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Comparative effects of explant sources and genotypes on microtuberization in yams (*Dioscorea* spp.)

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Abstract *Single node cuttings of two genotypes each of* Dioscorea alata *and* D. rotundata *from both plants grown in screen houses and in vitro plantlets were cultured in a tuberization medium. The screen house explants had significantly higher plantlet tuberization and primary nodal complex formation, and more tubers and primary nodal complexes per plantlet than* in vitro *explants, whereas* in vitro *explants performed better only in nodes per plantlet. It appears that* in vitro *tuberization is explant-, species- and genotype-dependent, the greatest variation being due to explant source. This is a first report of microtuber production from nodal explants of* D. rotundata *produced in a screen house.*

Keywords: *Dioscorea*, explant, genotype, microtuberization, yam.

Introduction

Yams are staple foods in much of West Africa, but productivity is hampered by pests and diseases (Emehute et al. 1998), and the limited availability and high cost of planting materials (Nweke et al. 1991). Plant tissue culture techniques have helped with disease elimination by heat therapy and meristem culture, higher rates of multiplication of virus-tested plantlets by micropropagation and the conservation of genetic diversity using *in vitro* plantlets without long-term losses of field collections. However, they require specialized handling during transplanting (Ng 1988), and their survival during international exchange depends on the conditions of transportation. Microtubers produced from *in vitro* plantlets could be less vulnerable to transportation conditions and easily established in the soil. They are also less bulky and can be kept for several months due to dormancy. Although *in vitro* tuberization has been reported in a number of *Dioscorea* species, there have been different degrees of success (Jean and Cappadocia 1992; Ng and Mantell 1996), and a generalized protocol for microtuberization is yet to be developed. This study investigated the types and amount of variation in microtuberization due to explant source, species and accessions in *D. alata* (water yam) and *D. rotundata* (white Guinea yam).

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Materials and methods

Two genotypes each of *D. alata* (TDa 297 and TDa 92-2) and *D. rotundata* (TDr 608 and TDr 93-23) were obtained from the IITA germplasm collection. Tubers of about 80 g each were planted in nursery pots 20.6 cm in diameter and 20.5 cm deep already filled with topsoil in a screen house. Each pot had received 23 g of compound fertilizer containing 15% each of N, P and K. The plants were staked with a 1.5 m long split bamboo. At the ten-node stage (two months after planting), single node cuttings 1.5 cm long were excised from each vine and disinfected with 70% ethanol for 5 min, followed by 10% NaOCl for 20 min and 5% NaOCl for 10 min inside a laminar flow hood. The cuttings were then rinsed three times in sterile distilled water. *In vitro* explants were obtained from meristem-derived plantlets multiplied for 75 days by subculturing into a liquid medium containing 4.43 g/l MS medium (Murashige and Skoog 1962), 30 g/l sucrose, 0.1 g/l myo-inositol and 5 mg/l kinetin, as described by Ng (1992). Single-node cuttings were dissected from the full-grown plantlets and introduced individually into vials containing the culture medium.

The culture medium was that of Chang and Hayashi (1995), containing 2.215 g/l MS basal medium, 60 g/l sucrose, 1.0 mg/l naphthalene acetic acid and 8 g/l agar, adjusted to pH 5.8. The medium was melted in a microwave oven, dispensed into 35 ml screwcap vials in 10 ml quantities and autoclaved for 15 min at 103.4 Kpa and a temperature of 121°C. The cultures were incubated in a culture room at 25 ± 2°C in the dark.

The experimental design was a 2 × 2 (explant × species) factorial with subsampling (Steel and Torrie 1980), each treatment having three replicates and ten units per replicate. Data were recorded on the cultures of each genotype 120 days after culturing, with microtubers, the primary nodal complexes, microtubers and primary nodal complexes and nodes per plantlet. Analysis of variance was performed on the data, and the means were separated at the 5% probability level using standard error. Variance component analysis was done, and estimates were made of the relative variation due to each factor.

Results

In the screenhouse (SH) explants, there were significant differences between the two accessions of each species in tuberization which was greater in the *D. rotundata* accessions (Table 1). However, the number of nodes per plantlet was higher in *D. alata*.

In explant by species interaction, significant results were observed in tuberization and the tubers and nodes per plantlet (Table 2). *D. alata* had higher values for SH explants, but for *in vitro* explants, there were no significant differences. For the nodes per plantlet *in vitro*, *D. alata* was significantly higher than *D. rotundata* but in the SH there was no significant difference.

For the SH explants, tuberization and the tubers per plantlet were about ten times those of *in vitro* explants, while the percentage of primary nodal complexes and their mean number per plantlet were about three times greater. On the other hand, the number of nodes per plantlet was significantly higher in *in vitro* than SH.

