IITA
Transforming African Agriculture

CGIAR

YIIFSWA-II
Yams for Livelihoods

YIIFSWA Research Brief

Improving Yam Micropropagation

Series 1

Effects of basal medium and plant growth regulator regimes on meristem and nodal cultures in white yam (*D. rotundata*)

Adeola Ajayi[2], [1], Morufat Balogun[2], [1], Norbert Maroya[1], and Robert Asiedu[1]

[1]International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan
[2]Department of Crop Protection and Environmental Biology, University of Ibadan

YIIFSWA Research Brief
Improving Yam Micropropagation Series 1

Effects of basal medium and plant growth regulator regimes on meristem and nodal cultures in white yam (*D. rotundata*)

Adeola Ajayi[2], [1], Morufat Balogun[2], [1],
Norbert Maroya[1], and Robert Asiedu[1]

[1]International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan
[2]Department of Crop Protection and Environmental Biology, University of Ibadan

International Institute of Tropical Agriculture
PMB 5320
Oyo Road
Ibadan, Nigeria
www.iita.org

ISBN 978-978-8444-79-4

Printed in Nigeria by IITA

Correct citation: Adeola Ajayi, Morufat Balogun, Norbert Maroya, and Robert Asiedu. 2017. YIIFSWA Research Brief: Improving Yam Micropropagation Series 1 . Effects of basal medium and plant growth regulator regimes on meristem and nodal cultures in white yam (D. rotundata), International Institute of Tropical Agriculture, Ibadan, Nigeria. 22 pp.

Contents

Tables

Annexes

Abstract

Yam production is constrained by the limited availability and high cost of quality seed due to its slow vegetative propagation ratio. Micropropagation using nodal and meristem explants have been used to produce clean plantlets, with a 1:4 multiplication ratio for nodal explants and 1:1 ratio for meristem explants, the latter taking 6 to 24 months before plantlet regeneration. The effect of six hormone-free basal medium compositions, made of Murashige and Skoog (MS) medium and Gamborg's B5 medium modified mainly in terms of nitrate to ammonium ratio on micropropagation ratio of four genotypes of white yam were investigated using single nodes from *in vitro* established plantlets. A second experiment was conducted on the effect of different concentrations of NAA (naphthalene acetic acid), GA_3 (Gibberellic acid), BAP (benzyl amino-purine), and UP (Uniconazole-p) at different combinations on micropropagation, using a completely randomized design with 20 replicates. Data were collected on number of nodes per plantlet every four weeks for twelve weeks. ANOVA was done and means were separated at $P = 0.05$. The average number of nodes per plantlet after 12 weeks was 3.76 in conventional MS medium which was the best for all the genotypes except TDr 95/19177 in which there were no significant differences among the different nitrogen regimes. The B5 medium produced the lowest number of 1.85 nodes after 12 weeks of culture. Nitrogen use efficiency seems to differ among genotypes, necessitating the use of an ion-specific nutrient test for increased optimization. Medium containing 0.2 mg/l BAP and 0.1 g/l UP in MS medium produced the highest mean number of nodes (6.5) after 12 weeks. Both mineral nutrition and hormones were effective in increasing the yam micropropagation ratio.

Keywords: Yam micropropagation, nitrogen nutrition, growth regulators, Uniconazole-p

Purpose of study

To investigate effects of basal medium and plant growth regulator regimes on meristem and nodal culture of white yam with a view to identifying optimum regimes for increased micropropagation ratio.

Background

Yam (*Dioscorea* spp.) is an annual or perennial tuber bearing and climbing plant. White yam *(D. rotundata*), yellow yam (*D. cayenensis*), water yam (*D. alata*), trifoliate yam **(**D**.** *dumetorum*), aerial yam (*D. bulbifera*), and Chinese yam (*D. esculenta*) (FAO 2013) are the most important staples out of more than 600 species of yams existing. Yam production is however constrained by the limited availability and high cost of quality planting materials due to the slow vegetative propagation methods used. Diseases also accumulate over generations due to continuous use of infected planting materials. Seed yam accounts for up to 63% of production costs (Agbaje and Oyegbami 2005; Ironkwe 2005). Micropropagation using meristems followed by nodal culture has been used to produce disease-free yam plantlets. The growth regulator content of culture media can have a dramatic impact on plant regrowth (Balogun et al., 2014) while morphogenesis is influenced by the form and total amount of nitrogen provided in the medium (Leblay et al., 1991; George and Klerk 2008) and other minerals (Niedz and Even 2007). However, a 1:4 multiplication ratio was reported for yam nodal cultures (Polzin et al*.,* 2013) while meristem cultures were characterized by blackening and premature senescence (Ng and Hahn 1985; Poornima and Ravishankar 2007) and shoot induction but no plantlet regeneration in *D. rotundata* (Adeniyi et al. 2008). The most commonly used basal medium is Murashige and Skoog (MS), which is characterized by high levels of nitrogen in the forms of ammonium nitrate and potassium nitrate. This study tested effects of other basal medium formulations and growth regulator regimes on nodal and meristem cultures of white yam.

