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Regeneration of Plantlet of Water Yam (*Dioscorea oppositifolia* L.) through *In Vitro* Culture from Nodal Segments

Kambaska Kumar BEHERA [1)], Santilata SAHOO [1)], Aratibala PRUSTI [2)]

[1)] *P. G. Deptt. of Botanty Utkal University, Vanivihar, Bhubaneswar, Orissa, India; kambaska@yahoo.co.in*

[2)] *P.N. College (Autonomous) Khurda, Orissa, India*

Abstract

The present investigation was carried out with a view to regenerate plantlet of *Dioscorea oppositifolia* L through in vitro culture. Nodal vine segments from 45days old plants of field grown *Dioscorea opositifolia* were used as explants for initial culture. The explants were cultured on MS (Murashige and Skoog's, 1962) medium supplemented with different concentration and combination of cytokinines and auxines for primary shoot proliferation. The best shoot proliferation was observed in MS medium containing 2.0 mg-l KINETIN +1.0 mg-l BAP+0.5mg-l NAA + 100mg-l ascorbic acid where 90 % of explants showed proliferation with highest rate of shoot multiplication (10.5 shoots per explant). For rooting, the *in vitro* micro shootlets were inoculated on to the half-strength MS basal media supplemented with 2.0 mg/l NAA and rooting was more profuse with micro tubers in the base of the root primordia. Rooted shoots were transplanted in the green house for hardening and their survival rate was 90 % in the field condition.

Keywords: *Dioscorea oppositifolia*, micro-tuber, nodal vine, shoot regeneration, *In vitro*-propagation, tissue culture, plant growth regulators

Introduction

Root and tuber crops are the most important food crops after cereals. They have the highest rate of dry matter production per day and are major calorie contributors. Tuber crops find an important place in the dietary habits of small and marginal farmers especially in the food security of tribal population. Tuber crops not only enrich the diet of the people but also possess medicinal properties to cure many ailments or check their incidence. Many tropical tuber crops are used in the preparation of stimulants, tonics, carminatives and expectorants. The tuber crops are rich in dietary fibre and carotenoids viz. carotene and anthocyanin. India holds a rich genetic diversity of tropical root and tuber crops especially the Yam *Dioscorea* (Edison *et al*., 2006). The *Dioscorea oppositifolia* is an edible tuber bearing species distributed, all most all jungles of Orissa and freely produce flowers. The male and female flowers synchronies and come to flowering in late autumn. In the nature the pollination and fertilization is perfect but seeds are not viable. As most of the *Dioscorea* species are highly heterozygous for their cultivars or elite clones and are propagated vegetatively by means of seed tuber. A single plant bears only a single or a few tubers and mass propagation through seed tuber is cost effective. Once tubers are infected by a virus, it is transmitted to later generations through tubers and is difficult to be removed. Single or multiple virus infections often cause reduction of yield and deterioration of quality of the tuber . Therefore, the regeneration of virus-free yam plantlet and their efficient propagation are required as a useful technology to increase production and to improve quality of the yams in tropical areas (Mitchell and Ahmad, 1999). There are many wild *Dioscoreas* are found in the forest patches of Orissa. Amongst them *Dioscorea oppositifolia* serve as a 'life saving' plant for the marginal farming and forest dwelling communities, during periods of food scarcity (Maharana, 1993; Arora and Pandey, 1996). Plant regeneration through in vitro propagation of some economically important *Dioscorea* species has been achieved by using nodal vine cuttings (Yan *et al*., 2002 ; Chen *et al*., 2003), bulbils (Asokan *et al*., 1983) meristem tips (Malaurie *et al*., 1995 a, b). Attention has been paid to the clonal propagation through *in vitro* production of microtubers in *Dioscorea abyssinica* (Martine and Cappadocia, 1991), *Dioscorea alata* (Jasik and Mantell , 2000), *Dioscorea batatas* (Koda and Kikuta , 1991) and *Dioscorea composita* (Alizadeh *et al* ., 1998) .

