

Production of mini-tubers from vine cuttings of white yam (*Dioscorea rotundata*)

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Abstract

The multiplication ratio for seed yam production is very low compared to other tuberous crops. Seven clones of *Dioscorea rotundata* Poir (white yam) were evaluated for production of mini tubers from their vine cuttings. Three to four nodes leafy vine cuttings were prepared from the middle portion of the lateral branches collected from mother plants 127, 134 and 141 days after planting (DAP). The lower portion of these nodes were wounded with a clean razor blade and then dusted with 1.0% Indole-3 butyric acid (IBA) powder in order to promote rooting. The mini tubers were harvested 115 DAP. The developed mini tubers varied in sizes among the tested cultivars from 1.9 to 4.2g. The weights were found to be genotype dependent. The survival rate of the planted vine cuttings ranged from 31.1 to 77.1% while the average total number of roots per vine ranged from 5.1 to 5.8. The average number of tubers per vine was 1.8 ± 0.8 . If these number and weights of mini tubers can be obtained from propagation of vine cuttings, there will be tremendous increase in propagating material thereby making yam cultivation less expensive and also allowing the ware yam only for consumption and other uses.

Key words: *D. alata*, *D. rotundata* Poir, cultivars, IBA, mini tuber, root formation, vine cuttings, survival, white yams.

Introduction

Yam thrives successfully from sea level up to 900m. A well-distributed rainfall (or water supply) of 1,500mm per annum is adequate for crop production and plants go dormant during period of extended drought. Short days between 10-11 hours promote tuber formation, while long days (greater than 12 hours) promote vine growth. Compared to other tropical tuber crops, yam requires deep, permeable soil of high fertility. Loose loamy soils are the best as heavy clays tend to be water logged and result in tuber rots and difficult harvesting. Gravel or rock soil tends to hinder tuber formation (O'Hair, 1984).

Limited availability and cost of planting material is a major constraint for yam production in Africa. Planting material account for about 50% of the cost of the production. Large amounts of material (about 10,000 seed yams) are needed to plant 1 hectare. If farmers do not buy new seed yams, they must set aside 30% of their harvest for the next year planting. In addition, seed yams are bulky and perish quickly.

Dioscorea rotundata (white yam) and *D. alata* (water yam) are two of the major food crops grown by small-scale farmers that provide a stable carbohydrate for a population in the humid/sub-humid tropics (Oyetunji *et al.*, 2003). It is an ancient crop in central West Africa (Coursey, 1967; Degras, 1993) and provides a promising avenue for alleviating the current food crises.

Despite this great potential, limited research has been carried out on agronomic techniques in the recent past to improve yam cultivation in sub-Saharan Africa (Budelman, 1987; Ekanayeke and Asiedu, 2002; Ikeorju *et al.*, 1995; Oyetunji *et al.*, 2003).

In most tropical countries, food yams are propagated vegetatively by planting small whole tubers or pieces cut from large tubers. This

method competes with yam availability for human consumption and at the same time makes the cultivation expensive for large scale production. Nevertheless, the propagation of yam through vine cuttings and aerial tubers (bulbils) could offer an even high multiplication rate than the minisett technique (Shiwachi *et al.*, 2002). Successful root development by vine cuttings has been reported but growth was limited and tuber production has been challenging (Shiwachi *et al.*, 2002).

Yam propagation using vine cuttings: The propagation of yam vine cuttings is a very useful technique for rapid multiplication of desirable clonal materials. Successful propagation of yams from vine cuttings was first reported in non-food yams. Using this method, Correll *et al.* (1995) obtained rooted cuttings of *D. floribunda* between 14-21 days. The first detailed and systematic study of propagation of yam vine cuttings was that of Preston and Haun (1962) with the species *D. spiculiflora*. Production of plants occurred most readily within 3-8 weeks on small rosetted shoots of immature plants.