Table 1. Basal medium* regimes used in the study.

Macronutrient	B5 (mg/L)	MS (mg/L)	N1 (mg/L)	N2(mg/L)	N3(mg/L)
NH4NO3	—	1650	—	4950	4950
(NH4)2SO4	134	—	—	—	—
NaH2PO4.2H2O	150	—	—	—	—
KNO3	2528	1900	5700	—	5700
CaCl2.2H2O	440	440	440	440	440
MgSO4.7H2O	370	370	370	370	370
KH2PO4. 2H2O	170	170	170	170	170

*The genotypes TDr 95/18544, TDr 95/19177, TDr 95/19158, and TDr 89/02565 were each grown in the above five media.

Materials and Methods

In vitro plantlets of four genotypes of yam (TDr 95/18544, TDr 95/19177, TDr 95/19158, and TDr 89/02565) obtained from the IITA's Genetic Resources Center and six hormone-free basal medium types (MS, N1: modified MS having double concentration of KNO_3 and without $NH_4NO_{3;}$ N2: modified MS with double concentration of NH_4NO_3 without KNO_3; N3: modified MS with double concentration of both KNO_3 and $NH_4NO_{3;}$ MS alternative having MS but with no L-Cysteine; and B5: Gamborg B5 (Gamborg and Eveleigh 1968; basal medium), which differed in nitrate to ammonium ratio (Table 1) were used in the first experiment. In another experiment, Naphthalene acetic acid (NAA), Gibberellic acid (GA_3), Benzyl amino-purine (BAP), and Uniconazole-p (UP) at different combinations were tested on nodal and meristem cultures in a completely randomized design. For meristem cultures, plantlets were precultured for twenty-one days, their meristems were excised and cultured into four media types containing per liter 4.43 g MS, 30 g sucrose, 0.1 g Myo-Inositol, 0.02 g L-Cysteine, 0.2 mg BAP, and 0.08 g Adenine hemisulphate. Media I, II, III, and IV differed in that they contained 1 mg GA_3 and 0.1 g UP; 0.5 mg GA_3 and 0.1g UP; 0.1g UP; 0.1 mg NAA and 0.04 mg $GA_{3,}$ respectively, per liter of medium. The same media (but without adenine hemisulfate) were tested for nodal culture except that 0.1 mg NAA and 0.04 mg GA_3 in medium IV was substituted with 1 mg kinetin. Data were collected on number of nodes and buds per plantlet every four weeks for 12 weeks. ANOVA was done using SAS 9.4 and means were separated at P = 0.05.

Results and Discussion

The average number of nodes per plantlet after 12 weeks was 3.76 in MS medium which was either the highest or not significantly different from cysteine-free MS, N1 or N2 for all the genotypes except TDr 95/18544 in which MS was significantly (Table 2, Annex 1) higher than other regimes. The B5 and N3 media had the lowest number of nodes per plantlet with 1.85 and 2.33 nodes, respectively, after 12 weeks of culture. This may be due to the low quantity of reduced nitrogen ($(NH_4)_2SO_4$) and absence of nitrate in Gamborg B5 medium and too much total nitrogen in N3. Nitrogen use efficiency seems to differ among genotypes, making it necessary to test this in more genotypes while also investigating effects of specific ions on micropropagation. Among plant growth regulator regimes added to MS medium, 0.2 mg BAP + 0.1 g UP produced the highest mean number of 6.5 nodes (Table 3, Annex 2) after 12 weeks. However, the plantlets had short internodes.