In vitro method of vegetative multiplication of *Dioscorea oppositifolia* would have considerable benefits for the medicinal trade and germplasm conservation. The application of plant tissue culture offers valuable ways to overcome all the problems that's found in natural propagation. The present study describes a suiTab. method for in vitro plantlet regeneration and induction of micro tubers in *Dioscorea oppositifolia* through nodal vine culture. The methodology definitely help full for obtaining large scale diseases free seedlings and ensure adequate supply of quality plantlets of *Dioscorea oppositifolia* to meet different purposes and conservation and domestication of the wild species.

Materials and methods

Explant source

Healthy vines with active buds were collected from 45days old plants of *Dioscorea opositifolia* maintained in the experimental garden, Postgraduate Department of Botany Utkal University. The vines were cut in to 1.5 cm to 2 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 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 and transferred to laminar air flow cabinet. In the laminar chamber the nodal segments were again treated with70% ethyl alcohol for 30 second to one minute followed by another treatment in 0.1% (w/v) mercuric chloride (HgCl2) for another 5 minutes. Finally, the nodal cut vines were washed thoroughly 3 to 4 times with sterile distilled water and soaked with sterile blotting paper and used as explants for in vitro cultures before the inoculation in to sterilized nutrient agar media pre-packed in culture tubes.

Culture medium and condition

The sterilized blotted explants were implanted 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 Kinetin + 0.25,0.5 and1.0 mg-l BAP and 0.25-0.5 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 length grown in multiplication medium were excised and cultured on half-strength MS basal medium supplemented with either NAA (α-Naphthalene acetic acid) or IBA (Indole3-butyricacid) in the concentration of 0.25,0.5,1.0,1.5,2.0,2.5 and 3.0 mg-l with ascorbic acid 100 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. Molten medium of 20 ml was dispensed into the culture tube and plugged with nonab-

Tab.1. Shoot formation in nodal explants of *Dioscorea opositifolia* L. cultured on semisolid MS medium supplemented with various concentration of Kinetin+ BAP and NAA with 100mg/l Ascorbic acid (20 explants per treatment, data scored after 6 weeks.)

Treatments	Hormonal, supplements (mg^{-1})			% 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	20	12-15	1.2 ± 0.18+	1.3 ± 0.14+	1.0 ± 0.23+
T3	0.5	0.25	0	30	10-12	2.0 ± 0.47+	2 ± 0.23+	1.0 ± 0.21+
T4	1.0	0.25	0	40	10-12	2.5 ± 0.12	2.2 ± 0.30+	1.5 ± 0.35+
T5	1.5	0.25	0	45	10-12	2.6 ±0.18	2.3 ± 0.14	1.6 ± 0.42
T6	2.0	0.25	0	60	8-10	4.5 ± 0.44	3.4 ± 0.33	2.1 ± 0.17
T7	2.5	0.25	0	50	8-10	3.1± 0.49+	3.2 ± 0.30+	2.0 ± 0.47+
T8	3.0	0.25	0	50	10-12	2.8 ±0.23+	3.0 ±0.33+	1.8 ±0.16+
T9	0.25	0.5	0.25	20	12-15	1.3 ± 0.23+	1.3 ± 0.09+	1.2 ± 0.18+
T10	0.5	0.5	0.25	30	10-12	1.4 ± 0.18+	1.5 ± 0.23+	1.3 ± 0.23+
T11	1.0	0.5	0.25	50	8-10	3.0 ± 0.16+	3.2 ± 0.20+	2.2 ± 0.18+
T12	1.5	0.5	0.25	60	8-10	3.5 ± 0.09	3.5 ± 0.42	2.3 ± 0.24
T13	2.0	0.5	0.5	50	8-10	4.2 ± 0.47	3.4 ± 0.18	2.1± 0.47
T14	2.5	0.5	0.25	50	10-12	2.6 ± 0.31+	2.2 ± 0.18+	1.4 ± 0.14+
T15	3.0	0.5	0.25	40	10-12	2.4±0.24+	2.0±0.34+	1.0±0.09+
T16	0.25	1.0	0.5	30	10-12	3.0 ± 0.52+	2.4 ±0.25+	1.0 ± 0.30+
T17	0.5	1.0	0.5	40	8-10	3.6 ± 0.30+	3.0 ± 0.12+	2.2 ± 0.20+
T18	1.0	1.0	0.5	45	7-9	4.0 ± 0.09	3.3 ± 0.16	2.0 ± 0.32
T19	1.5	1.0	0.5	70	6-8	5.5 ± 0.43	4.2 ± 0.21	3.5 ± 0.12
T20	2.0	1.0	0.5	90	6-8	10.5 ± 0.51	5.4 ± 0.24	5.2 ± 0.24
T21	2.5	1.0	0.5	60	7-9	3.2 ± 0.14+	2.6 ± 0.39+	1.5 ± 0.12+
T22	3.0	1.0	0.5	50	10-12	2.6±0.14+	2.3±0.20+	1.2±0.16+