In food yams, the establishment of plants through leafy vine cuttings has been extensively reported (Akoroda and Okonmah, 1982; Cabanillas and Martin, 1978; Njoku, 1963; Okonkwo *et al.*, 1973; van der Zaag and Fox, 1981). Cuttings of several yam species have been rooted in different medium without hormones (Akoroda and Okonmah, 1982; Cabanillas and Martin, 1978; Ferguson, 1971), although hormone treatment may accelerate root, shoot and tuber formation (Cabanillas and Martin, 1978; Kumar and Chacko, 1979).

The retention of leaves is also beneficial for root formation and further development of vine cuttings (Ferguson, 1971; Hartmann *et al.* 1990). Defoliation and heavy shade restrict growth and cause death of vine cuttings. The ease with which the cutting can

root and establish varies with species and cultivars, and is also influenced by physiological factors responsible for plant growth (Cabanillas and Martin, 1978; Hartmann *et al.*, 1990). *D. alata* for example roots more readily than *D. rotundata*, while *D. esculenta* and *D. trifida* are difficult to root (Onwueme, 1984).

The age of a plant also determines the ease with which its cuttings root. Cuttings from young plants (five weeks) have been reported to produce roots, tubers and shoots, while cuttings taken from old plants tend to develop tubers precociously and often fail to produce shoots (Cabanillas and Martin, 1978; Okonkwo *et al.* 1973). The multiplication ratio for seed yam production in the field is very low (less than 1:10), compared for instance to 1:300 for some cereals (IITA, 1999). However, there are indications that yam has great prospects of contributing to the alleviation of the projected food deficit in Africa in the 21st century, if efforts are made to identify and overcome the constraints to its production (Oyetunji *et al.*, 2003; Tetteh and Saakwa, 1994). There is, therefore, a current need to find methods of improving the multiplication ratio of yams, in order to increase the amount of seed available for seed and ware yam production, and to shorten the time for developing lines in breeding programs.

Propagation through rooting of vine cuttings and by layering of vines could offer higher multiplication rate than the miniset technique (Okoli *et al.*, 1982) and becomes viable alternatives in future. The objective of this study was to develop a method of mini seed tuber production using yam vines, which will reduce yam production cost.

Materials and methods

Field experiment: The study was conducted at the International Institute of Tropical Agriculture (IITA), Ibadan (7° 26'N, 3° 54'E) a transition zone between humid and sub-humid tropics. The soil of the area is derived from basement complex rocks with sandy loam surface texture overlying a layer of angular to sub-angular quartz gravel merging into an argillic horizon (Lal, 1974). Seed setts (100-150g) of seven cultivars of *D. rotundata* Poir, (TDr131, TDr 335, TDr 89/02665, TDr 99-21, TDr 93-49 and TDr 93-31) were planted in the field 50cm apart on ridges separated from each other 1m on the rows. The cultivars were arranged in a randomized complete block design. Sprouting took place averagely 21 days after planting (DAP). Plants were staked individually and thereafter weeding was done at due time.

Preparation of soil for controlled experiments: The soil was prepared by mixing rice husk collected from IITA rice field. The husk was processed using a big drum. The surface was perforated at intervals. The drum was eccentrically placed in a well constructed furnace of size 60 x 60 (cm) using cemented blocks. One side of constructed structure was free of blocks in order to ease the introduction of fire woods into the open side of the drum. The two extreme ends, that is, the top and bottom of the drum remain closed. After fire has been introduced to the fire woods in the drum, a good quantity of rice husk was then poured around the perforated side. The heat that radiated from the drum through the perforated holes carbonized the husk. As wood were continually introduced, the husk was continually turned using a wood stick until they were all homogeneously carbonized. The sterilized carbonized husk (20kg/bed) was then

filled into two prepared beds of depth of 9cm and size of 57 x 162.5cm, in the greenhouse. The yam cultivars were arranged in a complete randomized design. The beds were covered with a humidity chamber.

