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PATTERNS OF VEGETATIVE PROPAGATION OF STEM CUTTINGS OF THREE PHYSIOLOGICAL AGES OF *GONGRONEMA LATIFOLIA* BENTH OVER TWO SEASONS IN NSUKKA

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ABSTRACT

Investigations into the vegetative propagation pattern of stem cuttings of three physiological ages of Gongronema latifolia Benth were undertaken during two seasons in Nsukka. Stem cuttings of the three physiological ages (hardwood, semi-hardwood and soft wood) were taken from a clone in a forest in southeastern Nigeria. The study showed significant variability in shoot development and growth of shoots of the three physiological ages over the two seasons. Softwood stem cuttings recorded fewer days to opening of first bud and shoot initiation (9 and 12, respectively) during wet season as well as higher percentage of rooted cuttings of 75% and 56% in dry and wet seasons respectively. Similarly, hardwood and semi-hardwood cuttings showed fewer days to opening of first bud and shoot initiation in the dry season. Hardwood cuttings also had lower percentage of rooted cuttings (44%) during the wet season. However, it had longer vines in both seasons as well as higher number of shoots and leaves/cutting during the dry season. Vegetative stem cutting propagation of a selected clone of G. latifolia can thus be achieved with any of the physiological ages in both seasons at varying levels of success. Meanwhile, their propagation during the dry season when each (physiological age) gave more than seventy per cent (70%) rooted cuttings is more reliable.

Keywords: *Vegetative propagation, Physiological ages, Stem cutting, Gongronema Latifolia*

INTRODUCTION

Non-wood forest products (NWFPs) provide a safety net for the rural poor in many developing countries especially Nigeria, where these products serve as a dependable source of food security, income and medicare. In fact, poverty has led over 90% of rural dwellers in Nigeria to depend entirely on harvests of forest products for their livelihood and economic survival [UN, 2002; National Planning Commission,(NPC) 2004)].

Gongronema latifolia Benth is a non-wood forest product of West African origin (Nielsen, 1965) and known as "utazi" in southeastern Nigeria. It is used as a leafy vegetable as well as spice in soup making, and often eaten as a dessert with other preparations in southeastern Nigeria. The leaf extracts have been shown to be high in protein (62.66%) and vitamins (Okafor, 2005). The plant is equally used in the treatment of cough, worm, loss of appetite and stomach ache (Okafor, 1979). The use of the leaves in the management of diabetes mellitus, and high blood pressure is on the increase in Nigeria (Gamaniel and Akah, 1996 and Agbo *et al*, 2005).

Although the plant serves many nutritional and medicinal purposes, its availability is on the decline and in some places threatened to extinction. Osemeobo and Ujor (1999) reported that "Utazi" is one of the major NWFPs found in Nigeria, which is primarily harvested from forests and has become scarce and threatened. Therefore, in order to ensure sustainable conservation of *G. latifolia* there is need to develop a vegetative propagation method to ensure its continued availability. Vegetative propagation has the advantage of rapid dissemination of selected clones or new varieties resulting from breeding programmes, which are deemed desirable because of their quantitative and qualitative traits. It also helps to retain the heterotic nature of bred seedlings for a long time without fear of segregation because there is no further recombination process.

