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In vitro propagation and microtuber induction in dioscorea belophylla (Prain) Voigt ex Haine

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Abstract

A protocol was developed for the *in vitro* propagation and microtuber induction in *Dioscorea belophylla* using nodal explants at Central Tuber Crops Research Institute, Thiruvananthapuram, India. MS medium with benzyl adenine (BA) at 3 μM and Kinetin (Kin) at 4 and 5 μM were found to be the best concentrations for developing shoots with good number of nodes and leaves. The addition of 1-naphthaleneacetic acid (NAA) along with cytokinin resulted in healthier leaf production. Among the different concentrations of sucrose tested, MS medium with 4% sucrose was the best for shooting, having the highest shoot length of 11.8 cm and mean number of 17.4 nodes and 11.4 leaves/shoot within 15.4 days of inoculation. Microtuber induction was recorded in the presence of BA (3-5 μM), Kin (3-4 μM) and BA 3 μM along with NAA (1 μM). Addition of 3-8% sucrose in the medium was found to be the best concentration for inducing 1-3 microtubers/culture.

Key words: *Dioscorea belophylla, in vitro* propagation, microtuber induction, BAP, Kin, NAA

Introduction

Dioscorea belophylla (Spear-leaved-yam) commonly called as Hehkkukalasu (Narayanan et al., 2011) in Malayalam belongs to the family Dioscoreaceae with right twining vines. In the Himalayas, it occurs in Jammu and to some extent in Srinagar in Kashmir, where it grows up to 5000 ft elevation. It also grows in Jabalapur, Raipur, Godaveri and Devagiri at 2300 ft, Simla, below 5000 ft and Dehradun. In South India it occurs in Mangalore at sea level and it is distributed in Nilgiris also (Prain and Burkill, 1938). It grows very deep in the soil and is considered as the most delicious tuber, which is consumed in the form of liquid dishes (Balakrishnan et al., 2003). The tubers of *D. belophylla* are considered as good source of Vitamin C. Shoots are used for the treatment of heart troubles and fresh leaves are used to treat jaundice and mumps.

The species is characterised by brown coloured young stem and dark brown coloured mature stems with green patches. The young leaves have light brown shining appearance with elongated dark green tips and the mature leaves are green in colour with light brown veins and light purple leaf tips. The brown colour of veins is prominent on the abaxial side and less on the adaxial side. Both young and mature petioles are purple with green lines and dark purple at both ends. This species has the characteristic feature of having two ridged petiole. The leaves are simple and elongated cordate, alternately arranged with acute tips. The leaf length ranges from 11-15 cm, width 9-11 cm and petiole length 11-15 cm. The internode length varies from 16-20 cm (Fig. 1).

D. belophylla produces long cylindrical tubers often branching at the bottom, sometimes with 3-4 branches. The tubers are pale brown in colour, medium rough and slightly hairy (Fig.1a). The number of tubers/plant varies from 2-4, tuber length 40-55 cm and tuber thickness 2-4 cm. The tuber yield ranges from 800-900 g/plant. The tuber flesh is highly mucilaginous and white coloured. The cooked tuber flesh is soft and very light yellow in colour and the cooking quality is average.

Fig. 1 and 1a. *D. belophylla* plant and the underground tuber

The tubers of *D. belophylla* are delicate and good to eat. The species propagates vegetatively by tuber setts, but by this method large scale multiplication is limited. Bubils are never seen to occur in this species which could aid vegetative propagation. Since it is a shy flowering dioecious species, seed production is not common and thereby, propagation by fertile seeds is highly limited. Hence, to improve the multiplication rate, an *in vitro* protocol for rapid multiplication and microtuber induction was developed in *D. belophylla.*

Materials and methods

Nodes were used as explants in all the experiments. The nodes were washed in tap water to remove the surface contaminants and soaked in 1% teepol for 30 min and then washed in tap water. They were then disinfected with 0.1% mercuric chloride for 10 min and washed with sterile distilled water several times to remove mercuric chloride completely.

