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Variability in Propagation Potentials of Stem Cuttings of Different Physiological Ages of *Gongronema latifolia* Benth

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Abstract: Vegetative propagation potentials of stem cuttings of different physiological ages of *Gongronema latifolia* highly valued for its nutritional and medicinal properties in South-eastern Nigeria was studied during two seasons in Nsukka. A single source of stem cuttings of the different physiological ages (hardwood, semi-hardwood and soft wood) was taken from a clone in a forest in South-eastern Nigeria. Data collected on the attributes of the stem cuttings were analyzed using a computer software, Genstat 5 release (3.2). The study showed significant variability in rooting and shoot development and growth of shoots (seedlings) of the three physiological ages over the two seasons. Softwood stem cuttings had lower days to opening of apical bud and shooting (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 lower days to opening of apical bud and shoot development in the dry season. Hardwood cuttings also had lower percentage of rooted cuttings (44%) during the wet season. However, it had longer vine 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 percent (70%) rooted cuttings is more reliable.

Key words: Vegetative propagation · Physiological ages · Stem cutting · Season

INTRODUCTION

Non-wood forest product (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 forestry products for their livelihood and economic survival [1, 2].

Gongronema latifolia Benth is a non-wood forest product of West African origin [3] and known as "utazi" in South-eastern Nigeria. It is used as a leafy vegetable and spice in soup making and often eaten as a dessert with other preparations in South-eastern Nigeria. The leaf extracts have been shown to be high in protein (62.66%) and vitamins [4]. The plant is equally used in the treatment of cough, worm, loss of appetite and stomach ache [4]. The use of the plant leaves in the management of diabetes mellitus, and high blood pressure is on the increase in Nigeria [5, 6].

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 [7] 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 (vegetative plants) are classified based on physiological age of the wood from which they are taken. There are hardwood, semi-hardwood and soft wood cuttings [8, 9]. Hardwood cuttings are taken from dormant,

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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 [10]. 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 seedling plant (in the juvenile growth phase) have been shown to root much more readily than those taken from older plants [11, 12]. Reduced rooting potential as plants age in some species was reported to be caused as a result of lowering phenolic level that act as auxin co-factors in the root or shoot initiation [10]. 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 shooting in many cases, found in 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 [10].

Time of year in which cuttings are taken in some instances, have dramatic influences on the results obtained in rooting cuttings and could provide the key to timing of successful rooting during the year [10]. A notoriously hard to root plant, *Chionanthus refusus* have been shown to have high rooting percentages when cuttings are taken by the middle of its growing season Anonymous [13]. Softwood cuttings of the deciduous woody species taken during spring have been shown to root more readily then hardwood cuttings procured in winter [10]. However, the effects of timing is often merely a reflection of the response of the cuttings to existing environmental condition 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 cutting and appropriate season of the year when the cuttings should be taken. The specific objectives were:

- to determine the shooting potentials of the three physiological ages and
- the season when the cuttings give a better shooting percentages.

MATERIALS AND METHODS

A single source of stem cuttings were taken from a clone in a forest in South-eastern Nigeria. Healthy cuttings were taken early in the morning when the plants are 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 matured) and softwood (tender growing stems). Dark plastic bags with wet paper towels were used to store the cuttings from fresh until they were stuck. Ten cuttings of two opposite nodes were inserted in two rows into each polyethylene bag at 4 cm 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 [9]. 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 watered and misted on regular basis until cuttings shoot. The first experiment was conducted in dry season (January to March) and the repeat experiment was done in wet season (August to October). Both were done in 2004.

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

- Days to first opening of apical bud
- Days to first 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 vine 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) [14].

RESULTS

Hard wood and semi-hard wood cuttings had significantly /9p=0.05) lower number of days to opening of apical bud and shoot development during the dry season while softwood cuttings had significant (p=0.05) lower number of days to opening of apical bud and shoot

Table 1: Main effect of days to opening of apical bud and shoot development of planted cuttings

	Days to opening of apical bud		Days to shoot development	
	Dry	Wet	Dry	Wet
HW	3.40	22.90	5.40	32.40
SHW	3.30	17.70	5.40	21.30
SW	8.30	10.10	10.70	12.80
L.S.D.	2.30	7.80	2.50	8.02

Table 2: Percentage of cuttings with shoots and number of shoots/cutting over eight weeks of planting in both seasons

	Percentage of cuttings with shoot								Number of shoots/cuttings							
	Dry				Wet				Dry				Wet			
	2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
HW	41.0	70.0	71.0	71.0	0.0	4.0	29.0	44.0	1.8	2.9	2.9	2.9	0.0	0.4	1.8	2.6
SHW	43.0	73.0	74.0	74.0	9.0	21.0	54.0	57.0	1.6	2.5	2.5	2.5	0.7	2.0	2.4	2.7
SW	21.0	79.0	79.0	79.0	24.0	47.0	59.0	37.0	2.0	2.1	2.1	2.1	1.5	2.1	2.3	2.3
L.S.D.	6.80	4.50	38.00	3.80	12.00	10.20	10.00	7.40	0.32	0.40	0.40	0.40	0.41	0.62	0.5	-

