



BIOTECHNOLOGY

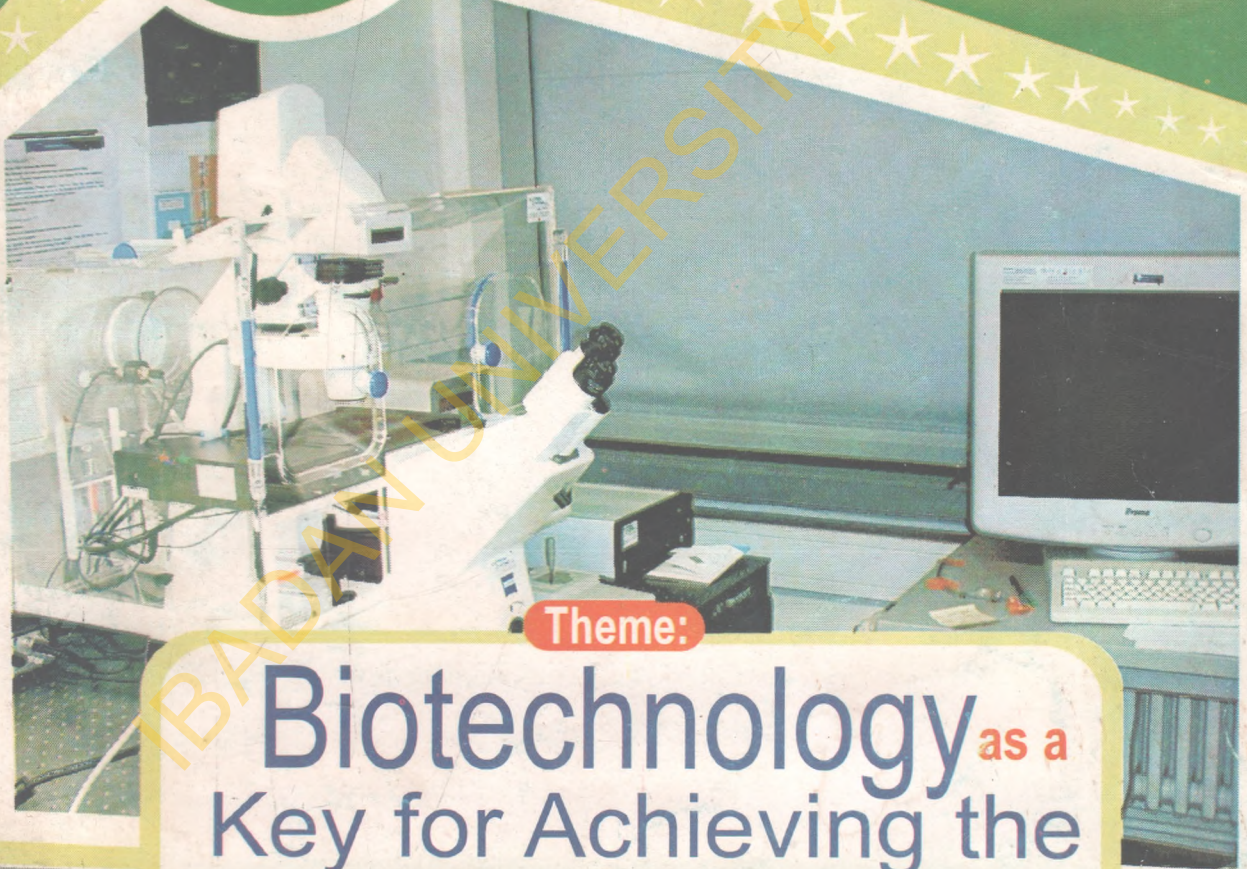
SOCIETY OF NIGERIA (BSN)

20th

Proceedings of the

ANNUAL CONFERENCE,

ABAKALIKI, 2007



Theme:

Biotechnology as a
Key for Achieving the

Millennium Development Goals (MDGS) In Nigeria

EDITORS: Ogunji, J.O., Ph.D
Ubi, B.E., Ph.D
Oselebe, H.O., Ph.D

COPYRIGHT © BIOTECHNOLOGY SOCIETY OF NIGERIA

All rights reserved. No part of this publication may be stored in a retrieval system or transmitted in any form or by any means, electronic, electrostatic, magnetic tape, mechanical, photocopying, recording or otherwise, without prior permission in writing from the Biotechnology Society of Nigeria.

ISBN NO: 97837772-7-0

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

Printed and Published By

Idealway Publishers
A Division Of RUGA ENT. NIG.
39 Nike Road
Abakpa, Enugu, Nigeria.
08064286101, 08036866716
e-mail idealway2008@yahoo.com.

