

SHOOT DEVELOPMENT OF THREE PHYSIOLOGICAL AGES OF *GONGRONEMA LATIFOLIA* AS INFLUENCED BY SOME OF THEIR CHEMICAL COMPOSITION

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ABSTRACT

Two experiments (one in the farm the other in the laboratory) were conducted at the University of Nigeria, Nsukka, between February and April 2006 to determine the shooting potentials of three physiological ages (hardwood, semi-hardwood and softwood) of the stem cuttings of *G. latifolia* in relation to their chemical composition. The results showed that opening of first buds and first shooting occurred in softwood cuttings in 3 and 6 days, respectively. Softwood recorded the least days to buds and shoots initiation than either hardwood or semi-hardwood. Softwood cuttings on the average had 61% shooting of cuttings by the second week of planting and this was significantly ($p < 0.05$) higher than values of semi-hardwood (21%) and hardwood (41%) during the study period. However, hardwood cuttings had vine lengths of 2.90cm by the second week of planting compared with 1.06cm recorded for softwood. Equally, softwood cuttings had 16.14 % protein, which was significantly ($p < 0.05$) higher than either semi-hardwood or hardwood throughout the study period. The higher level of protein in the softwood and significant positive correlation between softwood and percentage shooting ($r = 0.43$; $n = 24$) at two weeks after planting support earlier development of shoot and opening of leaves in softwood cuttings. On the other hand, low carbohydrate level in the softwood cuttings could not sustain the initial rate of shoot development and growth. Hardwood cuttings eventually had significantly ($p < 0.05$) higher shooting rate at the end of eight weeks. The positive and significant correlation ($r = 0.67$; $n = 24$) between carbohydrate level of parent cuttings and vine length suggest that carbohydrate has high influence on growth of vines and leaves. Also, the significant longer vines observed in the hardwood cuttings from second week after planting even though softwood cuttings started earlier showed the importance of carbohydrate level on growth of vines. The three physiological age groups of cuttings used in this study had showed varying levels of chemical composition. The internal chemical properties of the cuttings also had varying degrees of influence on the development of vines/shoots as well as growth potentials.

KEYWORDS: Shoot development, Chemical composition, Physiological ages and Stem cuttings

INTRODUCTION

Gongronema latifolia Benth, a non-wood forest product (NWFP) is a leafy vegetable harvested from forests in Southeastern states of Nigeria and some other parts of sub-Saharan Africa (Akpan, 2004 and Okafor, 2005). The forest vegetable is of West African origin (Nielsen, 1965) and called *Utazi* in Southeastern Nigeria. It is a common forest climber with hollow stems and broadly ovate elliptical leaves with a cordate base and belongs to asclepiadaceae family. The leaf extracts of the plant are high in protein (62.7%) (Okafor, 2005), vitamins and iron. The plant is equally used in the treatment of loss of appetite, cough, worm and stomach-ache (Okafor, 1979). It has been reported to be very useful in the management of diabetes mellitus (Gamaniel and Akah, 1996; Agbo *et al.*, 2005). Gamaniel and Akah (1996) showed that stem extracts of the plant have five bioactive compounds namely: alkaloids, saponins, tannins, flavonoides and glycosides. They suggested that the various bioactive compounds could make the plant have varied pharmacological effects.

There has not been adequate conservation of non-wood forest species in Nigeria and other developing countries regardless of the immeasurable benefits derivable from them in food, medicine and industrial raw

material provision. This attitude has led to extinction of some important species. UN (2002) reported that Nigeria has lost a great percentage of her luxurious vegetation and some species have gone extinct. *G. latifolia* has not been substantially domesticated. The species has become scarce and threatened in many places in Nigeria. Osemeobo and Ujor (1999) reported that *G. latifolia* is one of the major NWFPs found in Nigeria, which is mainly harvested from forest and has become scarce and threatened. Conservation of non-wood forest products can be *in-situ* or *ex-situ*. *In-situ* aims at conserving plant material and animal resources in their natural habitat while *ex-situ* conservation are essential for plant breeding and protection of population in danger of physical destruction (Reis, 1995). Laird (2002) described *ex-situ* conservation of biological resources as domestication, which Reis (1995) considered a means of ensuring economic success through sustained availability.

Propagation by stem cuttings is the most commonly used method to propagate many woody and herbaceous plants (Evans, 1999). Stem cuttings are classified based on the 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. 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.

