headaches, and of greatest benefit in the treatment of psychiatric and neurological disorders where there is a deficiency of neuro-serotonin receptors(Meltzer *et al.*, 2003).

Lescar *et al.*, (2002)also noted in their investigation on the plant that the seeds from the African legume shrub contain several lectins. Among them, Tetrameric lectin GSI-B(4) has a strict specificity for terminal alpha-Gal residues, whereas the closely related lectin GSI-A(4) can also bind to alpha GalNAc. These two lectins are commonly used as markers in histology or for research in Xenotransplantation. Lectin component of *G. simplicifolia* seeds and leaves is somewhat resistant to proteolysis. Despite the aforementioned, currently, no evidence exists as to whether or not these lectins pose a concern to a person with celiac diseases(Carnevale *et al.*, 2011; Jimoh *et al.*, 2019a, 2019b). Benton *et al.*, (2008) and Zhu *et al.*, (1996)also reported that *G.simplicifolia* contains an N-acetylglucosamine-specific legume lectin that has insecticidal activity when fed to the cowpea weevil *Callosobruchus maculatus* (F.). These results support the

hypothesis that legume lectins are encoded by genes that function in plant defence against herbivores (Zhu *et al.*, 1996).

Earlier reports suggest that much research studies have been carried out on the root, leaf, pod and seed of the plant. However, there is a dearth of information on its propagation for commercial cultivation. Probase record of the plant informed that propagation by seed gave poor results and different seed treatments did not improve germination, although fungicide treatment of the seed appeared beneficial for establishment. Aubreville, (1968) stated that the use of stem cuttings has not been successful and there are no indications that *G. simplicifolia* is currently being cultivated. Furthermore, Amujoyegbe *et al.*, (2012) and Işık, (2011) noted that rare and endemic medicinal plant species with a much-localized distribution are a particular problem and the need for conserving them cannot be over-emphasized. *G. simplicifolia* is endemic in Ghana, Cote d'Ivoire, Lome, Cameroon and Nigeria. Available literature shows that much work has not been carried out on the plant in Nigeria especially as it concerns its conservation. The plant has limited distribution and it is a rare plant species, with the dearth of information on its conservation. This research, therefore, aims at generating information on vegetative propagation of *G. simplicifolia*, to develop quality planting materials, standard techniques and cultivation practices necessary for its production. Also, this study focuses on determining the best soil medium for the crop and to monitor the plant for some growth parameters which include, shoot development, leaf formation and rooting.

MATERIALS AND METHODS

Experimental design

The study used the experimental type of research common in most agricultural researches. The study was a Two Factor experiment in a Randomized Complete Block Design (RCBD) replicated three times with the planting material (stem cuttings) as factor A, and the various soil media as factor B (Makinde et al., 2011; Soundy et al., 2008). The study was carried out in the nursery point within the Lagos State Rural Botanical Park Project Complex in Igbodu, Epe division of Lagos state (6°39'10'' N, 3°55'20'' E). Two growing environments within the fenced nursery point were used and these are Open Area and Under High Humidity Propagation Chambers. It focused only on nursery production of *G.simplicifolia* for three months.

Experimental layout

The study area measured 14 m x 5 m. The high humidity chambers consisted of eighteen (18) potting media. Half of the stated number contained topsoil and the remaining were washed river sand. Also, eighteen (18) treatment combinations were placed in the open area as well. Thus, a total of thirty-six (36) treatment combinations altogether were set. The high humidity propagator chambers were made air-tight (with polythene covering and nailed). These were left to the stand for 48 h and mist formation was observed underneath the polythene covering of each of the chambers.

Preparation of tags

Pieces of white plastic tags were cut into 20 cm lengths and used as the label for identifying each pot. The corresponding treatment combinations were inscribed on the tag.

