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In Vitro Growth Response of Different Varieties of Greater Yam *(Dioscorea alata* L.*)*

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Abstract

A simple, reproducible and efficient micropropagation protocol was developed for three different varieties of greater yam (*Dioscorea alata* L.) *viz*., Sree Roopa, Sree Keerthi and Sree Shilpa using nodal segments for axillary shoot proliferation at Central Tuber Crops Research Institute, Thiruvananthapuram, India. The effect of different explants, surface sterilants and different combinations and concentrations of Benzyl Adenine (BA) and Napthalene Acetic Acid (NAA) on different parameters *viz*., number of shoots, shoot length, number of roots, root length, number of leaves and number of nodal meristems were evaluated. The use of nodes from young, vigorously growing vines of sprouted tubers as well as 4 - 8 nodes from the tip of field grown plants proved to be the best explant source which gave a quick response. Minimal risk of contamination was observed with 0.08% mercuric chloride ($HgCl_2$), which was found to be an effective surface sterilant. For *in vitro* regeneration, MS media with 0.5 mg l^{-1} NAA and 0.75 mg l^{-1} BA with 0.1% activated charcoal was found to be the best. A high percentage of shoots were seen rooted in the same medium containing 0.1% activated charcoal (92%). Regenerated plants were successfully hardened and transplanted. About 90% of plantlets survived under net house conditions.

Key words: *Dioscorea alata*, micropropagation, plant regeneration, shoot induction, root development

Introduction

Greater yam or water yam (*Dioscorea alata* L.*)* of the family *Dioscoreaceae* is a tuber crop widely grown in the tropical and subtropical regions of the world. It has high production potential and plays an important role in food security. India holds a rich genetic diversity of tropical root and tuber crops, especially the yam, *Dioscorea* species (Edison et al., 2006). Production and distribution of healthy planting material of yams is one of the mandates of Central Tuber Crops Research Institute (CTCRI), Kerala, India. Some of the released varieties of *D. alata* which are mainly cultivated for edible purpose include Sree Roopa, Sree Karthika, Orissa Elite, Sree Keerthi and Sree Shilpa. The tubers of *D. alata* varieties produced at CTCRI are being distributed on a large scale to farmers and State Horticultural Departments throughout the country. But the tubers carry the risks of build-up of soil-borne diseases (fungi and nematodes) and transfer of yam diseases among plants within the propagation beds. Moreover, continuous vegetative propagation normally accumulates pathogens that cause degeneration of varieties and eventually loss of yield and degradation of quality. Hence, there is a need for development of practical *in vitro* approaches for cleaning the vegetative propagules to prevent disease spread.

High frequency regeneration of plants from *in vitro* culture is a pre-requisite for successful application of tissue culture technique for crop improvement. It has been noted in several reports of nodal cultures of yams that the shoot multiplication rates varied greatly among

different species and varieties *ie.*, 3 nodes after 5 weeks in *D. alata* (Borges et al., 2004); 10.5 shoots per explant after 6 weeks from nodal segments of *D. oppositifolia* (Behera et al*.,* 2009) and 7.5 shoots after 6 weeks from nodal segments of *D. oppositifolia* and *D. pentaphylla* (Poornima and Ravishankar, 2007).

In yams, plants from tissue cultures have been regenerated on an array of basal medium such as Murashige and Skoog (Lauzer et al.,1992; Alizadeh et al.,1998; Chen et al., 2003; Poornima and Ravishankar, 2007), Modified D-571 medium (Borges et al., 2004), Knop's modified medium (Malaurie et al., 1993). In the preliminary studies throughout our experiments, MS basal medium (Murashige and Skoog, 1962) was found to be more responsive.

Three released varieties of *D. alata viz.,* Sree Roopa, Sree Keerthi and Sree Shilpa were selected for the study considering the preference of these varieties by farmers due to its wide adaptation and resistance to scale insects on storage. The present study attempts to establish a rapid propagation system for the year round production of planting material in greater yam.

