African Journal of Biotechnology Vol. 7 (8), pp. 1003-1008, 17 April, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Shoot and plantlet regeneration from meristems of *Dioscorea rotundata* Poir and *Dioscorea alata* L.

O.J. Adeniyi[1]*, V.O. Adetimirin[2], I. Ingelbrecht[1] and R. Asiedu[1]

[1]International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria.
[2]Department of Agronomy, University of Ibadan, Ibadan, Nigeria.

Accepted 15 February, 2008

***In vitro* culture media capable of regenerating moderate to high shoots and/or plantlets from meristems of two yam species – *Dioscorea rotundata* and *Dioscorea alata* within comparable duration of 10 weeks as commonly obtained in other monocots and root and tuber crops were investigated. The study comprised 125 phytohormone combinations investigated in three factorial experiments each consisting of an auxin (NAA) and a cytokinin (BAP or kinetin), or two cytokinins only. The frequency of direct plantlet regeneration, though significantly (*P* < 0.05) higher for *D. alata* than for *D. rotundata*, was low and ranged from 0 to 10% at 3 weeks after culture (WAC) and 0 to 35% at 8 WAC. At 8 WAC, shoot regeneration of 42-75% was obtained in *D. rotundata* in MS medium supplemented with 0.1 µM NAA + 0.20 µM BAP, and shoot + plantlet regeneration of 60-82% obtained in media containing 0.05 µM + 0.20 µM BAP or 0.46 µM BAP + 0.50 µM kinetin in *D. alata*. Both shoot induction and plantlet regeneration were species-dependent. Induced shoots were successfully rooted in MS medium within 3 to 4 weeks, bringing time taken for plantlet regeneration to 11 to 12 weeks. Regenerants were morphologically similar to the mother plants. Results of the present study will facilitate regeneration of plantlets via meristem in *D. rotundata* and *D. alata*.**

Key words: Meristems, phytohormone combinations, plantlets, shoots, yam.

INTRODUCTION

Dioscorea rotundata Poir (white yam) and *D. alata* L. (water yam) are monocots that produce underground tubers that are edible. In Africa, yams are important not only as food but have been reported to have medicinal value (Coursey, 1967; Purseglove, 1972; Hegde, 1981). Yams are propagated vegetatively through the use of tuber sets. In general, vegetative propagation is associated with the rapid spread of diseases. In yams, yield loss due to fungal attack may be as high as 70 to 80% (Ghosh et al., 1988), while yam mosaic virus can reduce yield by as much as 50% (Craig, 1964). International germplasm exchange plays a unique role in crop improvement and has implications for food security. Plant materials for cross-border exchanges are required to be free from pests and disease-causing organisms. Meristems are usually free from diseases affecting the mother plant. Regeneration of plantlets from meristems, thus, offers the possibility of producing disease-free plantlets.

Phytohormones have been successfully used to induce plantlet regeneration from meristems. Mantell et al. (1980) reported the regeneration of plantlets from meristems of *D. alata* on modified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962). Ng and Hahn (1985) obtained plantlets from meristems of *D. rotundata* cultured on MS basal medium supplemented with 1 µM NAA (α-naphthaleneacetic acid), 0.6 µM BAP (6-benzylaminopurine) and 0.2 µM GA_3 (gibberellic acid). In the latter study, plantlets were obtained in 16 to 24 weeks. Malaurie et al. (1995) obtained 18% shoot regeneration at 12 weeks from cultured meristems of *D. cayenensis- D. rotundata* complex and *Dioscorea praehensilis* in modified MS medium supplemented with 2.69 µM NAA and 0.44 µM BAP. In elephant yam (*Amorphophallus campanulatus* var hortensis Backer),

*Corresponding author. E-mail: b.adeniyi@cgiar.org.