Preparation of soil media

Before the start of the study, the soil samples were tested for nutrient availability. Three soil samples were taken for laboratory analysis; topsoil, river sand and soil from the base of the mature plant stand. The soil samples were taken separately to the Chemistry Laboratory University of Lagos, Akoka for analysis. About 5 kg of wet river sand (washed with sterilized water) was measured on a labelled, clean, dry black poly-pot. These were repeated for the remaining poly pots, and same weight for the topsoil medium. A total of Thirty-six (36) bags (3 nodal positions x 2 potting media x 3 replicates) altogether were set as earlier stated. Thereafter, stem cuttings were firmly inserted on each poly-pot.

Preparation of planting materials

Using a very sharp cutlass and knife, young stems were cut from the apical portion of the mother plant. Leaves were gently cut off with a knife and stem were cut to 30 cm lengths. This was divided into 3 equal parts, taking note of at least 2 nodes and each node was respectively tagged terminal (T), middle (M), and distal (D). These were placed in sacks. The diameter of cuttings ranges from 1-1.5 cm, cuttings were transported to the nursery point in the plastic sacks same morning for planting.

Plant material

Stem cuttings obtained from the plant species within the park were used in the study. The species were identified at the University of Lagos Herbarium and assigned the voucher No.: LUH 6043. Stem cuttings of about 10cm long and 1-1.5 cm diameter from the apical portion of stems of the matured plant were used in the study. The soil media were limited to Topsoil (P1) collected within the park (mentioned above) and Washed River Sand (P2) collected from Itoikin sand point along Epe road, Lagos. Latitude: 6.6531N, longitude: 3.79334E.

Maintenance and observation

Plants were watered using a plastic sprinkler twice daily (morning and evening). Weekly recording of germination and sprouting was ensured throughout the study period. Weeding was done in alleys and pots routinely. Stated growth parameters were recorded during the second and last month of the study period.

Data collection

Primary data such as germination, shoot length, leaf number, leaf area, root number, root length and survival were taken from observations and measurement throughout the 3-months study period in the nursery. Shoot length, leaf length, leaf blade and root length were measured with a meter rule calibrated in centimetre. The number of roots was achieved by physical counting. Leaf area was measured with the use of graph sheet (leaf outlines/shape were drawn and the numbers of square meters added up to give respective leaf area in centimetre squared (cm²). The mean values of the measured growth parameters were taken per treatment combination in the third month of the study. Weekly observations on germination were noted per pot during the period of study. Shoot length was measured with a meter rule from the base of the plant to the top at the longest shoot for stem cuttings. For root length, seedlings were carefully removed from the polythene bag. The polythene bag was cut with a razor blade to expose the base soil and immersed in a bowl of water to shake-off stacked soil, exposing the root system. The longest root was measured with a meter rule from the base of the stem to its tip. The total number of plants that survived per treatment after the 3-months study period was noted as well. All data or measurements were recorded in a field notebook.

Statistical analysis

Data recorded in the three nodal positions in each treatment were averaged and subjected to one-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test using MINITAB 17 statistical software.

RESULTS

Soil analysis

Result of soil analysis shows variations in the physicochemical properties of the soil media used. It further indicated that the **percentage of sand** is higher in the topsoil than the river sand although the latter had more silt than the former (Table 1).

Table 1: physicochemical properties of the soil

Growth medium	Topsoil	River sand	Base of the plant stand
Description	Brown silty medium fine-grained sand	Dark silty sandy clay with gravels	Dark grey sand silty clay with gravels
Percentage gravel	Nil	5	3
Percentage of sand	94	55	40
Percentage silt	6	18	23
Percentage clay	Nil	22	34
Manganese (Mn)	0.13	0.16	0.21
Phosphorus (P)	3.4	2.29	3.14
Potassium (k)	1.6	1.77	1.82
Nitrogen (N)	0.07	0.45	0.56
pH	5.99	6.49	6.17
Total Organic matter	1.47	9.06	11.3

Open Area Top Soil

The longest shoot was recorded at the terminal nodal position while the shortest mean value was obtained at the distal nodal position. Statistical analysis indicated no significant difference in the mean values of shoot number, leaf number, leaf length, root number and root length recorded terminal, middle and distal nodal positions in the open area. However, the leaf area was highest at the distal nodal position although no significant difference was observed in leaf areas recorded at the terminal and middle nodal position (Table 2; Figure 2b).