Materials and Methods

The study was carried out at Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India, with the main objective to evaluate the effect of different explants, surface sterilants and the effect of Benzyl Adenine (BA) and Naphthalene Acetic Acid (NAA) at different concentrations and combinations on shoot regeneration and elongation, root induction and elongation, number of emerging leaves and number of nodal meristems using nodal segments of *D. alata* var., Sree Roopa, Sree Keerthi and Sree Shilpa.

Different explants *viz.,* nodes (1-3 and 4-8 from tip) and apical bud from young and fast growing vines of field grown plants (six months old) and nodes excised from vines of sprouted tubers (less than one week old) were selected for studying their response in cultures (Fig.1).

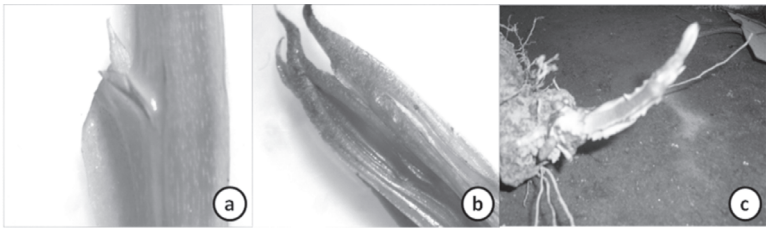

Fig. 1. Different explants used for the *in vitro* regeneration of *D. alata*. (a) Nodal segment from field grown plants (b) Apical bud from field grown plant (c) Node from sprouted tubers

In order to standardize suitable surface sterilization protocol, the explants were treated with surface sterilants like sodium hypochlorite NaOCl (3.5%) for 10, 15 and 20 minutes and mercuric chloride ($HgCl_2$) (0.08%) for 1, 2 and 3 minutes.

The nodal explants were placed on basal MS medium supplemented with 3% sucrose (w/v), 0.7% agar (w/v) and different concentrations and combinations of plant growth regulators (PGRs). Activated charcoal (0.1%, w/v) was uniformly added to all the media combinations to control phenolic oxidation and blackening of the explant. For both shoot and root induction, 36 combinations of NAA and BA in different concentrations (0.5 – 1.75 mg l^{-1}) along with a control (no PGRs), designated as D1-D37 was used in the present study.

The pH of the medium was adjusted to 5.8 using 0.1 N NaOH before autoclaving. The cultures were maintained at 25 ± 2°C for 8 h photoperiod, which was provided by cool-white fluorescent tubes (50 μmol m^{-2} s^{-1}). The response of different types of nodes was observed based on the number of days taken for initial shoot induction and the best responding explants were used as explant source for further studies.

Seven replicates were used for each treatment. All the experiments were repeated twice by employing a Completely Randomized Design. The data collected on the number and length of shoots, number of leaves, number of nodal meristems, number and length of roots were evaluated after 15 weeks by ANOVA. The variations among means were statistically analyzed using GENSTAT at the $P < 0.05$ level of significance.

Two methods were attempted for reducing the time of acclimatization process. 1) The plantlets were taken out and washed in running tap water to remove traces of agar and was treated with bavistin (0.1%, w/v) for 3-5 min and washed thoroughly in sterile distilled water. Then they were transferred to small plastic cups containing sand and soil in 1:1 ratio for about two weeks, then to big pots for two weeks and finally to big

cement troughs in the net house. The plastic cups were covered with polythene bags having holes for maintaining humidity. 2) The cultures after bavistin treatment were also directly planted out in big cement troughs and the survival percentage of both the methods of acclimatization was evaluated.

Results and Discussion

It was found that nodes taken from vines of sprouted tubers as well as 4 - 8 nodes from the tip of field grown plants gave a quick response within 3-4 days and 4-6 days respectively, when compared to other explants. For apical bud, there was a lag in the initiation of shoot induction, which took 4-5 weeks. It was noticed that nodes of size 0.5-0.8 cm with slightly initiating axillary buds gave better response when compared to others, which represents that age as well as the nature of the explants is crucial in the selection of explants. For surface sterilization, 0.08% of $HgCl_2$ for two minutes was found to be effective, since the percentage of survival of explants was found to be higher in $HgCl_2$ treatment (80%) compared to NaOCl treatments (20%) (Table 1). Diverse explants have been used proficiently to produce

