

Full Length Research Paper

Comparative Analysis of Macro-Propagation Techniques for *Gongronema latifolia* Benth Across Various Physiological Ages

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Non-wood forest products have been shown to be sustaining the rural poor in Nigeria and other developing countries. They are in most cases threatened to extinction because most species are not domesticated and thus harvested from forests. Macro-propagation of *Gongronema latifolia* Benth highly valued for its nutritional and medicinal qualities but has not been substantially domesticated was studied using three physiological ages (hardwood, semi-hardwood and soft wood) in Nsukka. Statistical analyses were performed on the attributes of the cuttings and nodal cuts. The study showed that large number of clones could be produced from a single stem cutting of *G. latifolia* with any of the three physiological ages. Hardwood and semi-hardwood nodal cuts produced five and four shoots/clones respectively and thus have higher potential of producing clones. Macro-propagation technique could thus be utilized to rapidly disseminate selected land races and new varieties of *G. latifolia* resulting from hybridization.

Key words: *Gongronema latifolia*, macro-propagation, nodal cuts and physiological age.

INTRODUCTION

Gongronema latifolia Benth is a forest leafy vegetable that grows in the forests of South-eastern Nigeria (Akpan, 2004). It is a non-wood forest product of West African origin (Nielsen, 1965) and called "utazi" in southeastern states of Nigeria. The forest vegetable is a good source of vitamins, minerals and proteins (Okafor, 2005). The non-wood forest vegetable is used for nutritional and medicinal purposes in Nigeria and other parts of sub-Saharan Africa. The plant is used in the treatment of loss of appetite, cough, worm, dysentery and malaria (Agbo et al., 2005). Okafor (2005) emphasized that the plant is a good source of iron, promotes pregnancy and effective in treatment of diabetes mellitus.

Macro-propagation technique was conceived as a means of rapid multiplication of *G. latifolia* because the plant can be vegetatively propagated (Agbo and Omaliko, 2005). The technique has the advantage of rapid dissemination of selected clones and new varieties that may result from breeding programmes which are deemed desirable because of their quantitative and qualitative attributes.

Stem cuttings are classified based on physiological age of the wood from which they are taken. There are hardwood, semi-hardwood and soft wood cuttings (Rice et al., 1990; Evans, 1999). Hardwood cuttings are taken from dormant, mature stems of more than one year old. The semi-hardwood cuttings are usually prepared from partially mature wood of the current season's growth while softwood cuttings are prepared from soft, succulent new growth of woody plants (Evans, 1999).

Many internal factors such as auxins, rooting co-factors, carbohydrate and nitrogen levels have been shown to influence root and shoot initiation in the rooting stock (Hartmann and Kester, 1975). They further explained that in some plants, marked differences in the chemical composition of different parts of the shoot are known to exist from base to tip with the highest shooting, in many cases, found in cuttings taken from the basal portions of the shoot. The influence of carbohydrate and some root promoting substances from buds have made the basal portions of such shoots to be the best cutting (Hartmann and Kester, 1975).

The availability of *G. latifolia* in Nigeria is on the decline and in some places threatened to extinction in spite of the nutritional and medicinal values of the plant. Osemeobo and Ujor (1999) reported that *G. latifolia* is mainly harvested from forests and has become scarce

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Table 1. Influence of physiological age of *Gongronema latifolia* cuttings on their rooting and shooting potentials after two weeks of panting in the boxes.