High Humidity Propagator Chambers Top Soil

None of the terminal nodal stem cuttings propagated in the humidity propagator chambers (Table 2). Also, the mean values of growth parameters recorded in the middle and distal nodal positions were not different significantly (Figure 1b).

Table 2: Mean values of growth parameters of *G. simplicifolia* cuttings from different nodal positions growing in topsoil

Growth Parameters (cm)	Open area			Humidity propagator chambers		
	Terminal nodal position	Middle nodal position	Distal nodal position	Terminal nodal position	Middle nodal position	Distal nodal position
Shoot number	1.67±0.47 ^a	1.00±0.00 ^a	1.17±0.24 ^a	-	1.75±0.25 ^a	1.25±0.25 ^a
Shoot length	11.93±3.23 ^a	9.60±0.00 ^{ab}	6.07±0.34 ^b	-	7.10±1.40 ^a	4.74±2.04 ^a
Leaf number	3.57±0.42 ^a	4.00±0.00 ^a	4.22±0.88 ^a	-	3.50±0.50 ^a	4.00±2.00 ^a
Leaf length	4.73±0.34 ^a	4.50±0.00 ^a	4.31±0.08 ^a	-	3.38±0.83 ^a	2.74±1.24 ^a
Leaf area (cm ²)	255.33±17.59 ^b	221.00±0.00 ^b	348.44±24.38 ^a	-	195.00±3.00 ^a	219.34±19.34 ^a
Leaf blade	2.74±0.11 ^a	2.40±0.00 ^b	2.57±0.05 ^{ab}	-	1.78±0.53 ^a	1.44±0.84 ^a
Root number	1.67±0.47 ^a	2.00±0.00 ^a	2.44±0.42 ^a	-	3.00±0.00 ^a	1.75±0.75 ^a
Root length	2.71±0.75 ^a	1.80±0.00 ^a	1.63±0.17 ^a	-	2.80±0.00 ^a	1.50±0.90 ^a

NOTE: All superscripts indicate a significant difference between the means at P<0.005. Values on the same row with the same superscripts indicate no significant difference and vice versa.

Open Area River Sand

The growth parameters recorded in plants grown on river sand in an open area showed no variabilities in the shoot number, leaf number, leaf length, leaf blade size and root number across the three nodal positions. However, variabilities occurred in leaf area and root length (Table 3; Figure 2a).

High Humidity Propagator Chambers River sand

The highest mean value of shoot length was recorded in the middle nodal position, while the mean shoot lengths recorded at the terminal and distal nodal positions were not significantly different. The highest mean value of the leaf length was recorded in the distal nodal position while the least mean leaf length was measured in the terminal nodal position. There was no variability in the number of leaves recorded, however; the highest leaf area was recorded at the distal nodal position followed by the mean value of the middle nodal position then, the terminal nodal position where the lowest mean leaf area value was recorded. The mean values of the root length also showed variabilities in river sand under high humidity propagator chamber. The longest root and the shortest were respectively recorded at the distal and middle nodal positions (Table 3; Figure 2b).

Table 3: Mean values of growth parameters of *G. simplicifolia* cuttings from different nodal positions growing in river sand