Table 1. Effect of surface sterilants on survival of node explants of *Dioscorea alata* (Sree Roopa)

Surface sterilants	Period of treatment (min)	*Survival of explants (%) Soft tissue	Hard tissue
Mercuric chloride (0.08%)	1	80	20
	2	85	80
	3	85	80
Sodium hypochlorite (3.5%)	10	10	15
	15	20	20
	20	20	30

*Average of ten plants

regenerable cultures of yam through *in vitro* morphogenesis or axillary bud from nodal vine cuttings (Borges et al., 2004; Poornima and Ravishankar, 2007; Behera et al., 2009), meristem (Mitchell et al., 1995; Adeniyi et al., 2008) and stem (Chen et al., 2003).

Effect of BA and NAA on shoot organogenesis

The MS media with growth regulators supplemented at different concentrations produced better results in terms of initial explant response, average shoot number, shoot length, number of nodal meristems, number of leaves, number of roots, length of root and percentage of survival when compared to control. Within 4 - 6 days, the nodal bud was fully visible and further young shoots with unopened leaves started emerging within 7 - 9 days. The overall percentage of shoot initiation at two weeks after culturing was found to be 62.16%. In most of the treatments, the cultures produced more number of shoots from the nodal portion than from the base of the tissue which is in contact with the medium.

In this study, we tested 36 different combinations of BA and NAA, to evaluate the best treatment for *in vitro* regeneration of three different varieties of *D. alata*. For Sree Roopa, out of the 36 combinations tested, D20 (1.25 mg l^{-1} NAA + 0.5 mg l^{-1} BA) elicited optimal response at 15 weeks of culturing with an average of 9.8 shootlets per explant. The second best shoot multiplication was obtained in D3 (0.5 mg l^{-1} NAA + 0.75 mg l^{-1} BA) with 0.1% activated charcoal giving an average of 8.4 shootlets, which was on par with the treatment, D20. Since the concentration of hormones was lower in D3 when compared to D20, the former can be taken as the best treatment for maximum shoot regeneration. The treatment, D3 (0.5 mg l^{-1} NAA + 0.75 mg l^{-1} BA) elicited an average growth of 6.8 cm per explants for Sree Roopa (Table 2). The growth response of axillary bud at two weeks after culture for Sree Roopa was 43.24%. Significant difference was observed between the treatments at 15 weeks of culture ($P < 0.05$). The number of leaves per explant was also recorded and the treatment, D3 (0.5 mg l^{-1} NAA + 0.75 mg l^{-1} BA) was found to be the best with an average of 8.4 leaves per explant (Table 2). Significant difference was observed in subsequent development of leaves during different duration of culturing and the percentage of emerging leaves differed significantly within and between treatments. Shoot multiplication was significantly improved by subculturing in the same media. Callus formation was not observed in any of the treatments.

On the treatment, D3 (0.5 mg l^{-1}NAA + 0.75

mg l^{-1} BA) Sree Keerthi elicited an average of 12 shootlets with an average length of 9.7 cm and Sree Shilpa elicited an average of 11.7 shootlets per culture with an average length of 10.14 cm (Table 2).The result shows that there was no significant difference between these two varieties in the number and length of shoots. Shoot regeneration was significantly different between and within duration in best treatments at 15 weeks of culture ($P < 0.05$).

Effect of BA and NAA on root organogenesis

The treatment D3 (0.5 mg l^{-1} NAA + 0.75 mg l^{-1} BA) resulted in the fastest root initiation within 5-7 days for Sree Roopa, with an average mean of 2.7 roots per explants at 15 weeks of culture and was on par with the other treatments, *viz.,* D2 and D13 (Table 3).

The same treatment D3 gave the fastest root initiation within 4-6 days for Sree Keerthi and Sree Shilpa, with an average mean of 4.4 roots and 7.4 roots per explant respectively at 15 weeks of culture and elicited a root length of 7.1 cm and 7.8 cm respectively (Table 2). A high percentage of shoots rooted in the same medium for all the three varieties studied (92%). Strong and long roots help the plant to survive for longer periods and also help in greater absorption of nutrients from the medium and thus increase the percentage of survival during the acclimatization period.

