Full Length Research Paper

**In vitro propagation of wild yams, Dioscorea oppositifolia (Linn) and Dioscorea pentaphylla (Linn)**

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**In vitro** propagation of two wild yams, *Dioscorea oppositifolia* and *Dioscorea pentaphylla*, is reported. Multiple shoots were initiated from nodal explants on Murashige and Skoog (MS) medium supplemented with 8.8 µM 6-benzylaminopurine (BAP) and 0.3% (w/v) activated charcoal. Root induction was also achieved simultaneously from the base of the shoots in the same medium. Individual shoots with a minimum of one node were excised and rooted **in vitro** on MS medium with 2.67 µM -naphthaleneacetic acid (NAA) or **ex vitro** rooted on by treatment with 49 µM indole-3-butyric acid (IBA) for 30 min. Regenerants acclimatized in soil-rite showed vigorous shoot growth (within 2 weeks) and after 5 - 6 months were suitable for planting. Plantlets also developed tubers on MS medium with 8.8 µM 6-benzylaminopurine (BAP).

**Key words:** Dioscorea, diosgenin, microtuber, nodal culture.

**INTRODUCTION**

The genus *Dioscorea* includes over 600 species (Ayensu, 1972), and is of considerable economic importance. A number of *Dioscorea* wild species are the source of compounds used in the synthesis of sex hormones and corticosteroids (Coursey, 1967) and cultivated species are the source of food in some tropical countries (Coursey 1976). These true yams are the source of agents used to treat such varied conditions as inflammation, joint pain, diabetes, infections and dysmenorrhea. The pharmacologically active components of the *Dioscorea* species include diosgenin, which is a steroidal saponin, and dioscin, a form of diosgenin with sugars attached (Ramberg and Nugent, 2002)

Plantlet regeneration **in vitro** for vegetative propagation of some economically important *Dioscorea* species has been achieved using nodal cuttings (Chaturvedi, 1975; Lakshmisita et al., 1976; Mantell et al., 1978; Alizadeh et al., 1998; Yan et al., 2002; Chen et al., 2003), bulbils (Asokan et al., 1983), zygotic embryos (Viana and Mantell, 1989), meristem tips (Maltaurie et al., 1995a, b), immature leaves (Kohmura et al., 1995) and roots (Twyford and Mantell, 1996). Attention has been paid to the clonal propagation through **in vitro** production of microtubers in *D. abyssinica* (Martine and Cappadocia, 1991), *D. alata* (Mantell and Hugo, 1989; Martine and Cappadocia, 1991; John et al., 1993; Jasik and Mantell, 2000), *D. batatas* (Koda and Kikuta, 1991), *D. composita* (Alizadeh et al., 1998) and *D. floribunda* (Sengupta et al., 1984)

The tubers of *D. oppositifolia* are used as an herbal tonic. It stimulates the stomach and spleen and has an effect on the lungs and kidneys. The tuber has been eaten for the treatment of poor appetite, chronic diarrhea, asthma, dry coughs, frequent or uncontrollable urination, diabetes and emotional instability. Externally, the tuber has been applied to ulcers, boils and abscesses. Leaf juice from *D. oppositifolia* can be used to treat snake bites and scorpion stings (Mandy, 2002).

In this paper, we describe the cultural conditions required to provide maximal **in vitro** shoot growth of two wild yams *D. oppositifolia* and *D. pentaphylla* and also to induce microtubers.

**MATERIALS AND METHODS**

Field grown plants of *D. oppositifolia* Linn and *D. pentaphylla* Linn, propagated from a wild tuber were used as source of explants for **in vitro** study. The nodal segments were kept in running tap water for 45 min. A few drops of Tween 20 were added following fungicide...
RESULTS AND DISCUSSION

Nodal explants of both the species were more responsive in terms of rapid bud break. The frequency and the rate of multiplication depended on the cytokinin and its concentration either alone or in combination. Enlargement and subsequent break of axillary buds was the initial response of nodal explants cultured on MS media supplemented with 8.8 µM BAP and 0.3% activated charcoal (Figure 1, Table 1). In both the species the nodal cuttings remained quiescent for about 20 days, after which swelling appeared at the site of axillary bud denting the formation of new tuberous tissue, from which roots came out first followed by the development of one or two shoots, as observed in D. floribunda (Chaturvedi, 1975; Lakshmisita et al., 1976). New shoots (six to eight) developed in this medium attained a mean length of 3 – 4 cm and two to three nodes within a span of 30 days in D. pentaphylla. While in D. oppositifolia, eight to ten nodes could be observed on the longest shoots.