Table 2. Number of nodes per plantlet in four yam genotypes cultured in different basal media at different ages after culturing

Genotypes	B5	MS	MS-Alt	N1	N2	N3	Mean (Age)
TDr 95/18544							
4 weeks	1.53	2.10	1.70	1.84	1.74	1.78	1.78c
8 weeks	1.55	3.85	1.75	3.25	3.42	3.06	2.81b
12 weeks	1.65	4.65	3.15	3.15	3.89	2.94	3.24a
Mean (medium)	1.58d	3.53a	2.20c	2.76b	3.02b	2.59b	
Mean (genotype)							2.61a
TDr 95/19177							
4 weeks	1.75	2.40	2.35	2.63	2.65	2.42	2.36c
8 weeks	1.80	2.85	2.75	2.68	3.20	2.47	2.63b
12 weeks	2.00	3.65	3.30	3.61	3.25	2.88	3.11a
Mean (medium)	1.85b	2.97a	2.80a	2.98a	3.03a	2.57a	
Mean (genotype)							2.70a
TDr 95/19158							
4 weeks	2.15	2.06	2.26	2.53	2.29	1.39	2.12b
8 weeks	1.84	2.22	2.65	2.42	2.16	1.81	2.20b
12 weeks	1.95	3.47	3.05	3.21	2.94	1.90	2.81a
Mean (medium)	1.98b	2.57a	2.66a	2.72a	2.44a	1.66c	
Mean (genotype)							2.36b
TDr 89/02565							
4 weeks	1.05	1.35	1.25	1.50	1.45	1.26	1.31c
8 weeks	1.15	2.42	1.85	2.70	2.15	1.58	1.97b
12 weeks	1.80	3.12	2.45	2.85	2.35	1.61	2.36a
Mean (medium)	1.33c	2.30a	1.85b	2.35a	1.96b	1.48c	
Mean (genotype)							1.88c

Means for medium, week and genotype followed by the same letters are not significantly different at $P = 0.05$
MS: Murashige and Skoog basal medium (1962); N1: MS modified with double the concentration of KNO_3 and without NH_4NO_3; N2: modified MS with double concentration of NH_4NO_3 without KNO_3; N3: modified MS with double concentration of both KNO_3 and NH_4NO_3; MS alternative having MS but with no L-Cysteine; B5: Gamborg B5 (Gamborg and Eveleigh, 1968) basal medium

Annexes

Annex 1.

Mean square values for effects basal medium type on the rate of micropropagation of yam nodal culture.

Source of Variation	DF	Number of nodes per plantlet
Rep	19	1.69
Genotype	3	46.66**
Age	2	106.31**
Medium	5	43.44**
Genotype*age	6	6.13**
Genotype*medium	15	4.56**
Medium*age	10	5.57**
Genotype*medium*age	30	1.38**
Error	1273	0.74

**Significant at $P = 0.01$.

Table 3. Number of nodes per plantlet in four yam genotypes cultured in MS medium at different growth regulator regimes

Genotype	Plant Growth regulator regimes				
TDr 95/18544	I	II	III	1V	Mean (Age)
4 weeks	2.65	2.30	3.05	1.75	2.44c
8 weeks	3.16	3.30	5.15	2.25	3.47b
12 weeks	8.11	6.18	8.46	3.45	6.35a
Mean (medium)	4.60b	3.81c	5.17a	2.48d	
Mean (genotype)					3.98a
TDr 95/19177					
4 weeks	2.20	1.95	2.50	1.50	2.04c
8 weeks	3.10	3.70	4.25	1.70	3.19b
12 weeks	5.45	6.20	6.25	3.40	5.33a
Mean (medium)	3.58a	3.95a	4.33a	2.20b	
Mean (genotype)					3.52b
TDr 95/19158					
4 weeks	1.65	1.65	2.32	2.05	1.91c
8 weeks	4.00	4.00	3.30	2.10	3.36b
12 weeks	6.48	5.90	5.17	4.10	5.43a
Mean (medium)	4.08a	3.85a	3.56a	2.75b	
Mean (genotype)					3.56b
TDr 89/02565					
4 weeks	2.45	1.90	2.05	1.80	2.05c
8 weeks	3.20	2.40	3.65	1.95	2.80b
12 weeks	3.75	2.95	6.50	3.20	4.10a
Mean (medium)	3.13b	2.42c	4.07a	2.32c	
Mean (genotype)					2.98c

Means for medium, week and genotype followed by the same letters are not significantly different at $P = 0.05$; Media I, II, III and IV: 1 mg GA_3 and 0.1 g UP; 0.5 mg GA_3 and 0.1 g UP; 0.1 g UP; 0.1 mg NAA and 0.04 mg GA_3, respectively per liter of medium.

Annex 2.

Mean square values from Analysis of variance for effects of plant growth regulators and genotype on rates of micropropagation of yam nodal culture.

