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EFFICIENT *IN VITRO* MICROPROPAGATION OF GREATER YAM (*DIOSCOREA ALATA* L. CV. HINJILICATU) THROUGH NODAL VINE EXPLANT

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SUMMARY

Nodal vine segments from 45 days old plants of *Dioscorea alata* L. (cv. Hinjilicatu) were cultured on Murashige and Skoog's (MS) medium supplemented with different concentration and combinations of BAP and Kn along with NAA. Explants cultured in MS basal medium supplemented with 2.0 mg/l Kn +1.0 mg BAP + 0.5 mg/l NAA showed highest rate of shoot multiplication. When *in vitro* shoots were inoculated on to the half-strength MS basal media supplemented with 2.0 mg/l NAA and rooting was more profuse. Rooted shoots were transplanted in the green house for hardening and their survival was 90% in the field condition without any morphological variation.

Key words: **Auxin, cytokinin, *Dioscorea*, micropropagation, nodal vine cuttings.**

INTRODUCTION

Among the tropical tuber crop yam occupy a very important position for their food, nutritional, medicinal and industrial significance. Considering the importance of yam the global research work started from the beginning of 20th century. The most notable work is collection and documentation of yams by Burkill (1960). He reported nearly 600 species of *Dioscorea*. The greater yam *Dioscorea alata* L. is a monocot belong to family *Dioscoreaceae*. It is consumed in almost all tropical and subtropical countries since it gives highest yield (Han *et al*.2000), produce corms and bulbils that are of economically important .The edible portion of most of the species are chiefly rich in carbohydrate along with good amount of protein and minerals (Mantell and Hugo 1989). They also play an enviable role in tropical food economy, especially of South Africa where it is taken as a staple food with a fairly per capita consumption of 0.5 to 1Kg (Ammirato 1976). In India tubers of *D. alata* were consumed mostly in Southern and North eastern states, and their economic importance is higher than the potato in some areas.

In Orissa thirteen species of *Dioscorea* have been reported (Maharana 1993) of which *D. alata* variety Hinjilicatu is the most abundant cultivated species of the state. The cultivated forms of the vegitatively propagated crop have a large genetic diversity .The basic propagation system of *Dioscorea* species is by tuber-seeds, a tuber fragment that sprouts each year, developing a new tuber, which is commercially used as vegetable in the local market. The long period required for obtaining usable tubers, the absence of viable seeds, the post harvest losses and the unknown life cycle are some of the factors that limit the economic exploitation of native species of *Dioscorea* found in Orissa (Chu and Ribeiro 2002,Tschannen *et al*. 2005). Although, there are reports on the *in vitro* micro propagation of some of the food yams such as *D. rotunda* , *D. alata* (Mantell and Hugo

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1989) and *D. trifida* using meristems (Saleil *et al*.1990). However, so far in the available literature no information was available on the micropropagation of *D. alata* variety Hinjilicatu of the native species of Orissa (Malurie *et al* .1995, Yuan *et al*. 2005). The main goal of this present study was to establish optimal conditions for *in vitro* propagation of *D. alata* L. (cv. Hinjilicatu), through nodal vine cuttings for obtaining large scale diseases free planting material in off season year round cultivation of the variety for the benefit of the farmers.

MATERIALS AND METHODS

Explant source: Healthy vines with active buds were collected from 45 days old plants of *D. alata* L. (cv. Hinjilicatu) maintained in the experimental garden of P.G. department of Botany Utkal University and were cut in to 1.5 cm to 2.0 cm length with single node intact . These nodal vine cuttings were washed with 5% (v/v) detergent solution (Teepol, Qualigen, Mumbai, India) for 10 minute and rinsed several times with running tap water. These active nodal cuttings were surface sterilized with bavistin 0.3 % (w/v) and streptomycin 0.2% (w/v) for 10 minutes each and then washed with sterile distilled water. In the laminar chamber the nodal segments were again treated with 70% alcohol for 30 second to one minute followed by 0.1% (w/v) mercuric chloride ($HgCl_2$) treatment for 5 minutes. Finally, the nodal cut vines were washed thoroughly 3 to 4 times with sterile distilled water and dried with sterile blotting paper and used as explants for raising *in vitro* cultures.