Abbreviations: NAA - α-Naphthalene acetic acid; BAP - 6-Benzylaminopurine; GA3 – Gibberellic acid; WAC – Weeks after Culture; MAP – Months after Planting; MS – Murashige and Skoog.

shoot regeneration was 22% at 24 weeks and 75% at 36 weeks using MS medium with 5 µM NAA and 0.05 µM kinetin (Irawati and Nyman, 1986). These studies indicate long period to shoot/plantlet regeneration or low shoot/ plantlet regeneration, or both in yams. Results of studies involving several monocots suggest that the level of shoot/plantlet regeneration reported for *Dioscorea* could be increased considerably and the time to regeneration drastically reduced. Multiple shoots from the meristem of sugarcane – a monocot like yam, were obtained in liquid MS medium supplemented with 0.8 µM BAP and 0.4 µM kinetin, both cytokinins (Ingelbrecht, 2001, unpublished). In *Musa* spp., another monocot, Gupta (1986) reported 97% shoot regeneration from meristems on MS basal medium supplemented with 3.1 µM BAP and 3.2 µM kinetin within 6 weeks. The study reported herein explored combinations of an auxin and a cytokinin as well as two cytokinins for high and rapid regeneration of shoot/plantlets from meristems of *D. rotundata* and *D. alata*.

MATERIALS AND METHODS

Mini-tubers of IITA elite lines TDr 179 (*D. rotundata*) and TDa 95/00005 (*D. alata*) were grown in pots in a screen house. At 4 to 5 months after planting (MAP), the apical and axillary buds of the yam vines were collected. Buds were surface sterilized, first in 70% (v/v) ethanol for 5 min, transferred into 5% (v/v) and 10% (v/v) sodium hypochlorite for 10 and 20 min, respectively, and finally rinsed thrice in sterile distilled water. Meristems were excised from both the apical and axillary buds. Murashige and Skoog (1962) medium was modified by supplementing with combinations of plant growth regulators at different concentrations. In the first study, three experiments were conducted involving pairs of phytohormones in factorial combinations. The first had eight levels each (0.00, 0.05, 0.10, 0.20, 0.40, 0.80, 1.60, and 3.20 µM) of NAA and BAP, the second had five levels each of NAA (0.00, 0.50, 1.00, 2.00 and 4.00 µM) and kinetin (0.000, 0.025, 0.050, 0.100 and 0.200 µM) and the third had six levels each of BAP (0.00, 0.23, 0.46, 0.92, 1.84 and 3.68 µM) and kinetin (0.00, 0.25, 0.50, 1.00, 2.00 and 4.00 µM). In all, a total of 125 media were formulated and studied. Media were sterilized for 15 min in an autoclave at a temperature of 121°C and 15 lb/in^2 pressure. A volume of 10 ml medium was dispensed per 60 x 15 mm Petri dish. Ten meristems of about 0.6 mm size were cultured per Petri dish. Cultures were incubated at 25°C with 12 h photoperiod, under 4,000 lux light intensity.

Experimental design was randomized complete block with two replications. Numbers of buds, shoots and plantlets were counted at 3 and 8 weeks after culture (WAC) and expressed as percentages of number of meristems plated. At 8 WAC, regenerated shoots were sub-cultured in a modified MS proliferation medium as described by Ng and Hahn (1985) to generate 25 plantlets from each genotype. Regenerated plantlets were observed for uniformity as well as compared to their respective mother plants. Field acclimatization of regenerated plantlets was done using the yam post-flask technique described by Ng et al. (1994). At 5 MAP, the observations made were on vigour, stem colour, stem diameter, internode length, stem waxiness, leaf colour, leaf waxiness, leaf shape, petiole length and petiole colour using the yam descriptors of the International Plant Genetic Resources Institute (IPGRI) and the International Institute of Tropical Agriculture (IITA) (IPGRI/IITA, 1997).

The best phytohormone treatments/combinations for shoot/plantlet induction, four for *D. rotundata* and seven for *D. alata*, were selected from the first study and their effectiveness evaluated in a second study. Data generated from the *in vitro* study and in the green house were arcsine-transformed for statistical analysis. Data were analysed using analysis of variance (ANOVA). If results of statistical analysis for transformed and non-transformed data were similar, results of non-transformed data are presented.

RESULTS

Plantlet and shoot induction in the first study

NAA + BAP

Direct plantlet induction at 8 WAC was, in general, poor. The highest level of plantlet induction in *D. rotundata* was 30%, and this was obtained in the medium containing only NAA at 0.10 µM, while plantlets were not induced in *D. alata* in any of the 64 phytohormone combinations investigated.