[20 replicate per treatment; repeated twice. 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]

Tab. 2. Influence of different levels of NAA and IBA on rooting response of invitro generated shoot lets of *Dioscorea opositifolia* L. with 100mg/l Ascorbic acid (20 replicates/treatment, data scored after 4 weeks)

Different treatments		Growth regulators augmented with1/2 strength MS basal medium(mg/l) IBA	% of Explant Response	Days to root initiation	Mean root numbers ±S.E.
NAA	NAA				
T1	0	0	-	-	-
T2	0.25	0	-	-	-
T3	0.5	0	30	10-12	1.0 ± 0.21+
T4	1.0	0	45	8-10	4.2 ± 0.32+
T5	1.5	0	80	6-8	4.5 ± 0.12
T6	2.0	0	90	6-8	6.5 ± 0.30
T7	2.5	0	50	8-10	2.7 ± 0.23+
T8	3.0	0	40	8-10	2.4 ±0.16+
T9	0	0.25	30	12-15	1.0 ± 0.14+
T10	0	0.50	40	12-15	1.0 ± 0.31+
T11	0	1.0	45	10-15	2.1 ± 0.24
T12	0	1.5	55	10-15	2.2 ± 0.34
T13	0	2.0	60	10-15	2.4 ± 0.21
T14	0	2.5	50	10-12	2.6 ± 0.37+
T15	0	3.0	40	10-15	2.5 ± 0.37+

[20 replicate per treatment; repeated twice. 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]

sorbent cotton wrapped in one layer of cheesecloth. All cultures were incubated in 16 h light/8 h dark photoperiod (cool, white fluorescent light, -30μmol m-2S-1. The cultures were incubated at 25 ± 3°C in diffused light under 60 - 70% relative humidity in the culture room. Each treatment had 20 culture tubes and the experiment was repeated thrice. The cultures were maintained by regular subcultures at 2 week intervals on fresh medium with the same compositions. For micro tuber induction different percentage of sucrose were taken in the culture media as a source of carbon.

Acclimatization

Rooted micro-propagules were removed from the culture tube and the roots were washed under running tape water to remove the agar .Than the plantlets were transferred to sterile poly pots (small plastic cups) containing pre-soaked vermiculite (TAMIN ,India) and maintained inside growth chamber set at temperature 28°C and70-80% 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.

Observation of cultures and presentation of results

Twenty cultures 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 analyzed by the Post- Hoc Multiple Comparison test at the $P < 0.05$ level of significance (Marascuilo and McSweency, 1977).

Results

Shoot proliferation and multiplication

The response of *Dioscorea opositifolia* nodal vine explants cultured on different shoot proliferation media over a period of six weeks is presented in Tab.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 bud break was noticed within 6-8 days of culture (Fig.1.A&B, Fig. 2. A, Tab. 1). Of the combination tested MS+ Kinetin (2.0 mg-l) +BAP (1.0 mg/l) + NAA (0.5 mg/l) with ascorbic acid 100mg/l, elicited optimal response in which an average of 10.5 ± 0.51 shoot lets (Fig.1A,Tab-1) with a mean shoot length of 5.4 ± 0.24 cm per explant was recoded. The second best shoot multiplication 5.5 ± 0.43 was obtained in the medium MS + Kinetin (1.5mg/l) + BAP (1.0 mg-l) + NAA (0.5 mg-l) + 100 mg-l ascorbic acid with a mean shoot length of 4.2 ± 0.21 cm .Higher concentration of Kinetin (3.0 mg-l) + BAP (1.0 mg-l) with NAA (0.5mg-l) +100mg-l ascorbic acid showed callusing explants with fewer number of

Tab. 3. Influence of sucrose concentration on in vitro micro tuber production.