For micro-propagation, Murashige and Skoog medium (MS medium) (1962) supplemented with various concentrations of plant growth hormones, viz., benzyl adenine (BA) (1-10 μM) and Kinetin (Kin) (1-10 μM) individually as well as combinations of BA (1-6 μM) or Kin (1-6 μM) with 1-naphthaleneacetic acid (NAA) (1 μM), sucrose 30 g l^{-1} and activated charcoal 1 g l^{-1} were used (Table 1). For microtuber induction, MS basal medium with varying concentrations of sucrose (1-10 %) were used. The pH of the medium was adjusted to 5.8 and autoclaving was done at 120°C for 15 min. The cultures were grown at 26 ± 2°C, under an 16:8 hr light:dark regime (3000 lux). For hardening the cultures, the rooted plantlets were washed to remove the adhering medium and transferred to plastic cups filled with vermiculite and nurtured by adding Hoagland's solution

Table 1. Concentrations of additives used in MS medium for micropropagation and microtuber induction in *D. belophylla*

BA (μM)	Kin (μM)	NAA (μM)	Sucrose (%)
1-10	..	..	..
..	1-10	..	..
1-6	..	1	..
..	1-6	1	..
..	..	..	1-10

at weekly intervals. After the development of new leaves, the plantlets were transferred to pots, which were later transplanted to the field.

The experiments were conducted with 5 replicates for each treatment using a Completely Randomized Design. Response variation in terms of number of nodes, roots and shoots developed as well as shoot length after 16 weeks were recorded. Number of days for shoot initiation was also recorded. The variation among the means was statistically analyzed using SAS 9.3 (SAS, 2010).

Results and Discussion

Micropropagation

Effect of benzyl adenine and kinetin on shooting in nodal cultures

In *D. belophylla*, BA 1-6 μM and Kin 1-7 μM resulted in shoot production. BA 3 μM elicited the development of lengthy shoots of 11.8 cm, 9.4 leaves and 11.6 nodes/shoot followed by BA 4 μM (Fig. 2). The higher concentration of 5 μM was less effective, whereas, the lower concentrations of 1-2 μM were effective, but induced smaller shoots with fewer nodes and leaves/shoot (Table 2).

Kin at 4 and 5 μM induced good shoots but the latter produced smaller, fewer leaves (Fig. 3). With Kin 7 μM, shoot length, number of nodes and leaves were reduced and the same, at 8-10 μM, resulted in callusing of cultures.

Both BA (3 μM) and Kin (4 and 5 μM) were found to be the best concentrations for shoot induction with good number of nodes and leaves, but shoot length was slightly reduced in the presence of Kin. Higher concentrations of cytokinin showed absence of shoot induction. In *D. nipponica* the rate of shoot induction and shoot height increased with increasing concentrations of BA up to 2

Table 2. Effect of different concentrations of BA/Kin alone and in combination with NAA on shoot induction in nodal cultures of *D. belophylla*