REPS	Number of nodes with roots/cutting			Number of nodes with shoot/cutting			Length of the longest root/cutting			Height of shoot/cutting			Number of root/cutting			Number of shoot/cutting		
	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW
1	0.67	1.00	0.17	3.00	3.67	4.17	2.08	2.17	1.08	0.42	0.75	1.25	1.33	2.67	1.33	3.67	4.33	3.30
2	2.00	0.67	0.17	3.00	3.37	3.67	2.33	1.50	1.33	0.75	1.08	0.67	2.67	1.17	0.33	4.00	4.67	317
3	1.80	1.67	0.17	3.67	4.67	4.00	3.50	2.83	1.17	0.08	1.42	1.33	3.17	3.83	0.50	5.00	4.37	4.83
4	2.17	0.33	0.33	3.33	5.50	4.50	3.67	1.75	1.92	4.50	1.17	1.83	6.00	1.50	1.17	4.17	5.20	5.33
5	0.17	0.17	0.17	4.00	3.17	1.33	0.50	0.33	1.25	1.58	2.00	0.33	2.17	0.17	0.83	4.83	3.03	2.50
6	1.00	1.67	0.33	3.50	3.00	3.33	1.25	4.17	2.50	2.25	1.00	0.08	2.17	4.50	1.33	5.17	3.50	3.67
7	0.50	1.17	0.67	3.50	3.33	4.50	1.17	0.58	1.08	1.58	1.92	0.91	0.67	1.33	1.33	3.50	4.50	5.00
8	1.33	1.33	0.67	2.83	3.67	3.33	1.08	2.00	1.67	2.67	2.42	0.17	1.67	2.83	0.83	2.83	4.27	4.50
9	1.17	1.17	1.00	3.17	3.50	3.83	1.50	1.17	4.50	0.17	0.17	0.67	1.83	2.33	2.33	3.33	4.17	6.00
10	1.33	0.33	1.17	3.33	4.00	4.00	1.83	0.33	2.91	1.33	0.33	0.42	2.50	0.67	1.33	4.00	5.03	5.33
Mean	1.21	0.95	0.48	3.33	3.78	3.67	2.08	1.68	1.94	1.53	1.23	0.77	2.42	2.10	1.13	4.05	4.31	4.36
F-LSD (p = 005)	0.49			NS			NS			NS			1.10			NS		

HW, hardwood; SHW, semi-hardwood; and SW, softwood cuttings.

Table 2. Influence of physiological age of *G. latifolia* cuttings on their rooting and shooting potentials after two weeks of planting in the boxes

REPS	Number of nodes with roots/cutting			Number of nodes with shoots/cutting			Length of the longest root/cutting			Height of shoot/cutting			Number of roots/cutting			Number of shoots/cutting		
	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW
1	1.33	1.17	0.17	3.83	4.33	2.67	6.33	5.00	4.50	4.00	3.42	1.75	4.00	3.83	2.33	6.67	5.83	3.67
2	2.67	0.50	0.33	3.83	4.50	2.83	6.86	4.75	4.33	6.50	2.75	1.83	6.50	2.00	1.83	6.83	6.00	3.67
3	1.83	1.33	0.83	4.00	4.83	2.83	4.00	6.58	7.00	4.67	2.08	2.50	5.67	4.00	2.67	6.17	5.00	3.17
4	1.83	0.67	1.67	4.00	4.67	4.50	6.83	6.50	6.08	8.50	3.17	3.50	7.00	5.83	2.67	5.17	5.83	5.83
5	0.67	0.83	0.33	5.00	3.33	2.67	6.83	4.50	1.75	4.17	2.00	1.33	3.50	4.00	1.83	6.67	4.00	3.17
6	1.83	1.33	0.50	4.17	3.50	3.33	4.50	4.75	3.25	5.92	4.83	0.67	4.33	4.83	1.83	6.00	4.17	4.00
7	0.83	0.83	0.67	4.17	4.17	4.67	2.42	1.33	2.50	0.50	4.92	2.42	1.00	1.50	2.67	6.83	5.67	5.00
8	1.83	1.50	0.67	4.17	3.50	3.50	5.17	3.00	2.00	3.67	6.25	0.33	3.83	4.67	1.00	4.67	4.67	4.83
9	2.83	1.17	1.17	4.83	4.50	3.83	5.70	1.67	7.25	0.67	1.67	1.58	3.17	2.33	4.00	7.67	5.00	6.00
10	1.83	0.83	0.67	4.67	4.50	3.83	4.58	3.67	3.17	4.92	2.00	1.67	4.50	1.67	2.50	6.17	6.50	5.67
Mean	1.75	1.02	0.70	4.27	4.18	3.47	5.32	4.18	4.18	4.35	3.31	1.76	4.35	3.46	2.33	6.29	5.67	4.50
F-LSD (p = 0.05)	0.47			0.53			0.20			1.61			0.99			NS		

HW, hardwood; SHW, semi-hardwood; and SW, softwood cuttings.