Stem cuttings are classified based on physiological age of the wood from which they are taken. There are hardwood, semi-hardwood and softwood cuttings (Rice *et al*., 1990 and Evans,1999). Hardwood cuttings are taken from dormant, mature stems of more than one year old. The semi-hard wood cuttings are usually prepared from partially mature wood of the current season's growth while soft wood cuttings are prepared from soft, succulent new growth of woody plants. Many internal factors have been shown to influence root initiation and shoot development in stem cuttings. Such factors are auxins, rooting co-factors, carbohydrate and nitrogen levels in the rooting stock (Hartmann and Kester, 1975). They explained further that the easily rooted cuttings have high correlation with carbohydrate level in the stems. In plants difficult to root, stem cuttings taken from young seedlings (in the juvenile growth phase) have been shown to root much more readily than those taken from older plants (Sax, 1962; Gardener, 1929). Reduced rooting potential as plants age in some species was reported to be as a result of lowering phenolic levels that act as auxin co-factors in the root or shoot initiation (Hartmann and Kester, 1975). However, in some woody plants, marked differences in the chemical composition of different parts of the shoot are known to exist from base to tip. Variations in root and shoot production on cuttings taken from different portions of the shoot are often observed, with the highest shoot formation in many cases, found among cuttings taken from the basal portions of the shoot. It has been suggested that the influence of carbohydrate and some root promoting substances from buds and leaves have made the basal portion of such shoots to be the best cutting (Hartmann and Kester, 1975). Development and growth of shoots which translates into cuttings that can be transplanted into a field were emphasized in this research because some hardwood cuttings of this species can root and stay for six months without developing shoots. Such a rooted cutting is not useful because it cannot be transplanted. This has been observed in the preliminary studies carried out with this species.

Time of year in which cuttings are taken in some instances, have dramatic influences on the results obtained in rooting cuttings. Such results could provide the key to timing of successful rooting during the year (Hartmann and Kester, 1975). A notoriously hard-to-root plant, *Chionanthus refusus* has been shown to have high rooting percentages when cuttings are taken by the middle of its growing season, (Anonymous,1942). Softwood cuttings of the deciduous woody species taken during spring have been shown to root more readily than hardwood cuttings procured in winter (Hartmann and Kester, 1975). However, the effects of timing are often merely a reflection of the response of the cuttings to existing environmental conditions at the different times of the year. For any given plant species, empirical tests are therefore required to determine the optimum time of taking cuttings which reflects physiological condition of the cuttings rather than the given calendar date.

This study was therefore conducted to ascertain the potentials of the different parts of the stem cuttings and appropriate season of the year when they should be taken. The specific objectives were:

- to determine the sprouting pattern of the three physiological ages and
- the season when the cuttings give a better shoot production percentages.

MATERIALS AND METHODS

A single source of stem cuttings was taken from a clone in a forest in southeastern Nigeria. Healthy cuttings were taken early in the morning when the plants were turgid and the cuttings were grouped based on their physiological ages. The chosen ages were hardwood (more than a year old), semi-hardwood (current season's growth but fairly mature) and softwood (tender growing stems). Dark plastic bags with wet paper towels were used to store the cuttings from the forest until they were stuck. Ten cuttings of two opposite nodes were inserted in two rows into each polyethylene bag at 4cm intervals in each row. The sawdust medium used for the study was composted for four weeks 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 volume by volume as recommended by Evans (1999). The medium was used to fill the polyethylene bags to three quarter full and laid out under 65% shading in completely randomized design replicated ten times. After inserting the cuttings, each rooting container was moistened on a regular basis until the cuttings sprouted. The first experiment was conducted in the dry season (January to March) and the repeat experiment was done in the wet season (August to October) of the same year (2005).

Data were collected from the following traits of the ten cuttings planted in each polyethylene bag

- Days to opening of first bud
- Days to shoot development
- Percentage of cuttings with shoots up to 8 weeks after planting (WAP)
- Number of shoots per cutting up to 8 WAP
- Length of vines per cutting up to 8 WAP and
- Number of opposite leaves per shoot up to 8 WAP

The data collected were subjected to analysis of variance using Genstat 5.0 release (3.2) (2003). Fisher's least significant difference (LSD) as outlined by Obi (2002) was used for mean comparisons, and t-test was used to compare the two seasons.

RESULTS

Hard wood and semi-hard wood cuttings had significantly ($P=0.05$) higher number of days to opening of first bud and shoot initiation while softwood cuttings had significantly ($P=0.05$) lower number of days to opening of first bud and shoot development (Table 1).

