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Advances in Applied Science Research, 2016, 7(3): 114-119

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ISSN: 0976-8610
CODEN (USA): AASRFC

Differential response of synthetic plant hormones and inorganic fertilizer on root initiation in Yam Vines (*Dioscorea rotundata*) in diverse media

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ABSTRACT

This study was conducted to evaluate differential response of synthetic plant hormones and chemical fertilizer (Phostrogen) on rooting ability of vine cuttings of two cultivars (TDr 95/18544 and TDr 89/02565) of Dioscorea rotundata in two different planting media (solution and carbonized rice husk). Three leafy nodes cuttings were prepared from healthy mother plants. Using a clean razor, the leaves of the two lower nodes were detached and then immersed into 270ml cups capacity containing two sets of planting media (i) solution medium of different treatments vis. Chemical fertilizer (CF), CF + IBA, CF + NAA, Water (W) +IBA, and W + NAA (ii) Solution of different concentrations of 10 mg, 50 mg and 100 mg/ 100 ml for 1% IBA and 0.4% NAA powder each, mixed with 100 g of carbonized rice husk. The vine cuttings were sampled for rooting 3WAP. The rooting percentage in all hormone treatments varied from 27.44% to 98.95% for the 2 cultivars of D. rotundata evaluated. TDr 95/18544 had an overall better response of all hormone treatments in both media compared to TDr 89/02565. The rooting percentage in carbonized rice husk was better compared to solution medium ranging from 72.4 to 99.9 %.

Key words: *Dioscorea rotundata*, plant hormones, root formation, vine cuttings

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INTRODUCTION

Yams belong to the family *Dioscoreaceae* within the order Dioscoreales, and are members of the genus *Dioscorea*, which produce tubers and bulbils that are economically important [1]. The genus *Dioscorea* is by far the largest genus of the family and is very important throughout coastal West Africa where approximately 60 million people obtain critical calories of energy, about 800 KJ day-1 from this food source.[2].

Yams are major staple and important source of income for small-scale farmers in West Africa. This region accounts for 90-95 % of the world's yam production [3], [4], [5] of which 71% is grown in Nigeria, the largest single producer in the world [6]. Yam production is, however, relatively expensive compared with other root and tuber crops. This is attributed to costly input requirements; especially labor and planting material [7].Of all yams, the white yam is one of the most essential in the diet of the people of West Africa especially those living in the yam growing zone, from Cote d'Ivoire to Cameroon [8].

Traditionally, food yams are propagated vegetatively by means of tubers. Whole tubers called 'seed' yam ranging from 100 to 1,500 g, are used as planting materials. Alternatively, larger tubers are often cut into approximately 200 g pieces and used to establish the new crop [9]. The practical implication is that the cost of planting material constitutes over 33 % of the total outlay for yam production. [6], [10], [11]. This method of propagation competes with human consumption and at the same time makes its cultivation expensive for large-scale production.

Commercial scale of yam production meant for marketing or in domestic purpose, most farmers depend on different sources of seed tubers as planting material. Among these vine cuttings, mini setts and micro tubers are the preferred seed tuber for yam planting [12]. In traditional practices, rural farmers generally use100 to 600 gm larger tuber pieces as seed tubers, which constitutes 10 to 30% of the total yam yield. As a result, the use of seed tubers in conventional farming is more costly than using vine cuttings, mini sett method, or micro tuber for yam planting. Also, the transportation of high volumes of planting material for field planting is difficult. About 2,500 to 3,000 kg of planting material is required to plant 1 ha. Higher transportation costs increases the total cost of production [13].

The aim of this study was to determine the response of vine cutting to rooting in different media using synthetic plant hormone and chemical fertilizer of two cultivars of *Dioscorea rotundata* as principal and rapid method for production quality planting material for large-scale propagation in yam cultivation.

MATERIALS AND METHODS

The study was conducted at the International Institute of Tropical Agriculture (IITA), Ibadan (7^0 26'N, 3^0 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.