First trial: Healthy vines of three cultivars of *D. rotundata* (TDr 131, TDr 335 and TDr 89/02265) were excised from plant 127 days after planting (DAP) using a razor blade. In order to maintain moisture, vines of each clone were placed in a moist transparent polyethylene bag immediately after collection. The collection was done in the morning between 8.30 and 9.30am and taken to the green house for further processing. From the middle portion of each vine 20cm long, cuttings with three nodes each were prepared. Using a sterilized clean razor blade, vines of each cultivar were carefully wounded by scraping to remove the lower epidermis at the cut ends near the nodes.

These vines were wounded with a sterilized clean razor blade to promote root initiation. The cut surfaces were dusted with 1% Indole-3-butyric acid (IBA) (Okishibiran, Sionogi, CO, Japan). Shallow ditches of 2-3cm depths were made on the bed and filled up with sterilized carbonized rice husk. The treated vines with IBA were layered horizontally 1cm apart between the row and 4cm within the row, allowing the lower sides of the nodes dusted with IBA come in contact with the soil. The nodes were then covered with the soil and leaves were left upright to trap sunlight in order to produce more assimilates that will be translocated to the rooting zone. The planted cuttings (Table 1) were watered sparingly. A transparent polythene sheet was used to construct a humidity chamber in order to maintain a higher relative humidity within the chamber. Cheesecloth was used to maintain the shade above the humidity chamber in order to reduce the incidence of sunlight. Air temperature averaged 25.8°C and mean humidity was 85.2% while cuttings were in the rooting chamber.

Second trial: Vine cuttings of seven cultivars of *D. rotundata* (TDr 131, TDr 335, TDr 89/02265, TDr 93-49, TDr 99-12, TDr 99-21 and TDr 93-31) were planted on carbonized rice husk in the greenhouse on 6 August, 2003 as in the first trial. Healthy vines were collected from plant 134 DAP between 10.30 and 11.30am. They were taken to the green house in a transparent polyethylene bag. To promote root initiation, the cut at lower surface of the nodes were dusted with 1% IBA powder. Cuttings treated with IBA were planted as in the first trial.

Third trial: The above experiments were repeated for the same seven cultivars of *D. rotundata*. Vines were excised from the field 141 DAP between 9.20 and 10.10am. Vines were put into transparent polythene bags as done in the previous trials.

Cuttings were sampled for root initiation at 14 DAP stage. Wetting of the cuttings continued every two days. Plant food supplement (NPK: 14-10-27, phostrogen, UK) at a concentration of 0.52g L⁻¹ was applied to the stems avoiding as much as possible the leaves in order to minimize the spread of fungi which may be enhanced by the aid of nitrogen when in contact with the leaves.

Inspection for root formation was done 28 DAP by careful digging out the cutting from the carbonized rice husk. The transparent polyethylene bag used for the construction of the humidity chamber was removed 44 DAP when the roots were formed.

Fungicide (Benlate) was applied to the three trials 15 DAP at

Table 1. Number of cuttings planted for each cultivar in the three trials

Trials	TDr131	TDr335	TDr93-31	TDr93-49	TDr99-12	TDr99-21	TDr89/02665
First	45	36	-*	-	-	-	31
Second	50	36	45	43	15	31	56
Third	24	22	41	30	20	35	25

*Some cultivars did not have branches at the time of first trial.

concentration of 2.5g L⁻¹. Visual observations of the leaves were carried out for any disease occurrence. A fungal disease was observed to affect the plants. Affected leaves were removed to avoid contamination of the healthy ones. The application of Benlate fungicide continued once a week until a week to harvest. The bare soil by the bed side was wetted intermittently to prevent the spread of soil borne fungi.

Statistical Analysis: The data were analyzed using fixed model analysis of variance (ANOVA) for individual experiments using the windows version of Statistical Analysis System (SAS) package (SAS, 1991). The means were separated with t-test for significant variables.