Source of variation	Df	Number of nodes
Rep	19	3.11
Age	2	833.58**
Medium	3	157.75**
Genotype	3	50.14**
Medium*age	6	21.68**
Genotype*age	6	16.85**
Genotype*medium	9	15.30**
Genotype*medium*age	18	9.10**
Error	878	2.94
Corrected Total	945	

**Significant at $P = 0.01$.

A multiplication ratio of 3.76 achieved without hormones after 12 weeks of culture was reached only after 8 weeks with the use of growth regulators. The multiplication ratio of 1 plantlet to yield 6.5 new plantlets is near double the 1:4 hitherto reported for yam. Ezeibekwe et al. (2009) reported 4.8 nodes from a single node after 12 weeks of culture of *D. rotundata* in BAP combined with NAA medium. The effect of genotype was most significant for meristem regeneration into plantlets (Table 4, Annex 3). In TDr 95/19158, regeneration occurred after 12 weeks but in TDr 95/19177, up to six buds per meristem (Plate 1) were obtained in 12 weeks in media containing 0.2 mg BAP + 0.1 g UP with or without 1 mg GA_3. There was high variation among individual meristems, with some having a mean of 0, less than 1 bud and others having up to 3 (Table 4). Meristem excision is not limited to only the shoot apex of the mother plant. It is also done on the lateral branches causing variations in the metabolic activities and rate of plantlet regeneration from meristems.

Table 4. Number of buds regenerated per meristem in four yam genotypes cultured at four plant growth regulator regimes

Genotype	Plant growth regulator regimes				Mean (Age)
	I	II	III	IV	
TDr 95/18544					
4 weeks	0.00	0.00	0.00	0.00	0.00b
8 weeks	0.10	0.10	0.00	0.00	0.05a
12 weeks	0.00	0.00	0.10	0.00	0.03a
Mean (medium)	0.03a	0.04a	0.03a	0.00b	
Mean (genotype)					0.03c
TDr 95/19177					
4weeks	0.00	0.00	0.00	0.00	0.00c
8weeks	1.40	0.57	0.90	0.10	0.76b
12weeks	1.90	2.71	2.50	0.50	1.84a
Mean (medium)	1.10a	0.96b	1.13a	0.20c	0.84a
Mean (genotype)					
TDr 95/19158					
4 weeks	0.00	0.00	0.00	0.00	0.00
8 weeks	0.00	0.00	0.00	0.00	0.00
12 weeks	0.00	0.00	0.00	0.00	0.00
Mean (medium)	0.00	0.00	0.00	0.00	0.00
Mean (genotype)					0c
TDr 89/02565					
4weeks	0.00	0.00	0.00	0.00	0.00b
8weeks	0.50	0.63	0.78	0.44	0.589a
12weeks	0.625	0.33	1.00	0.375	0.59a
Mean (medium)	0.35b	0.30b	0.57a	0.26c	
Mean (genotype)					0.37b

Means in each row followed by the same letters are not significantly different at $P = 0.05$
Media I, II, III and IV: 1 mg GA_3 and 0.1 g UP; 0.5 mg GA_3 and 0.1 g UP; 0.1 g UP; 0.1 mg NAA and 0.04 mg GA_3, respectively per liter of medium

Annex 3.

Mean square values from analysis of variance for effects of Plant growth regulators (PGR) and genotype on rates of plantlet regeneration from yam meristems.

Source of variation	Df	Number of buds
Age	2	14.28**
PGR	3	2.3
Rep	9	1.03
Genotype	3	18.57**
Age*PGR	6	0.97
Genotype*age	6	7.79**
Genotype*PGR	9	1.46
Genotype*age*PGR	17	0.81
Error	387	0.46
Corrected Total	442	.

**Significant at $P = 0.01$.

1

2

3

4

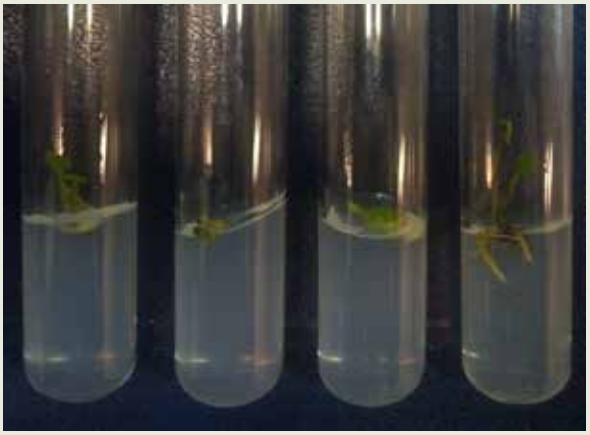

Plate 1: (1,2,3,4): Multiple shoots from meristem of TDr 95/19177 in medium I, II, III (see Table 3 for definitions), respectively and regenerated plantlets from meristem of TDr 95/19177 transferred to multiplication medium.