Culture medium and condition: The sterilized blotted explants were inoculated on to the Murashige and Skoog's (1962) agar-gelled medium fortified with various concentrations/combinations of growth hormones. For shoot induction, the medium was supplemented with 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l Kn + 0.25, 0.5, 1.0, 1.5 mg/l BAP and 0.25, 0.5, 1.0 mg/l α-naphthalene acetic acid (NAA), either individually or in combination with ascorbic acid 100 mg/l as an antioxidant. For root induction *in vitro* raised shoots measuring about 4–5 cm grown in multiplication medium were excised and cultured on half-strength MS basal medium supplemented with either NAA (α-naphthalene acetic acid) or IBA (indole 3-butyricacid) at the concentration of 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l. The pH of the medium was adjusted to 5.8 before autoclaving at 1.04 kg/cm^{-2} pressure and 121°C temperature for 15 - 20 minute. All cultures were maintained in 16 h photoperiod (cool, white fluorescent light 30µmol $m^{-2}s^{-1}$) at 25 ± 3°C in diffused light under 60 - 70% relative humidity in the culture room. Each treatment comprised of 20 culture tubes and the experiment was repeated thrice. The cultures were maintained by regular subcultures at 4 week interval on fresh medium of the same composition.

Acclimatization: Rooted micropropagules were removed from the culture tube and the roots were washed under running tap water to remove agar .The plantlets were transferred to sterile poly pots (small plastic cups) containing vermiculite (TAMIN ,India) and maintained inside the growth chamber at 28^{0}C and 70-80% relative humidity. After three weeks they were transplanted to earthen pots containing mixture of soil + sand + manure (FYM) in 1:1:1 ratio and kept under shade house for a period of three weeks for acclimatization. The potted plants were irrigated with Hoagland's solution every 3 days for period of 3 weeks. Survival rate of the plantlets were recorded after 3 weeks

Observation of cultures and presentation of results: A set of twenty cultures tubes were used per treatment and each experiment was repeated at least three times. The data pertaining to mean percentage of cultures showing response, number of shoots/culture and mean percentage of rooting were statistically analysed by the post-hoc multiple comparison test at the $P < 0.05$ level of significance (Marascuilo and Mc Sweency 1977)

RESULTS AND DISCUSSION

Shoot proliferation and multiplication: The response of *D. alata* L. (cv. Hinjilicatu) nodal vine explants cultured on different shoot proliferation media over a period of six weeks is presented in Table 1, culture medium devoid of growth regulators (control) failed to stimulate the bud break response in the cultured explants even when the cultures were maintained beyond the normal observation period of four weeks. MS medium with growth regulator supplements produced better results in terms of percentage explants response, shoots /explant, average shoot length and average number of nodes produced per shoot. In such media combinations

Table 1. Shoot formation in nodal explants of *Dioscorea alata* L.(cv. Hinjilicatu) cultured on semisolid MS medium supplemented with various concentration of kinetin+ BAP and NAA.

