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DIRECT PLANTLET REGENERATION FROM MALE INFLORESCENCES OF MEDICINAL YAM (*DIOSCOREA FLORIBUNDA* MART. & GAL.)

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Summary

Segments of male inflorescences of medicinal yam (*Dioscorea floribunda*) cultured on Murashige and Skoog (MS) medium supplemented with 13.94 μ*M* kinetin (Kn) resulted in the conversion of floral buds into vegetative buds and these later developed into plantlets. Growth and multiplication of shoots could be obtained by culturing individual shoots in MS modified basal medium, replacing the MS standard three vitamins with 10.0 mg l^{-1} thiamine in addition to 13.94 μ*M* Kn. Root induction was also obtained simultaneously from the base of the shoots in the same medium. Such plantlets have been successfully transferred to potted soil, where they grew normally. Plantlets were also made to develop tubers on MS medium with 18.91 μ*M* abscisic acid (ABA) and also with 2.68 μ*M* α-naphthaleneacetic acid (NAA) and 40–50 g l^{-1} sucrose.

Key words: Dioscorea; diosgenin; direct shoot regeneration; male inflorescences; medicinal yam.

Introduction

Medicinal yam (*Dioscorea floribunda* Mart. & Gal.) of Dioscoreaceae is a dioecious perennial climbing herb which yields diosgenin, a major steroidal drug precursor as well as a corticosteroid drug used as an anti-inflammatory and anti-arthritic agent (Marker et al., 1947; Correll et al., 1955; Wall et al., 1961). Micropropagation of plants has many advantages over conventional methods of vegetative propagation which suffer from several limitations (Martin and Gaskins, 1968; Martin and Delpin, 1969). Micropropagation studies through excised leaves and nodes of *Dioscorea floribunda* have been reported (Chaturvedi, 1975; Lakshmisita et al., 1976; Sinha and Chaturvedi, 1979). Direct plantlet formation from inflorescence cultures are rare and this communication is the first report of a successful regeneration of plantlets from inflorescence explants of *Dioscorea floribunda*.

Materials and Methods

Inflorescences of a homogeneous male clone of the medicinal yam (*Dioscorea floribunda*) planted in the experimental farm of the Regional Research Laboratory, Jorhat were used as explant. Immature inflorescences measuring 2–5 cm after initiation from the base were separated and placed aseptically in culture vessels. MS medium (Murashige and Skoog, 1962) was used as the basal medium for initiation of culture. The basal medium was further supplemented with kinetin (Kn), 6-benzyladenine (BA), α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA) alone or in combination at various concentrations. During shoot growth, modification was made to the vitamin supplements of the MS mineral salt solution. Thiamine at 10.0 mg l^{-1} was added to the MS basal medium in place of the MS standard three vitamins, this was termed modified MS basal medium (MMS). For *in vitro* tuberization individual rooted shoots were separated and cultured on MS media supplemented with 15.13–18.91 μ*M* abscisic acid (ABA). The effect of different concentrations of sucrose (40–50 g l^{-1}) along with 2.68 μ*M* NAA was also tested separately. The pH of the media was adjusted to 5.8 before autoclaving. The cultures were incubated at 23 ± 2°C under cool white fluorescent light (40 μmol m^{-2} s^{-1}) with a 16-h day photoperiod.