Table 3. Influence of physiological age of *Gongronema latifolia* nodal cuts on their shooting potentials at two weeks after planting in polyethylene bags.

Reps	Number of shoots/nodal cut			Number of shoots with leaves			Number of opposite leaves			Height of shoots		
	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW
1	5.00	2.00	0.00	3.50	1.50	0.00	2.00	2.50	0.00	5.50	2.50	0.00
2	3.50	3.50	1.50	2.50	3.50	1.00	2.50	6.00	1.50	5.00	7.50	4.25
3	2.50	3.00	1.50	1.50	2.50	1.00	1.50	5.50	2.50	5.00	10.00	4.00
4	1.00	1.50	2.50	0.50	1.50	2.00	3.00	3.00	3.00	15.25	4.00	4.00
5	3.00	3.50	1.00	2.50	3.50	0.50	5.00	6.00	1.50	19.00	7.50	4.00
6	3.50	2.00	2.00	3.50	1.50	2.00	7.50	2.00	3.50	9.67	4.50	9.00
7	3.00	1.00	2.00	3.00	1.00	1.50	3.00	2.00	2.00	12.00	3.50	3.50
8	4.00	1.50	1.00	3.50	1.50	1.00	6.00	3.00	2.50	0.72	7.00	3.00
9	4.00	2.50	1.00	4.00	2.00	1.00	7.00	3.00	2.50	1.42	7.00	6.00
10	2.00	4.00	1.50	2.00	3.00	0.50	4.50	6.00	0.50	6.00	11.00	0.50
Mean	3.15	2.45	1.40	2.65	2.15	1.05	4.20	3.90	1.85	7.95	6.45	3.83
F-LSD (p = 0.05)	0.91			0.82			1.57			3.73		

HW, hardwood; SHW, semi-hardwood; and SW, softwood cuttings

and threatened. In order to ensure sustained conservation and continued availability of identified clones and varieties with quality genetic potentials, there is a need for development of rapid dissemination system for such clones and varieties. Macro-propagation technique was therefore conceived to achieve the desire to generate seedlings for rapid propagation of selected clones. The objective of the research was to study the rooting and shooting potentials of the three physiological ages of the stem cuttings in pre-nursery planting boxes and nursery polybags.

MATERIALS AND METHODS

Stem cuttings of five opposite nodes were taken within one kilometer radius of a forest in southeastern Nigeria for the study. The sawdust medium used for rooting in this study was composted for six months and moistened with sodium hypochlorite (3.5%) for sterilization. The sodium hypochlorite was diluted with water at a ratio of 1:9 of sodium hypochlorite : water (v:v) as recommended by Evans (1999).

In the first experiment, the medium was used to fill rectangular planting boxes, each measuring 120 cm x 28 cm x 15 cm. The base of the boxes was perforated to create room for drainage. Healthy cuttings were taken early in the morning when the plants are turgid and the cuttings were grouped based on their physiological ages. Six cuttings of each age (treatment) were placed 4 cm apart and 10 cm into the rooting medium in each planting box. The boxes were spaced 50 cm x 50 cm and arranged in completely randomized design with ten replications under 65% shading. Transparent cellophane was cut and fastened on the top of the boxes with a masking tape to create a humid environment in the boxes. Data were collected on the stem cuttings by removing and placing them back after thorough moistening. Data were collected at two and three weeks after planting on number of nodes with roots/shoots per cutting, number of roots/shoots per cutting, length of the longest root per cutting and highest shoot per cutting.