The shoot induction responses of the two species studied to combinations of NAA and BAP were significantly different ($P < 0.01$) at 8 WAC. In *D. rotundata*, a total of 55 treatments had less than 30% shoot induction, while 8 and 1 treatments had shoot induction values of 30-60 and > 60%, respectively. The highest shoot induction of 75% was obtained in the medium containing 0.10 µM NAA + 0.20 µM BAP (Table 1). The most promising medium for *D. alata* had 60% shoot induction. The number of treatments with shoot induction in the ranges < 30% and 30-60% were 53 and 11, respectively. Relatively high (60%) shoot induction was achieved in *D. alata* with only NAA in the medium, although at a high level (0.20 µM NAA). A 75% reduction in the concentration of NAA, however, required between 0.20 to 0.40 µM BAP to produce the same level of shoot induction as in the medium with sole NAA at 0.20 µM. Among the three phytohormone combinations that gave the highest shoot induction of 60%, the medium with 0.05 µM NAA + 0.20 µM BAP was best. This was evidenced by the standard deviation value of zero for the mean, indicating high consistency in the results produced by the treatment across replications.

There was significant NAA x BAP interaction, indicating that the effectiveness of each of the phytohormones in inducing shoots and plantlets was influenced by the presence or absence of the other.

NAA + kinetin

Plantlet induction was significantly ($P <0.01$) higher in *D. alata* than in *D. rotundata*, although it was, in general, low. No plantlet was induced in *D. rotundata* while it averaged 4.1% in *D. alata*. In *D. alata*, 24 phytohormone treatment combinations had plantlet regeneration values less than 30% while only one (0.50 µM NAA + 0.025 µM kinetin) had greater than 30% (35%) at 8 WAC. Plantlet

Table 1. Combinations of NAA and BAP with the highest percentages of plantlets and shoots in *D. rotundata* and *D. alata* in the first study.

Species	Phytohormone Concentration (µM)			Mean (%)±SD		
	NAA	BAP	WAC[a]	Bud	Shoot	Plantlet
D. rotundata						
Best combination for shoot induction						
	0.10	0.20	8	0.0±0.00	75.0±7.07	0.0±0.00
Best combination for plantlet regeneration						
	0.10	0.00	8	10.0±14.14	0.0±0.00	30.0±28.28
D. alata						
Best combinations for shoot induction						
	0.05	0.20	8	15.0±7.07	60.0±0.00	0.0±0.00
	0.20	0.00	8	0.0±0.00	60.0±14.14	0.0±0.00
	0.05	0.40	8	25.0±7.10	60.0±28.28	0.0±0.00

[a]WAC = weeks after culture.

Table 2. Combinations of NAA and kinetin with the highest percentages of shoots and plantlets in *D. rotundata* and *D. alata* in the first study.

Species	Phytohormone concentration (µM)			Mean (%)±SD		
	NAA	Kinetin	WAC[a]	Bud	Shoot	Plantlet
D. rotundata						
Best combination for shoot induction						
	0.0	0.050	3	20.0±14.14	40.0±56.57	0.0±0.00
D. alata						
Best combinations for shoot induction						
	0.5	0.025	8	20.0±28.28	65.0±35.36	35.0±21.21
	0.0	0.050	3	20.0±14.14	60.0± 0.00	5.0± 7.07
	0.5	0.100	3	10.0±14.14	60.0±28.28	0.0± 0.00
Best combinations for plantlet regeneration						
	0.5	0.025	3	20.0±14.14	20.0±14.14	35.0±21.21
	0.5	0.025	8	20.0±28.28	65.0±35.36	35.0±21.21

[a]WAC = weeks after culture.

induction was mainly influenced by NAA as evidenced by the significant differences (*P <0.01*) obtained among the five levels of NAA and the non-significant differences among the five levels of kinetin. The effectiveness of NAA for plantlet induction was, however, influenced by the level of kinetin in the medium.