Results

Field experiment: The seed setts planted (100-150g) on 20 March 2003, were harvested on 29 October, 2003. Different sizes and weights of tuber were observed. The average weight of tuber ranged from 1.03 to 1.99kg for TDr 131 and TDr 89/02665 respectively (Table 2). There were significant differences among cultivars, with the highest average weight of 1.99kg for TDr 89/02665 and lowest average weight of 1.03kg for TDr 131 (Table 2). The tuber weight of TDr 335 was similar to that of TDr 89/02665. Also the weight recorded for TDr 99-12 was at par with TDr 131.

Vine cutting survival: It was observed that all the planted vines could not survive (Table 3). The percentage survival was between 31.0% for TDr335 which was the lowest and 77.1% for TDr99-12 as highest. The average percentage vine survival of the 7 cultivars was 56.7% with a deviation of 16.6. It was observed that the survival rates of two cultivars (TDr 335 and TDr 89/02665) were below 50%, while the survival rates of the remaining five cultivars were above average (50%). TDr 99-12 had highest survival rate of 77.1% at par with TDr 93-49 (76.7%) and TDr 99-12. The survival rates of the vines were similar in TDr 335 and TDr 89/02665 and were the lowest. The rest shared similar vines' survival rates (Table 3).

Root formation on cuttings: Root formation on cuttings of the 7 cultivars used in this study showed no genotypic difference. Root initiation on cuttings was visible 2 weeks after planting (WAP). The number of roots per vine ranged between 5.4 and 5.8 for TDr 93-49 and TDr 99-12, respectively and with average rooting per vine of 5.55 (Table 3). The numbers of roots produced from the vine cuttings were higher in TDr 89/02665, 99-12, and 99-21. The lowest was observed in TDr 335.

Mini tuber development through vine cutting of 7 cultivars of *D. rotundata*: Mature mini tubers were finally harvested from the cuttings of all cultivars at 115DAP. Total number of mini tubers ranged from 31 for TDr335 and 82 for TDr93-49 while the number of mini tubers per vine cutting ranged from 1.2 to 2.1 for TDr93-31 and TDr 99-21, respectively (Table 4).

The data show that each cultivar produced more than one mini tuber per vine. TDr 93-31 produced the least average number (1.2) of mini tubers per vine followed by TDr 93-49 with an average of 1.5 per vine. TDr 99-21 produced the highest number of mini tubers per vine with an average of 2.1 per vine. This was closely followed by TDr 99-12 and TDr 89/02665 with the average of 1.8 per vine. TDr. 335 had an average of 1.7 of mini tubers per vine (Table 4).

The weights of the mini tubers produced varied from one cultivar to another. The least tuber weights were produced by TDr 99-12 and TDr 99-21 with 1.1 and 1.2g per tuber, respectively. The higher tuber weights were recorded among cultivars TDr 93-31 and TDr 93-49 with 4.2g. TDr 131 and Tdr 335 produced mini tubers with average weight of 2.9 tuber each, while TDr 89/02665 produced mini tuber weight of 2.19 (Table 4). These trends of results were also recorded in the field trial. The total average weight was 2.7g whereas the error varied from 1.1g for TDr 99-12 to 3.3g for TDr 93-31 and TDr93-49 (Table 4). The mini tubers had average size of 1.9cm in height, width long of 1.3cm, width short of 0.9cm and error of 0.9, 0.5 and 0.4cm, respectively (Table 4). The moisture content ranged from 67.2% for TDr 93-49 to 72.5% TDr 87/02665 (Table 4).

The vines were observed for vegetative growth. All the cultivars produced shoots profusely with many branches (Table 5). The

Table 2. The fresh weight (kg) of tuber yield per plant of tested cultivars

Cultivar	Tuber weight
TDr 131	1.03e
TDr335	1.76ab
TDr89/02665	1.99a
TDr 93-31	1.54bc
TDr93-49	1.69b
TDr99-12	1.23de
TDr99-21	1.49cd

The means followed by the same letter in a column are not statistically different at $P=0.05$.

