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Propagation of *Asparagus racemosus* through tissue culture

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Abstract. Murashige and Skoog's medium with 2, 4-D and kinetin induced callus in the shoot segments of *Asparagus racemosus*. Regeneration of shoot buds and clonal multiplication of excised shoots through proliferation of nodal buds could be achieved by the use of IAA and BAP in the medium. Rooting was achieved with half strength MS basal medium plus IBA. Complete plants with cladode, crown and root systems were developed in hormone free medium. The plants were successfully transferred to soil.

Introduction

The Liliaceous species *Asparagus racemosus* is important for its sapogenin content [7] which is used as precursor of many pharmacologically active steroids [12]. The plant is perennial and usually propagated by seeds. Although any one plant can be lifted and divided to produce several new plants, this is a very slow and laborious technique of vegetative propagation. The in vitro method is an effective tool for propagation of plants. Most of the in vitro work on propagation has been carried out on the European species *A. officinalis* [1, 2, 8–11, 13, 14]. In view of the lacuna existing on this aspect of Indian species of *Asparagus* and its medicinal importance the in vitro propagation of this plant was investigated.

Materials and methods

The plant *Asparagus racemosus* Willd. was obtained from a local nursery (Calcutta, West Bengal) and grown at the Experimental Garden, Department of Botany, University of Calcutta. Rapidly elongating 15–20 cm tall spears were used as the source of explant. 3 cm length of the apex was discarded and the subjacent 5 cm section of each spear, freed from lateral buds and leaf scales, were taken. After washing with 5% teepol (Commercial Laboratory grade neutral liquid detergent), the explant was surface sterilized with 0.1% $HgCl_2$ solution for 8–10 minutes and washed thoroughly with sterile distilled water. The stem was cut into small discs of 4–6 mm thick each of which was cultured in tubes (150 mm x 25 mm) containing

20 ml of Murashige and Skoog's (MS) basal medium [6] with 3% sucrose and 2, 4-dichlorophenoxy acetic acid (2, 4-D) + Kinetin [3] for callus induction. The pH of media was adjusted to 5.6–5.8 using lN KOH/HC1. Media were solidified with 0.8% Bacto agar (BDH) and autoclaved for 15 mins at 1.05 kg/cm^2 pressure. Cultures were incubated at 25 ± 1 °C and 50–60% relative humidity with 16 hours of light at approximately 20×10^8 μ moles sec^{-1} m^{-2} from Philips fluorescent lamps. Calli were maintained by subculturing at intervals of 30–35 days.

Two months old callus weighing 600 ± 50 mg were aseptically inoculated on MS basal medium with 3% sucrose supplemented with Indole-3-acetic acid (IAA) + 6-benzyl amino purine (BAP) [4] for shoot regeneration. Shoots regenerated from callus were used as source of explants for rapid multiplication. Shoots, 3–4 cm long, were excised from the apical region and placed vertically on solidified MS basal medium with 3% sucrose and different hormone combinations (Table 1) for multiplication. After multiplication, shoots were planted into solid MS basal medium with 3% sucrose and α-naphthalene acetic acid (NAA) or Indole-3-butyric acid (IBA) for rooting (Table 2). Rooted plants were transferred to simpler media and finally to soil.

Results

Regeneration of shoots from callus cultures

Callus was obtained from 80% of the shoot segments within 8–12 days on medium supplemented with 2, 4-D and kinetin, 1 mgl^{-1} each. The callus was friable and greenish yellow in colour (Figure 1). Appearance of green shoots (Figure 2) was noted from callus within 40 days when grown in presence of IAA (0.5 mgl^{-1}) + BAP (1.0 mgl^{-1}). The regenerated shoots attained a length of 5 cm within one month after their appearance (Figure 3) and used for rapid multiplication.

Multiplication of shoots

When shoots from callus were individually recultured on medium containing IAA (0.1 mgl^{-1}) and BAP (1.0 mgl^{-1}) 12.0 ± 0.4 (Mean ± S.E.) new shoots per shoot was recorded at 6 weeks (Table 1, Figures 4 & 5). This degree of multiplication has been recorded up to fourth successive cultures. Growth and elongation of shoots continued in the medium.

Initiation of root in excised shootlets and regeneration of a complete plant

Roots were initiated (Figure 6 & 7) on excised shoot grown in half strength MS basal medium supplemented with 0.5 mgl^{-1} of IBA (Table 2). Roots were formed on 70% of the explants within 25 days. Root formation was initiated in a swollen mass of callus-like tissue at the base of the shoot.