Species	Sucrose (gm/l)	Tuber number	Tuber weight (mg)
D. oppositifolia	20	1.4± 0.14	16.6±1.82
	30	2.2±0.14	55.8±7.94
	15	1.5±0.19	12.25±1.95
	25	0.83±0.20	25.83±5.56

[10 replicate per treatment; repeated twice. 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]

shoots. In such cultures shoots were stunted with a mean shoot length of 2.3 ± 0.20 cm (Tab.1).

Induction of rooting from micro shoots

The well developed elongated shoots were excised from the shoot clump and transferred to half strength MS medium containing NAA or IBA .The rooting responses (Fig.2.B) of shoots on different media, which included rooting percentage, days required for root initiation mean number of roots/shoot and mean root growth over a period of three weeks were different (Fig.1.B&C, Tab-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 four weeks of observation period. However higher concentration of NAA (1.5&2.0 mg-l) and IBA at all concentration tested respond well. Rooting was better in the culture which had combination of 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

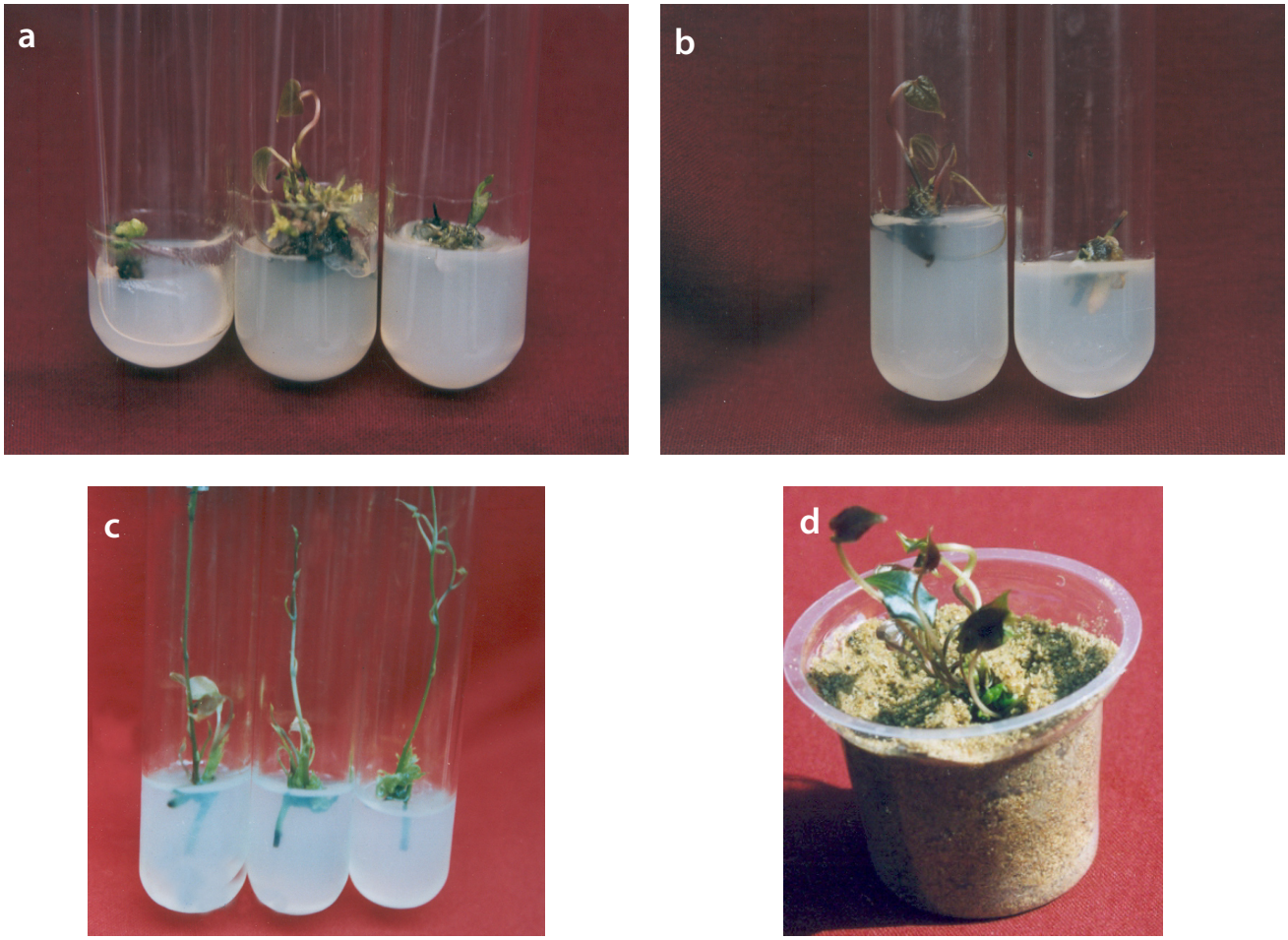

Fig. 1. (A-D) *In vitro* regeneration and plant establishment of *D. opositifolia* (A) Multiple shoot emergence in MS+2mg/l Kn+1mg/lBAP+0.5mg/l NAA +100mg/l Ascorbic acid.(B) Root and microtuber induction.(C) Microshoots rooted in 1/2MS+NAA(2mg/l). (D)Hardening of rooted plant lets in plastic pots

Fig.1. (E-G).Field grown plant of D.oppositifolia ,(E) Three month Plant in field condition.(F) Growth of tuber at the time of harvest .(G) Tuber after harvesting

