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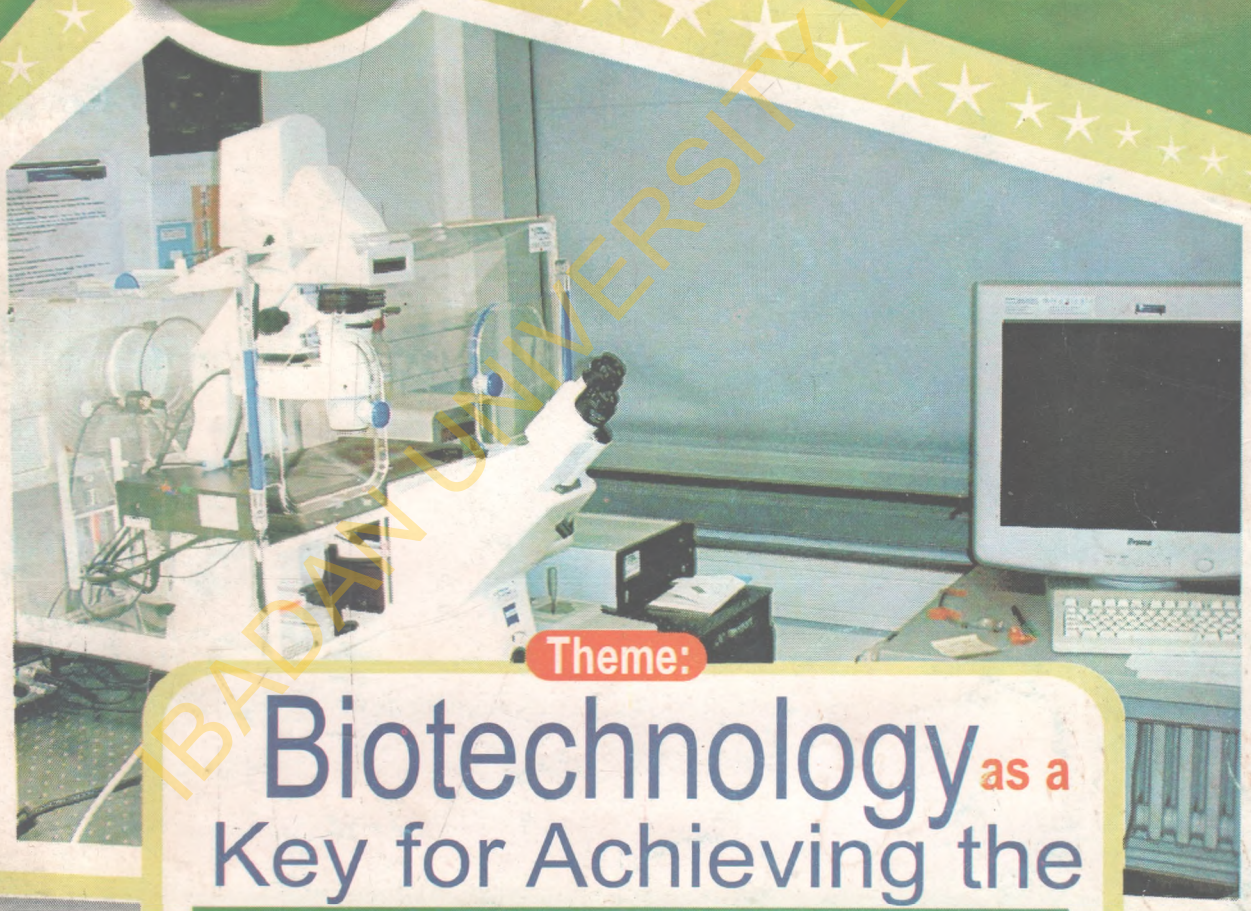

Theme:

# Biotechnology as a Key for Achieving the Millennium Development Goals (MDGS) In Nigeria


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**Typesetting and Formatting By**