BA (μM)*	Kin (μM)*	NA A (μM)*	% of survival of explants	No. of days for initial sprouting[1]	No. of shoots[1]	No. of nodes[1]	No. of leaves[1]	Shoot length (cm)[1]
1	..	..	60	12.4±0.13	1.0±0	2.2±0.11	2.8±0.11	2.6±0.13
2	..	..	60	11.4±0.13	1.2±0.11	4.6±0.13	5.4±0.13	4.4±0.13
3	..	..	100	7.6±0.13	1.0±0	11.6±0.13	9.4±0.13	11.8±0.11
4	..	..	80	9.4±0.13	1.0±0	7.4±0.13	5.6±0.21	7.2±0.11
5	..	..	80	10.4±0.13	1.0±0	6.8±0.11	3.8±0.11	5.2±0.11
6	..	..	40	11.2±0.2	1.0±0	2.4±0.13	3.4±0.13	2.6±0.13
..	1	..	40	8.6±0.21	1.0±0	1.2±0.11	1.6±0.13	1.6±0.13
..	2	..	60	7.0±0.17	1.0±0	2.8±0.11	3.6±0.13	3.2±0.11
..	3	..	60	6.4±0.13	1.2±0.11	5.6±0.13	9.6±0.13	5.4±0.13
..	4	..	60	5.4±0.13	3.0±0.17	9.8±0.11	12.2±0.11	7.4±0.13
..	5	..	80	6.2±0.11	1.0±0	15.6±0.13	7.8±0.11	10.2±0.11
..	6	..	80	6.6±0.21	1.0±0	7.4±0.13	7.0±0.17	7.4±0.13
..	7	..	60	8.8±0.2	1.0±0	4.8±0.11	4.8±0.11	4.6±0.13
1	..	1	60	18.4±0.13	1.0±0	2.2±0.11	2.0±0.17	2.8±0.11
2	..	1	60	17.2±0.11	2.2±0.11	9.4±0.11	2.6±0.13	8.6±0.13
3	..	1	80	16.8±0.11	1.2±0.11	7.4±0.13	8.6±0.13	6.6±0.13
4	..	1	60	16.8±0.11	1.0±0	3.6±0.13	6.6±0.13	3.6±0.13
5	..	1	60	19.2±0.11	1.0±0	1.8±0.11	3.6±0.13	1.6±0.13
..	1	1	40	18.2±0.11	1.2±0.11	0±0	0.8±0.11	8.8±0.11
..	2	1	60	18.2±0.11	2.2±0.11	4.2±0.11	2.2±0.11	9.4±0.13
..	3	1	60	16.6±0.13	2.2±0.11	5.8±0.11	7.6±0.13	7.2±0.11
..	4	1	60	17.4±0.13	1.2±0.11	5.0±0.17	4.6±0.13	6.4±0.13
..	5	1	40	18.8±0.2	1.0±0	3.4±0.13	3.4±0.13	2.8±0.11

*The concentrations of BA, Kin and NAA which did not respond to shoot induction are excluded from the table. [1]Each value is Mean ± SE of five replications.

LSD for number of days for initial sprouting = 0.732; LSD for number of shoots = 0.256; LSD for number of nodes = 1.142; LSD for number of leaves=0.805; LSD for shoot length = 0.685

Fig. 2. Good shoot induction in MS medium with BA 3 μM; Fig. 3. Effect of MS medium with Kinetin 4 μM in inducing good shoots

mg l^{-1} and then dropped at 4 mg l^{-1} as the BA became superoptimal (Chen et al., 2007). In *D. oppositifolia* and *D. pentaphylla* the shoot induction was more in the presence of BA than Kin (Poornima and Ravishankar, 2007). Lakshmisita et al. (1976) reported Kin to be an effective cytokinin in nodal cultures of *D. floribunda*. They reported that Kin supplied at either 11.6 or 46.4 μM significantly enhanced the shoot development. The effect of cytokinins in reduced shooting at higher concentrations was reported in *D. bulbifera* (Forsyth and Staden, 1982). Cytokinins at excessively high or low concentrations could result in formation of fewer and shorter shoots, or no shoot at all as in *D. nipponica* (Chen et al., 2007). The presence of cytokinins in the medium was found to increase the shoot induction potentiality. Growth and morphogenesis *in vitro* are regulated by the interaction and balance between the growth regulators supplied in the medium and the growth substances

produced endogenously by cultured cells. In tissue culture, cytokinins are necessary for plant cell division. They regulate the synthesis of proteins involved in the formation and function of mitotic spindle apparatus. To encourage the growth of axillary buds and to reduce apical dominance in shoot cultures, cytokinins are incorporated into the medium (George et al., 2008).

Effect of cytokinin and auxin on shooting

An auxin NAA (1 μM) was added along with Kin and BA (1-6 μM) separately to study the effect of auxin and cytokinin on shoot induction. Healthy leaf production was commonly observed when the auxin was used in the presence of both BA and Kin.