and an average root length 4.5 ± 0.16 cm was recorded (Fig. 1. B&C, Fig. 2.B, Tab. 2) .The second highest response (80%) was recorded at 1.5mg/l of NAA. It was observed that root primordial emerged from the shoot base starting from day 6 to 8 days after shoot inoculation and soon after that the root growth was rapid. NAA has more effective then IBA in induction of rooting as days required to rooting was only 6-8 days as against the 10 to15 days required for similar response in case of IBA.

Induction of Micro tuber

For micro tuber induction a range of sucrose (15-30 gm-l) was tested to the basal medium. Around 40-50% of the cultures produced one to two tubers at the base of their rooted shoots. Highest number of micro tubers are produced with the media composition of 30 gm/l sucrose and Kinetin (2.0 mg/l) +BAP (1.0 mg-l) + NAA (0.5 mg-l) with average number of micro tuber 2.2 ± 0.14 and average weight 55.8 ±7.94 mg per micro tuber (Fig.1.B, Tab. 3).

Acclimatization and field establishment

Rooted plantlets grown *in vitro* were washed thoroughly to remove the adhering gel, transplanted to sterile poly pots (small plastic cups) containing pre-soaked vermiculite (TAMIN ,India) and maintained inside growth chamber set at temperature 280C 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 3days for period of 3 weeks. Survival rate of the plantlets were recorded after 3 weeks. About 90% of the rooted plantlets established in the greenhouse within 2-3 weeks of transfer. The plants grew well and attained a 6-8 cm height within 4