The second experiment started three weeks after planting of stem cuttings in the planting boxes. The rooted stem cuttings were

cut into five nodal sizes and the nodal cuts from each stem cutting were placed in black polyethylene bags measuring 30 cm x 20 cm and filled to 75% of volume with the sawdust rooting medium. The polyethylene bags containing the nodal cuts of the physiological ages were arranged in completely randomized design (CRD) with ten replications giving eighteen (18) polyethylene bags in each replication. The bags were mulched with dead grasses and spaced 50 cm x 50 cm. The rooting medium was moistened regularly. Data were collected at 2, 4, and 6 weeks after planting in the polyethylene bags on number of shoots, number of shoots with leaves, number of opposite leaves and height of shoots per nodal cutting. Data collected were analyzed using computer software, Genstat 5 release (3.2), 1995. Separate analysis was done for each experiment in a CRD format.

RESULTS AND DISCUSSION

The results (in Table 1) show that hardwood cuttings had significantly higher ($P < 0.05$) number of nodes with roots and number of roots per cutting. There was no significant difference on other attributes of the cuttings during the early growth of the cuttings in the boxes. Hardwood cuttings had significantly ($P < 0.05$) higher number of nodes with roots and shoots and number of roots in each stem cutting (Table 2). It also had longer and higher roots and shoots, respectively. Nodal cuts from hardwood stem cuttings had significantly ($P < 0.05$) higher shoots, number of shoots, shoots with leaves and number of opposite leaves and they were consistent at 2, 4 and 6 weeks of establishment in the polyethylene bags (Tables 3-5).

The progressive growth observed in nodal cuts was relatively higher in hardwood cuttings throughout the period they were assessed. At the fourth and sixth week after planting of nodal cuts in polyethylene bags, shoots of hardwood cuts are twice and thrice the heights of semi-hardwood and softwood cuts, respectively. Similarly, the nodal cuts of hardwood had double number

Table 4. Influence of physiological age of *Gongronema latifolia* nodal cuts on their shooting potentials at four weeks after planting in polyethylene bags.

Reps	Number of shoots/nodal cut			Number of shoot with leaves			Length of opposite leaves			Height of shoot		
	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW
1	5.00	4.00	1.50	4.50	4.00	1.50	7.50	7.50	2.50	8.50	4.50	4.00
2	5.00	5.00	3.00	5.00	5.00	3.00	13.50	18.00	7.00	8.00	10.00	8.50
3	4.50	4.50	4.50	4.00	4.00	4.50	13.75	8.50	8.50	8.50	17.50	3.50
4	3.00	2.50	4.50	3.00	2.50	4.00	8.00	7.00	7.00	25.50	9.50	5.50
5	5.50	5.50	3.00	5.50	5.00	2.50	16.00	12.50	5.00	26.00	17.00	8.50
6	6.00	3.50	4.00	5.50	3.50	4.00	20.00	6.00	10.00	31.00	6.00	13.00
7	6.00	2.50	4.00	4.00	2.50	4.00	13.50	5.00	9.50	19.00	5.00	6.50
8	6.00	4.50	1.50	6.00	4.50	1.50	17.00	8.00	3.50	8.20	22.50	3.50
9	7.00	4.00	2.50	7.00	4.00	2.50	15.00	10.00	4.50	1.30	7.00	8.00
10	3.50	5.50	3.00	3.50	5.50	2.50	10.50	16.00	4.00	18.40	14.00	4.50
Mean	5.15	4.15	3.15	4.8	4.50	3.00	13.45	9.85	6.60	15.44	11.60	6.55
F-LSD (p = 0.05)	1.05			1.44			3.39			6.33		

HW, hardwood; SHW, semi-hardwood; and SW, softwood cuttings

Table 5. Influence of physiological age of *Gongronema latifolia* nodal cuts on their shooting potentials at six weeks after planting in polyethylene bags.