In the presence of BA (3 μM) and NAA (1 μM) good leafy shoots of 6.6 cm length were produced having 7.4 nodes and 8.6 thick leaves/shoot (Fig. 4). At higher concentrations of BA (4 μM), good leafy shoot was induced, but shoot length was reduced. Beyond 5 μM, no shoot induction was observed. BA at lower concentrations of 2 μM, induced lengthy shoots with fewer leaves and plenty of roots (Table 2).

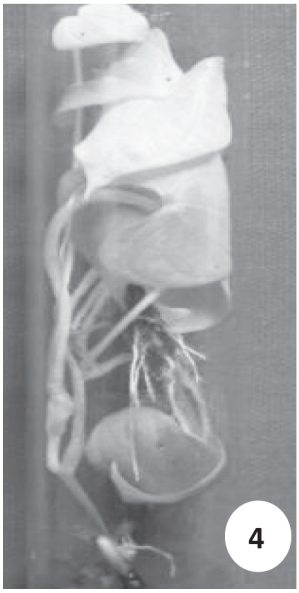

Fig. 4. Development of leafy shoot with thick leaves in MS medium with BA 3 μM and NAA 1 ìM

In the presence of Kin at 3 μM and NAA at 1 μM, development of shoots of 7.2 cm length with 5.8 nodes and 7.6 normal/shoot were observed. Kin at 4 μM also produced similar lengthy shoots with normal leaves but the number of leaves was reduced. In the presence of 5 μM Kin, smaller shoots with fewer leaves and nodes were developed. MS medium with 6 μM Kin resulted in cultures without shoots. Lower concentrations of Kin (1 and 2 μM) and NAA (1 μM) containing medium developed lengthy shoots with fewer leaves and reduced number of nodes/shoot with slight callus development.

In *D. belophylla* response to shoot induction in terms of shoot length, number of nodes and number of leaves were high in the presence of cytokinin alone but the leaves developed in medium with NAA (1 μM) along with BA/Kin (3 μM) were big and healthy. Higher concentrations of Kin (10 mg l^{-1}) along with IAA (2 mg l^{-1}) was reported to be good for leaf development in *D. polystachya*, *D. sansibarensis* and *D. japonica* (Islam et al., 2008). They also reported better leaf development at lower concentrations of BA (0.5 mg l^{-1}) and NAA (0.2 mg l^{-1}) in *D. polystachya*, *D. japonica* and *D. bulbifera*. Ezeibekwa et al. (2009) reported the importance of both the phytohormones, auxin and cytokinin in *in vitro* propagation of *D. rotundata* wherein, BA (0.2 mg l^{-1}) and NAA (0.5 mg l^{-1}) showed an increase in almost all the parameters. The synergistic effect of BA and NAA in shoot induction was also reported in *D. nipponica*, where, the percentage of node forming shoots increased from 52.81% (BA at 2.0 mg l^{-1}) to 85.71% in the presence of BA (2.0 mg l^{-1}) and NAA (1.0 mg l^{-1}) (Chen et al., 2007). Explants grown in media with 0.5 mg l^{-1} BA and 0.01 mg l^{-1} NAA showed the highest rate of multiplication and survival in *Dioscorea prazeri* (Thankappan and Patell, 2011). These results indicate that interaction of auxin and cytokinin is synergistic for plant *in vitro* organogenesis.

Effect of sucrose levels on shoot induction

MS medium with 4% sucrose was the best for shoot induction in which shoot bud initiation occurred within 15.4 days with 80% survival having the highest shoot length of 11.8 cm with 17.4 nodes and 11.4 leaves/shoot (Fig.5). Sucrose at 5, 6 and 7% produced similar lengthy shoots but with reduced number of leaves. At higher concentrations (9 and 10%) no shoot induction was observed. At lower concentrations of 3 and 2% sucrose, fairly good response was observed with the development of smaller shoots with fewer nodes and leaves (Table 3).