Treatments	Hormonal supplements (mg/l)			% of Explant response	Days to bud break	Mean No of shoot/explant ±S.E.	Mean shoot length (cm) ±S.E.	Mean No of nodes/shoot ±S.E.
	Kinetin	BAP	NAA					
T1	0	0	0	-	-	-	-	-
T2	0.25	0.25	0	5	12-15	1.2 ±0.04+	1.0 ±0.09+	0.88 ±0.07+
T3	0.5	0.25	0	10	12-15	1.2 ±0.09+	1.1 ±0.12+	0.96 ±0.02+
T4	1.0	0.25	0	10	12-15	1.3 ±0.09+	1.4 ±0.09+	1.0 ±0.09+
T5	1.5	0.25	0	15	12-15	1.5 ±0.21	1.6 ±0.18	1.0 ±0.04
T6	2.0	0.25	0	20	12-15	2.0 ± 0.47	2.0 ± 0.23	1.0 ± 0.21
T7	2.5	0.25	0	20	10-12	2.5 ± 0.12	2.2 ± 0.30	1.5 ± 0.35
T8	3.0	0.25	0	20	12-15	2.0 ± 0.33+	2.2 ± 0.21+	2.0 ± 0.29+
T9	0.25	0.5	0.25	30	10-12	3.0 ± 0.16	3.2 ± 0.20	2.2 ± 0.18
T10	0.5	0.5	0.25	40	10-12	3.2 ±0.14	3.3 ±0.29	2.2 ±0.16
T11	1.0	0.5	0.25	50	8-10	3.5 ± 0.09+	3.5 ± 0.42+	2.3 ± 0.24+
T12	1.5	0.5	0.25	60	8-10	5.5 ± 0.12	4.2 ± 0.21	2.5 ± 0.12
T13	2.0	0.5	0.25	70	6-8	7.5 ±0.41	5.2 ±0.33	3.5 ±0.32
T14	2.5	0.5	0.25	60	7-9	3.2 ± 0.14	2.6 ± 0.39	1.5 ± 0.12
T15	3.0	0.5	0.25	40	8-10	2.5±0.18+	2.3±0.34+	1.4±0.16+
T16	0.25	1.0	0.5	25	10-12	3.0 ± 0.52	2.4 ±0.25	1.0 ± 0.30
T17	0.5	1.0	0.5	30	10-12	3.6 ± 0.30+	3.0 ± 0.12+	2.2 ± 0.20+
T18	1.0	1.0	0.5	40	8-10	4.0 ± 0.09+	3.3 ± 0.16+	2.0 ± 0.32+
T19	1.5	1.0	0.5	50	7-9	4.3 ±0.14	3.8 ±0.16	2.4 ±0.24
T20	2.0	1.0	0.5	90	6-8	12.5 ± 0.51	6.4 ± 0.24	4.5 ± 0.24
T21	2.5	1.0	0.5	50	10-12	2.6 ± 0.31+	2.2 ± 0.18+	1.0 ± 0.14+
T22	3.0	1.0	0.5	45	12-15	2.4±0.23	2.3 ± 0.20+	1.0± 0.09+

[20 culture tubes per treatment; repeated thrice. Means are calculated by post-hoc multiple comparisons tests at $P < 0.05$ level of significance ,+ callusing at the basal end , S.E.: standard error of mean].

bud break was noticed within 6-8 days of culture (Fig. 1b, c, d, Table 1). Of the combination tested MS +Kinetin (2.0 mg/l) + BAP (1.0 mg/l) + NAA (0.5 mg/l) elicited optimal response with an average of shoot lets (12.5 ± 0.51) (Fig. 1c, Table 1) with a mean shoot length of 6.4 ± 0.24 cm per explant. The second best shoot multiplication 7.5 ± 0.41 was obtained in the medium MS+Kinetin (2.0 mg/l) +BAP (0.5 mg/l) + NAA (0.25mg/l) with a mean shoot length of 5.2 ± 0.33cm. Higher concentration of Kinetin (2.5 mg/l) +BAP (1mg/l) with NAA (0.5 mg/l) showed callusing explants with less number of shoots. In such cultures shoots were stunted with a mean shoot length of 2.2 ± 0.18 cm, some produced a compact callus at the base of the explants. Prolonged culture on the proliferation and multiplication media resulted in the blackening of the basal ends of the developing shoots.

Induction of rooting from micro shoots: The well developed elongated shoots were excised from shoot clump and transferred to half strength MS medium containing NAA or IBA. The rooting responses of

shoots in terms of rooting percentage , days required for root initiation, mean number of roots/shoot and mean root growth over a period of three weeks were different on different media (Fig. 1e, Table 2).There was no rooting in case of shoot planted on auxin free basal medium (control). Similarly, at lower level of NAA (0.5 mg/l) treatments, there was hardly any rooting in the cultured shoots during the 4 weeks of observation. However NAA at higher concentration (1.5 & 2.0 mg/l) and IBA at all concentration responded well. Rooting was highest in $^{1}/_{2}$ MS+2.0 mg/l NAA where about 90 % cultures responded with an average number of 6.5 ± 0.30 roots per plantlet and an average root length 5.0 ± 0.16 cm (Fig. 1e, Table 2). The second highest response (70%) was recorded at 1.5 mg/l of NAA. Emergence of root primordia was observed from the shoot base from day 6 to 8 days after inoculation followed by a rapid root growth. NAA was found more effective than IBA in induction of rooting as days required to induce rooting was only 6-8 as against 10 to15 in case of IBA.

Acclimatization and field establishment: About 90% of the rooted plantlets exhibited establishment in the greenhouse within 2-3 weeks of transfer. The plants grew well and attained 6-8 cm height within 4 weeks of transfer (Fig. 1d). The acclimatized plants were established in the field condition and grew normally without any morphological variation.