Table 3. Rooting performance and percentage survival of vine cuttings of seven cultivars of *D. rotundata* treated with 1% IBA

Cultivar	Number of planted vines	Average number of roots/vine	Survival (%)
TDr 131	74	5.7b	56.8b
TDr 335	58	5.1c	31.0c
TDr 93-31	86	5.4b	60.5b
TDr 93-49	73	5.4b	76.7a
TDr 89/02665	56	5.8ab	35.7c
TDr 99-21	57	5.8ab	57.9b
TDr 99-12	35	5.9a	77.1a
Mean \pm SD	62.7 \pm 5.8		

The means followed by the same letter in a column are not statistically different at $P=0.05$.

Table 4. Results of mini-tuber development through vine cuttings of seven cultivars of *D. rotundata* harvested at 115 DAP

Cultivar	Tuber characteristics						
	Total number of tubers	Number of tubers per vine	Height (mean±SE)	Width long (mean±SE)	Width short (mean±SE)	Weight/tuber	Moisture content (%)(mean±SE)
TDr 131	69cd	1.6c	1.9±1.1	1.3±0.6	0.9±0.4	2.9b	68.5(0.9)
TDr 335	31f	1.7bc	1.9±0.9	1.3±0.6	0.9±0.4	2.9b	69.8(1.1)
TDr 93-31	62d	1.2d	2.2±0.9	1.5±0.5	1.0±0.4	4.2a	69.2(1.8)
TDr 93-49	82a	1.5c	2.5±0.9	1.6±0.5	1.1±0.4	4.2a	67.2(1.7)
TDr 89/02665	36ef	1.8b	1.5±0.8	1.1±0.6	0.8±0.4	2.1b	72.5(1.8)
TDr 99-21	70b	2.1a	1.5±0.7	1.1±0.5	0.9±0.4	1.2c	72.1(1.9)
TDr 99-12	48e	1.8b	1.5±0.7	0.9±0.4	0.7±0.4	1.4cb	71.4(2.1)

The means followed by the same letter in a column are not statistically different at $P = 0.05$.

Table 5. Average number of branches in 5 randomly selected plants of each cultivar

Cultivar	1 ^o branches	2 ^o branches
TDr 131	2.2c	3.2b
TDr 335	2.8cb	2.1c
TDr 89/02665	3.6b	4.5a
TDr 99-12	4.0b	4.8a
TDr 99-21	5.6a	4.5a
TDr 93-49	2.2c	2.7b
TDr 93-31	1.9c	3.2b

The means followed by the same letter in a column are not statistically

higher vegetative growth was recorded in TDr 99-12, TDr 99-21 and TDr 335, while the lowest was recorded in TDr 93-31

Discussion

Cultivated food yams are usually propagated vegetatively by planting whole setts or cut pieces of tuber. A considerable proportion of the tuber yield of each yam crop must therefore be reserved for planting. This often competes with supply for human consumption and at the same time makes its cultivation expensive for large scale production. The use of stem cuttings has the advantage of permitting the rapid multiplication of limited quantities of planting materials. The propagation of food yams using stem cuttings represents a departure from the conventional method of propagation using tuber pieces.

Recommendation on sett weights required for planting do not differentiate whole and cut tubers, although whole sett yams are superior planting material compared with cut setts (Onwueme, 1978). Farmers, however, plant whole and cut setts of varying weights. The recommendation of setts does not appear to be based on multiplication ratio of the planted sett. The ratio of sett weight to yield is a measure of the efficiency with which sett yams of a specified weight category produces sett or ware yams. In Our study, the cut setts of *D. rotundata* weighing 100-150g gave an average yield of 1.9kg per cultivar. The yield observed was within the ranges reported in previous studies for African average with setts between 500g to 1kg.