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 further showed that percentage of rooted cuttings have high correlation with carbohydrate level in 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 and Gardener, 1929). Reducing rooting potential as plants age in some species was reported to be caused by lowering of phenolic content that act as auxin co-factors in 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 or 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 the best cutting (Hartmann and Kester, 1975).

Significance differences in rooting and shooting potentials of different physiological ages of stem cuttings of woody and herbaceous plant species have been reported (Steward and Krikorian, 1971; Hartman and Kester, 1975; Stomquist and Harnsen, 1980; Agbo and Obi, 2006). In most of the reports, the differences in rooting potentials of cutting from base to tip were attributed to differences in the composition of rooting hormones, phenols, carbohydrates and protein contents of the cuttings. There is paucity of literature on chemical composition of the different physiological ages of stem cuttings of *Gongronema latifolia*. The study was conceived to quantify some rooting and growth factors of stem cuttings of the different physiological ages of *G. latifolia*. The specific objective was to determine the shooting potentials of the three physiological ages of the stem cuttings in relation to their chemical composition.

MATERIALS AND METHODS

The two experiments were conducted in the research farm and laboratory of the Faculty of Agriculture, University of Nigeria, Nsukka to determine the shooting potentials of three physiological ages of *G. latifolia* in relation to their chemical composition. Nsukka is in the derived savanna agro-ecology. It is located on latitude 06°52'N, longitude 07°24'E and 447m altitudes. Rainfall is bimodal with an annual total of about 1500mm. Rainy season starts in March and stabilizes in May and ends in October while dry season is experienced between November and March. The experiment on shoot development was done in a nursery shade in the research farm, protected with wire netting against rodents. Weeding was frequently done and the surroundings were cleared against neighbour effect. Bamboo poles were used to erect the nursery structure and dried palm fronds were used to shade the thatched roof to 65% level.

Treatments Used and Layout of Experiments

Three physiological ages of *G. latifolia* used were hardwood (a year and six months old), semi-hardwood (current season's growth but fairly mature) and softwood (tender growing stems) cuttings, constituted the treatments. The two experiments were performed simultaneously and were done between 20 February to 22 April, 2006. The dry period was chosen because of reported significant higher shoot development in *G. latifolia* during dry season as compared to rainy season (Agbo and Obi, 2006).

The medium of rooting was sawdust as it has been reported by Agbo and Omaliko (2006) to be a better rooting medium for the stem cuttings of *G. latifolia*. The sawdust medium was mixed with poultry manure at the ratio of 1.00:0.25 of sawdust to poultry manure volume-by-volume and composted for four weeks. The composted medium was 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. Thirty polyethylene bags filled with the rooting medium were placed on three rows for the three treatments. Each row comprising ten polyethylene bags which served as the replicates. The polyethylene bags were arranged in a complete randomized design (CRD) and were spaced 1m x 1m. The plot size for each treatment measured 1m² and each plot served as the sampling area because each stem cutting has the potential to shoot or not. A single source of stem cuttings was taken from a clone in germplasm conservation field of Department of Crop Science, University of Nigeria, Nsukka. Healthy cuttings were taken early in the morning when the plants are turgid and the cuttings were grouped based on their physiological ages. Ten cuttings of two opposite nodes of the physiological ages were inserted in two rows in each polyethylene bag. The cuttings were spaced at 4cm intervals in each row. After inserting the cuttings, each rooting container was watered on regular basis until cuttings shoot.

Sample Preparation for Laboratory Analysis

Two days after the stem cuttings for shoot development were taken; stem cuttings of the three physiological ages were taken for determination of their chemical compositions. Fresh stem cuttings of hardwood, semi-hardwood and softwood (physiological ages) were taken from the field to the laboratory. Forty stem cuttings of two nodes each of every physiological age were grouped into eight places. The eight groups of each physiological age served as replicates. The stem cuttings were oven dried at about 70° C until permanent dry weights were obtained. Dry samples were ground to pass through one-millimeter mesh sieve using Thomas Wiley laboratory Mill (model 4). The proximate (protein, oil, ash, fibre, water and carbohydrate) composition and level of phenolics (phytate and tannin) of the physiological ages were determined following the official methods of AOAC (1980). The percent dry matter was determined as fresh weight of samples minus oven dry weight divided by fresh weight and expressed in percentages.