TABLE OF CONTENTS

	Pages
WELCOME ADDRESS	10
GOODWILL MESSAGES	11
KEYNOTE PAPERS	16
Prof. Dora Akunyili, DG, Natl. Agency for Food & Drug Administration & Control (NAFDAC), Abuja	17
Prof. E. Ene-Obong, Dept. of Genetics & Biotechnology, University of Calabar, Calabar	24
Prof. A. Okpokwasili, Dept. of Microbiology, University of Port Harcourt, Rivers state	29
Prof. Olawole Olatunji, DG, Federal Institute of Industrial Research, Oshodi, Lagos	36
PROFESSOR J. E. ASIEGBU, Coordinator; South East Zonal Biotechnology Center (SEZBC) University Of Nigeria; Nsukka	39
SUBTHEME 1: Agricultural biotechnology as a catalyst for food security and accelerated economic development in Nigeria	43
Variations in microtuberization among local, improved and exotic yam accessions in Nigeria – Balogun, M. O., N. Q. Ng, I. Fawole & H. Kikuno	44
Macropropagation of <i>Musa</i> genotypes on non – soil media – Oselebe, H. O., Okporie, E.O. & Nwosimiri, K.	48
Apparent digestibility coefficients of differentially processed <i>mucuna cochinchinensis</i> (lour.) seed meal by hybrid catfish (<i>Heterobranchus longifilis</i> x <i>Clarias gariepinus</i>) fingerlings – Osuigwe, D. I. and Okoro, A. C.	55
Preliminary evaluation of housefly maggot meal (magma) as an alternative protein source in the diet of carp (<i>Cyprinus carpio</i> L.) – Ogunji, J. O., Slawski, H. & Kloas, W.	58
Effect of disease incidence and severity of bacterial of soft rot wilt on seven varieties of sweet pepper – Opara, E.	64
potential of communication process in enhancing adoption of genetically modified maize varieties: implications for food security in southeast Nigeria – Eze, S. O., & Okporie, E. O.	69
SUBTHEME 2: Harnessing biotechnology for healthcare delivery in Nigeria.	72
Isolation and characterization of natural microorganisms of <i>Lactuca Sativa</i> and <i>Brassicaceae</i> <i>Ou Brassica</i> : – Maduesike D. W., Olabode, A. O., Molokwu, J. U. J., Echeonwu G.O.N., Nwosu, C., Chukwedo T., Ogbonna, L. N., Okeke, I. & Kwatjel, J.S.	73
Preliminary antimicrobial activities of crude extracts of some medicinal plants on otitis media pathogen – Nwafor, I.B., Amadi, E.S., Nwaziri, A.A. & Nwuzo, A.C.	77
Effectiveness of probiotic <i>Lactobacillus species</i> in the treatment of dextran sulphate sodium (DSS) – induced ulcerative colitis in mice. – Umeh, C. N., Aniето, U. O. And Onyiorah. V.	80
The susceptibility of <i>Pseudomonas species</i> to some medicinal herbs – Mgbabu, C. N., Ogunji, J. O. & Ogbu, O.	85
Effects of leaf extracts of <i>Draceana aborea</i> L. and <i>Vitex doniana</i> Sweet (<i>V. cienkowskii kotschy</i> & <i>peyr.</i>) on the larvae of anopheles mosquito. – Nnamani, C. V., Oselebe, H. O. & Ogbonna, A. N.	90
Biotechnology a key tool to breakthrough in medical and veterinary research – Soetan, K. O. & Abatan, M. O.	93
SUBTHEME 3: Application of biotechnology tools in sustainable bio-resources utilization in Nigeria.	102
Extraction and characterization of oil from <i>Afzelia africana</i> (<i>Afzelia</i>) and <i>Aucuna sloanei</i>	103

Proc. 20th Annual Conf., Biotechnology Society of Nig (BSN) 14th – 17th Nov. 2007, Ebonyi State University Abakaliki, Nigeria

(horse-eye bean) from Ebonyi State – Ibiam, A., Agbor G. & Igwenyi, I. O.	
Proximate analysis of <i>Hibiscus sabdarriffa</i> L. (Zobo Plant) seed and leaf) – Nweke, F. N., Nworie, O. & Ebede, O. T.	107
Ethnobotany of indigeous leafy vegetables Of Izzi clan in Ebonyi State of Nigeria – Nnamani, C.V., Oselebe, H.O. & Okporie, E.O.	111
SUBTHEME 4/5: Research and development for biotechnology industries in Nigeria/ Biotechnology and clean environment	115
Fortified Mushroom Broth: An Acceptable Alternative Growth Medium for <i>Staphylococcus aureus</i> – Egonu, E. C. And Eke, L. O.	116
Investigation of Trace Element Levels in Water Sources At Abakaliki and its Environs – Edeogu, C.O., Ifemeji, J. C. & Afiukwa, J .N.	119
Determination of Protein Contents and Potassium Bromate Levels in Different Brands of Breads Sold in Abakaliki Metropolis, Ebonyi State – Ibiam, U., Oluigbo E., & Gwenyi, I. K.	122
The effects of crude oil and its products on blood glucose level of catfish – <i>Clarias gariepinus</i> – Nwamba, H. O.	127
Effects of the consortium of <i>Pseudomonas bacillus</i> and <i>Micrococcus</i> on polycyclic aromatic hydrocarbons in crude oil. – Ifeanyi, Virginia O.	129
Studies on the ability of some bacteria genera in the assimilation of heavy metals contained in crude oil. – Ifeanyi, Virginia O.	135
Conference Communiqué	140