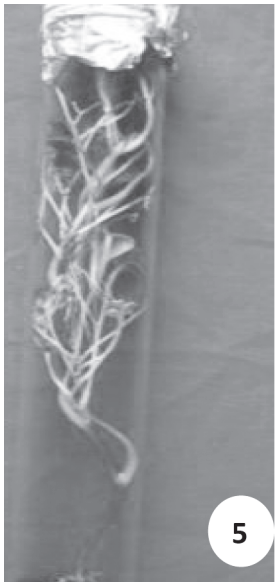

Fig. 5. Very good shoot induction in MS medium with 4 % sucrose

Table 3. Effect of different concentrations of sucrose on shoot induction in *D. belophylla*

Sucrose (%)*	% of survival of explants	No. of days for initial sprouting[1]	No. of shoots[1]	No. of nodes[1]	No. of leaves[1]	Shoot length (cm)[1]
1	40	15.6±0.13	1.0±0	2.8±0.11	3.6±0.13	2.4±0.13
2	60	14.4±0.13	1.0±0	3.6±0.13	5.8±0.11	5.0±0.17
3	80	12.4±0.13	1.0±0	4.6±0.13	7.6±0.13	6.4±0.13
4	80	15.4±0.13	1.0±0	17.4±0.13	11.4±0.13	11.8±0.11
5	80	16.4±0.13	1.0±0	10.2±0.11	5.6±0.13	11.4±0.13
6	80	17.4±0.21	1.0±0	9.4±0.13	5.4±0.13	9.6±0.13
7	60	18.0±0.17	1.0±0	4.6±0.21	5.0±0.24	8.8±0.2
8	60	18.4±0.21	1.0±0	2.6±0.21	2.2±0.11	8.0±0.29

*The concentration of sucrose which did not show shoot induction response are excluded from the table.
[1]Each value is Mean ±SE of five replications.
LSD for number of days for initial sprouting = 0.734; LSD for number of shoots = 0.213; LSD for number of nodes = 1.853; LSD for number of leaves=0.842; LSD for shoot length = 0.851

Asha and Nair (2007) reported that sucrose at 3% was more effective for nodal segment cultures of *D. pentaphylla*. High concentration of sugar source was found to be ideal for *in vitro* propagation in several species as sucrose is an essential carbon source under *in vitro* conditions where photosynthesis could not sufficiently support growth and development of the explants. In *D. alata* increasing the sucrose concentration from 2 to 5% enhanced shoot and root development with increase in the production of complete plantlets from 56-89%. In *D. bulbifera* and *D. olfersiana*, 3 to 5% sucrose was reported as the optimal concentrations for producing maximum number of leaves (Acedo, 2003; Chu and Ribeiro, 2002). The increase in the shoot induction potentiality with increasing concentrations of sucrose may be mainly due to the high sugar levels available in the culture medium which speeds up cell division. Mean shoot height and root formation were highest in *D. rotundata* cultures on full-strength MS medium containing 10% sucrose (Asare et al., 2011).

Microtuber induction

Effect of BA and Kin on microtuber induction

Among the different concentrations of BA used in the medium, microtuber induction was observed in the presence of 3, 4 and 5 μM. BA at 3 μM produced a single brown elongated microtuber from the lower node after 7.8 months of culture, while BA (4 μM) produced a single black oval microtuber from the basal node after 9.2 months. BA at 5 μM induced the development of a single, smaller, black oval microtuber from the middle node, after 9.2 months (Table 4).

Of the different concentrations of Kin, 3 and 4 μM induced microtubers. In both cases the microtubers were smaller. While Kin 3 μM induced oval microtubers from the basal node after 7.6 months, Kin 4 μM induced round microtubers from the middle node after 9.2 months.

Effect of cytokinin and auxin on microtuber induction

BA at 3 μM along with NAA (1 μM) induced the development of a single small, oval black microtuber from the middle node after 9.2 months of culture (Fig. 6).

In *D. belophylla*, BA in the medium was more effective than Kin for microtuber induction. BA along with NAA also promoted

Fig. 6. Oval black microtuber produced in the presence of BA 3 μM and NAA 1 μM in MS medium

microtuber induction. BA 8.87 ìM alone with 3% sucrose induced microtubers in *D. oppositifolia* and *D. pentaphylla*, (Poornima and Ravishankar, 2007). The promotive effect of NAA on microtuber induction was reported in *D. composita* (Alizadeh et al., 1998) and *D. floribunda* (Sengupta et al., 1984). Levels of nitrogen and NAA in the media influenced tuberization in *D. rotundata* (Klu, 2002).