First experiment:
In vitro plantlets of white yam (*Dioscorea rotundata*) cultivars (TDr 95/18544 and TDr 89/02565) derived from meristem were collected from the tissue culture laboratory of IITA for this study. These cultivars were then transferred in the greenhouse for acclimatization for 30 days. A transparent polythene sheet was used to construct a humidity chamber over the plantlets in order to maintain a higher relative humidity within the chamber. The acclimatized plantlets were then transplanted into pots in the greenhouse. Light watering was carefully and continually applied, with the frequency of watering depending upon the soil moisture content within the pot. About 5 months later, healthy cuttings were excised and subjected into 6 treatments for root initiation. Healthy plants were clipped from the grown transplanted plants and then washed clean for the preparation of the cuttings. Three leafy nodes cuttings of 20 cm long were prepared.

Using clean scissors, the leaves of the two lower nodes of the cuttings were removed by detaching their petioles from the vine, leaving four leaves on the two upper nodes for the assimilates production. The lower ends of the cuttings were cut in a slant form in order to increase the absorption surface area of the cuttings. The treatments included Chemical fertilizer (CF) (Phostrogen consist of NPK: 14-4.4-22.4), CF + IBA, CF + NAA, Water (W)+IBA, and W + NAA. All treatments had the same concentration of 100 mg of IBA or NAA/ 200 ml of solution. The solutions were separately poured into different 270 ml capacity transparent cups and then covered with pieces of paper in order to reduce the rate of evaporation of the solution and to prevent impurities from entering into the solution. The prepared cuttings were immersed into the solution via the perforated paper on the cup. Each cup contained five cuttings. The cups were then placed into baskets (48.5 cm long x 32.5 cm wide x 13 cm deep) in a complete randomized design with 4 replications. The observation of the roots development was done three weeks after treatment (WAT).

Second experiment:
The established plantlets of TDr 95/18544 and TDr 89/02565 from meristem in the experiment1 were transplanted into pots in the green house. The cuttings were excised 6 months after transplanting for root initiation. Solution of different concentrations (5mg, 10mg, 50mg and 100 mg/ 200 ml of tap water) of IBA and NAA each were prepared and then transparent cups (270ml) were filled with solution to ¼ of the cups total capacity. The prepared cuttings of the two cultivars, as in above experiment, were then submerged into transparent cups containing different solution of IBA and NAA with the cut nodes totally immersed in the solution through the perforated paper, in order to promote root initiation. The experiment was equally laid out in a complete randomized design with 3 replications. Three WAT, cuttings were sampled for root initiation and other variables.

Third experiment:
Preparation of carbonized rice husk
Rice husk collected from an IITA rice field was processed using a large drum. The surface was perforated at intervals. The drum was eccentrically placed in a well-constructed furnace, size 60 x 60 (cm), of cemented blocks.

One side of the structure was free of blocks in order to allow the introduction of fire woods into the open side of the drum. The top and bottom of the drum remain closed. After fire has been introduced to the wood 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 husks. As fire woods were continually added, the husks were constantly turned using a wooden stick until they were all homogeneously carbonized.

Solutions of different concentrations (10 mg, 50 mg and 100 mg/ 100 ml of tap water) for 1% IBA and 0.4% Naphatelene Acetic Acid (NAA) powder, each were prepared. Each of the concentrations were thoroughly mixed with 100 g of carbonized rice husk in 3/4 filled small cups (270ml). One hundred liters of water was as well thoroughly mixed with 100 g of carbonized rice husk as a control experiment. The cuttings of acclimatized cultivars of TDr 95/19544 and TDr 89/02565 in the greenhouse were prepared as in the previous experiments. Five healthy cuttings were then planted in the treated carbonized rice husks in the cups. For each cultivar, a total of 140 cuttings were used with 5 cuttings per cup. The cups were kept in baskets in a complete randomized design in the green house for the root initiation. 20 ml of water was applied to the cuttings in each cup twice a week, depending on the weather conditions and rice husk moisture content. 3 WAT, data collection was carried out.