Conclusion and way forward

Up to six new nodes and six buds were produced from a single node and meristem, respectively, in 12 weeks. Buds regenerated into plantlets 2 months later, making a total of 5 months. Taking this plantlet through two cycles of nodal propagation of 3 months each at a ratio of 1:6, 36 clean plantlets will be produced in another 6 months. Basal medium and growth regulator increased micropropagation ratio although this varied with genotype. Protocols should be optimized for end-user preferred genotypes. Work is also ongoing to investigate the effects of other cultural factors for better performance.

Acknowledgement

YIIFSWA colleagues and team members.

References

Adeniyi, O.J., V.O. Adetimirin, I. Ingelbrecht, and R. Asiedu. 2008. Shoot and plantlet regeneration from meristems of *Dioscorea rotundata* Poir and *Dioscorea alata* L. African Journal of Biotechnology 7(8): 1003–1008.

Agbaje, G.O. and A. Oyegbami. 2005 .Survey on the adoption of yam minisett technology in South-Western Nigeria. Journal of Food Agriculture and Environment 3(2): 134–137.

Balogun, M.O. S.Y. Ng, I. Fawole, H. Shiwachi, and H. Kikuno. 2014. Relative effects of Uniconazole–p and other growth regulators on micropropagation ratio of yam (*Dioscorea* spp.) plantlets at different growth phases. Journal of Root Crops 40(2): 5–11.

Ezeibekwe, I.O., C.L. Ezenwaka, F. N. Mbagwu and C.I.N. Unamba. 2009. Effects of combination of different levels of auxin (NAA) and Cytokinin (BAP) on in vitro propagation of *Dioscorea rotundata* L. (White Yam). Journal of Molecular Genetics 1(2−4): 18−22.

FAO. 2013. Food and Agriculture Organization of the United Nations. www.fao.org/statistics/en/ FAO, Rome

Gamborg, O.L. and D.E. Eveleigh. 1968. Culture methods and detection of glucanases in suspension cultures of wheat and barley. Canadian Journal of Biochemistry 46: 417–421.

George, E. and G.J. Klerk. 2008. The components of plant tissue culture media I: macro- and micro-nutrients. Pages 65–113 *in* Plant Propagation by Tissue Culture, edited by E. George, A.H. Micheal, and G.J. Klerk. 3rd Edition. Springer, The Netherlands.

Ironkwe, A.G. 2005. Adoption of yam minisett technology by women farmers in Abia State, Nigeria. Unpublished MSc. Thesis in the Department of Rural Sociology and Extension, Michael Okpara University of Agriculture, Umudike, Abia State. Pp 75–81.

Murashige, T. and F.J. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiologia Plantarum 15: 473–497.

Leblay, C., E. Chevreau, and L.M. Raboin. 1991. Adventitious shoot regeneration from leaves of several pear cultivars (*Pyrus communis* L.). Plant Cell, Tissue and Organ Culture. 25: 99–105.

Niedz, R.P. and T.J. Even. 2007. Regulating plant tissue growth by mineral nutrition. In vitro Cellular and Developmental Biology-Plant, 43: 370–381.

Ng, S.Y. and S.K. Hahn. 1985. Application of tissue culture to tuber crops at IITA. Pages 29–38 *in* Biotechnology in International Research, IRRI, Manila.

Polzin, F, I. Sylvestre, E. Dechampe P. Ilbert, H. Etienne, and F. Engelmann (2013). Effect of activated charcoal on multiplication of African Yam *(Dioscorea cayenensis–rotundata)* nodal segments using a temporary immersion bioreactor (RITA). In vitro Cellular and.Developmental Biology-Plant 50(2) 210−216. DOI 10.1007/s11627–01309552–6.

Poornima, G.N. and R.V. Ravishankar. 2007. *In vitro* propagation of white yams, *Dioscorea oppositifolia* (Linn) and *Dioscorea pentaphylla* (Linn.). African Journal of Biotechnology. 6 (20): 2348–2352.