Days to opening of first bud and shoot development were significantly ($P=0.05$) lower during the dry season as compared to the wet season (Table 1). There was incremental progress in the percentage of shoots produced by the cuttings from two to eight weeks after planting (Table 2). Semi-hardwood cuttings had significantly ($P=0.05$) higher percentage number of shoots by the second week after planting.

Table 1: Effect of physiological age of cuttings and season on opening of first bud and shoot development

Cuttings	Days to opening of first bud	Days to shoot development
Hardwood	13.01	18.90
Semi-hardwood	10.50	13.40
Softwood	9.20	11.80
$LSD_{(p=0.05)}$	1.01	0.96
Season		
Dry	5.00	7.20
Wet	16.90	22.20
$t_{(0.05)}$	0.82	0.78

Table 2: Effect of physiological age of cuttings and season on percentage of cuttings with shoots, number of shoots/cutting, length of vines (shoots)/cutting and number of leaves/vine over the eight weeks of planting

Cuttings	Percentage of cuttings with shoot				Number of shoots /cutting				Length of vines /cutting				Number of leaves/vine			
	2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
HW	20.50	37.00	50.00	57.50	0.90	1.65	2.35	2.75	0.69	3.50	8.50	11.54	1.20	3.45	6.20	7.40
SHW	26.00	47.00	64.00	65.50	1.15	2.25	2.45	2.60	0.35	2.17	3.90	4.95	1.40	3.60	5.05	6.40
SW	22.50	63.00	69.00	72.50	1.75	2.10	2.20	2.20	0.34	2.00	2.03	3.70	1.80	3.65	4.90	5.75
$LSD_{(p=0.05)}$	-	4.91	7.64	5.92	0.31	0.40	0.40	0.40	-	-	4.33	5.26	0.39	-	0.76	0.86
Season																
Dry	35.00	74.00	74.70	74.70	1.80	2.50	2.50	2.50	0.80	4.40	9.40	11.80	2.00	5.20	7.60	8.30
Wet	11.00	24.00	47.30	55.70	0.73	1.50	2.20	2.50	0.12	0.80	1.30	2.20	0.90	1.93	3.17	4.80
$t_{(0.05)}$	5.77	4.01	6.24	4.83	0.25	0.33	0.30	-	0.42	2.18	3.53	4.30	0.32	0.57	0.62	0.70

Hardwood cuttings had the lowest (P=0.05) percentage number of shoots arising from the cuttings. Percentage shoot formation of cuttings was significantly (P=0.05) higher and more rapid during the dry season. This parameter was found to be slow and gave only 56% by the eighth week after planting.

Softwood cuttings had significantly (P=0.05) higher number of shoots per cutting by the second week after planting but could not increase reasonably and had lower number of shoots after eight weeks from planting (Table 2). Although hardwood initially had a lower percentage of cuttings producing shoots, it had higher number of shoots by the eight week. This implies that the cutting develops more shoots probably because of its expected higher carbohydrate reserve (Hartmann and Kester, 1975). Rate of shooting was rapid and abrupt during the dry season. It almost ended by the fourth week after planting while it was prolonged during wet season and extended to the eighth week after planting. Hardwood cuttings had significantly (P=0.05) longer vines than the other two groups of cuttings (Table 2). Dry season had significantly (P=0.05) longer vines due to higher growth rate of vines during the period. The different stem cuttings and seasons of the year thus had significant variations in their ability to influence shoot development. Wet season tripled the days it took the cuttings to initiate shoot development during the dry season. Softwood cuttings had significantly higher percentages of cuttings that produced shoots in both seasons (Table 2). Softwood cuttings had significantly (P=0.05) higher number of opposite leaves by the second week after planting (Table 2) because it had higher percentage of cuttings with shoots coupled with its higher number of shoots at the period. The increased shoot development coupled with higher food reserve in hardwood, which accelerated growth rate, resulted in significantly (P=0.05) higher number of leaves in the cuttings from the sixth to the eighth week after planting. The higher growth rate observed in the dry season also resulted in increased number of opposite leaves during the same period. The number of opposite leaves by dry season at eight weeks after planting was almost double the value by the wet season.