Statistical analysis
Data was analyzed using SAS, version 9 (SAS institute, 1987). Analysis of variance was performed for all the traits measured. Significant means were separated using Student-Newman-Keuls multiple-range test.

RESULTS

1. Response of CF, NAA and IBA interactions on root formation on cuttings of two cultivars of *D. rotundata* in solution medium
Root formation on the cuttings of the two cultivars used in this study showed significant differences among treatments. Root initiation was visible 3 weeks after planting (WAP) on the cuttings of the two clones used in this study. The mean rooting had the highest response of 87.78 % (W+ NAA) and the lowest response of 43.08% (CF + NAA and CF + IBA), which were not different, for TDr 95/18544 and with an average rooting of 60.62% (Table 1). In the second cultivar TDr 89/02565, Water (control) had the highest response of 75.61% and the lowest response of 27.44 % in (CF + IBA) (Table 1) with average rooting of 45.83%. The root length had the highest mean of 4.57 cm (W + IBA) and the lowest mean of 2.23 cm (CF + NAA) for TDr 95/18544 with an average length of 3.53 cm, whereas TDr 89/02565 had the highest mean of 4.10 cm (Water-control) and the lowest mean of 2.70 cm (CF) with an average length of 3.19 cm (Table 1). This result indicates that the two cultivars differ markedly in their ability to root when subjected to different treatment interactions. However, cultivar TDr 95/18544 had a better overall performance.

Table 1. Rooting ability of two cultivars (TDr 95/18544 and TDr 89/02565) of *D. rotundata* treated with chemical fertilizer (CF-phostrogen), NAA and IBA in solution medium

Treatments	**TDr 95/18544**			**TDr 89/02565**		
	Root Number	Mean Rooting (%)	Root length (cm)	Root Number	Mean Rooting (%)	Root length (cm)
CF	11	59.22abcd	3.67abc	5	35.01de	2.70cd
CF + NAA	7	43.08cde	2.23d	5	35.01de	3.03bcd
CF + IBA	7	43.08cde	3.87abc	4	27.44e	3.13cd
W + NAA	14	87.78a	3.77abc	9	46.92bcde	2.87cd
W + IBA	12	63.44abcd	4.57a	10	54.99cde	4.10ab
Water (control)	11	67.16abc	3.04bc	13	75.61ab	3.33bcd
Mean ± SD	10.3±2.8			7.7±3.6		

Means with same letter(s) are not significantly different at P≤ 0.05 (Student-Newman-Keulsmultiplr-range test); CF- Chemical fertilizer, NAA- Naphthalene acetic acid; IBA- Indole-3-butyric acid; W- Water

2. Effect of NAA and IBA concentrations on root development using solution medium.
There were no significant differences among treatments on the root numbers, but significant differences were observed among treatments on the root lengths. However, there were variations on rooting of cuttings of the 2 cultivars with the highest mean of 99.95% (IBA-0.05 gl^{-1}) and 67.16% (NAA-0.25 gl^{-1}) and the lowest mean of 71.39% (NAA-0.25 gl^{-1}, 0.5 gl^{-1}, IBA-0.5 gl^{-1}) and 35.51% (NAA-0.5 gl^{-1}) for TDr 95/18544 and TDr 89/02565 respectively (Table 2). The root lengths had the highest mean of 3.37 cm (IBA-0.5 gl^{-1}) and 3.33 cm (NAA-0.25 gl^{-1}) and the lowest mean of 2.20 cm (NAA-0.5 gl^{-1}) and 1.33 cm (NAA-0.5 gl^{-1}) for TDr 95/18544 and TDr 89/02565 respectively (Table 2). This indicates that the two cultivars used in this study can produce roots in solution medium when treated with plant hormones, though they did not differ in their ability to root. However, high concentration of NAA (NAA-0.5 gl^{-1}) may hamper growth and root elongation. Figure 1 shows the rooting response of TDr 95/18544 and TDr 89/02565 on different concentrations of IBA and NAA in solution medium. The analysis of variance indicated no significant difference among the treatments mean but TDr 95/18544 cuttings treated with IBA-0.05 gl^{-1} had the highest mean root number and best overall performance.

