Effect of plant growth regulator on in vitro micropropagation of ‘Bitter Yam’ (*Dioscorea hispida* Dennst.)

Kambaska Kumar Behera1,*, Santilata Sahoo1, Aratibala Prusti2

1. P. G. Dept. of Botany, Utkal University, Bhubaneswar, Orissa, India
2. P.N. College, Khurda, Orissa, India

Submitted: 26 Nov. 2008; Accepted: 30 Dec. 2008

Abstract

The present study reports a highly efficient and cost effective protocol for high frequency plantlet regeneration from nodal explants of *Dioscorea hispida*. Nodal vine segments from 40 days old plants of *Dioscorea hispida* were cultured on Murashige and Skoog’s (MS) medium supplemented with different concentration and combination of cytokinin i.e. 6-Benzyl Aminopurine (BAP) and auxin i.e. a-naphthalene acetic acid (NAA) to evaluate optimum condition for multiple shoot proliferation. The highest number of multiple shoot proliferation was obtained with explants cultured on MS medium supplemented with 2.0 mg/l BAP+0.5 mg/l NAA +100mg/l ascorbic acid. Shoot multiplication rate was maintained by repeated sub culturing on to fresh media containing same growth regulators. When elongated *in vitro* shootlets were inoculated to half-strength MS basal media supplemented with 2.0 mg/l NAA+2 g/l activated charcoal, rooting was more profuse. Rooted shoots were transplanted in the green house for hardening and their survival was 90% in the field conditions.

**Keywords:** *Dioscorea hispida*, Growth regulators, Nodal vine, *in vitro* propagation, Shoot regeneration, Tissue culture.

INTRODUCTION

Yams belong to the oldest monocotyledonous family *Dioscoreaceae*, genus *Dioscorea* that contains over 600 species. The family *Dioscoreaceae* is a natural group of tuber-forming tropical vine and is usually allied with Liliales and placed near the Amaryllidaceae (Ben, 1937). Asia, South America and West Africa are the major yam growing region of the world (Coursey, 1967; Ayensu and Coursey, 1972). The six most cultivated species in Africa are *D. alata*, *D. bulbifera*, *D. cayenensis*, *D. esculenta*, *D. rotundata* and *D. trifida*. The tubers have a dual agricultural function: first, as source of food and secondly, as planting material (Hahn, 1995; Craufurd et al., 2006). The cultivated forms of this vegetative propagated crop have a large genetic diversity. *Dioscorea hispida* was considered as one of the most neglected species of *Dioscorea* because of the presence of poisonous alkaloids known as Dioscorine. *Dioscorea hispida* has several names all over India but the common names used in Orissa are Kulia, Kolhua, Kolokanda, Baikanda etc., (Burkill, 1960) and the Sanskrit name is ‘Marpa shpoli’. *D. hispida* is a toxic tuber bearing Dioscorea well distributed in jungles, all over the state of Orissa in dryer areas. Moderate seed production is found in this species because they live for a short period and decline by the end of winter. Among the *Dioscoreas*, *D. hispida* is fast to flower in Orissa climatic condition because of persistent large tubers on soil. Due to toxic nature, tubers are hardly removed from the soil but consumed during rainy season in lean period. The processing methods is difficult to practice as they require slicing and soaking in running water for several days (Burkill, 1960; Coursey, 1967). The basic propagation system of *Dioscorea* species is by tuber seeds, a tuber fragment that sprouts each year, developing a new tuber, which is traded 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* (Chu and Ribeiro, 2002; Tschannen et al., 2005). There are reports on the micropropagation of some food yams *in vitro* such as *D. alata* and *D. rotundata* (Mantell and Hugo, 1989; Jean and Cappadocia, 1991; Alizadeh et al., 1998) and *D. trifida* using meristems (Saleil et al., 1990). However, little information were available on the micropropagation of *D. hispida* (Malaurie et al., 1995; Yuan et al., 2005). The aim of this study is thus to...
Effect of plant growth regulator

standardize the in vitro multiplication method of this species for production of disease free quality planting material for large scale propagation and conservation of the genetic stock of the plant.

MATERIALS AND METHODS

Explant source

Healthy vines with active buds were collected from 35 days old plants of *D. hispida*, maintained in the experimental garden of P.G. department of Botany Utkal University and 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 min 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 and transferred to laminar air flow cabinet. In the laminar chamber the nodal segments were again dip with 70% alcohol for 30 second to one minutes 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 BAP and 0.25 and 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 microshoots 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 (Indole 3-butyric acid) in the concentration of 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/l with 2g/l activated charcoal. The pH of the medium was adjusted to 5.8 before autoclaving at 1.04 kg/cm² pressure and 121°C temperature for 15-20 minute. Molten medium of 20 ml was dispensed into the culture tube and plugged with nonabsorbent cotton wrapped in one layer of cheesecloth. All cultures were incubated in 16 h light (cool, white fluorescent light - 30µmol m⁻²S⁻¹) and 8 h dark period. 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 required hormonal combination.

Acclimatization

Rooted micro-propagules were removed from the culture tube and the roots were washed under running tap water to remove agar. Then 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 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.

Observation of cultures and statistical analysis

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 analysed by the Post- Hoc Multiple Comparison test at the P < 0.05 level of significance (Marascuoio and McSweeney, 1977).