Johnny O. Ogunji, Ph.D.
Department of Fisheries and Aquaculture
Ebonyi State University
P.M.B. 053 Abakaliki, Nigeria

Email: Ogunjijo@yahoo.com

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# VARIATIONS IN MICROTUBERIZATION AMONG LOCAL, IMPROVED AND EXOTIC YAM ACCESSIONS IN NIGERIA

**Balogun, M.O.[1], Ng, N.Q.[2], Fawole, I.[3] and Kikuno, H.[4]**

[1]Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, P.M.B. 5029, Ibadan, Nigeria, [2]FAO Regional Office for Asia and Pacific, Bangkok, Thailand. [3]Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria, [4]International Institute of Tropical Agriculture, P.M.B. 5320, Ibadan, Nigeria.

Contact: kemtoy2003@yahoo.com.

## INTRODUCTION

The constraints of yam (*Dioscorea* spp.) production, imposed by pests, diseases and abiotic factors can be alleviated by development of improved varieties (Emehute *et al.* 1998). This will require a broad germplasm base for selection. The conservation of yam genetic resources using field genebanks on one hand, and pollen and seed storage on the other, are constrained by high losses and space requirements, and irregular flowering, respectively (Ng and Ng, 1997; Daniel *et al.* 2002, 2003). Also, conservation using embryo, callus and suspension cultures are constrained by the requirement for successful regeneration protocols, while cryopreservation is still in its infancy for yam (Ng and Ng, 1997). Meristem culture combined with heat therapy have been used to produce virus-free plantlets which are not only conserved in *in vitro* genebanks but also used in rapid multiplication of superior clones (Ng, 1992). However, the stress of transportation which causes low survival rates during transplanting and germplasm exchange (Ng, 1988) and the need for frequent subculturing are major limitations.

Microtubers (MTs) produced from *in vitro* plantlets are possible means of germplasm conservation, being less vulnerable to transportation hazards, less bulky and can be kept for long due to dormancy. They can also be easily established in the soil, not requiring acclimatization and transplanting (Ng, 1988). Knowledge of the genetic variation in microtuberization (TUB) is thus critical, since the use of MTs in conservation will require high, regular MT production across genotypes. This will enhance development of optimum MT production systems while aiding selection and breeding for this trait. This paper describes the variation in tuberization among accessions of *D. alata* and *D. rotundata* plantlets when conserved *in vitro.*

## MATERIALS AND METHODS

The International Institute of Tropical Agriculture, Ibadan, Nigeria provided the accessions that were evaluated. These accessions were meristem-derived plantlets conserved in Murashige and Skoog (1962) medium containing (per litre) 20mg cystein, 100mg myo-inositol, 0.5g kinetin, 30g sucrose and 7g agar (Ng and Ng, 1997), and they constitute an *in vitro* back-up for the field genebank. The conservation room was set at 18-22 °C, 12 hours photoperiod and 4000lux of light.

The age of each plantlet, taken as the number of months in culture differed among the accessions. This was because plantlets were subcultured onto fresh medium based on the rate of senescence. The number of samples per accession varied from 1-10. Sixty-five and 320 accessions of *D. alata* and *D. rotundata* respectively were evaluated. Data were collected on seven parameters (Table 1). A structure in the nodal axis of yam plantlets, with a bulge and at least two roots was taken as a primary nodal complex (PNC) from which MTs were produced (Wickham *et al.*, 1982; Plate 1). Number of MTs per plantlet (NTUB), percent microtuber (%TUB) and aerial microtuber (%ATUB) formation were jointly referred to as TUB parameters. A microtuber was taken to be 'aerial' if formed on a node other than the initial explant. Data were subjected to stepwise regression and analysis of variance in a nested design (of accessions within species) using the Statistical Analysis System and age taken as a regressor with one degree of freedom. Correlation and stepwise regression analyses were performed. A grouping of the accessions was done with the fastclus procedure and considered in relation to their geographical origin.