Similar results were obtained for shoot induction, except that significant differences in shoot induction were obtained among the different levels of kinetin. Averaged over the 25 phytohormone combinations, shoot induction was 2.1% for *D. rotundata* and 10.3% for *D. alata*. There was no shoot induction at 8 WAC for 22 out of the 25 treatments in *D. rotundata* while three phytohormone combinations had values between 10 and 25%. In *D. alata*, the highest shoot induction of 65% was obtained in 0.50 µM NAA + 0.025 µM kinetin and 60% in 0.50 µM NAA + 0.10 µM kinetin (Table 2). Medium without NAA but containing 0.05 µM kinetin also had 60% shoot induction. A total of 19 out of the 25 treatments had shoot induction values less than 30% while shoot induction for three treatments was 30%.

BAP + Kinetin

The different levels of each of BAP and kinetin had poor levels of plantlet induction (less than 30% in both *D. rotundata* and *D. alata*). There was significant species x BAP x kinetin interaction for shoot induction. Only four phytohormone combinations has shoot induction values ranging between 30 and 60% in *D. rotundata*, with the highest shoot induction of 40% obtained in 0.23 µM BAP

Table 3. Combinations of BAP and kinetin with the highest percentages of shoots and plantlets in *D. rotundata* and *D. alata* in the first study.

Species	Phytohormone concentration (µM)			Mean (%) ± SD		
	BAP	Kinetin	WAC[a]	Bud	Shoot	Plantlet
D. rotundata						
Best combination for shoot induction						
	0.23	0.50	8	30.0±14.14	40.0±56.57	0.0±0.00
D. alata						
Best combination for shoot induction						
	0.46	0.50	8	5.0±7.07	75.0±7.07	0.0±0.00

[a]WAC = weeks after culture.

Table 4. Shoot and plantlet regeneration of promising media containing pairs of phytohormones in *D. rotundata* at 8 weeks after culture in the second study.

NAA (µM)	BAP (µM)	Kinetin (µM)	Shoot (%)*
0.10	-	-	8.3 b
0.10	0.20	-	41.7 ab
-	-	0.050	3.3 b
-	0.23	0.500	40.0 a

*Means in a column followed by similar letters are not significantly different at $P < 0.05$.

+ 0.50 µM kinetin. In *D. alata*, two and one treatment combinations had shoot induction values between 30 and 60% (0.23 µM BAP + 0.25 µM kinetin, 45%; 0.46 µM BAP + 1.00 µM kinetin, 50%) and > 60% (0.46 µM BAP + 0.50 µM kinetin, 75%), respectively (Table 3).

Comparison of regenerants and mother plants

All induced shoots were successfully rooted in modified MS proliferation medium within 3 to 4 weeks. Stem and leaf colour, stem and leaf waxiness, leaf shape and petiole colour of regenerants and mother plants were not significantly different, indicating that the phytohormone combinations that induced shoots/plantlets did not cause changes in the plant characteristics of the yam genotypes used.

Plantlet and shoot induction of selected promising media in the second study

In *D. rotundata*, no plantlets were induced in any of the media, including the medium containing 0.10 µM NAA in which 30% plantlets were induced in the first study. With the exception of the medium containing 0.23 µM BAP + 0.50 µM kinetin that had similar shoot induction as in the first study, all the phytohormone combinations investigated had considerably lower shoot induction than the levels obtained in the first study (Table 4). Shoot induction in the media containing 0.23 µM BAP + 0.50 µM kinetin and 0.10 µM NAA + 0.20 BAP were not significantly different.

In *D. alata*, three phytohormone combinations with 60% shoot induction in the first study had shoot/plantlet induction ranging between 66.7 and 86.7%. (Table 5) The optimal combination of phytohormones was 0.05 µM NAA + 0.20 µM BAP with shoot/plantlet induction of 81.7%. The concentration of BAP in the latter combination is half that in the medium that produced 86.7% plantlets/shoots. With the exception of the medium containing 0.46 µM BAP + 0.500 kinetin, media containing kinetin alone or in combination with NAA had plantlet/shoot induction values considerably lower than values obtained in the first study, with two media not showing any shoot/plantlet induction.