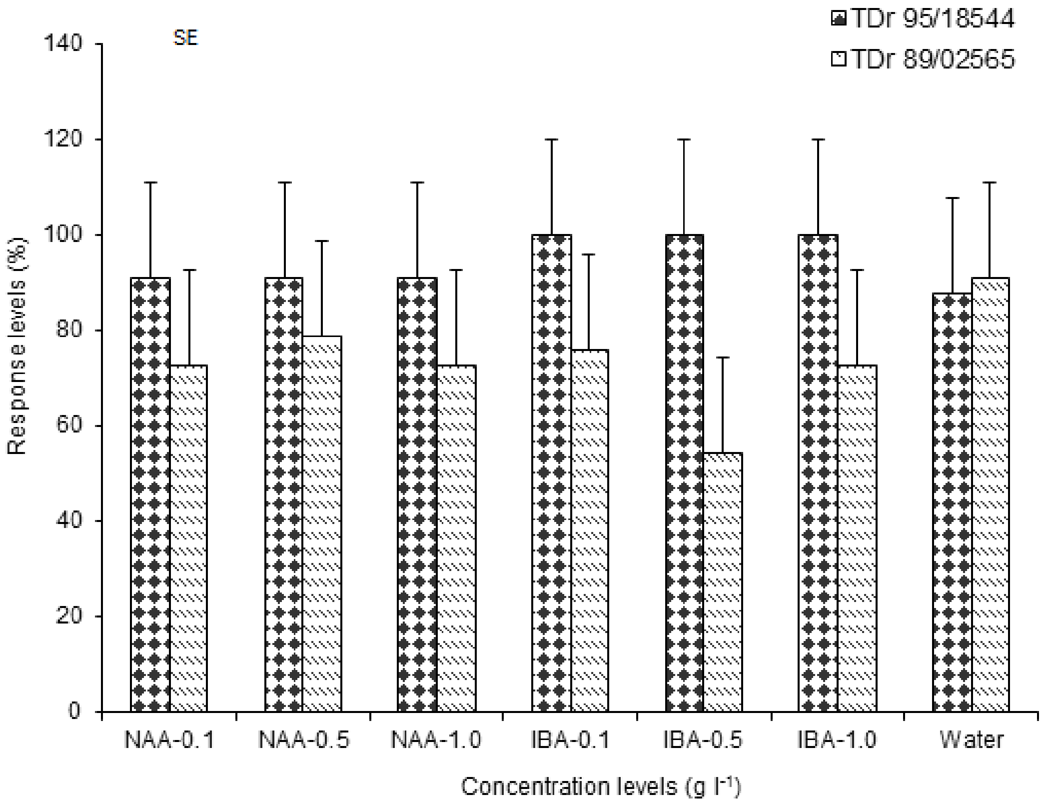

Figure 1. Rooting response of TDr 95/18544 and TDr 89/02565 on different concentration of IBA and NAA in carbonized rice husk

3. Rooting response of TDr 95/18544 and TDr 89/02565 on different concentrations of IBA and NAA in carbonized rice husk.
Figure 1 shows the rooting response of the TDr 95/15844 and TDr 89/02565 in carbonized rice husk mixed with different concentrations of IBA and NAA. The analysis of variance indicated no significant difference among the treatments means. However, the treatments of TDr 95/18544 cuttings with IBA- 0.1 gl^{-1}, IBA-0.5 gl^{-1}, IBA- 1.0 gl^{-1} had the best rooting performance of up 99.95% and the least performance with water (control) up to 87.66%. The cuttings of TDr 89/02565 gave better rooting response with water (control) and least performance with IBA-0.5 $gl^{-1}$with average values of 90.82% and 54.22% respectively. The rest of the treatments had a good response in the medium range.