## RESULTS AND DISCUSSION

The effect of age of the plantlet was significant for the three TUB parameters. Although the average age of the plantlets of

both species was about 15 months, the range in *D. rotundata* was higher than in *D. alata* (Table 1). The significant, positive correlation and regression of age with TUB in *D. rotundata* showed that it took longer time to TUB than *D. alata.* Under *in vivo* conditions however, the crop growth duration of *D. alata* is longer than that of *D. rotundata* (Sobulo, 1972). This disparity is probably due to the origin of *in vitro* plantlets from axillary buds and *in vivo* plants from tubers. The leaf formation habits of yams differed between seed- and tuber- originated plants (Okezie *et al.*, 1981). Thus, the performance of both species as tuber- and axillary bud-originated plantlets under *in vivo* and *in vitro* conditions should be compared.

All parameters significantly differed among accessions within species. *D. rotundata* had significantly higher PNC formation than *D. alata* while it was vice versa for shoot vigour. Percent PNC and microtuber formation ranged from 0% to 100%. Mean values for *D. alata* accessions ranged from 0.0 to 4.0 and 1.12 to 3.0 for number of PNCs per plantlet and shoot vigour respectively. In *D. rotundata,* the values ranged from 0.0 to 3.0 and 1.0 to3.0 for the same parameters respectively. NTUB ranged from 0.0 to 2.67 in *D. alata* and 0.0 to 3.0 in *D. rotundata*.

Regression analysis showed high root vigour to significantly increase TUB in both species. This is probably due to greater penetration of culture media and absorption of available nutrients during tuber expansion (Alhassan and Mantell, 1991). Large numbers of small roots also covered the surfaces of developing tubers in field studies (Ferguson and Gumbs, 1976). TUB depended on more parameters in *D. rotundata* than *D. alata.* The latter has been reported to be highly adaptive to diverse environmental conditions (including the *in vitro* environment) due to its wider geographical distribution and adaptation (Shiwachi *et al.*, 1995).

Shoot vigour significantly reduced TUB in *D. rotundata*. This suggests competition for nutrients between meristems in vegetative shoot and tuber tissues, since yam tubers are of stem origin (Alhassan and Mantell, 1991). Tuber bulking continued even after leaf senescence due to assimilate translocation totally directed to tubers (Okezie *et al.,*1981). The level of nutrients in the medium should be high enough to allow for both shoot growth and tuberization, or permit a high level of tuberization that will compensate for the reduced number of nodes and hence propagules. Balogun *et al* (2006) reported a 50% increase in TUB of *D. rotundata* when sucrose concentration was increased from 50 to 80%.

Clustering into three groups based on TUB parameters (Table 2) revealed that about half of the accessions of both species had less than 10% tuberization as shown by membership of cluster 1. The other half had at least 50% MTZ. Thus, both species are amenable to microtuberization. In *D. alata,* about 60% of the accessions from each location (except Ghana) were low in TUB (cluster 1). All the Ghanaian accessions were medium in TUB. Togo, improved lines and Nigeria in a decreasing order, were most represented among the high microtuber formers. The prospects are high that if cultural conditions are optimized, TUB can be increased in more of the accessions of both species for use in germplasm conservation and exchange.

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Table 1: Means of microtuberization parameters in of *D. alata* and *D. rotundata*.

| | ***D. alata*** | | ***D. rotundata*** | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| %TUB | 27.81a | 0.00-100 | 37.02a | 0.00-100 |
| %ATUB | 23.98a | 0.00-100 | 27.20a | 0.00-100 |
| NTUB | 0.44a | 0.00-2.67 | 0.54a | 0.00-3.00 |
| %PNC | 36.55b | 0.00-100 | 57.60a | 0.00-100 |
| NPNC | 0.57b | 0.00-4.00 | 0.87a | 0.00-3.00 |
| RT | 1.85a | 1.00-3.00 | 1.76a | 1.00-3.00 |
| SHT | 2.39a | 1.12-3.00 | 2.23b | 1.00-3.00 |
| Age (Months) | 15.22 | 9.00-23.30 | 15.52 | 8.00-24.00 |