Table 3: Season x physiological age of cutting interaction on days to opening of first bud and shoot development of the cuttings.

Season	Cuttings	Days of opening of first bud	Days to shoot development
Dry	HW	3.40	5.40
	SHW	3.30	5.40
	SW	8.30	10.70
Wet	HW	22.90	32.40
	SHW	17.70	21.30
	SW	10.10	12.80
	LSD (p = 0.05)	1.43	1.35

Table 4: Season x physiological age of cutting interaction on percentage of cuttings with shoots and number of shoots/cutting over the eight weeks of planting

		Percentage of cuttings with shoots over 8 WAP				Number of shoots/cutting over 8 WAP			
Season	**Cuttings**	**2**	**4**	**6**	**8**	**2**	**4**	**6**	**8**
Dry	HW	41.00	70.00	71.00	71.00	1.80	2.90	2.90	2.90
	SHW	43.00	73.00	74.00	74.00	1.60	2.50	2.50	2.50
	SW	21.00	79.00	79.00	79.00	2.00	2.10	2.10	2.10
Wet	HW	0.00	4.00	29.00	44.00	0.00	0.40	1.80	2.60
	SHW	9.00	21.00	54.00	57.00	0.70	2.00	2.40	2.70
	SW	24.00	47.00	59.00	67.00	1.50	2.10	2.30	2.30
	LSD(p = 0.05)	10.00	6.95	10.80	8.37	0.43	0.57	-	-

Table 5: Season x physiological age of cuttings interaction on length of vine and number of opposite leaves over the eight weeks of planting

		Length of vine over the eight weeks of Planting				Number of opposite leaves over the eight weeks of planting			
Season	**Cuttings**	**2**	**4**	**6**	**8**	**2**	**4**	**6**	**8**
Dry	HW	1.39	6.39	15.98	20.43	2.40	6.40	9.80	10.90
	SHW	0.56	3.32	6.14	7.52	1.80	5.20	7.40	7.90
	SW	0.46	3.01	6.09	7.61	1.80	4.00	5.60	6.00
Wet	HW	0.00	0.25	1.04	2.64	0.00	0.50	2.60	3.90
	SHW	0.15	1.02	1.56	2.39	0.90	2.00	2.70	4.90
	SW	0.21	1.04	1.40	1.61	1.70	3.30	4.20	5.50
	$LSD_{(p=0.05)}$	-	-	6.12	7.45	0.55	0.98	1.07	1.22

The significant (P=0.05) combined effect of season by age of cutting interaction on days to opening of first bud and shoot development gave an insight to the better timing of propagation of the right physiological age (Table 3). Semi-hardwood and hardwood cuttings showed significant earliness to opening of first bud and shoot development during the dry season. Opening of first bud and subsequent shoot development during wet season took thrice the number of days by which it was accomplished during dry season. There was rapid shoot formation on the cuttings by dry season resulting in almost all the cuttings developing shoots by the fourth week of planting whereas in the wet season, there was gradual shoot development of cuttings up to the eighth week.

Even though hardwood cuttings showed earlier shoot development, the softwood and semi-hardwood had higher percentage shoot development from the second week after planting during the dry season (Table 4).

The combined interaction of season by percentage shoot formation of cuttings observed indicated that softwood and semi-hardwood cuttings would have higher percentage shoot development when propagated during the dry season. There was significant (P=0.05) season by age of cutting interaction on number of shoots per cutting from the second to the fourth week after planting.

Hardwood cuttings had significantly (P=0.05) longer vines from the sixth week after planting (Table 5).