In this study, among the 7 cultivars of *D. rotundata* evaluated, the survival rate of vines planted was between 31.0 and 77.1% with total number of roots per vine between 5.11 and 5.76. These were comparable with the findings of Akoroda and Okonmah

(1982) and van der Zaag and Fox (1981) who worked with other varieties of yam. The percentage rooting was within the ranges reported in their previous studies. For good root initiation, the sand or the medium in which the vine cuttings are planted must have good aeration and the root callus on the leafless nodes. This was the main reason why carbonized rice husk was mixed with the soil. The survival rates of the vines were noticeable from the percentage survival recorded. The root production was also very good among all the varieties. Only TDr 335 and 89/02665 had very low survival rates of the vines.

Cuttings from several yam species have been rooted in sand media without hormones (Ferguson, 1972). Application of hormone (IBA) was found to accelerate rooting in this study. It also increased the percentage rooting of the vines compared to the previous reports. This corroborates the findings of Carpenter and Cornell (1992) with *Hibiscus* stem cuttings, Schrader and Graves (2000) with cuttings of *Alnus maritima* and Al-Salem and Karam (2001) with cuttings of *Arbutus andrachne*.

Our study revealed that for the best results, cuttings must be taken between 4 and 14 weeks after sprout emergence from the setts planted and also the position from which the cuttings are collected. However, previous studies have shown that the position of cuttings on a branch determines the ease of root formation (Al-Salem and Karam, 2001; Cabanillas and Martin, 1978). The propagation of these clones using vine cuttings from different positions on a lateral branch is necessary in order to determine the cutting age that most responsive to rooting. The age of vine cuttings determine the ease with which shoots develop on rooted cuttings. With age, branches become woody and meristems show reduced activity, the node is very important in the regeneration of plantlets (Preston and Haun, 1962) and the buried nodes should be healthy and have opposite leaves.

Emphasis has not been laid yet on mini tuber (tubercles) production. Small tubercles, whose size and quantity were not specified, were reported in previous studies of Akoroda and Okonmah (1982). Also, Acha *et al.* (2002) observed the formation of mini tubers when studying the effect of auxins on root development in yam (*D. rotundata*) vine. In this study, the formation or production of min tubers ranged from 1.2 to 2.1 per vine with average size of 1.9 cm in height, 1.3 cm in width (long), 0.9 cm in width (short) and weight of 2.7g. The ease of mini tuber formation on cutting appears to be genotype dependent. The cultivars with higher

vegetative growth (TDr99-21 and TDr 99-12) produced the least tuber weights while those with low vegetative growth (TDr 93-31 and TDr 93-49) produced the highest tuber weights. This suggests that those with low tuber weights converted most of their photo-assimilates for vegetative growth, while those with low vegetative growth diverted more photo-assimilates to the storage tissue (*i.e.* tubers). These attributes help cultivars with high vegetative growth rates to possess higher survival rates than those with low vegetative growth rates as indicated in our results.

The small tuber sett weights produced from this technology can provide suitable materials for research in physiology, botany or entomology where the emphasis is not on commercial tuber yield evaluations *per se*. The small size (compact shape), wholeness and reduced weight of tubercles make them easier to pack and transport, being less prone to injury than tubers, and are therefore suitable specimens for easy transfer of genetic material in tuber form.

Tubercles can be left also to re-grow for one or more seasons to enable the observations of a treatment for several seasons or the production of sizeable tubers. From the mother plant, there is a total average number of 68.7 vines (both primary and secondary branches) per mother plant. If this number of vines can be collected per plant to produce mini-tubers (tubercles), there will be a tremendous increase in number of propagating materials, thereby allowing the ware yam only for consumption and other uses. Also, its cultivation will be less expensive.

The study indicates that vine cutting treated with 1% IBA can survive when propagated in soils with well sterilized carbonized rice husk. This study also suggests that a quantity of quality mini tubers can be produced from vine cuttings when treated with 1% Indole-3-butyric acid powder.

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