Effect of sucrose levels on microtuber induction

In *D. belophylla* 3-8% sucrose in the medium was found to be the best concentration for inducing 1-3 microtubers/culture. The microtubers produced from 3 and 4% sucrose were single and oval in shape, borne on the middle node of the developed shoots. Sucrose at 5 to 8 % resulted in the development of 2-3 microtubers, varying in size, shape, colour and nodal positions (Table 4; Figs.7 and 8).

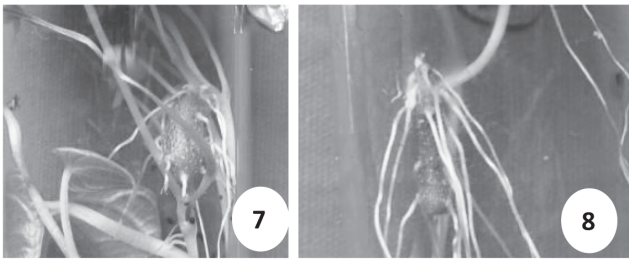

Fig. 7 & 8. Brown oval microtuber from upper node and thin slender microtuber from middle node in MS medium with 5 % sucrose

There are several reports which indicate the need to increase or decrease the sucrose concentrations for the induction of microtubers in yams. In *D. oppositifolia*, microtuber induction in the presence of 7 and 8% sucrose and absence of microtuber development at lower sucrose concentrations were reported (Maheswari et al., 2012). The positive effect of 2-8% sucrose in inducing microtuber in *D. abyssinica* was reported (Lauzer et al., 1992). In *D. nipponica*, at a high concentration of 7%

Table 4. Effect of different concentrations of BA/Kin and sucrose concentrations on microtuber induction

BA (μM)*	Kin (μM)*	NA A (μM)*	Sucrose (%)*	No. of microtubers	Size of microtuber		Initiation time of microtuber[1] (months)	Nature of microtuber
					Length[1] (cm)	Width[1] (cm)		
3	..	..	..	1	3.8±0.1	0.48±0.01	7.8±0.11	Brown elongated from lower node
4	..	..	..	1	2.6±0.13	0.46±0.01	9.2±0.11	Black oval from very basal node
5	..	..	..	1	0.72±0.02	0.32±0.01	9.2±0.11	Black oval from middle node
..	3	..	..	1	0.72±0.02	0.38±0.02	7.6±0.13	Brown oval from basal node
..	4	..	..	1	0.28±0.01	0.26±0.01	9.2±0.11	Black round from middle node
3	..	1	..	1	0.66±0.01	0.34±0.01	9.2±0.2	Black oval from middle node
..	..	..	3	1	1.24±0.02	0.48±0.01	9.8±0.11	Oval dark brown from middle node
..	..	..	4	1	1.4±0.02	0.46±0.01	9.6±0.13	Oval brown from middle node
..	..	..	5	2	1.66±0.06	0.38±0.03	8.4±0.13	Brown oval from upper and middle nodes
..	..	..	6	2	0.92±0.02	0.48±0.01	8.0±0	Black oval from middle nodes
..	..	..	7	3	1.7±0.04	0.26±0.01	7.4±0.13	Dark brown oval from middle nodes
..	..	..	8	2	1.66±0.06	0.38±0.01	7.0±0.17	Black oval from basal and middle nodes

*The concentrations of BA, Kin, NAA and sucrose which did not show microtuber induction are excluded from the table. [1]Each value is Mean ±SE of five replications.