In vitro shooting: The dependence of cultured explants on bud break response and shoot multiplication has already been established and extensively discussed (George and Sherrington 1984). This has also been recently reported in the case of micropropagation of other Yams like *D. composita* (Alizadeh *et al*.1998), *D. floribunda* (Sengupta *et al*.1984), *D. batatas* (Koda and

Table 2. Influence of different levels of NAA and IBA on rooting response of *in vitro* generated shoot lets of *Dioscorea alata* L. (cv. Hinjilicatu)

Treatments	Growth regulators augmented with1/2 strength MS basal medium (mg/l)		% of rooting response	Days to root initiation	Mean root numbers ±S.E.	Mean root length (cm) ±S.E.
	NAA	IBA				
T1	0	0	-	-	-	-
T2	0.25	0	-	-	-	-
T3	0.5	0	25	8-10	2 ± 0.21+	2.0 ± 0.32+
T4	1.0	0	45	8-10	4.2 ± 0.32+	2.2 ± 0.18+
T5	1.5	0	70	6-8	4.5 ± 0.12	3.5 ± 0.12
T6	2.0	0	90	6-8	6.5 ± 0.30	5.0 ± 0.16
T7	2.5	0	35	10-12	2.2 ±0.32+	2.3 ±0.14+
T8	3.0	0	30	10-12	1.4 ±0.24+	1.7 ±0.28+
T9	0	0.25	20	12-15	1.0 ±0.14+	2.3 ± 0.19+
T10	0	0.50	35	12-15	1.0 ± 0.31+	2.4 ± 0.04+
T11	0	1.0	50	10-15	2.1 ± 0.24	2.5 ± 0.14
T12	0	1.5	60	10-12	2.2 ±0.32	2.6 ±0.18
T13	0	2.0	70	10-12	2.4 ± 0.21	3.0 ± 0.24
T14	0	2.5	50	10-15	1.8 ±0.38+	2.2 ±0.14+
T15	0	3.0	40	10-15	1.6 ±0.14+	1.5 ±0.14+

[20 culture tubes per treatment; repeated thrice. Means are calculated by post-hoc multiple comparisons tests at $P < 0.05$ level of significance, + basal callusing at the cut end, S.E.:-standard error of mean]