D. alata had significantly more tuberization and more tubers and nodes per plantlet than *D. rotundata*. In contrast, the percentage of primary nodal complexes and their number per

Table 1. Mean values of *in vitro* tuberization parameters

	Screen house explants				In vitro explants				
Species	*D. rotundata*		*D. alata*		*D. rotundata*		*D. alata*		
Variety	TDr	TDr	TDa	TDa	TDr	TDr	TDa	TDa	s.e.
	608	93-23	297	92-2	608	93-23	297	92-2	
Tuberization (%)	49.23b	10.26c	50.47b	82.11a	10.00c	0.00c	0.00c	10.00c	8.08
Tubers/plantlet	0.60a	0.13b	0.68a	0.97a	0.10b	0.01b	0.00b	0.10b	0.13
Primary nodal complex formation (%)	55.56a	51.85a	46.86ab	20.36bc	10.00b	26.57b	0.00b	26.57b	7.71
Primary nodal complexes/plantlet	0.80a	0.75a	0.62ab	0.24b	0.20b	0.27b	0.00b	0.27b	0.12
Nodes/plantlet	1.70c	1.66c	2.12b	1.52c	1.60c	1.70c	2.60ab	3.00a	0.22

Values in a row with the same letters are not significantly different at $P = 0.05$.

Table 2. Mean square values of *in vitro* tuberization parameters of nodal explants

Source	Df	Tuberization (%)	Primary nodal complex formation (%)	No. of Tubers	No. of primary nodal complexes	No. of nodes/plantlet
Replicate	2	0.01ns	0.05ns	0.03ns	0.09ns	0.01ns
Explant	1	1.11***	0.47***	1.78***	1.06***	1.33***
Species	1	0.20**	0.09*	0.31*	0.30*	2.47**
Explant × species	1	0.20**	0.03ns	0.31*	0.09ns	1.55**
Accession within explant × species	4	0.10**	0.06*	0.12ns	0.08ns	0.20ns
Orthogonal contrasts						
TDa 297 versus TDa 92-2	1	0.15*	0.11*	0.12ns	0.21ns	0.54ns
TDr 608 versus TDr 93-23	1	0.23**	0.0021ns	0.34*	0.004ns	0.004ns
Error	14	0.02	0.02	0.05	0.05	0.15

*, **, ***: Significant at the 5, 1 and 0.1% p levels respectively; ns: not significant.

plantlet were significantly higher in *D. rotundata*. Differences between the two explant sources and between species were significant in all the parameters (Table 2).

Variance component analysis showed that except for nodes per plantlet, about half of the total variance is due to explant source (Table 3). For the nodes per plantlet, explant by species interaction has the highest variation. Species differences did not contribute any variation to tuberization or tubers per plantlet, and only little to the rate or number of primary nodal complexes or nodes. Explant source, accessions within species and species contributed high, medium and low variability respectively to *in vitro* tuberization.

Table 3. Contribution of some factors to total variance (%)

Factor	Tuberization (%)	Primary nodal complex formation (%)	Tubers/plantlet	Primary nodal complexes/ plantlet	Nodes/plantlet
Explant source	51	45	54	50	0
Species	0	5	0	12	19
Explant × species	14	0	14	1	45
Accession	21	20	11	7	4
Error	14	30	21	30	32

Discussion

The differences in microtuberization between the two explants may depend on the environmental conditions. *In vitro* plantlets were particularly exposed to vitrification, a stress condition in tissue-cultured plants, manifested mainly as abnormal leaf functioning (Ziv 1991). Protein and photosyntheses, gas exchange, cellulose and lignin synthesis, and ethylene production, all critical in tuberization, may have been adversely affected, resulting in fragile plantlets (Ziv 1986, 1991). Also, *in vitro* plantlets were limited in space by the culture container, so the nutrients available to them were lower. All these may reduce the food reserves of *in vitro* explants relative to SH explants. Using *in vitro* explants, our unpublished studies have shown that the optimum concentration of naphthalene acetic acid for microtuberization in *D. alata* was 0.1 mg/l, in contrast with an optimum of 1.0 mg/l for SH explants of *D. alata* (Chang and Hayashi 1995). This emphasizes the need to optimize culture conditions in vitro (Debergh 1987). This is a first report of microtuber production of nodal explants of *D. rotundata* produced in a screen house.

The significant differences observed among genotypes (species and accessions within species) with respect to microtuberization agree with earlier reports. Different species respond differently to basal medium type and sucrose concentration (Mantell and Hugo 1989), ammonium nitrate deficiency, hormones and photoperiod (Jean and Cappadocia 1991, 1992). For a full understanding of yam tuberization, more sources of explants and accessions within species should be studied. However, as screen house explants have higher tuberization frequencies, they could be used for microtuber propagation of *D. alata* and *D. rotundata*.

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