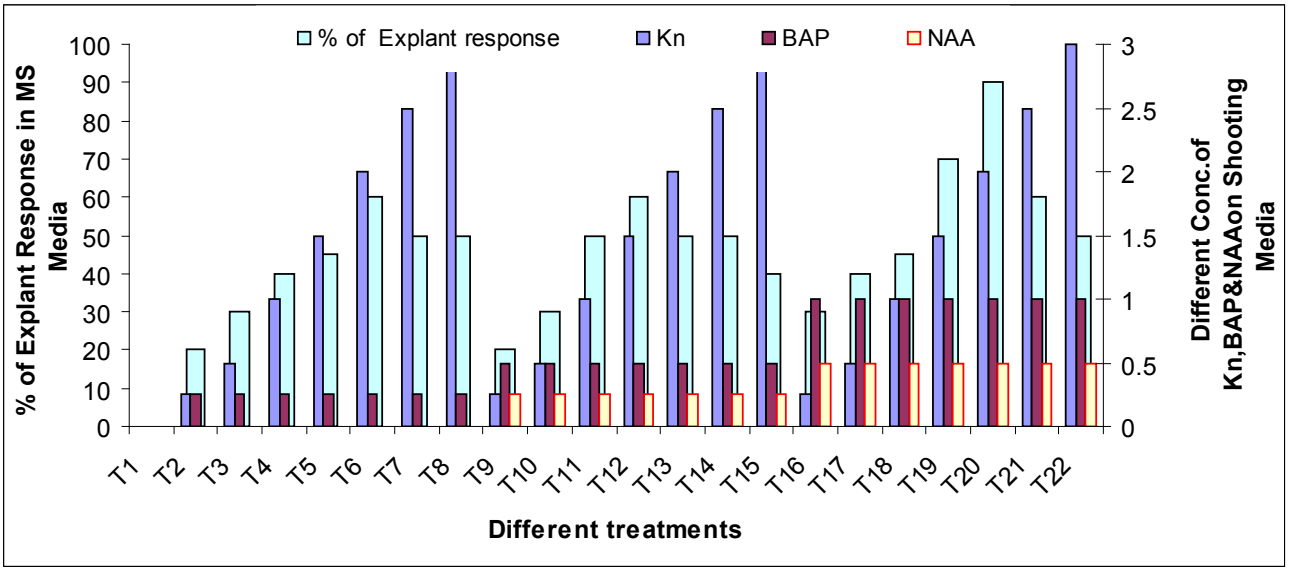

Fig. 2.A. Shooting Medium: Showing % of Explant Response in Different Treatments of Kinetin,BAP and NAA in MS medium with 100 mg/l Ascorbic acid.

weeks of transfer (Fig.1.D, Tab-2). The acclimatized plants were established in the field condition and grew normally without morphological variation (Fig.1.E, F&G).

Discussion

Micro propagation of other yam species in a solid medium has been reported (Jean and Cappadocia, 1992; Mitchell et al., 1995; Borthakur and Singh, 2002; Chu and Ribeiro, 2002). 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 micro propagation of other Yams like *Dioscorea composita* (Alizadeh et al., 1998), *Dioscorea floribunda* (Borthakur and Singh, 2002), *Dioscorea batatas* (Koda and Kikuta,1991) and *Dioscorea abyssinica* (Martine and Cappadocia,1991).

In the present study, nodal vine explants of of *Dioscorea opositifolia* showed significantly higher response in the medium with the combination of Kinetin (2.0 mg-l) +BAP (1.0 mg-l) +NAA (0.5mg-l) with 100mg/l ascorbic acid.