Table 2. Effect of NAA and IBA concentration on root development in solution medium

Treatment levels	TDr 95/18544			TDr 89/02565		
	Number Rooted	Mean Rooting (%)	Root length (cm)	Number Rooted	Mean Rooting (%)	Root length (cm)
NAA - 0.025gl^{-1}	13	83.56ab	2.33c	12	63.44abc	3.07abc
NAA - 0.05gl^{-1}	13	75.61abc	3.07abc	10	63.32abc	3.13abc
NAA - 0.25gl^{-1}	12	71.39abc	3.17abc	11	67.16abc	3.33ab
NAA - 0.5gl^{-1}	12	71.39abc	2.20c	6	35.51c	1.33d
IBA - 0.025gl^{-1}	13	75.61abc	3.60a	8	46.92bc	2.97abc
IBA - 0.05gl^{-1}	15	99.95a	3.40ab	8	46.92bc	3.07abc
IBA - 0.25gl^{-1}	14	87.78ab	2.67abc	7	43.08bc	2.60bc
IBA - 0.5gl^{-1}	12	71.39abc	3.37ab	10	63.32abc	2.73abc

Means with same letter(s) are not significantly different at $P \leq 0.05$ (Student-Newman-Keuls multiple-range test); NAA- Naphthalene acetic acid; IBA- Indole-3-butyricacid

DISCUSSION

Propagation of yam by vine cutting is not yet practiced in commercial plantings because of the intensive care required for rooting and the low tuber yield from rooted cuttings, but it has a future. Vine cuttings can be established very quickly, and are an effective means of not only rapid multiplication of limited quantity of planting materials, but also of producing disease and nematode free planting materials.

In this study, the vine cuttings of the two cultivars of *D. rotundata* treated with root promoting substances rooted. The rooting percentage in all hormone treatments varied from 27.44% to 98.95% for the 2 cultivars of *D. rotundata* evaluated. [14] had a similar result, of 0% to 87% for *D. alata* and 0% to100% for *D. rotundata* grown in washed coarse sand treated with 10% formalin. The percentage rooting observed in this study was within the ranges reported in previous studies. For good root initiation, the sand or the medium in which the vine cuttings are planted must have good aeration around the root callus on the leafless nodes. This might be the main reason why carbonized rice husk as a medium had a good rooting percentage compared to liquid medium in this study.

Varying the hormone concentrations has been reported to significantly increase the percentage rooting of *Hibiscus* stem cuttings [15], cutting of *Alnusmaritina*[16] and also of cuttings of *Arbutus andranchne* (Al-Salem and Karam 2001). Our study also showed that variation in hormone concentration produced a different response in root formation in the vine cuttings of the two cultivars with lower concentration giving better performance. Thus, application of hormone in higher concentration may hamper root initiation in *Dioscorea sp.* Treating vine cuttings of responsive cultivars with different hormone concentrations before planting in a suitable rooting medium could be valuable in determining the optimum hormone concentration required for effective rooting.

There was significant difference among the hormone treatments means of the 2 cultivars (TDr 95/18544 and TDr 89/02565) derived from tissue culture materials with regards to the mean rooting percentage and root length. Nevertheless, TDr 95/18544 had an overall better response of all hormone treatments, in all respect, compared to TDr 89/02565. This could be because the ease of root formation on cuttings appears to be genotype dependent.

In treatment of chemical fertilizer or a combination of chemical fertilizer and NAA or IBA, the rooting of vine cuttings was lower than water or a combination of water and NAA or IBA. Therefore, when attempting to initiate rooting in vine cuttings, chemical fertilizer (phostrogen) should be avoided because of the poor outcome.

However, for the best results, cuttings must be taken between 4 and 14 weeks after sprouts emerge from the setts planted and also the position from which the cuttings are collected [17]. Other studies have shown that the position of cuttings on a branch determines the ease of root formation [15], [18]. The age of vine cuttings determines 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 [19] and the buried nodes should be healthy and have opposite leaves.

Acknowledgement
I am indebted to the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for providing the research grant, facilities/resources, and enabling environment to carry out this research.

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