LSD for microtuber length = 0.163; LSD for microtuber width = 0.0215; LSD for duration for microtuber induction = 0.531

sucrose, the production of heavier microtubers were promoted (Chen et al., 2007). In *D. cayenensis*, greater number and size of microtubers were achieved when 40 g l^{-1} sucrose was used (Jasik and Mantell, 2000) whereas, raising sucrose concentration from 30 to 80 g l^{-1} inhibited tuberization rate in *D. fordii* and *D. cayenensis-D. rotundata* complex (Yan et al., 2011; Ovono et al., 2007). In *D. rotundata*, a decrease in the percentage of microtuberization with 8 or 10 % sucrose and 2.5 ìM Kin was reported by Ng (1988).

Hardening of cultures

Shoots with roots developed in MS medium, was transferred to plastic cups with vermiculite, which showed 90% survival and development of new leaves after 20-25 days (Fig. 9). After two months the plantlets

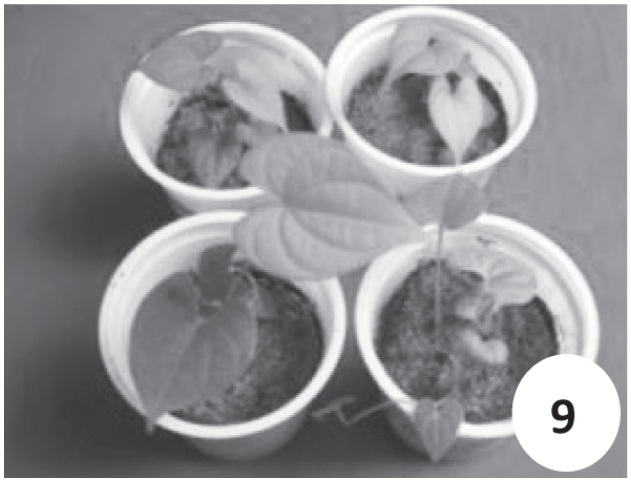

Fig. 9. Hardened *in vitro* plantlets.

were transplanted to pots and kept in green house for establishment and tuberisation. The plants showed 90% survival with underground tuber formation.

Conclusion

The preferred medium for axillary shoot proliferation in *D. belophylla* was MS basal medium supplemented with BA at 3 μM and Kin at 4 and 5 μM. For healthier leaf production presence of auxin (NAA) along with cytokinin was required. Sucrose concentration was found to be the most important factor for microtuber induction. Sucrose at 3-8% was found to be the best concentration for microtuber induction. Using this protocol, unlimited number of shoots can be produced, independent of the growing season. It can also be used for conservation of *Dioscorea belophylla.*

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References

Acedo, V.Z. 2003. Propagation of purple yam (*Dioscorea alata*) through tissue culture. *J. Root Crops*, **29** (2): 7-16.

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 Organ Cult.*, **53**: 107-112.

Asare, A.T., Acheampong, E., Galyuon, I.K.A., Aboagye, L.M. and Takrama, J.F. 2011. Effects of sucrose on development of cultures of some accessions of *Dioscorea* spp. *J. Ghana Sci. Ass.*, **13** (2): 12-19.

Asha, K.I. and Nair, G.M. 2007. *In vitro* bulbil induction in *Dioscorea* species. *J. Root Crops*, **33** (2): 81-87.

Balakrishnan, V., Narayanan. M.K.R. and Anil Kumar, N. 2003. Ethnotaxonomy of *Dioscorea* among the Kattunaikka people of Wayanad District, Kerala, India. *Plant Genetic Resour. Newsl.*, **135**: 24-32.

Chen, F.Q., Fu, Y., Wang, D.L., Gao, X. and Wang, L. 2007. The effect of plant growth regulators and sucrose on the micropropagation and microtuberization of *Dioscorea nipponica* Makino. *J. Plant Growth Regul.*, **26**: 38-45.

Chu, E.P. and Ribeiro, R.C.L.F. 2002. Growth and carbohydrate changes in shoot cultures of *Dioscorea* species as influenced by photoperiod, exogenous sucrose and cytokinin concentrations. *Plant Cell Tissue Organ Cult.,* **70**(3): 241-249.

Ezeibekwa, I.O., Ezenwaka, C.L., Mbagwu, F.N. and Unamba, C.I.N. 2009. Effects of combination of different levels of auxin (NAA) and cytokinin (BAP) on *in vitro* propagation of *Dioscorea rotundata* L. (White Yam). *New York Sci. J.*, **2**(5): 1-8.