REPS	Number of shoots/nodal cut			Number of shoots with leaves			Number of opposite leaves/shoot			Height of shoots		
	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW	HW	SHW	SW
1	5.50	5.00	2.00	5.50	5.00	2.00	21.00	10.00	3.00	7.50	5.50	5.00
2	5.50	5.00	4.00	5.50	5.00	4.00	16.50	18.00	8.50	13.00	10.00	9.00
3	4.50	5.00	4.50	4.00	5.00	4.50	13.75	11.00	12.50	8.50	17.50	7.50
4	3.50	3.00	4.50	3.50	3.00	4.00	9.50	7.00	8.50	31.50	9.50	5.50
5	6.00	5.50	3.00	6.00	5.50	2.50	17.00	13.00	6.50	26.50	18.50	6.50
6	5.50	4.00	4.00	5.50	4.00	4.00	20.00	8.50	10.30	46.50	10.00	20.00
7	6.50	3.00	4.00	4.00	3.00	4.00	21.50	6.00	9.50	26.00	5.50	10.00
8	6.00	4.50	1.50	6.00	4.50	1.50	19.00	11.00	3.50	43.50	22.50	3.50
9	7.00	4.50	3.00	7.00	4.50	2.50	15.50	17.00	5.00	25.00	10.00	8.00
10	4.00	5.50	3.00	4.00	5.50	2.50	11.50	16.00	4.00	26.50	14.00	4.50
Mean	5.40	4.50	3.35	5.80	4.50	3.15	16.48	11.75	7.08	25.40	12.60	7.35
F-LSD (p<0.05)	0.93			1.35			3.47			8.01		

HW, hardwood; SHW, semi-hardwood; and SW, softwood cuttings.

of fully opened opposite leaves as compared to nodal cuts of softwood.

The results in Table 1 indicated that the activation of hormones or growth regulators for root initiation at two weeks of planting in the boxes commenced simultaneously in the different physiological ages even though it was graded. Hardwood cutting had higher potential of root production by possession of more nodes with roots and eventual higher number of roots/cutting. With increased activation of growth hormones and stored carbohydrates by the third week of planting in boxes, hardwood cuttings showed higher potentials in all but one of the attributes of the cuttings measured. This may

suggest that the hardwood cutting may contain higher levels of growth hormones than others. It has been confirmed in many instances that a number of internal factors (growth hormones, carbohydrates, etc) affects the initiation of roots on stem cuttings (Steward and Krikorian, 1971; Hartmann and Kester, 1975; Stomquist and Harnsen, 1980).

The significant higher number of roots and nodes with roots and shoots in hardwood cuttings showed that it had higher potential for use in macro-propagation and that was evident in Tables 3-5 where they had significantly higher number of shoots/cutting. This implies that reasonable number of shoots (5) could be obtained

through macro-propagation from a nodal cut of hardwood after activation of the growth hormones and other internal factors in the planting boxes. Hartmann and Kester (1975) had earlier reported differential rooting and shooting of different parts of a shoot in herbaceous and woody plants due to differing concentrations of hormones and carbohydrates on the sections.

The longer growth of roots and better development of shoots in hardwood nodal cuts suggest that they had more growth hormones and reserved carbohydrates as the rooting medium for the three ages were from a single source. This implies that the internal composition of the nodal cuts contributes to the growth and development of its shoots. The earlier development of some functional opposite leaves in hardwood cuttings enhanced and sustained their shoot growth because of *photosynthate* accumulation. This was so evident in the sharp increment of shoot growth between the fourth and sixth week of planting in polyethylene bags. Remove the long sentence upto polyethylene bags. An increment of 10 cm was observed within the period in hardwood while the other two recorded about 1 cm increment and were yellow in colour.

This study has shown that macro-propagation technique could be utilized to rapidly disseminate selected landraces and new varieties of *G. latifolia* resulting from breeding programmes without considerable damage to such clone(s). Large number of clones can thus be produced from a single cutting. This is possible with five, four and three shoots/clones that could be obtained from each opposite node of hardwood, semi-hardwood and softwood, respectively. Hardwood and semi-hardwood nodal cuts thus have higher potential of

producing shoots/clones. Even though, hardwood cuttings that contain sections of semi-hardwood and soft wood gave higher number of shoots/clones, it becomes imperative that softwood section could also be utilized to save damage of such selected parent-clone.

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