TUB: Microtuberization, ATUB: aerial TUB, NTUB: Number of MTs per plantlet, PNC:Primary nodal complex formation, NPNC: Number of primary nodal complexes per plantlet, RT: Root vigour on a scale of 1 - 3 (1: 1-20 roots, 2: 21-40 roots, 3: more than 40 roots per plantlet), SHT: Shoot vigour (1: 1-5 nodes, 2: 6-10 nodes, 3: more than 10 nodes per plantlet), Age of plantlet. S.E.: Standard error. Means in each row followed by the same letter(s) are not significantly different p=0.05

**Table 2. Mean microtuber yields and cluster groups of yam plantlets.**

| Cster | % TUB | NTUB | % ATUB | Geographic origin of accessions | | | | | | No. of Accessions |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Togo | BEN | CV | NG | GH | IMP. | |
| ***D. alata*** | | | | | | | | | | |
| 1 | 7.17 | 0.09 | 5.42 | 18 | 3 | 2 | 4 | 0 | 11 | 38 |
| 2 | 54.92 | 0.82 | 49.22 | 10 | 2 | 1 | 3 | 2 | 6 | 24 |
| 3 | 100 | 2.31 | 91.67 | 2 | 0 | 0 | 1 | 0 | 0 | 3 |
| Total | | | | 30 | 5 | 3 | 8 | 2 | 17 | 65 |
| ***D. rotundata*** | | | | | | | | | | |
| 1 | 6.80 | 0.08 | 2.81 | 73 | 3 | 12 | 36 | 3 | 20 | 147 |
| 2 | 49.38 | 0.66 | 31.79 | 42 | 4 | 8 | 28 | 1 | 26 | 110 |
| 3 | 87.51 | 1.42 | 78.05 | 23 | 0 | 8 | 15 | 0 | 17 | 63 |
| Total | | | | 138 | 7 | 28 | 79 | 4 | 63 | 320 |

BEN: Benin; CV: Cote d'Ivoire; NG: Nigeria; GH: Ghana; IMP.: Improved accessions. %TUB: Percent TUB, %ATUB: Percent aerial TUB, NTUB: Number of MTs per plantlet.

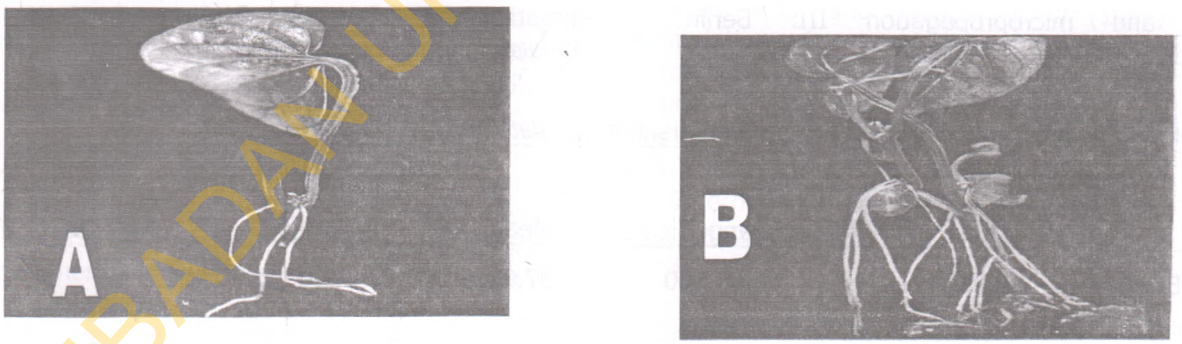


Plate 1: Microtuberization on a yam plantlet. A: Primary nodal complex, B: Mature microtuber