Data Collection and Statistical Analysis

In the shoot development experiment, data were collected from the following traits of the ten cuttings in the ten replicates for each treatment. The traits included: days to opening of first bud, days to first shooting, days to full opening of leaves, percentage of cuttings that shoot at 2, 4, 6 and 8 weeks after planting (WAP), number of shoots per cutting at 2, 4, 6 and 8 WAP, number of opposite leaves on a vine at 2, 4, 6 and 8 WAP and length of vine per cutting at 2, 4, 6 and 8 WAP. Data were also collected on the proximate and phenolic compositions of the ground stem cuttings of the 8 replicates for each physiological age in the laboratory. Data collected on the two experiments were subjected to analysis of variance following the procedures outlined for completely randomized design (CRD). Fisher's least significant difference was used to separate the means as outlined by Obi (2002). Also multiple correlation tests was conducted on the two sets of data to establish the relationship between shoot development parameters and the chemical composition of the different physiological ages. Data were analysed with GENSTAT 5.0 Release (3.2) (GENSTAT, 2003).

showed that there were no significant differences in days to opening of first bud among the three physiological ages of the cuttings. Semi-hardwood cuttings however, had significantly higher number of days to first shooting and opening of leaves than either softwood or hardwood. Softwood cuttings had lower days to shooting and opening of leaves than either semi-hardwood or hardwood. There was a progressive increment in shooting two weeks after planting (WAP) to eight weeks after planting (Table 2). Semi-hardwood had lower rate of percentage shooting than either softwood or hardwood throughout the period of study. Softwood cuttings had higher percentage shooting than either semi-hardwood or hardwood up to the sixth week of planting. Hardwood cuttings showed significantly ($p < 0.05$) higher number of shoots per cutting by the eight week of planting than either softwood or semi-hardwood. The result in Table 3 showed that hardwood cuttings had significantly ($p < 0.05$) longer vine length from second to eight weeks of planting than either softwood or semi-hardwood. The softwood cuttings had significantly lower vine length and number of opposite leaves throughout the eight weeks.

RESULTS

Shoot Development

The results of shoot development as influenced by the treatments are presented in Table 1. The results

Table 1: Influence of physiological age of stem cuttings to days to opening of first bud, shooting and opening of leaves

Physiological ages	Days to opening of first bud	Days to first shooting	Days to first full opening of leaves
HW	4.38	6.75	9.13
SHW	5.50	9.38	12.00
SW	4.32	6.00	8.63
LSD _(P=0.05)	-	2.45	2.65

Where HW = Hardwood, SHW = Semi-hardwood and SW = Softwood

Table 2: Percentage of cuttings with shoots and number of shoots per cutting of the three physiological ages of stem cuttings over eight weeks after planting (WAP)

Physiological ages	Percentage of cuttings with shoots				Number of shoots/cutting			
	2	4	6	8 (WAP)	2	4	6	8 (WAP)
HW	41.25	52.50	60.00	69.50	.46	1.57	1.76	2.19
SHW	21.25	48.50	58.25	64.50	1.45	1.62	1.63	1.63
SW	61.25	65.00	66.25	66.25	1.41	1.51	1.65	1.65
LSD _(p = 0.05)	20.87	10.00	6.52	3.17	-	-	-	0.48

Where HW = Hardwood, SHW = Semi-hardwood and SW = Softwood

Table 3: Length of vine (shoots) and number of opposite leaves per vine of the three physiological ages of stem cuttings over eight weeks after planting (WAP)

Physiological ages	Length of vine (cm)				Number of opposite leaves/vine			
	2	4	6	8 (WAP)	2	4	6	8 (WAP)
HW	2.85	10.16	27.38	50.21	1.53	2.21	2.98	3.82
SHW	1.00	4.00	5.63	8.16	0.37	1.88	2.47	2.93
SW	1.06	2.80	3.90	5.34	0.61	1.35	2.17	2.51
LSD _(p = 0.05)	0.66	3.77	0.77	13.92	0.73	-	-	0.76

Where HW = Hardwood, SHW = Semi-hardwood and SW = Softwood

Chemical Compositions of the Cuttings

Hardwood cuttings had significantly higher percentage ash, fibre, carbohydrate and dry matter (Table 4) but, had low levels of oil, water, protein, phenol and tannin contents. On the other hand, softwood cuttings had significantly higher percentage oil, water, protein, phenol and tannin.