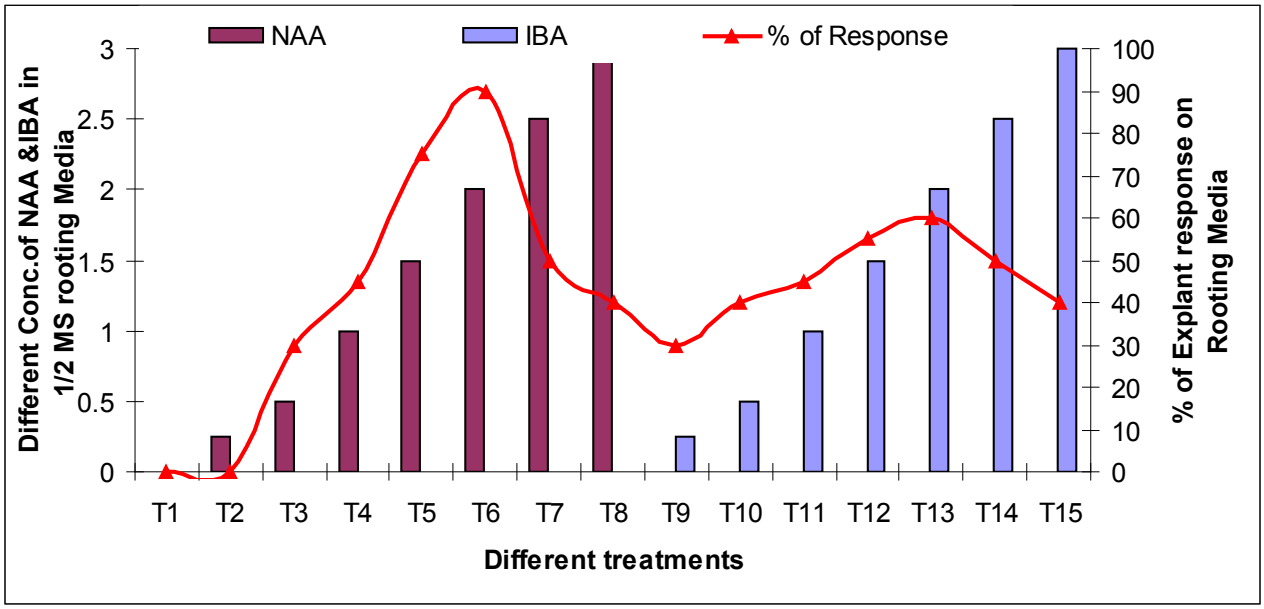

Fig. 2.B. Rooting Medium: Showing % of Micro shoots Response in Different Treatments of NAA and IBA in ½ MS medium with 100mg/l Ascorbic acid.

The quality of shoots and the over all growth response in terms of average shoot length was better in 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 the response in a number of species in terms of shoot growth. Adeniyi et al. (2008) reported that shoot regeneration of 42-75% was obtained in *Dioscorea rotundata* in MS medium supplemented with 0.1 μM NAA + 0.20 μM BAP, and shoot + plantlet regeneration of 60-82% obtained in media containing 0.05 μM + 0.20 μM BAP or 0.46 μM BAP + 0.50 μM kinetin in *Dioscorea alata*. Similarly Shin et al., (2004) reported that the combination and interaction of BA and NAA plays important role for *in vitro* propagation of nodal explant for multiple shoot induction. MS medium supplemented with 1.0 mg/l NAA and 0.5-1.0 mg-l BA is the best concentration for multiple shoot bud induction in *Dioscorea opposita*. Behera et al., (2008) reported that in *Dioscorea hispida* , BAP (2.0 mg-l) + NAA (0.5 mg-l) with ascorbic acid 100 mg/l , elicited optimal response in which an average of 6.0 ± 0.18 shoot lets with a mean shoot length of 5.0 ± 0.29 cm per explant was recorded and the best combination for multiple shoot production. Hussain et al., (2008) reported that in *Sterculia urens* addition of ascorbic acid 100mg-l to the shooting media enhanced the production of multiple shoot.

In our study two cytokinins were taken for higher shoot multiplication .Some authors also suggested that the combination of two cytokinins were needed for producing multiple shoots on *Aristolochia* bracteolate (Rameshree et al., 1994), *Lavandula* species (Jordan et al., 1998). Nodal explants of *Dioscorea oppositifolia* were more responsive in terms of rapid bud break. The frequency and the rate of multiplication depend on the concentration of KINETIN+BAP+NAA, either alone or in combination. Ascorbic acid was observed to be more effective in reducing phenolic exudation from the explant and better growth responses of plant tissues, as it act as antioxidant and removes inhibitory substances from the media produced either on autoclaving (Weatherhead et al., 1978) or by the tissue itself (Fridborg et al., 1978).