Acclimatization

The survival rates of the regenerated plants were found to be high in the first method as compared to the second. The rooted plantlets were transferred to small plastic cups which was punched and filled with sand: soil (1:1). The cups were covered with polythene bags with holes for maintaining proper humidity for less than two weeks. Later, after removing the cover, they were maintained in mud pots for another two weeks in the net house and about 90% of the regenerated plantlets survived and grew vigorously within three weeks. For increasing the percentage of survival of these regenerated plants, they can be maintained in mud pots for another one month and later transferred to net house or directly to the field. The second method of direct planting of the tissue culture plants did not bring any significant improvement in the hardening process and the survival rate was found to be less when compared to the first method. The result of the first method showed high rate of survival (90%) of the regenerated plantlets which grew vigorously within three weeks after they were transplanted to net house for large scale multiplication of yam plantlets (Fig. 2).

It can be concluded that MS medium with 0.5 mg l^{-1} NAA and 0.75 mg l^{-1} BA and 0.1% activated charcoal was found to be the best for outgrowth of axillary bud, shoot elongation, multiplication and rooting in three varieties *viz.*, Sree Roopa, Sree Keerthi and Sree Shilpa of *D. alata*, which may be used for further large scale multiplication of these elite varieties.

Table 2. Effect of best hormone combination on regeneration and growth of nodal culture of *D. alata* varieties after 15 weeks of culturing

Varieties	Treatment NAA:BA (mg l^{-1})	No. of shoots	Shoot length (cm)	No. of leaves	No. of roots	Root length (cm)	No. of nodal buds
Sree Keerthi	0.50 : 0.75	12.1 ± 1.2	9.7 ± 1.0	12.4 ± 0.9	4.4 ± 0.5	7.1 ± 1.1	2.1± 0.5
Sree Shilpa	0.50 : 0.75	11.7 ± 1.3	10.1 ± 0.8	12.0 ± 1.4	7.4 ± 0.7	7.8 ± 0.9	1.8 ± 0.4
Sree Roopa	0.50 : 0.75	8.40 ± 0.6	6.8 ± 0.6	8.40 ± 0.6	2.7 ± 0.5	10.8 ± 0.5	0.2 ± 0.1
Control	0.00 : 0.00	04.5 ± 0.8	6.8 ± 0.8	4.50 ± 0.8	1.7 ± 0.4	5.2 ± 1.0	0.4 ± 0.2

*Data represents mean of seven replicates per treatment scored at 15 weeks; LSD of number of shoots (T) = 0.734; LSD of shoot length (T) = 0.737; LSD of number of roots (T) = 0.480; LSD of root length (T) = 1. 215; LSD of number of leaves (T) = 0.741; LSD of number of nodal buds (T) = 0.289

Table 3. Effect of NAA and BA on root induction and elongation, number of leaves and number of nodal buds of *Dioscorea alata* (var. Sree Roopa) in the initial study

Treatments NAA:BA (mg l^{-1})	Average number of roots /explant[a] (± SE)	Average root length[a] (cm ± SE)	Days to initiate rooting	No. of leaves	No. of nodal buds
D1 (0.00 : 0.00)	1.7 ± 0.4	5.2 ± 1.0	12-14	4.5 ± 0.8	0.4 ± 0.2
D2 (0.50: 0.50)	2.4 ± 0.2	6.2 ± 0.7	6-8	6.7 ± 0.7	0.4 ± 0.2
D3 (0.50 : 0.75)	2.7 ± 0.5	10.8 ± 0.5	5-7	8.4 ± 0.6	0.2 ± 0.1
D4 (0.50:1.00)	1.5 ± 0.2	7.9 ± 1.0	6-8	5.8 ± 0.5	0.4 ± 0.2
D5 (0.50: 1.25)	1.0 ± 0.2	6.1 ± 1.5	6-8	3.8 ± 0.7	0.4 ± 0.2
D6 (0.50:1.50)	2.1 ± 0.4	8.2 ± 1.3	6-8	5.1 ± 0.4	0.4 ± 0.1
D13 (0.75:1.75)	2.2 ± 0.2	8.7 ± 1.2	8-10	7.5 ± 0.6	0.2 ± 0.1