Growth parameters (cm)	Open area			Humidity propagator chambers		
	Terminal nodal position	Middle nodal position	Distal nodal position	Terminal nodal position	Middle nodal position	Distal nodal position
Shoot number	1.67±0.47 ^a	3.17±0.24 ^a	2.17±0.85 ^a	2.17±0.24 ^a	1.00±0.00 ^b	2.17±0.24 ^a
Shoot length	10.70±0.51 ^a	7.11±1.97 ^a	10.51±0.69 ^a	5.80±0.50 ^b	11.10±0.50 ^a	7.64±1.63 ^b
Leaf number	5.67±1.25 ^a	5.33±4.11 ^a	4.11±0.16 ^a	4.33±0.47 ^a	5.25±0.25 ^a	5.33±1.89 ^a
Leaf length	5.11±0.83 ^a	3.93±0.25 ^a	5.91±0.77 ^a	2.91±0.01 ^c	4.59±0.12 ^b	5.40±0.08 ^a
Leaf area (cm ²)	308.22±62.03 ^a	150.78±1.29 ^b	338.89±61.78 ^a	76.22±1.10 ^c	190.25±1.75 ^b	292.89±10.22 ^a
Leaf blade	2.78±0.49 ^a	2.10±0.08 ^a	2.84±0.11 ^a	1.22±0.03 ^c	2.08±0.08 ^b	2.89±0.08 ^a
Root number	2.17±0.24 ^a	2.78±0.32 ^a	2.17±0.24 ^a	3.00±0.82 ^a	3.25±0.75 ^a	3.89±0.83 ^a
Root length	1.83±0.29 ^b	2.12±0.16 ^{ab}	2.80±0.22 ^a	2.52±0.13 ^{ab}	1.93±0.23 ^b	3.43±0.82 ^a

NOTE: All superscripts indicate a significant difference between the means at P<0.005. Values on the same row with the same superscripts indicate no significant difference and vice versa.



Fig 1a. Humidity propagator chamber distal nodal position river sand



Fig 1b. Humidity propagator chamber distal nodal position topsoil



Fig 2a. Open area distal nodal position river sand



Fig 2b. Open area distal nodal position topsoil

DISCUSSION

The development of propagation method is the first step in any domestication effort (Jimoh *et al.*, 2020; Leakey and Simons, 1998). The results of the present study indicated that *G. simplicifolia* stem cuttings can be successfully propagated (Figures 1a, 1b, 2a and 2b). The possibility of rooting stem cuttings of *G. simplicifolia* is crucial to its domestication strategy. The results of this study indicate the important role of determining the optimal rooting conditions in the process of vegetative propagation. The ability of cuttings to survive and produce long and massive shoots is very important. River sand followed by garden topsoil was able to serve this

purpose in the studied species. The results across the two media conform to those reported by Hartmann *et al.*, (2010) that an ideal propagation medium is known to provide sufficient porosity to allow good aeration and this ensures adequate oxygen availability for the developing roots system. It is important to select a medium with the correct characteristics as there can be marked differences between root formations by the same species on different media (Gruber *et al.*, 2013). According to Farrell *et al.*, (2013) and Boshoff *et al.*, (2004), different media differ in air-water ratios usually to enhance the rooting potential of nodal cuttings of plant species. Basal cuttings rooted better than apical cuttings in this study and this disagrees with the results reported for *Lippia javanica* by Soundy *et al.*, (2008) but agrees with results of Zalesny *et al.*, (2003) where the similar trend was reported in *Populus* spp. The result also agrees with the findings of Sadhu (1989) and Dolor, (2011) who stated that quartz sand is most suitable for propagation purpose. This assertion contrasted with the findings of Okunomo *et al.*, (2009) who obtained a higher germination percentage in topsoil with *Dacryodes edulis*, citing the presence of adequate nutrients for germination and growth in topsoil. Sand warms up more rapidly leading to increased biochemical activities which translate to increased germination (Brady and Weil, 1999). These findings were earlier attested to by Tisdale and Nelson, (1975) where it was stated that temperature directly affects the plant's functions of photosynthesis, respiration, cell wall permeability, protein coagulation, absorption of water and nutrients transpiration, and enzyme activity.

CONCLUSION

In conclusion, this study demonstrates that *G. simplicifolia* can be reproduced through macro propagation using stem cuttings. The cuttings require a medium that allows for good drainage and sufficient space to prevent waterlogging and subsequent rooting of the cutting. Rooting was

enhanced in the high humidity propagator chambers and this is serving as a good nursery condition for raising the species.

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