The cuttings had a rapid vine growth during the dry season, which tripled the length of other cuttings. The combined influence of season and age of cutting on number of leaves per shoot showed that the cuttings had significantly higher number of leaves during the dry season. Hardwood and softwood cuttings had higher number of leaves during the dry and rainy seasons respectively. Higher number of leaves in hardwood would be attributed to their longer vines and higher number of shoots per cutting while softwood might have been due to its higher percentage shoot formation of cutting and shorter internodes (data not shown).

DISCUSSION

Earlier opening of first bud and shoot development in softwood in wet season when the plants are growing actively indicates greater presence of growth promoting hormone(s) at the growing points. In the other vein, during the dry season (inactive growth) period, the presence of growth hormones on the older part (hardwood and semi-hardwood) might have been due to the presence of tips of axils ready to shoot. This is consistent with the report of Sax (1962) and Gardener (1929) on the behaviour of some plants during rooting. The rapid and higher percentage (73%) shoot formation of softwood in this study suggests higher concentration and influence of root promoting substances from the tips of *G. latifolia* stem cuttings. The reduced rooting potentials in hardwood may be due to lowering phenolic levels as reported by Hartmann and Kester (1975) in some plant species as they age.

The significantly higher number of shoots in softwood above others by second week after planting could be due to earlier shoot development in the cuttings. The progressive development of shoots in hardwood resulted in non-significance number of shoots in the cuttings by sixth week and a significant effect on number of shoots in the cuttings by the eighth week. Although hardwood cuttings had lower percentage shoot formation by the cuttings, the significant higher number of shoots above others implies that they eventually developed more shoots may be because of the higher carbohydrate food reserve, which Dick (2001) suggested contributes to shoot development. The influence of season on number of shoots only caused highly increased number of shoots up to the sixth week and became same by the eighth week. This suggests earlier utilization of growth factors by cuttings during the dry season. The non-significant differences in the length of vines up to the fourth week after planting might be due to higher levels of stored food in hardwood, which has been shown by Onwuneme and Sinha (1999) to promote propagule growth. This is most probable as the other cuttings developed shoots earlier but were growing at a lower rate.

The significantly higher number of opposite leaves by the second week in softwood cuttings could be as a result of low shoot rate by hardwood during the period, which translates into low leaf number. However, number of opposite leaves was same on the stem cuttings by the fourth week, which may be because of increased rate of shoot development in hardwood (83%) without a corresponding increment in softwood (2%). The significantly higher leaf number of hardwood from the sixth week was as a result of increased shoot initiation and growth in hardwood more than other cuttings coupled with higher food reserve which accelerates growth rate. Significant earliness to opening of first bud and shoot development and higher attributes of other parameters in the cuttings during the dry season was an indication of higher prevalence of growth hormones and stored starch in the plant during the season. Higher number of leaves during the dry season would imply greater availability of photosynthates for utilization by the plant. This is indicated in the three-fold length of vines of hardwood when compared to softwood during the dry season. Meanwhile, the shorter internodes applicable to softwood vines (data not shown) would have contributed to the vines not being long especially when they had higher number of leaves. The dry season was also a resting period for the plant when it sheds its leaves and grows minimally which may imply that available growth hormones may be activated as the cutting is stuck for rooting.

The present study has shown significant variability in shoot development and growth of shoots of the three physiological ages over the two seasons. Softwood stem cuttings had lower days to opening of first bud and consequent shoot development during wet season as well as higher percentage of cuttings that produced shoots during both seasons. Similarly, hardwood cuttings showed lower days to opening of first bud and shoot growth as was applicable to semi-hardwood during the dry season. Hardwood stem cuttings also had the lowest percentage of cuttings that formed shoots during both seasons. However, the shoots had longer vines during both seasons as well as a higher number of shoots and leaves/cutting during the dry season. Vegetative stem cutting propagation of a selected clone of *Gongronema latifolia* can thus be achieved with any of the physiological ages in both seasons at varying levels of success. However, their propagation during the dry season when each (physiological age) gave more than seventy per cent (70%) of cuttings that produced shoots is more reliable

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