Identification of Optimum Media for *In-vitro* Shoot Multiplication of *Dioscorea pentaphyla*

S.V. GUNAWARDENA[1], E.S.C. EDIRISINGHE[2], S.K. WASALA[2] and N. KOTTEARACHCHI[1]

[1]*Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), 60170, Sri Lanka*

[2]*Plant Genetic Resource Center, Gannoruwa, Peradeniya, Sri Lanka*

ABSTRACT

***Dioscorea pentaphyla* is a medicinally important plant which has antibacterial and antifungal properties. It is one of the important food crops in the diet of people living in rural areas of Sri Lanka. Since it is a seasonal crop, *in vitro* multiplication is an efficient method for quick and large scale propagation. *In vitro* shoot initiation and shoot multiplication of *D. pentaphyla* were investigated by identifying optimum media compositions. Immature shoot tips of field grown *D. pentaphyla* were used as explant source for shoot initiation. The explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of 6-Benzylaminopurine (BAP) with constant concentration of Gibberellic acid (GA_3), Naphthalene acetic acid (NAA) and Ascorbic acid. The number of days to bud break and explant response percentage were observed. The number of days to bud break was not significantly different in the three culture media. The highest explant response (56.25%) was observed in MS+0.5 mg/L BAP with 0.1 mg/L GA_3+0.1 mg/L NAA+100 mg/L Ascorbic acid. For shoot multiplication, explants were cultured on MS medium with different concentrations of NAA with constant concentration of BAP. Significantly high number of leaves and high shoot length were observed in MS+0 mg/L NAA+2 mg/L BAP. Therefore, this medium can be selected for shoot multiplication via nodal cuttings.**

KEYWORDS: *Dioscorea pentaphyla, In-vitro* propagation, Shoot Initiation, Shoot Multiplication

INTRODUCTION

Dioscorea pentaphyla is an edible tuber crop which belongs to the family Dioscoreacea. It is native to southern and eastern Asia (China, India, Indonesia *etc.*) as well as New Guinea and Northern Australia. It is a widely cultivated food crop in Cuba and Hawaii. It is grown in tropical areas of the world. It is known by the common name Buck yam and known as *Katu ala* in Sri Lanka. It is a seasonal monocot bearing aerial bulbils which is a left twining climber that has 3-5 foliate leaves (Kumar *et al.*, 2013). They produce 5-6 feet long root tubers and often tuberiferous in the leaf axils (Rajapaksha, 1998). The genus *Dioscorea* contains 600 known species. The most cultivated *Dioscorea* spp. in Sri Lanka are *Dioscorea alata*, *Dioscorea esculenta* and *Dioscorea bulbifera* (Department of Agriculture, 2006). In Sri Lanka, majority of *Dioscorea* spp. are found in home gardens, especially in wet and intermediate zones (Sangakkara and Frossard, 2014). But, *Dioscorea pentaphyla* is not cultivated commercially in Sri Lanka. It is found in natural habitats in the warmer, moist areas in Sri Lanka (Rajapaksha, 1998).

Dioscorea spp. play a major role in food security (Department of Agriculture, 2006) and has medicinal values (Prakash and Hosetti, 2012). In times of food scarcities, yams provide a reliable source of food (Sangakkara and Frossard, 2014). It produces more dry matter and energy than rice where yams produce 2.4 mg dry matter ha^{-1} and 182 MJ of energy ha^{-1} day^{-1} when compared to rice which produce 1.9 mg dry matter ha^{-1} and 151 MJ of energy ha^{-1} day^{-1} (Sangakkara and Frossard, 2014). Starch is the main component in yams (60-85% dry basis) which are important food items in the diet of people living in rural areas of Sri Lanka (Jayakody *et al.*, 2007). In addition, a number of wild species of *Dioscorea* are sources that are used to produce sex hormones and corticosteroids (Coursey, 1976). Diosgenin is the pharmacologically active component in wild *Dioscorea* species. *Dioscorea pentaphyla* is also a medicinally important plant which has antibacterial and antifungal properties (Prakash and Hosetti, 2012).