A few cultures growing on medium containing BAP and kinetin initiated lateral shoots in the axils of leaves. All newly produced shoots in 8.8 µM BAP augmented media exhibited characteristic growth. Shoot multiplication was significantly improved by sub-culturing into same shoot-containing medium, while the growth response of the cultured nodes to exogenous kinetin varied with the concentration of kinetin. At the lower concentrations, growth was normal while at higher concentration callus formation was induced as recorded in D. bulbifera (Uduebo, 1971), D. alata and D. rotundata (Mantell et al., 1978). Growth inhibitory effect of kinetin on shoot numbers of D. oppositifolia and D. pentaphylla microplants was observed. Lakshmisita et al. (1976), however, reported that the kinetin supplied at either 11.6 or 46.4 µM significantly increased the shoot development in D. floribunda shoots cultures. Also the promotive effects of kinetin (46.4 µM) on plantlet growth for D. bulbifera, which increased the number of shoots per plantlet, were shown by Forsyth.
Table 1. Response of nodal explants of Dioscorea oppositifolia and Dioscorea pentaphylla on different concentrations of cytokinins after 6 weeks.

| Species         | Growth regulators (µM) | No. of shoots/nodal explant | Shoot formation (%) |
|-----------------|------------------------|----------------------------|---------------------|
| *D. oppositifolia* | MS+Kn (2.32)           | 1.50±0.15                 | 63.5                |
|                 | MS+Kn (4.65)           | 1.83±0.27                 | 58.3                |
|                 | MS+Kn (9.29)           | 3.41±0.31                 | 25.0                |
|                 | MS+BAP (2.22)          | 2.41±0.22                 | 65.5                |
|                 | MS+BAP (4.44)          | 5.16±0.50                 | 78.0                |
|                 | MS+BAP (8.87)          | 7.50±0.79                 | 89.3                |
| *D. pentaphylla*  | MS+Kn (2.32)           | 1.25±0.13                 | 56.8                |
|                 | MS+Kn (4.65)           | 1.41±0.14                 | 43.6                |
|                 | MS+Kn (9.29)           | 1.44±0.18                 | 33.3                |
|                 | MS+BAP (2.22)          | 1.41±0.14                 | 68.6                |
|                 | MS+BAP (4.44)          | 2.50±0.19                 | 79.5                |
|                 | MS+BAP (8.87)          | 5.16±0.61                 | 87.8                |

Values represent mean ±SE (n=12).

and Van Staden (1982). In our present study, we found that BAP was more responsive than kinetin in inducing multiple shoot formation. In most cases, however, shoots that BAP was more responsive than kinetin in inducing vigorous root systems. The plantlets were transplanted to plastic pots containing soil rite and hardened by exposing them gradually to an increased duration of daylight and temperature. No morphological abnormalities were visible in the transplanted plants.