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Results and Discussion

Of each of the 2-cm long floral buds of the immature inflorescences, 10–45% differentiated into small shoots directly from the base when cultured in various concentrations of Kn-augmented media (Table 1). Immature inflorescences as a whole, when cultured on MS medium supplemented with 0.46–18.59 μ*M* Kn, remained quiescent for about 15–20 d and then swelled at their bases. The size of the explants was also elongated after 25 d due to increase in inter-nodal length. Later on, opening of the bract and development of leaf-like structures were observed from the basal portion of the explants. By the sixth week 80% of the cultures containing 13.94 μ*M* Kn showed development and emergence of green vegetative buds directly from the axils of the bracts instead of the appearance of floral buds (Fig. 1*A*). Appearance of shoot buds was confirmed when well-developed shoots emerged and grew like normal shoots in most cases. The number of multiple shoots could be increased by implanting the individual shoots or shoot branches in MMS fortified with 13.94 μ*M* Kn. In this medium a maximum of five shoots per shoot explant were obtained within 8 wk of culture (Fig. 1*B*). Media containing 4.44–11.10 μ*M* BA alone did not show any floral bud conversion. Rather, explants became pale greenish, gradually turned brown and died shortly after. Addition of auxin, 0.53 μ*M* NAA or 0.57 μ*M* IAA, to these media stimulated callus growth but failed to convert the floral buds into vegetative ones.

Rooting. Simultaneous rooting could be induced from the shoots by culturing them in both shoot initiation and shoot growth media for a 2-mo. period. Rooting could also be induced by placing the shoots in MS basal medium +10.74 μ*M* NAA. Similar responses have been

TABLE 1

CONVERSION OF FLORAL BUDS TO VEGETATIVE SHOOTS FROM MALE INFLORESCENCES OF *DIOSCOREA FLORIBUNDA* UNDER THE INFLUENCE OF KINETIN

Kinetin (μ*M*)	Conversion of floral buds per explant (%)	Mean number of shoots per explant	Mean length of shoots (cm)	Number of nodes per shoot
0.46	–	–	–	–
0.92	–	–	–	–
10.62	10	2.7	1.6 ± 0.24	1.6 ± 0.5
13.94	45	6.2	1.7 ± 0.12	4.4 ± 1.1
16.26	40	4.5	0.7 ± 0.07	3.0 ± 1.0
18.59	40	4.0	0.2 ± 0.03	2.4 ± 0.5

Values are means ± SE for 20 replicates.