Table 3: Length of vines (shoots) and number of opposite leaves/vines over eight weeks of planting in both seasons

	Length of vine								Number of leaves/vine							
	Dry				Wet				Dry				Wet			
	2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
HW	1.39	6.75	15.98	20.43	0.0	0.25	1.04	2.64	2.40	6.40	9.80	10.90	0.00	0.50	2.60	3.90
SHW	0.56	3.32	6.14	7.52	0.15	1.02	1.56	2.39	1.80	5.20	7.40	7.90	0.90	2.00	2.70	4.90
SW	0.46	3.01	6.09	7.61	0.21	1.04	1.40	1.61	1.80	4.00	5.60	6.00	1.70	3.30	4.20	5.50
L.S.D.	0.62	2.42	5.46	8.63	0.18	0.35	-	0.23	0.29	0.74	0.92	2.40	0.48	0.86	1.53	1.86

development during wet season (Table 1). Software cuttings had significant higher percentages of rooted cuttings in both seasons (Table 2). These was rapid rooting of the cuttings by dry season resulting in almost all the cuttings rooting by fourth week of planting. Whereas in the wet season, there was gradual rooting of cuttings up to the eight week. Hardwood cuttings head significantly lower vines in both season (Table 3), there was longer vines in dry season as compared to wet season in all the cuttings. In dry season, hardwood cuttings had higher number of leaves while softwood cuttings had significantly higher number during wet season. Days to opening of apical bud and shoot development was significantly (p=0.05) lower during the dry season as compared to the wet season. The different stem cuttings and seasons of the year thus had significant variations in their ability to initiate shoot development. Wet season tripled the days it took the cuttings to initiate shoot development during the dry season.

Table 4: Effect of physiological age of cuttings and season on opening of apical bud and shoot development

Cuttings	Days to opening of apical bud	Days to shoot development
Hardwood	13.01	18.90
Semi-hardwood	10.50	13.40
Softwood	9.20	11.80
L.S.D. (p=0.05)	1.01	0.96
Season		
Dry	5.00	7.20
Wet	16.90	22.20
L.S.D. (p=0.05)	0.82	0.78

There was incremental progress in the percentage shooting of the cuttings from second to eight weeks after planting (Table 5). Semi-hardwood cuttings had a significant (p=0.05) higher percentage shooting by the second week after planting. Hardwood cuttings had significantly (p=0.05) lowest percentage shooting of cuttings while softwood cutting had significant highest

Table 5: Effect of physiological ages of cutting and season on percentage of cuttings with shoots, number of shots/cutting, vine (shoot)/cutting and number of leaves/vine over the eight weeks of planting

	Percentages of cutting with shoot				Number of shoots/cutting				Length of vine/cuttings				Number of leaves/vine			
Cuttings	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
L.S.D. (p=0.05)	-	4.91	7.64	5.92	0.31	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
L.S.D. (p=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

Table 6: Season x physiological age of cutting interaction on day to opening of apical bud and shoot development of the cuttings

Season	Cuttings	Days of opening	Days to shoot
Dry	HW	3.40	5.40
	SHW	3.30	5.40
	SW	8.30	10.70
	Mean	5.00	7.20
Wet	HW	22.90	32.40
	SHW	17.70	21.30
	SW	10.10	12.80
	Mean	16.90	22.20
	L.S.D. (p=0.05)	1.43	1.35

percentage of shooting of cuttings than the other two. Percentage shooting of cuttings was significantly (p=0.05) higher and more rapid during the dry season. Percentage shooting of cuttings by wet season was slow and gave only 56% by the eight week after planting.

Softwood cuttings had significantly (p=0.05) higher number of shoots by the second week after planting but could not increase reasonably and lower number of shoots by the eight weeks after planting (Tables 3 & 5) Even though, hardwood had lower percentage shooting of cuttings (Table 5), it had higher number of shoots by the eight week. This implies that the cutting develops more shoots probably because of its higher carbohydrate reserve [10]. 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 eight week after planting. Hardwood cuttings had significantly (p=0.05) longer vines than the other two cuttings (Table 5). Dry season had significantly (p=0.05) longer vines due to its higher growth rate.

Softwood cuttings had significantly (p=0.05) higher number of opposite leaves by the second week after planting (Table 5) because it had higher percentage of cuttings with shoots (Table 2) coupled with its higher number of shoots at the period. The increased shoot development coupled with higher food reserve in hardwood which accelerate growth rate resulted in significant (p=0.05) higher number of leaves in the cutting from the sixth to eight weeks after planting the higher growth rate observed in the dry season also resulted in increased number of opposite leaves in the same period. The number of opposite leaves by dry season at eight weeks after planting was almost double of the value by the wet season.