Days to opening of first bud, first shooting and opening of leaves negatively correlated with water and protein levels of the cuttings (Table 5). Conversely, they are positively related to other constituent of the cuttings with the exception of oil. Days to opening of first bud were positively and significantly correlated to days to first shooting and opening of leaves. Vine length at eight weeks after planting was positively and significantly related with number of opposite leaves on vine, ash, fiber, dry matter and carbohydrate levels of the stem cuttings. On the other hand, vine length at eight weeks after planting was negatively and significantly correlated

with oil, water, protein, phenol and tannin levels of the cuttings. Percentage of shooting of cuttings at two weeks after planting was negatively and significantly related to dry matter and carbohydrate but positively correlated with water and protein levels of the cuttings. However, at eight weeks after planting, percentage shooting of cuttings became positively related to ash, dry matter, protein and carbohydrate levels of the cuttings. Ash level of the cuttings was positively and significantly correlated with fiber, dry matter and carbohydrate contents of the cuttings. Oil had significant and negative relation to dry matter and carbohydrate levels of the cuttings. Meanwhile, dry matter had positive and significant correlation with carbohydrate. Similarly, water was positively and strongly related to protein, phenol and tannin and negatively related to carbohydrate level in the cuttings. The strongest relationship of 0.99 occurred between phenol and tannin.

Table 4: Chemical compositions of the three physiological ages of stem cuttings

Physiological ages	Proximate analysis (%)					Phenolics (mg/100g)			
	Ash	Fibre	Oil	Carbohydrate	Water	Protein	Phenol	Tannin	Dry (%) matter
HW	3.63	3.19	2.03	27.22	52.18	12.05	6.49	14.89	28.19
SHW	3.12	2.95	3.03	22.99	54.61	12.93	17.37	27.15	25.83
SW	1.75	2.19	3.57	14.41	61.93	16.14	19.13	21.11	18.00
LSD _(p = 0.05)	0.65	0.22	0.21	1.33	0.79	1.29	0.27	0.66	0.94

Where HW = Hardwood, SHW = Semi-hardwood and SW = Softwood

DISCUSSION

The significant differences in the chemical compositions of the three different physiological ages of the cuttings were evident in their performance in the nursery. Hardwood and softwood were similar with respect to first shooting and full opening of leaves. This indicated earlier starting of independent life of the vine(s) as the opened leaves will manufacture its own food through photosynthesis. However, the softwood cuttings showed some limitations in the use of opened leaves for photosynthesis as its vine length did not grow as fast as that of semi-hardwood or hardwood that it shoot and opened leaves earlier on. This could be attributed to the carbohydrate and dry matter content of the softwood that was low when compared to hardwood and semi-hardwood. The positive and significant correlation between vine length and carbohydrate

validates the importance of carbohydrate level of the stem cutting to the performance of the vine in the nursery as earlier reported by Hartmann and Kester (1975). In another vein, softwood had higher water, protein, phenol and tannin contents. The negative correlation between protein level and days to first shooting and opening of leaves showed that higher level of protein and phenol in softwood caused reduced number of days to first shooting and opening of leaves. Lowering levels of phenolic compounds have been suggested by researchers to reduce rooting potentials in older plants (Hartmann and Kester, 1975). The significant higher percentage shooting of cuttings of softwood at early stage of planting of cutting typifies the contributions of protein and phenol in early shooting of the cuttings. The positive and significant correlation between percentage shooting at two weeks after planting and protein support the contributions of protein

in earlier shooting in softwood cuttings. This is most likely as percentage shooting at two weeks was positively and significantly related to days to opening of first bud and shooting. However, sustained shooting is most applicable with high concentration of carbohydrate. This is evident with significant higher percentage shooting of the hardwood cutting at eight weeks after planting when the little reserve in softwood cuttings would have been used up. This is in agreement with the report of Hartmann and Kester (1975) on the high correlation between carbohydrate level and percentage of rooted cuttings.

The significant longer vines from hardwood cuttings from second week after planting further indicated the influence of carbohydrate reserve in the cuttings on the subsequent growth of the emerging vines. The highly significant correlation of 0.67 between carbohydrate and vine length at eight weeks after planting validated the suggestion of high influence of carbohydrate on growth of new vines/shoots. The report of high influence of carbohydrate level and other growth factors of parent stem cuttings on the growth of emerging vine(s) have been reported by Agbo and Obi (2006); Stomquist and Harnsen (1980); Hartmann and Kester (1975). The negative high correlation between vine length and phenol, tannin, protein, water and oil contents of parent cutting which are significantly higher in softwood cuttings supports the low growth observed in vines from softwood cuttings. Low growth rate in softwood of *G. latifolia* regardless of earlier shootings of vines in them has been reported by Agbo and Obi (2006). Number of shoots/vines per cutting had the highest positive correlation of 0.32 with vine length. This indicates that components that promote growth of vines also promotes emergence of more shoots on the cuttings. Number of