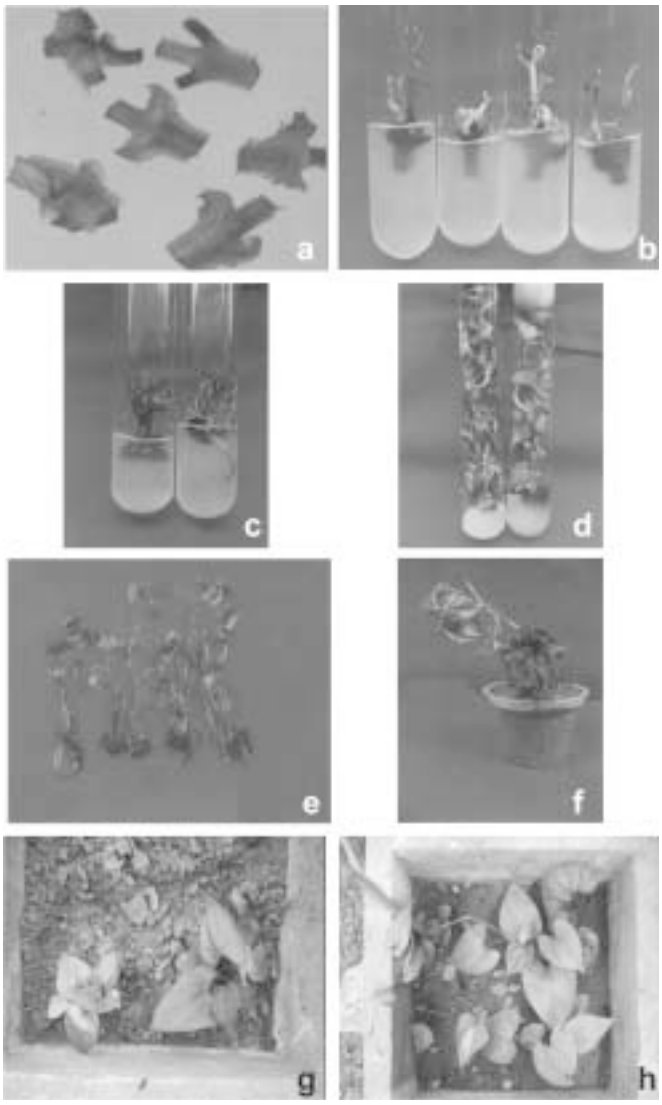

Fig. 1(a-h). ***In vitro*** **regeneration and plant establishment of** ***D. alata*** **L. (cv. Hinjilicatu), (a) nodal vine explant ready for inoculation, (b) shoot emergence in MS+ 2 mg/l Kn + 1.0 mg/l BAP + 0.5 mg/l NAA+100 mg/l ascorbic acid, (c) multiple shoot emergence in MS+2mg/l Kn+1mg/l.0BAP + 0.5 mg/l NAA + 100 mg/l vitamin C, (d) shoots rooted in 1/2MS+NAA (2.0 mg/l), (e) micropropagules ready for hardening, (f) hardening of rooted plant lets in plastic pots, (g) 20 days** ***in vitro*** **plantlet in cement pot, (h) growth of** ***in vitro*** **generated plantlet after 40 days of transplantation.**

Kikuta 1991) and *D. abyssinica* (Martine and Cappadocia 1991). In the present study, nodal vine explants of *D. alata* L. (cv. Hinjilicatu) showed significantly higher response in the medium with the combination of Kinetin (2.0 mg/l)+BAP (1.0 mg/l) + NAA (0.5 mg/l).The quality of shoots and the over all growth response in terms of average shoot length was better at this growth regulator combination. A comparatively lower response was recoded when BAP or Kinetin was added alone in the medium. Review of literature indicates that the addition of either IAA or NAA in the culture medium improved shoot growth. It has been reported that when *Spathiphyllum floribundam* cultured on media with BA supplement alone, a limited proliferation of explants with a maximum of average of 1.8 shoots per cultured explants was observed ,while addition of IAA produced an average number of 11.6 shoots per explant (Ramierez-Magon *et al*. 2001). In our study two cytokinins was taken for higher shoot multiplication. Some authors also suggested that the combination of two cytokinins were needed for better production of multiple shoots on *Aristolochia bracteolate* (Rameshree *et al.*1994), *Lavandula* species (Jordan *et al*. 1998).

In vitro rooting and plant establishment: Production of plantlets with profuse rooting *in vitro* is important for successful establishment of regenerated plants in soil (Ohyam 1970). The auxins NAA and IBA were used singly to induce rooting from *in vitro* raised shootlets. A range of concentration was tested (0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) for rooting. In the present study $^{1}/_{2}$ strength MS basal medium and the two different auxins (NAA and IBA) were tried, the superior results on rooting were obtained on half strength MS with NAA (2.0 mg/l) than IBA (2.0 mg/l). Our observations are in accordance with the result of Chen *et al*. (2003) in *D. zingiberenesis*. The well rooted plantlets were transferred to plastic cups containing vermiculite for hardening and kept under controlled condition ((Fig. 1f, g & h). Plants started producing fresh shoots and roots after one week of transplanting. Later they were transferred to the field condition and the survival rate was 90% (Sedigeh *et al*. 1998).The efficient micropropagation technique described here may be highly useful for raising disease free quality planting material of *D. alata* L. (cv. Hinjilicatu) for commercial and off season cultivation which not only help in socioeconomic development of the farmers but also fulfill the food value and market demand of the species.

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REFERENCES

Alizadeh, S., Mantell, S.H. and Viana, A.M. (1998). *In vitro* shoot culture and microtuber induction in the steroid yam *Dioscorea composite* Hemsl. *Plant Cell Tiss Organ Cult.* **53:** 107-112.

Ammirato, P.V. (1976). Hormonal control of tuber formation in cultured axillary buds of *D. bulbifera* and *D. alata*. *Plant Physiol*. (suppl). 57-66.

Burkill, I.H. (1960). The organography and the evolution of the *Dioscoreaceae*, the family of the yams. *J. Linn. Soc.* (London) **56:** 319-412.

Chen, Y.Q., Fan, J.Y., Yi, F., Luo, Z.X. and Fu, Y.S. (2003). Rapid clonal propagation of *Dioscorea zingiberensis*. *Plant Cell Tiss Org. Cult*. **73:** 75-80.