DISCUSSION

The present study explored the possibility of a one stage, high and rapid *in vitro* plantlet regeneration from meristems of *D. rotundata* and *D. alata*. The highest plantlet regeneration for any of the phytohormone combinations was lower than 40% in each of the two studies for both species. In general, plantlet regeneration was more easily induced in *D. alata* than in *D. rotundata*. While the highest plantlet regeneration in *D. rotundata* was obtained in the medium with 0.10 µM NAA, it was

Table 5. Shoot and plantlet regeneration of promising media containing pairs of phytohormones in *D. alata* at 8 weeks after culture in the second study.

NAA (µM)	BAP (µM)	Kinetin (µM)	Shoot (%)*	Plantlet (%)**	Plantlet + Shoot (%)*
0.20	-	-	38.3 b	28.3 a	66.6
0.05	0.20	-	55.0 ab	26.7 a	81.7
0.05	0.40	-	71.7 a	15.0 ab	86.7
-	-	0.050	0.0 c	0.0 b	0.0
0.50	-	0.025	11.7 c	36.7 a	48.4
0.50	-	0.100	0.0 c	0.0 b	0.0
-	0.46	0.500	63.3 a	0.0 b	63.3

*Means in a column followed by similar letters are not significantly different at $P < 0.05$.

obtained in the medium with 0.500 µM NAA + 0.025 µM kinetin in *D. alata*. There were media with 2 to 4.5 times shoots than plantlets, and given that induced shoot were successfully rooted in MS media, a two-stage process involving (i) shoot induction and (ii) rooting, has the greatest potential in the two species investigated. A two-stage *in vitro* culture in one container in which MS medium containing the optimal phytohormone combination for shoot induction is layered over an MS medium without phytohormones would reduce the possibility of contamination that may be occasioned by sub-culturing.

With respect to shoot induction, the best phytohormone combinations for *D. rotundata* were 0.10 µM NAA + 0.20 µM BAP and 0.23 µM BAP + 0.500 µM kinetin while the optimum combination for *D. alata* was 0.05 µM NAA+ 0.20 µM BAP. These results suggest differences in endogenous phytohormone production by the two species. Malaurie et al. (1995) had earlier reported differences in the endogenous auxin and cytokinin levels between *D. praehensilis* and the *Dioscorea cayenensis-D. rotundata* complex. It is, thus, probable that the different species in the genera have different phytohormone requirement for optimum regeneration.

In media containing NAA and BAP, *D. alata* exhibited lower NAA requirement for shoot induction (half that for *D. rotundata*), while 0.20 µM was the optimum concentration of BAP for both species. In media with BAP and kinetin, the optimum kinetin concentration was 0.50 µM for both species; doubling the BAP concentration from 0.23 µM to 0.46 µM considerably increased shoot induction only in *D. alata*.

Considerable differences in the shoot/plantlet regeneration between the first and second studies are indicative of yet-to-be-verified factors influencing the level of *in vitro* regeneration success. Among these factors would be the age of plants from which meristems were collected. Differences between results obtained in the two studies were more pronounced in *D. rotundata.* Media containing kinetin varied considerably in the level of shoot/plantlet regeneration between the two studies while results of media containing NAA and BAP were more consistent.

The highest shoot production of 73% obtained in five months in the *D. cayenensis-D. rotundata* complex with NAA/BAP by Malaurie et al. (1995) was similar to the shoot induction levels obtained in the present study in *D. rotundata* (first study) and *D. alata* (second study) at 8 WAC. In the present study, this similar level of success was achieved at much lower concentrations of NAA and the cytokinin (BAP) used and at a considerably shorter time. Results of the present study indicate that high plantlet regeneration in *D. rotundata* and *D. alata* could be achieved in 11 to 12 weeks.

Although, *D. rotundata* and *D. alata* were included in the studies by Ng and Hahn (1985) and Malaurie et al. (1995), the lower concentrations of phytohormone combinations for high shoot regeneration obtained in the present study compared to those of the latter studies suggest genotypic differences for phytohormone requirement even within the same species. The effectiveness of the phytohormone combinations identified as promising in this study in a large number of *D. rotundata* and *D. alata* genotypes, therefore, needs to be ascertained.

ACKNOWLEDGEMENT

This study constituted the M.Phil. dissertation research of the first author and was carried out with facilities and materials provided by the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

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