opposite leaves of the vines had a positive significant correlation of 0.71 with vine length and it shows that number of leaves on the vine is dependent on vine length. This further implies that carbohydrate level in the parent cutting enhances more production of opposite leaves. Parent cuttings high in water contents, which had positive and significant correlation with protein, tannin and phenol, started shooting of vine earlier. This is applicable to softwood cuttings that had significantly higher water, protein, tannin and phenol and also had higher early development of vines/shoots as well as percentage shooting of cuttings.

This study showed that softwood cutting had lower days to opening of first buds, development of shoots/vines and opening of leaves due to higher concentration of protein and phenols in the cuttings. The higher level of protein in the softwood also caused higher percentage of shooting of vines at the early stage of establishment. However, low carbohydrate level in the softwood cuttings could not sustain the initial rate and hardwood cuttings eventually had significant higher shooting rate at the end of eight weeks. The positive and significant correlation ($r=0.67:n=24$) between carbohydrate level of parent cuttings and vine length showed that carbohydrate has high influence on growth of vines as well as development of leaves. This was shown by the significant longer vines observed in the hardwood cuttings from second week after planting even though softwood cutting started earlier. The three physiological age groups of cuttings used in this study had significant varying levels of chemical compositions. The internal chemical properties of the cuttings also had varying degrees of influence on the development of vines/shoots as well as their growth.

Table 5: Correlation matrix of the traits of the cuttings

	Dofb	Dts	Deol	Pcs 2	Pcs 8	VI8	Spc 8	Olpc 8	Ash	Fiber	Oil	Dm	Water	Protein	Phenol	Tannin	CH ₂ O
Dofb	-	0.77**	0.59**	0.74**	-0.37	0.016	-0.18	0.19	0.01	0.05	-0.03	0.12	-0.16	-0.05	0.05	0.12	0.12
Dts		-	0.67**	0.65*	-0.43*	-0.06	-0.48*	0.08	0.17	0.23	0.02	0.23	-0.28	-0.24	0.09	0.16	0.22
Deol			-	-	0.44*	-0.10	-0.04	0.18	0.10	-0.18	0.05	0.17	-0.21	-0.07	0.14	0.24	0.16
Pcs 2				-	0.87**	0.02	0.05	-0.01	-0.35	-0.37	0.30	-0.47*	0.46*	0.43*	0.08	-0.01	-0.42*
Pcs 8					-	0.21	0.08	0.11	0.23	0.24	-0.02	0.28	0.16	0.29	-0.26	-0.33	0.43*
VI8						-	0.32	0.71**	0.43*	0.62**	-	0.59**	-0.62**	-0.47**	-0.85**	-0.85**	0.67*
Spc 8							-	0.24	0.15	-0.06	-0.33	0.04	-0.01	0.08	-0.35	-0.35	0.05
Olpc 8								-	0.27	0.54*	-	0.53*	-0.55**	-0.27	-0.61**	-0.56**	0.57**
Ash									-	0.62**	-	0.79**	-0.79**	-0.79**	-0.64**	-0.56**	0.76**
Fiber										-	-	0.90**	-0.91**	-0.72**	-0.70**	-0.63**	0.89**
Oil											-	-	0.85**	0.68**	0.93**	0.90**	-0.86**
Dm												-	0.83**	-0.98**	-0.83**	-0.75**	-0.66**
Water													-	0.81**	0.77**	0.68**	-0.97**
Protein														-	0.64**	0.57**	-0.88**
Phenol															-	0.99**	-0.81**
Tannin																-	-0.73**
CH ₂ O																	-

*Sig at 5%

**Sig at 1%

Where Dofb, DTS, Deol, Pcs 2, Pcs 8, VI8, Spc 8, Olpc 8, DM and CH₂O means: Days to opening of first bud, days to first shooting, days to opening of leaves, percentage shooting of cuttings at 2 weeks after planting (WAP), percentage shooting of cuttings at 8 WAP, vine length at 8 WAP, number of shoot/vines per cutting at 8 WAP, number of opposite leaves at 8 WAP, dry matter and carbohydrate, respectively

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