Micro tubers developed at the base of the rooted shoots, around 40-50% of the cultures produced one to two tubers at the base of their rooted shoots. Using nodal cutting as explants, the phenomenon of *in vitro* tuberization has been observed in *Dioscorea bulbifera* (Forsyth and Van Staden, 1982b), *Dioscorea alata* (Jean and Cappadocia, 1992; Alhassan and Mantell, 1994), *Dioscorea rotundata* (Ng, 1988), and *Dioscorea cayenensis* (Ng and Mantell, 1992).The nodal shoots of *Dioscorea oppositifolia* produced tubers even on the shooting media. These tubers were found to be bigger and more uniform in size in comparison with other treatment. A combinations of 15 -30 gm-l sucrose and Kinetin (2.0 mg-l) +BAP (1.0 mg-l) + NAA (0.5 mg-l) and 100 mg-l ascorbic acid added to the MS basal medium which induced the micro tubers. However, these tubers were smaller and not uniform in size. Effect of sucrose on micro tuberization has also been observed in *Dioscorea bulbifera* (Forsyth and Van Staden, 1982a), *Dioscorea rotundata* (Ng, 1988), *Dioscorea alata* (Mantell and Hugo, 1989) and *Dioscorea opposita* (Kohmura et al., 1995). The nodal shoot cultures of *Dioscorea alata* and *Dioscorea bulbifera* showed maximum micro tuber formation with 2% sucrose (Mantell and Hugo, 1989). When Kohmura et al. (1995) compared sucrose concentrations (3 and 6%) in *Dioscorea opposita* with 8.9 μM BAP alone in the medium, 6% sucrose was found to be more efficient for tuberization. In the present study, two cytokines (Kn+BAP) and NAA with 3% sucrose can successfully induce in vitro micro tuber formation (Tab-3). In *Dioscorea rotundata*, a decrease in the percentage of microtuberization with 8 or 10% sucrose and 2.5 μM kinetin was reported by Ng (1988), and our findings is a new result in the current study on *Dioscorea oppositifolia* . Increase in the sucrose amount in culture media from 2 to 8%, in the presence of higher levels of kinetin (23.2 to 46.4 μM), raised microtuber frequencies in *Dioscorea bulbifera* (Forsyth and Van Staden, 1982 b).

Production of plantlets with profuse rooting in *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 shoot lets . 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 maximum results on rooting were obtained on half strength with NAA (2.0 mg-l) then IBA.(2.0 mg-l). The auxins, NAA and IBA were used by Behera et al., (2008) singly to induce rooting from *in vitro* raised shootlets of *Dioscorea hispida*. In their study 1/2 strength MS basal medium with NAA (2.0 mg-l) + Ac 2g-l has profuse rooting response than IBA (2.0 mg-l)+ Ac 2g-l. Matsubara and Ishihara (1988) reported the in *Dioscorea .opposita* cv Nagaimo and Tsukeneimo higher concentration of NAA 2 mg-l in combination with 0.2 and 0.5 mg-l BA led to root formation and similar findings also reported in *Dioscorea esculenta* by Belarmino and Rosario (1991). Our observation are in accordance with the result of Yongqin Chen et al. (2003) in *Dioscorea zingiberenesis*.

The well rooted plants were transferred to plastic cups containing vermiculite for hardening and kept under controlled condition (Fig.1.D). Upon transferred to vermiculite medium plants started producing fresh shoots and roots after one week of transplanting. Later they were transferred to the field and the survival rate was 90%. The efficient micro propagation technique described here may be highly use full for raising quality planting material of *Dioscorea oposotifolia* for commercial and off season cultivation which is not only help the ex-situ conservation

but also help full in the restoration of genetic stock of the species.

Conclusion

Clonal propagation through tissue culture (popularly called micro propagation) can be achieved in a short time and space. Thus, it is possible to produce plants in large numbers starting from a single individual protoplast to different plant parts as an explant. Micro propagation has, wide commercial application, starting from conservation of genetic stock of threatened species to secondary metabolite production in important plant taxa and year round supply of disease free quality planting material for commercial cultivation. Since than, several crop species have been micro propagated and recipes are now available which can be adopted by growers trained in aseptic manipulations in a new era of plant husbandry. The results obtained in our experiment suggested that *in vitro* plantlet regeneration using nodal vine may be used for direct clonal propagation and conservation with a low risk of generating disease free quality planting material in large scale for the said species.

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