Forsyth, C. and Staden, V.J. 1982. An improved method of *in vitro* propagation of *Dioscorea bulbifera*. *Plant Cell Tissue Organ Cult.*, **1**: 275-281.

George, E.F., Hall, M.A. and Klerk, G.J.D. 2008. Plant growth regulators II: Cytokinins, their analogues and antagonists. In: *Plant Propagation by Tissue Culture*. 3rd Edn., Volume 1. *The Background*. George, E.F., Hall, M.A. and Klerk, G.J.D. (Eds.). Springer, The Netherlands. pp. 205-226.

Islam, M.T., Keller, E.R.J. and Dembele, D.P. 2008. Effects of growth regulators on *in vitro* propagation and tuberization of four *Dioscorea* species. *Plant Tissue Cult. Biotech.*, **18**(1): 25-35.

Jasik, J. and Mantell, S.H. 2000. Effects of jasmonic acid and its methyl ester on *in vitro* microtuberisation of three food yam (*Dioscorea*) species. *Plant Cell Rep.,* **19**: 863-867.

Klu, G.Y.P. 2002. Conservation of *Dioscorea rotundata*: effect of basal medium type and naphthalene acetic acid on growth and microtuberization. *West Afr. J. Applied Ecology,* **3**: 99-103.

Lakshmisita, G., Bammi, R.K. and Randhawa, G.S. 1976. Clonal propagation of *Dioscorea floribunda* by tissue culture. *J. Hort. Sci.,* **51**: 551-554.

Lauzer, D., Laublin, G., Vincent, G. and Cappadocia, M. 1992. *In vitro* propagation and cytology of wild yams, *Dioscorea abyssinica* Hoch. and *D. mangenotiana* Miege. *Plant Cell Tissue Organ Cult.,* **28**(2): 215-223.

Maheswari, R.U., Prabha, A.L., Nandagopalan, V. and Anburaja, V. 2012. *In vitro* rhizome production from nodal explants and callus formation of the medicinal plant *Dioscorea oppositifolia* L. *J. Pharmacy Biol. Sci.*, **1**(6): 17-21.

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

Narayanan, M.K.R., Anilkumar, N., Balakrishnan, V., Sivadasan, M., Alfarhan, H.A. and Alatar, A.A. 2011. Wild edible plants used by the Kattunaikka, Paniya and Kuruma tribes of Wayanad district, Kerala, India. *J. Med. Plant Res.*, **5**(15): 3520-3529.

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

Ovono. P.O., Kevers, C. and Dommes, J. 2007. Axillary proliferation and tuberisation of *Dioscorea cayenensis-D.rotundata* complex. *Plant Cell Tissue Organ Cult.*, **91**: 107-114.

Poornima, G.N. and Ravishankar Rai, V. 2007. *In vitro* propagation of wild yams, *D. oppositifolia* (Linn) and *D. pentaphylla* (Linn). *African J. Biotechnol.*, **6**(20): 2348-2352.

Prain, D. and Burkill, I.H. 1938. An account of the genus *Dioscorea* in the east. The species which twine to the right with addenda to part I and a summary, Royal Botanic Garden, Calcutta, part II.

SAS. 2010. SAS Institute, Inc., Cary, NC, USA.

Sengupta, J., Mitra, G.C. and Sharma, A.K. 1984. Organogenesis and tuberization in culture of *Dioscorea floribunda. Plant Cell Tissue Organ Cult.,* **3**(4): 325-331.

Thankappan, S.S. and Patell, V.M. 2011. *In vitro* propagation studies and genetic fidelity assessment of endangered medicinal wild yam- *Dioscorea prazeri. Plant Omics J.*, **4**(4): 177-189.

Yan, H., Yang, L. and Li, Y. 2011. Axillary shoot proliferation and tuberisation of *Dioscorea fordii* Prain et Burk. *Plant Cell Tissue Organ Cult.*, **104**: 193-198.