Microtubers developed at the base of their rooted shoots. 60% of the cultures produced one to three tubers at the base of their rooted shoots. Using nodal cutting as explants, the phenomenon of in vitro tuberization has been observed in *D. bulbifera* (Uduebo, 1971; Ammirato, 1976, 1984; Mantell et al., 1987; Forsyth and Van Staden, 1982). *D. alata* (Ammirato, 1976; Mantell et al., 1987; Jean and Cappadocia, 1991; Alhassan and Mantell, 1994), *D. rotundata* (Mantell et al., 1987; Ng, 1988), *D. abyssinica* (Jean and Cappadocia, 1991), *D. opposita* (Mantell and Hugo, 1986) and *D. cayenensis* (Ng and Mantell, 1996). Both *D. oppositifolia* and *D. pentaphylla* nodal shoots produced tubers on the shooting media. These tubers were found to be bigger and more uniform in size in comparison with other treatment. A combination of 20 g l⁻¹ sucrose and 8.8 µM BAP added to the MS basal medium induced tubers. However, these tubers were smaller and not uniform in size. Effect of sucrose on microtuberization has also been observed in *D. bulbifera* (Forsyth and Van Staden, 1984; Forsyth, 1982), *D. rotundata* (Ng, 1988), *D. alata* (Mantell and Hugo, 1989) and *D. opposita* (Kohmura et al., 1995). The nodal shoot cultures of *D. alata* and *D. bulbifera* showed maximum microtuber formation with 2% sucrose (Mantell and Hugo, 1989). When Kohmura et al. (1995) compared sucrose concentrations (3 and 6%) in *D. opposita* with 8.9 µM BAP alone in the medium, 6% sucrose was found to be more efficient for tuberization. In the present study, 8.87 µM BAP alone with 3% sucrose can successfully induce in vitro microtuber formation (Table 3). In *D. rotundata*, a decrease in the percentage of microtuberization with 8 or 10% sucrose and 2.5 µM
kinetin was reported by Ng (1988), which contrasted with the results obtained in the current study on *D. oppositifolia* and *D. pentaphylla*. Increase in the sucrrose 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 *D. oppositifolia*, which contrasted with Staden, 1984). In higher levels of kinetin (23.2 to 46.4 µM), raised microtuber induction in the steroidal yam *Dioscorea alata* increased microtuber size and frequency in shoot cultures of food yam, *D. pentaphylla*. Increase in the sucrose concentration on *in vitro* tuber production.

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### Table 2. Rooting of shoots of *D. oppositifolia* and *D. pentaphylla*.

| Species       | Method | Growth regulators (µM) | No of roots/shoot | Root length (cm) | Rooting (%) |
|---------------|--------|------------------------|-------------------|------------------|-------------|
| *D. oppositifolia* | In vitro | MS+NAA (0.0) | 2.91±0.28b | 4.08±0.31b | 83.8 |
|               |        | MS+NAA (0.54) | 2.66±0.18b | 3.50±0.26b | 89.6 |
|               |        | MS+NAA (2.69) | 5.25±0.44b | 7.83±0.84b | 76.9 |
|               | Ex vitro | IBA (49) 30 min | 6.75±0.41a | 6.33±0.35a | 91.7 |
|               |        | IBA (49) 60 min | 2.33±0.22b | 3.50±0.28b | 82.2 |
|               |        | IBA (49) 120 min | 2.08±0.19b | 2.25±0.25c | 67.0 |
| *D. pentaphylla* | In vitro | MS+NAA (0.0) | 3.33±0.25b | 3.08±0.28b | 87.9 |
|               |        | MS+NAA (0.54) | 2.41±0.22c | 2.08±0.19b | 82.3 |
|               |        | MS+NAA (2.69) | 5.25±0.39b | 3.50±0.28b | 78.4 |
|               | Ex vitro | IBA (49) 30 min | 4.66±0.28a | 4.50±0.23d | 89.9 |
|               |        | IBA (49) 60 min | 2.50±0.26b | 2.50±0.19b | 83.3 |
|               |        | IBA (49) 120 min | 2.0±0.17b | 1.75±0.17c | 54.8 |

Values represent mean ± SE (n = 12). Data recorded after 60 days.

### Table 3. Influence of sucrrose concentration on *in vitro* tuber production.

| Species       | Sucrose (g l⁻¹) | Tuber number | Tuber weight (mg) |
|---------------|-----------------|--------------|-------------------|
| *D. oppositifolia* | 20 | 1.33±0.14c | 16.6±1.68c |
|               | 30 | 2.41±0.14c | 55.8±7.92b |
| *D. pentaphylla* | 20 | 1.58±0.19c | 28.25±1.96c |
|               | 30 | 2.83±0.20c | 65.83±5.56a |

Values represent mean ±SE (n = 12). Data recorded after 60 day.
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