RESULTS

Shoot proliferation and multiplication

The response of *D. hispida* nodal vine explants cultured on different shoot proliferation media over a period of six weeks is presented in Table 1. [Supplementary data], 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 of explants responded to MS media, number of shoots per explant, average shoot length and average number of nodes produced per shoot. Bud break was noticed within 8-10 days of culture when MS media was supplemented with BAP (1.0, 1.5, 2.0 mg/l) with NAA 0.5 mg/l, where as other combination of BAP and NAA took 12 to 15 days. (Fig. 1A, Table 1, Fig. 2). Of the combinations tested 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 shootlets (Fig. 1A, Table 1) with a mean shoot length of 5.0 ± 0.29 cm per explant were recorded. The second best shoot multiplication 4.5 ± 0.12 was...
Effect of plant growth regulator obtained in the medium BAP (1.5 mg/l) + NAA (0.5 mg/l) + 100 mg/l ascorbic acid with a mean shoot length of 4.0 ± 0.29 cm. Higher concentration of BAP (3.0 mg/l) with NAA (0.5 mg/l) + 100 mg/l ascorbic acid showed callusing explants with fewer number of shoots. In such cultures shoots were stunted with a mean shoot length of 1.7 ± 0.08 cm.

Induction of rooting from microshoots

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 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. 1B). 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 and 2.0 mg/l) and IBA at all concentration tested respond well for root development. Rooting was better in the culture which had combination of 1/2 MS+2.0 mg/l NAA+2.0g/l activated charcoal where about 90% cultures responded with an average number of 5.2 ± 0.28 roots per plantlet and an average root length 3.5 ± 0.12 cm was recorded (Fig. 1B, Table 2 [Supplementary data], Fig. 3). The second highest response (75%) was observed in the MS medium supplemented with 1.5 mg/l of NAA+2g/l activated charcoal. It was observed that root primordia emerged from the shoot base starting from 8 to 10 days after shoot inoculation and soon after that the root growth was rapid. Rooting was better in MS medium when supplemented with NAA + Ac (Activated charcoal) than IBA+ Ac (Activated charcoal) (Table 2 [Supplementary data]).

DISCUSSION

The dependence of cultured explants on bud break response and shoot multiplication has already been established and extensively discussed (George and Sherrington, 1984; Ammirato, 1976). This has also been recently reported in the case of micro propagation of other Yams like D. abyssinica (Martine and Cappadocia, 1991), D. batatas (Koda and Kikuta, 1991), D. composita (Alizadeh et al., 1998), and D. floribunda (Sengupta et al., 1984). In the present study, nodal vine explants of D. hispida show significantly higher response of multiple shoot in the medium with the combination of BAP (2.0 mg/l) + NAA (0.5 mg/l) + Ascorbic acid 100g/l. 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 in shoot development was recorded when BAP 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. It has been reported that, Spalthyllum floribundam when cultured on media with BA supplement alone, a limited proliferation of explants with an average of 1.8 shoots per cultured explants was observed, while addition of IAA produced an average number of 11.6 shoots per explant (Ramirez-Magon et al., 2001). Similar observation was reported in Hovenia dulcis nodal culture (Echeverrigaray et al., 1998). In our study only single cytokinins are taken for shoot multiplication but some authors suggested that the combination of two cytokinins were needed for producing higher number of multiple shoots on Aristolochia bracteolata (Ramashree et al., 1994), Lavundula species (Jordan et al.,1998), Canavalia virosa (Kathiravan and Ignacimuthu, 1999) and Enicostemma littorale (Shanthi and Anne Xavier, 2003; Han et al., 2000). 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.

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 shootlets. In the present study 1/2 strength MS basal medium and the two different auxins (NAA and IBA)+Activated charcoal (Ac) was tried. The maximum results on rooting were obtained on half strength MS media with NAA (2.0 mg/l) + Ac 2mg/l than IBA (2.0 mg/l)+Ac 2mg/l. Our observation are in accordance with the result of Chen et al. (2003) in D. zingiberensis. Bennett (1987) reported that activated charcoal in combination with growth regulators was advantageous for rooting in vitro. Wayne et al. (1995) reported that in Cercis canadensis var. mexicana rooting of microshoots in vitro was best on half strength woody plant medium containing 6.71 µM naphthalenecetic acid and 0.1% activated charcoal. Rukiye (2003) reported that in Galanthus ikariae, rooting was better in the combination of 1/2MS medium containing 0.5% AC and 0.5 mg/l NAA but in the present study 2g/l activated charcoal is more effective for profuse rooting in D. hispida.

Acclimatization and field establishment

The acclimatization procedure describe here resulted in a high survival rate of D. hispida plantlets. There was 90% survival rate of the plantlets that were rooted in NAA supplemented medium where the plantlet exhibited healthy growth (Fig. 1C). After three weeks in vermiculite medium the plants were transferred to field, where the plant shows luxurious growth (Fig. 1D), while shoots rooted in IBA supplement did not survive much. This may be due to improper development of root system in such cultures.

CONCLUSION

The in vitro propagation protocols developed in the present study, thus can be effectively utilized for commercial cultivation and domestication of the valuable species as well as for further biotechnological and molecular research as the species has quick bulking capacity of tuber production as compared to other Dioscoreas of Orissa.

Acknowledgement

Authors are thankful to Prof. T. Moharan, OUAT, Bhubaneswar, Orissa for providing seed tuber and valuable suggestion for this work.

References


Effect of plant growth regulator