The significant (p=0.05) combined effect of season by cutting interaction on days to opening of apical bud and shoot development gave an insight of the better timing of propagation of the rightly physiological age (Table 6). Semi-hardwood and hardwood cuttings showed significant earliness to opening of apical bud and shoot development during the dry season. Opening of apical bud and subsequent shoot development during wet season took thrice the number of days by which it was accomplished during dry season.

Even though, hardwood cuttings, showed earlier shoot development, the softwood and semi-hardwood had higher percentage shooting from the second week after planting during the dry season (Table 7). The combined interaction of season by percentage shooting of cuttings observed indicated that soft and semi-hardwood cuttings would have higher percentage shooting when propagated during the dry season. There was significant (p=0.05) season by cutting interaction on

Table 7: 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 shoot/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
	Mean	35.00	74.00	74.70	74.70	1.80	2.50	2.50	2.50
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
	Mean	11.00	24.00	47.30	56.00	0.73	1.50	2.10	2.53
	L.S.D. (p=0.05)	10.00	6.95	10.80	8.37	0.43	0.57	-	-

Table 8: 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				No. of opposite leaves over the eight weeks of plating			
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
	Mean	0.80	4.36	9.37	11.85	2.00	5.20	7.60	8.30
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
	Mean	0.12	0.77	1.33	2.21	0.89	1.93	3.20	4.77
	L.S.D. (p=0.05)	-	-	6.12	7.45	0.55	0.98	1.07	1.22

number of shoots per cutting from the second to fourth week after planting.

Hardwood cuttings had significantly (p=0.05) longer vines from the sixth week after planting (Table 8). The cutting had a rapid vine growth during the dry season which tripled the length of other cuttings. The combined influence of season and 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 cuttings while softwood might have been due to its higher percentage shooting of cutting and shorter internodes (data not shown).

DISCUSSION

Earlier opening of apical bud and shoot development in softwood in wet season when the plants are growing (active) indicates greater presence of growth promoting hormone(s) at the growing points. In the other vein, during the dry season (inactive) period, the presence of growth hormones on the older part (hardwood and semi-hardwood) may be because of presence of tips of axils ready to shoot agrees with the report of Sax [11] and Gardener [12] on behaviour of same plants that are difficult to root. The rapid and higher percentage (73%) shooting of softwood in this study suggests higher concentration and influence of root promoting substances from the tips of *G. latifolia s*tem cutting. The reduced rooting potentials in hardwood may be due to lowering phenolic levels as reported by Hartmann and Kester [10] in some plant species.

The significant higher number of shoots in softwood above others by second week after planting could be due to earlier shoot development in the cutting. The progressive development of shoots in hardwood resulted in non-significance in number of shoots in the cuttings by sixth weeks and a significant effect on number of shoots in the cuttings by the eight week. Even though hardwood cuttings had lower percentage shooting of cuttings, the significant higher number of shoots above others implies that it eventually developed more shoots may be because of the expected higher carbohydrate food reserve which Jan [15] increased number of shoots up to the sixth weeks and became same by eight week which 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 may be due to higher levels of stored food in hardwood which has been shown by Onwuneme and Sinha [16] to promote propagule growth. This is most probable as the other cuttings developed shoots earlier but were growing at a slower rate.

The significantly higher number of opposite leaves by the second week could be as a result of low shoot rate by hardwood by the period which translates into low leaf number. However, number of opposite leaves was same on the stem cuttings by the forth week which may be because of increased rate of shoot development in hardwood (83%) without a corresponding increment in softwood (2%). The significant higher leaf number of hardwood from sixth week was a result of increased shoot initiation and growth in hardwood more than other cuttings coupled with higher food reserve which accelerates growth rates.

Significant earliness to opening of apical 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 dry season implies more photosynthesis and more photosynthate will be produced 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) contributed to it's vines not being long especially when it had higher number of leaves. The dry season was also resting period for the plant when it sheds it's leaves and does not grow which may imply that available growth hormones may be activated as the cutting is stuck for rooting.

The study has been significant variability on rooting and shoot development and growth of shoots (seedlings) of the three physiological ages over the two seasons. Softwood stem cuttings had lower days to opening of apical bud and shooting during wet season as well as higher percentage of rooted cuttings in both seasons. Similarly, hardwood cuttings showed lower days to opening of apical bud and shooting as was applicable to semi-hardwood during the dry season. Hardwood stem cuttings also had lowest percentage of rooted cuttings in both seasons. However, it had longer cines in both seasons as well as higher number of shorts and leaves/cutting during the dry season. Vegetative stem cuttings 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. Although, their propagation during the dry season when each (Physiological age) gave more than seventy percent (70%) rooted cuttings is more reliable.

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