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

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

⁴International 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 to 3.0 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.

ACKNOWLEDGEMENTS

The author is grateful to the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria, for providing a Visiting Research Studentship for the study.

REFERENCES

- Alhassan A.Y. and Mantell A.H. (1991). Manipulation of cultural factors to increase microtuber size and frequency in shoot cultures of food yam *Dioscorea alata* L. cv. Oriental Libson. In: Ofori F and Hahn SK (Eds.) Proceedings of the ninth Symposium of the International Society for Tropical Root crops. 20-26 October 1991, Accra, Ghana, pp. 342-348.
- Balogun, M. O., Fawole, I., Ng, S.Y.C., Ng, N.Q. Shiwachi, H. and Kikuno. H. (2006). Interactions among cultural factors in microtuberization of white yam *Dioscorea rotundata*. *Tropical Science*. 46(1): 55-59
- Daniel I. O., Ng N. Q., Tayo T. O. and Togun. A. O. (2002) Wet-cold preservation of West African yam (*Dioscorea* spp.) pollen. *J. of Agricultural Science*.138: 57-62.
- Daniel, I. O., Ng, N. Q., Tayo, T. O. and Togun, A. O. (2003) Storage of West African

yam (*Dioscorea* spp.) seeds: Modelling seed survival under controlled storage environment. *Seed Science and Technology*. 31:139-147.

Emehute, J. K. U., Ikotun T., Nwaüzor E. C. and Nwokocha, H. N. (1998) Crop Protection. In: Orkwor, G. C., Asiedu R and Ekanayake, I. J. (Eds.) Food yams. *Advances in research*. IITA / NRCRI. Pp. 143-186.

Ferguson, T. U. and Gumbs, S. A. (1976) Effect of soil compaction on leaf numbers and area, and tuber yield of white Libson yam. In: Cock J., MacIntyre, R. and Graham M. (Eds.) Proceedings of Fourth symposium of International Society of Tropical Root Crops. CIAT, Cali, Columbia. IDRC-080E. pp 89-93.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473-497.

Ng, S. Y. C. (1988) *In vitro* tuberization in white yam (*Dioscorea rotundata* Poir). *Plant Cell, Tissue and Organ Culture* 14: 121-128.

Ng S.Y.C. (1992) Micropropagation of white yam (*D. rotundata*), In: Bajaj YPS (Ed.) Biotechnology in Agriculture Forestry, High-tech and micropropagation III. Berlin Heidelberg, Springer -Ver lag, Vol 19 pp. 135-

159.

Ng S. Y. C. and Ng, N. Q. (1997) Germplasm conservation in food yams (*Dioscorea spp*): Constraints, Application and Future prospects. In: Razdan MK and Cocking EC (Eds.) Conservation of plant Genetic resources *in vitro*. Volume 1: General Aspects. Science publishers Inc. U.S.A. pp 257-286.

Okezie, C. E. A., Okonkwo S. N. C. and Nweke F. I. (1981) Growth pattern and growth analysis of the white Guinea yam raised from seed. In: Terry, E. R, Oduro K. A and Caveness F. (Eds.) Tropical Root Crops: Resear Strategies for the 1980s. IDRC. Ottawa, Canada. pp. 180-188.

Shiwachi, H., Chang K and Hayashi, M. (1995) Ecological and Morphological characterization and general evaluation of the introduced yams (*Dioscorea* spp.). *Bulletin of the Faculty of Agriculture, Kagoshima University*. 45: 1-17.

Sobulo, R. A. (1972). Studies on the white yam (*Dioscorea rotundata*) I. Growth analysis. *Experimental Agriculture* 8: 99-106.

Wickham, L. D., Passam, H. C. and Wilson, L. A. (1982) The origin, development and sprouting of bulbils in two *Dioscorea* species. *Annals of Botany* 50: 621-627.

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

	<i>D. alata</i>		<i>D. rotundata</i>	
	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.	
<i>D. alata</i>										
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
<i>D. rotundata</i>										
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