However, *Dioscorea* spp. (yam tuber) are one of the least studied tuber crops in Sri Lanka. There are a number of studies carried out on cassava, sweet potato and potato due to their prevalence in Sri Lanka (Sangakkara and Frossard, 2014). Therefore, there is an urgent need to initiate studies on *Dioscorea* spp. in order to popularize yams among the society. But, yam production has been limited due to seasonal sprouting of yams and slow multiplication rate of planting materials.

Dioscorea flowers are unisexual and plants are dioecious where they rarely set fruits. They can be vegetatively propagated by tubers which is a slow process (Shukla and shukla, 2014). *In vitro* multiplication of *Dioscorea pentaphyla* would overcome the problems in vegetative propagation and produce a large number of planting materials throughout the year. In addition, *in vitro* method of multiplication of *Dioscorea pentaphyla* would give considerable benefits for germplasm conservation and medicinal trade. The present study was conducted to find out optimum media for the initiation and multiplication of *Dioscorea pentaphyla* under *in vitro* conditions.

MATERIALS AND METHODS

The study was carried out at the Plant Genetic Resource Center, Gannoruwa during the period from December, 2015 to May 2016.

Identification of the Optimum Medium for Shoot Initiation

Immature shoots of *Dioscorea pentaphyla* were selected as the explant source which were obtained from the plant house maintained at Plant Genetic Resource Center.

Shoot buds were excised from immature shoots, kept in water after adding two drops of teepol (detergent solution) for 30 min and then kept under running tap water for 1 h. Shoots were washed with sterilized distilled water for 3 times, followed by washing with 70% alcohol for one minute. Then, the explants were repeatedly washed with sterilized distilled water for three times. The shoots were disinfected with 20% Clorox and two drops of Tween 20 by shaking the shoots for 20 min in a shaker, followed by washing with sterilized distilled water for several times until the foam removed. Shoots were dipped in 0.1 g/L Streptomycin Sulphate (bactericide) for 5 min followed by dipping in 0.1 g/L Topsin (fungicide) solution for 5 min.

Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with different combinations of plant growth regulators (PGRs) were used for shoot initiation. Different concentrations of 6-Benzylaminopurine (BAP) were used and concentration of Gibberellic acid (GA_3) and Naphthalene acetic acid (NAA) were kept constant. Concentrations of BAP were 0 (T_1), 0.1 (T_2), 0.5 (T_3) mg/L with 0.1 mg/L of GA_3 and 0.1 mg/L NAA. Sucrose (3%) and 100 mgL^{-1} ascorbic acid were added and pH was adjusted between 5.7 and 5.8. The media was gelled with 0.8% (w/v) agar and media (7 ml) were dispensed into small capped culture vials and autoclaved at 121 °C at 105 Kpa for 20 min.

The surface sterilized shoots were sized to 1-1.5 cm length containing a single axillary bud and placed vertically in sterilized media containing culture tubes. Sixteen replicates per treatment were used. All cultures were incubated at 25 °C under 16 h light and 8 h dark photoperiod provided by Philips 36 W/54 day light tubes. The cultures were incubated under 65-70% relative humidity and 2500 Lux light intensity. Culture tubes were arranged according to Completely Randomized Design (CRD). The data were recorded after 30 days.

Identification of the Optimum Medium for Shoot Multiplication

Already initiated cultures were used for shoot multiplication. Murashige and Skoog medium supplemented with different combinations of plant growth regulators (PGRs) were used. Different concentrations of NAA were used and the concentration of BAP was constant (Poornima and Ravishankar, 2007). Concentrations of NAA are 0 (T_4), 0.5 (T_5) and 1.0 (T_6) mg/L with 2 mg/L BAP. Five replicates per treatment were used. Sucrose (3%) was added, pH was adjusted between 5.7 and 5.8 and the media was gelled with 1.1% (w/v) agar. The media (25 ml) were dispensed into culture bottles, capped with heat resistant polypropylene and autoclaved at 121 °C at 105 Kpa for 25 min.