Chu, E.P. and Ribeiro, R.D.L.F. (2002). Growth and carbohydrate changes in shoot cultures of *Dioscorea* species as influenced by photoperiod, exogenous sucrose and cytokinin concentrations. *Plant Cell Tiss Organ Cult*. **70:** 241-249.

George, F.E. and Sherrington, P.D. (1984). Plant Propagation by Tissue Culture-Hand Book and Directory of Commercial Laboratories. Exegetics Ltd, Eversly U.K.

Han, M., Yang, L.M. and YH, Yu. (2000). A study on *Dioscorea nipponica* distinguishing age and cutting propagation. *J. Jilin Agr. Univ*. **22:** 65-68.

Jordan, A.M., Calvo, M.C. and Seguraj (1998). Micro propagation of adult *Lavandula dentate* plants *J. Horti. Sci. & Biotech*. **73:** 93-96.

Koda, Y. and Kikuta, Y. (1991). Possible in volvement of jasmonic acid in tuberization of yam plants. *Plant Physiol*. **32:** 629-633.

Malaurie, B., Thouvenel, J.C. and Pungu, O. (1995b). Influence of meristem-tip size and location on morphological development in *D. cayenensis–D. rotundata complex* 'Grosse Caille' and one genotype of *D. praehensilis*. *Plant Cell Tiss Organ Cult*. **42:** 215-218.

Maharana, T. (1993). Productivity and quality study in *Dioscorea* of Orissa. Ph.D thesis Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India.

Mantell, S.H. and Hugo, S.A. (1989). Effects of photoperiod, mineral strength, inorganic ammonium, sucrose and cytokinin on root, shoot and micro tuber development in shoot cultures of *Dioscorea alata* L and *D. bulbifera* L yams. *Plant Cell Tiss Org Cult*. **16:** 23-37.

Marascuilo, L.A. and McSweeney, M. (1977). Post-hoc multiple comparisons in sample preparations for test of homogenesity, In: M. McSweeney and L.A. Marascuilo (eds.), Non-parametric and Distribution Free Methods the Social Sciences, pp. 141-147. Books/Cole, U.S.A.

Martine, J. and Cappadocia, M. (1991). *In vitro* tuberization in *Dioscorea alata* L. Brazofuerte and Florido and *D. abyssinica* Hoch. *Plant Cell Tiss Org Cult*. **26:** 147-152.

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

Ohyama, K. (1970). Tissue culture in mulberry tree. *Jpn. Agric. Res. Quart*. **5:** 30-34.

Ramirez-Magon, R., Borodanenko, A., Barrera-Guerra, J.L. and OchoaAlejo, N. (2001). Shoot number and shoot size as affected by growth regulators in *in vitro* cultures of *Spathyphyllum floribundum* L. *Scin. Horti*. **89:** 227-236.

Remeshree, A.B., Hariharan, M. and Unnikrishana, K. (1994). Micropropagation and callus induction of *Aristolochia bracteolate* Lam. A medicinal plant. *Phytol*. **44:** 247-252.

Saleil, V.L., Degras, L. and Jonard, R. (1990). Obtention de plantes indemnes du virus de la mosaique de l'igname (YMV) parla culture *invitro* des apex chez l'igname ame´ricaine *Dioscorea trifida* L. *Agronomie* **10:** 605-615.

Sedigeh, A., Mantell, S.H. and Viana, A.M. (1998). *In vitro* shoot culture and microtuber induction in the steroid yam *Dioscorea composite* Hemsl. *Plant Cell Tiss Org Cult*. **53:** 107-112.

Sengupta, J., Mitra, G.C. and Sharma, A.K. (1984). Organogenesis and tuberization in cultures of *Dioscorea floribunda*. *Plant Cell Tissue Organ Cult*. **3:** 325-331.

Tschannen, A.B., Escher, F. and Stamp, P. (2005). Post-harvest treatment of seed tubers with gibberellic acid and field performance of yam (*Dioscorea cayenensis-rotundata*) in Ivory Coast. *Expl Agric*. **41:** 175-186.

Yuan, S., Yan, Y.C. and. Lin, H.H. (2005). Plant regeneration through somatic embryogenesis from callus cultures of *Dioscorea zingiberensis*. *Plant Cell Tiss Org Cult*. **80:** 157-161.