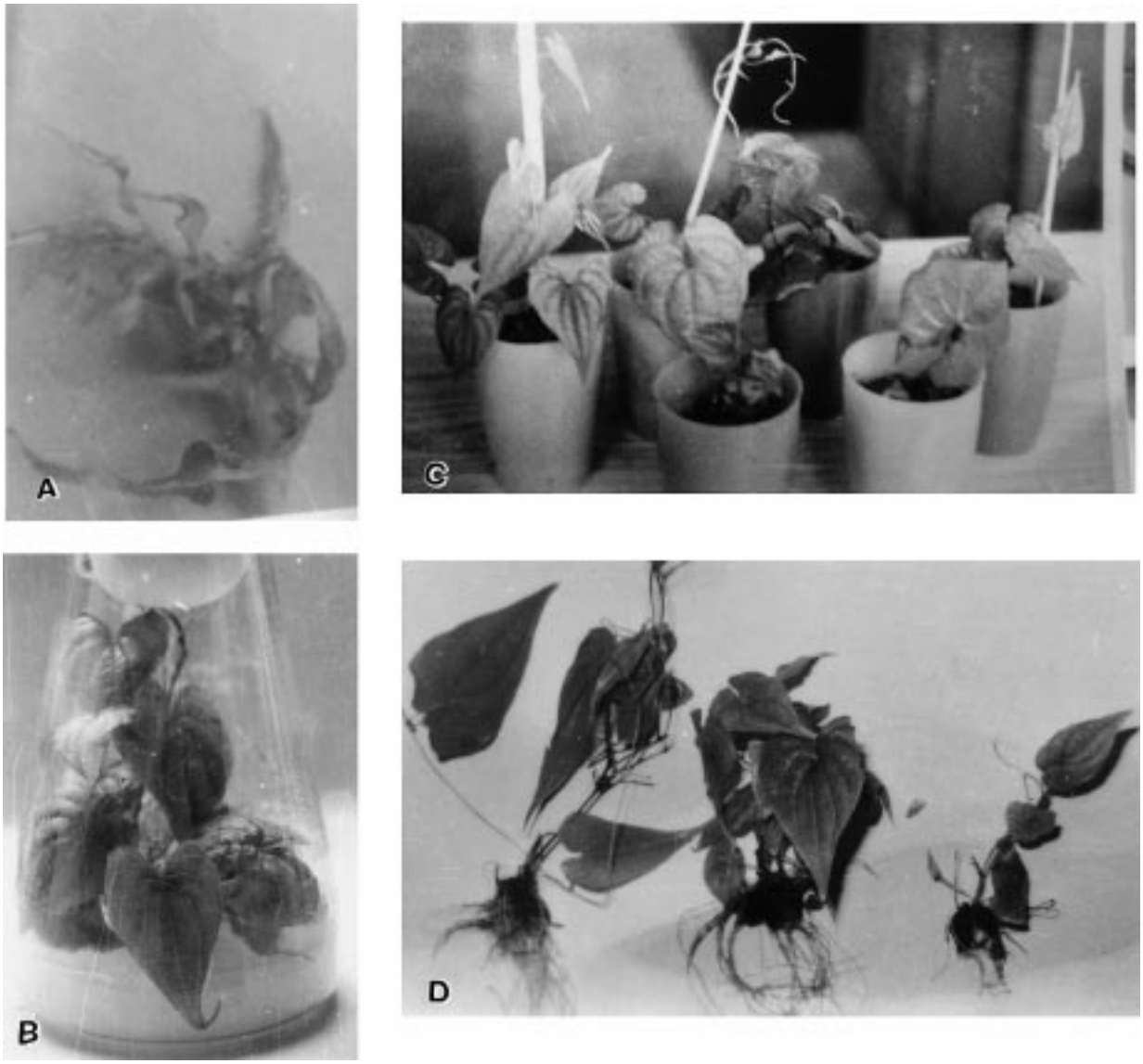

FIG. 1. Regeneration of plantlets in *Dioscorea floribunda*. *A*, Vegetative shoots emerging from the axil of bracts after 6 wk of culture. *B*, Formation of well-developed shoots on MS inorganic basal + 10.0 mg l^{-1} thiamine + 13.94 μ*M* Kn. *C*, Regenerated plants established in potted soil. *D*, Formation of tubers at the bases of rooted shoots on MS + 17.02 μ*M* ABA after 50 d of culture.

TABLE 2

EFFECT OF GROWTH REGULATORS ON ROOTING OF *IN VITRO*-REGENERATED SHOOTS OF *DIOSCOREA FLORIBUNDA*

Culture media (μM)	Culture period (d)	Shoot with root (%)	Root length (cm)
MS + Kn 13.94	32	90	4.7 ± 1.0
MS + NAA 10.74	25	85	4.2 ± 1.0
MS + IAA 11.41	32	70	9.0 ± 0.7
MMS + Kn 13.94	35	100	6.6 ± 0.9

Values are means ± SE for 20 replicates.

reported in the shoot culture of *D. floribunda* (Chaturvedi, 1975). Transfer of shoots to MS basal medium containing 11.41 μM IAA also induced root initiation. This combination resulted in a long and slender root system (Table 2). All these plantlets were hardened by exposing them gradually to an increased duration of daylight and increased temperature. Finally, they were transplanted to potted soil (1:1 non-sterile sandy loam and farmyard manure). All the plants transplanted in this way grew vigorously (Fig. 1*C*). Using this protocol it will be possible to produce about 500 plantlets within 6 mo. in three cycles.

In vitro *tuberization*. With the incorporation of 17.02 μM ABA into the MS basal medium, 60% of the cultures produced one to three tubers at the bases of their rooted shoots (Fig. 1*D*). These tubers were found to be bigger and more uniform in size in comparison with other treatments. A combination of 40–50 $g\,l^{-1}$ sucrose and 2.68 μM NAA added to the medium induced tubers (20% of the cultures) above the rooting zone of the plantlets. However, these tubers were smaller and not uniform in size.

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