All cultures were incubated at 25 °C under 16 h light and 8 h dark photoperiod provided by Philips 36 W/54 day light tubes. The cultures were incubated under 65-70% relative humidity and 2500 Lux light intensity by employing the Completely Randomized Design (CRD). The data were recorded after 40 days.

Data Recording

For the identification of the optimum medium for shoot initiation, days to bud break (from the establishment of the shoots in the media until 2 mm of bud formation) and explant response (number of shoots of bud break to the number of total shoots established as a percentage) were recorded. For the identification of the optimum medium for shoot multiplication, shoot length (cm), number of leaves, number of shoots and length of axillary shoots (cm) were recorded.

Data Analysis

The data were analyzed using ANOVA and mean separation was done according to the Turkey's test, using Minitab 15 version. $P \leq 0.05$ level of significance was used to compare the differences among means of the treatments.

RESULTS AND DISCUSSION

Identification of the Optimum Medium for Shoot Initiation

Number of days to bud break and explant response of *Dioscorea pentaphyla* on shoot initiation, in three different initiation media, are presented in Table 1. According to the analysis, days to bud break in the three treatments were not significantly different. However, significant differences were identified in explant response (Figure 1). Murashige and Skoog medium supplemented with highest BAP concentration (0.5 mg/L-T_3) elicited better explant response in which the percentage of explants of bud break to the total number of explants cultured were 56.25% which is more than other two treatments (T_1 and T_2).

Identification of the Optimum Medium for Shoot Multiplication

Numbers of leaves, number of shoots, shoot length and length of axillary shoots recorded in three different tested media for shoot multiplication are presented in Table 2. Treatment four produced an average of 9.4 number of leaves, while an average of 3.8 and 2.0 number of leaves were produced by T_5 and T_6 respectively. There were significantly different responses in terms of number of leaves and shoot length ($p<0.05$). However, tested treatments did not significantly affect the length of axillary shoots and number of shoots. MS+NAA (0 mg/L) +BAP (2 mg/L) produced a significantly higher number of leaves than other two treatments (T_5 and T_6). With regards to the shoot length, Treatment 4 showed 1.89 cm long shoot length compared to 1.03 cm and 1.02 cm long shoot lengths in media T_5 and T_6 respectively. According to statistical analysis, MS+NAA (0 mg/L) +BAP (2 mg/L) significantly increased the shoot length over the other two treatments. Based on these results, T_4 medium enhanced the growth of main shoot than shoot proliferation.

For the micropropagation of *Dioscorea* spp., MS medium has been commonly used (Das *et al.*, 2013; Behera *et al.*, 2009; Poornima and Ravishankar, 2007). BAP is more responsive than kinetin for shoot multiplication in *Dioscorea pentaphyla*. Kinetin has a growth inhibitory effect on shoot numbers of *Dioscorea pentaphyla* and *Dioscorea oppositifolia* (Poornima and Ravishankar, 2007).

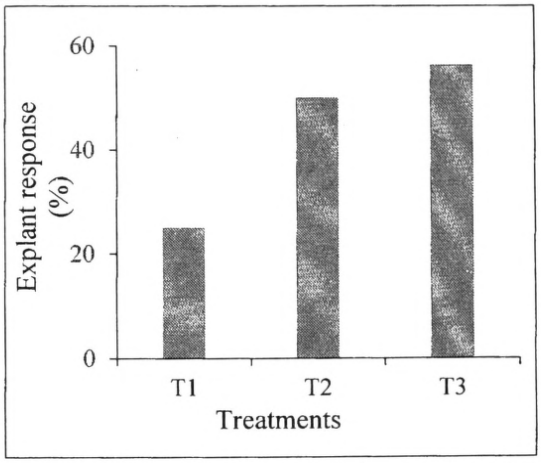
In this study, immature shoot tips were used as explants for shoot initiation. Das *et al.*, (2013) has reported that shoot tips are the best explants for micropropagation of *Dioscorea* spp. In the present study, explant response was comparatively higher in the MS medium