[a]Data represents mean of seven replications per treatment scored after 10 weeks of culture, LSD of number of roots (T) = 0. 480; LSD of root length (T) = 1.215

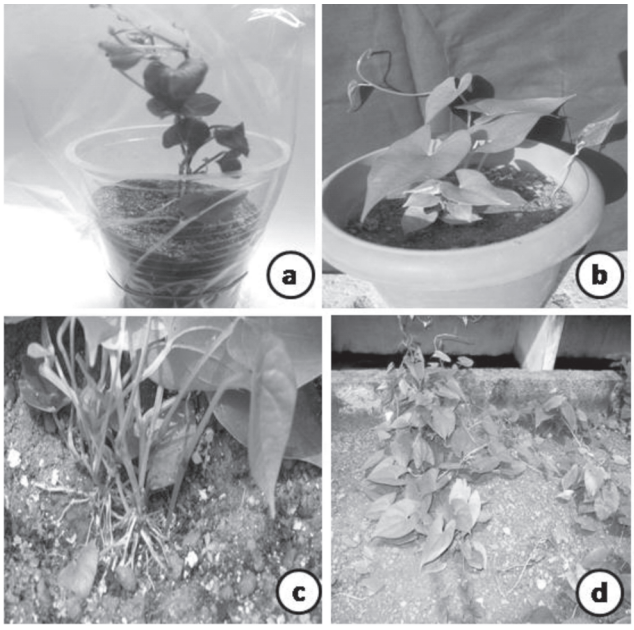

Fig. 2. Different stages of hardening of *in vitro* regenerated *D. alata* (a) Regenerated plantlets in plastic cups covered with polythene bags (b) Plantlets in big pots (c) Well developed plants (d) Plants in cement trough

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References

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

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

Behera, K.K., Sahoo, S. and Prushi, A. 2009. Regeneration of plantlet of water yam (*Dioscorea oppositifolia* L.) through *in vitro* culture from nodal segments. *Not. Bot. Hort. Agrobot. Cluj.*, **37** (1): 94-102.

Borges, M., Ceiro, W., Meneses, S., Aguilera, N., Vazquez, J., Infanta, Z. and Fonseca, M. 2004. Regeneration and multiplication of *Dioscorea alata* germplasm maintained *in vitro*. *Plant Cell Tissue Organ Cult.*, **76**: 87-90.

Chen, Y., Fan, J., Yi, F., Luo, Z. and Fu, Y. 2003. Rapid clonal propagation of *Dioscorea zingiberensis* Wright. *Plant Cell Tissue Organ Cult.*, **73**: 75-80.

Edison, S., Unnikrishnan, M., Vimala, B., Santha Pillai, V., Sheela, M. N., Sreekumari, M.T. and Abraham, K. 2006. *Biodiversity of Tropical Tuber Crops in India*. NBA Scientific Bulletin Number 7. National Biodiversity Authority, Chennai, TamilNadu, India. p.60.

Lauzer, D., Laubiln, G., Vincent, G. and Cappadocia, M. 1992. *In vitro* propagation and cytology of wild yams, *Dioscorea abyssinica* Hoch. and *Dioscorea mangenotiana* Miege. *Plant*

Cell Tissue Organ Cult., **28**: 215-223.

Malaurie, B., Pungu, O., Dumont, R. and Trouslot, M.F. 1993. The creation of an *in vitro* germplasm collection of yam (*Dioscorea* spp.) for genetic resources preservation. *Euphytica*, **65**: 113-122.

Mitchell, S.A., Asemota, H.A. and Ahmad, M.H. 1995. Effects of explant source, culture medium: Strength and growth regulators on the *in-vitro* propagation of three Jamaican yams: (*Dioscorea cayenensis*, *D. trifida* and *D. rotundata*). *J. Sci. Food Agric.*, **67** (2): 173–180.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Pl.*, **15**: 473-497.

Poornima, G.N. and Ravishankar, R.V. 2007. *In vitro* propagation of wild yams, *Dioscorea oppositifolia* (L.) and *Dioscorea pentaphylla* (L.). *African J. Biotech.*, **6** (20) : 2348-2352.