Table 1. Effect of different treatments on shoot initiation of *Dioscorea pentaphyla*

Treatments	BAP (mg/L)	Number of days to bud break	Explant response (%)
T_1	0.00	20.00	25.00
T_2	0.10	18.37	50.00
T_3	0.50	21.22	56.25

T_1-0 mg/L+0.1 mg/L of GA_3+0.1 mg/L NAA, T_2-0.10mg/L+0.1 mg/L of GA_3+0.1 mg/L NAA, T_3-0.50 mg/L+0.1 mg/L of GA_3+0.1 mg/L NAA

Figure 1. Explant response of three culture media



T_1-0 mg/L+0.1 mg/L of GA_3+0.1 mg/L NAA, T_2-0.10mg/L+0.1 mg/L of GA_3+0.1 mg/L NAA, T_3-0.50 mg/L+0.1 mg/L of GA_3+0.1 mg/L NAA

supplemented with 0.5 mg/L BAP. Very little explant response was observed in 0 mg/L BAP where only 25% of the explants were able to break the bud in a 30 day of period. Poornima and Ravishankar (2007) stated that, the presence of BAP is important for shoot initiation and they demonstrated that nodal explants of *Dioscorea pentaphyla* cultured on MS medium supplemented with 2 mg/L BAP with 0.3% activated charcoal enlarged and broke the axillary buds after 20 days.

Dioscorea spp. are rich in phenolic compounds. Poornima and Ravishankar (2007) have reported that activated charcoal was effective in reducing phenolic exudation. Behera *et al.* (2009) used ascorbic acid to reduce phenolic exudation. However, in this study, phenolic exudation in the multiplication medium was effectively controlled by frequent transferring into fresh medium.

In the present study, shoot multiplication in MS medium supplemented with 0 mg/L NAA and 2 mg/L BAP was the most effective treatment and these results are compatible with the results reported by Poornima and Ravishankar (2007). In this medium, a significantly high number of leaves and shoot length were recorded. But, it failed to increase the number of shoots per explant significantly.

Table 2. Effect of different treatments on shoot multiplication of *Dioscorea pentaphyla*

Treatment	NAA mg/L	No. of leaves	No. of shoots	Shoot length (cm)	Length of axillary shoots (cm)
T_4	0.00	9.4^{a*}	2.2 ns	1.89^{a}	0.572 ns
T_5	0.50	3.8^{ab}	1.4 ns	1.03^{bc}	0.396 ns
T_6	1.0	2.0^{b}	1.6 ns	1.02^{c}	0.260 ns

**Means of the same letters are not significantly different at 0.05 probability level. T_1-0 mg/L+2 mg/L BAP, T_2-0.5+2 mg/L BAP, T_3-1.0 mg/L+2 mg/L BAP*

In this study, MS+2 mg/L BAP produced mainshoots with an average length of 1.89 cm and average number of shoots of 2.2. However, Poornima and Ravishankar, (2007) observed that MS medium with same BAP concentration and 0.3% activated charcoal produced high shoot length and high number of shoots per explants.

Though the number shoots were not increased significantly, a significant growth of the main stem was observed in MS+2mg/L BAP. Significant increment of the number of leaves indicates the significant increment of number of nodes because a leaf arises from a node. Therefore, MS+2 mg/L BAP can be selected for shoot multiplication via nodal cuttings.

CONCLUSIONS

Based on the results, shoot initiation was not affected significantly by the three tested culture media. For shoot multiplication, culture medium with high BAP concentration significantly enhanced the growth of the main stem by increasing number of leaves and shoot length, and thus suitable for shoot multiplication via nodal cuttings. However, further studies are necessary to optimize media composition for shoot initiation and shoot multiplication.

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