Table 1. Effect of different hormones on multiplication of shoots (M)

Experimental set	MS basal medium with growth substances (mgl^{-1})	Response (% of explants)	No. of shoots developed per explant within six weeks (Mean of 20 replications ± S.E.)
M_1	NAA (0.5) + Coconut water (15% v/v) + adenine sulphate (50)	Poor multiplication (30)	3.0 ± 0.3
M_2	NAA (0.5) + Kinetin (0.1) + adenine sulphate (40)	Poor multiplication (30)	3.7 ± 0.3
M_3	NAA (0.1) + Kinetin (1.0)	No response	–
M_4	NAA (0.1) + BAP (0.5)	No response	–
M_5	IAA (0.5) + Kinetin (1.0)	Multiplication (50)	4.1 ± 0.3
M_6	IAA (0.5) + BAP (1.0)	Multiplication (80)	7.0 ± 0.4
M_7	IAA (0.1) + BAP (1.0)	Vigorous multiplication (100)	12.0 ± 0.4

Table 2. Effect of different hormones on rooting of excised shoots (R)

Experimental set	MS basal medium with growth substances (mgl^{-1})	Response (% of explants)	No. of roots developed per shoot within six weeks (Mean of 20 replications ± S.E.)
R_1	IBA (0.5)	Root initiation (40) and continuation of growth	2.6 ± 0.2
R_2	NAA (0.1)	Rooting (30)	2.0 ± 0.2
R_3	NAA (0.1) + Kinetin (0.05)	Rooting (30)	2.3 ± 0.3
R_4	[a]IBA (0.5)	Best root initiation (70)	4.2 ± 0.4
R_5	[a]NAA (0.1)	Root initiation (50)	3.0 ± 0.3

[a]Basal medium used was half strength MS

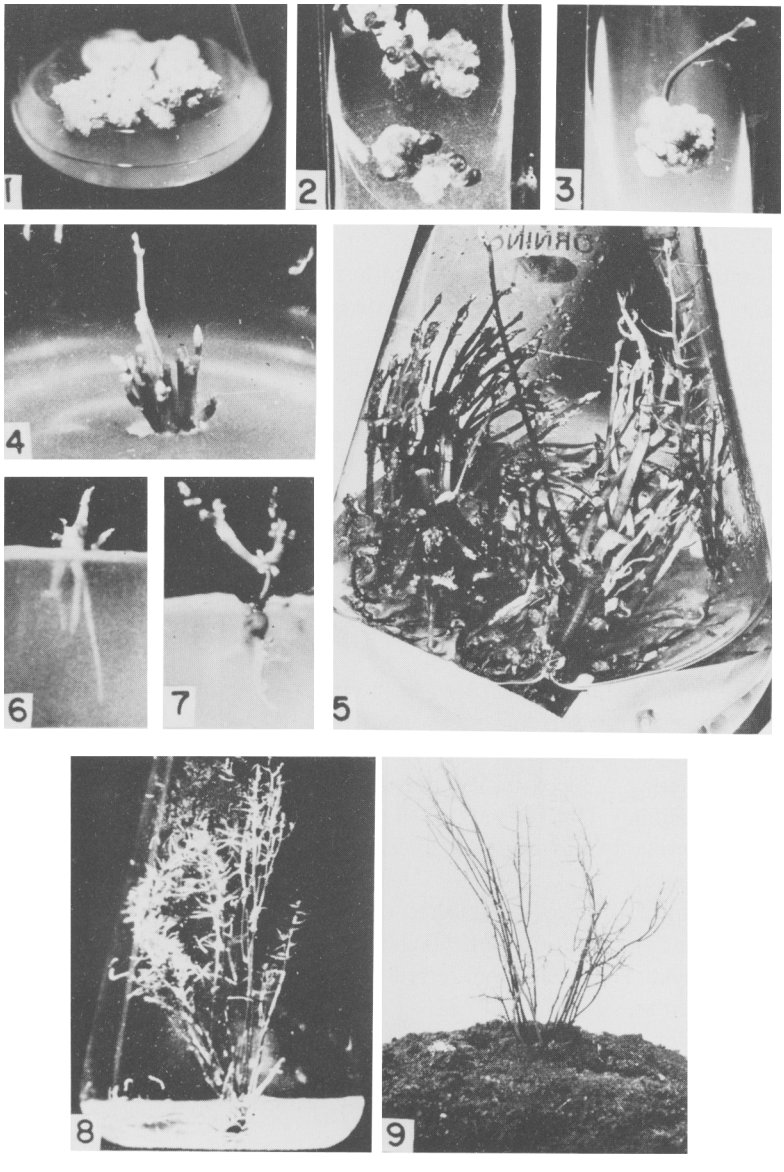

Figure 1. Callus derived from shoot segments.
Figure 2. Regeneration of shoot buds from callus.
Figure 3. Growing regenerated shoot.
Figures 4 & 5. Multiplication of excised shoot.
Figures 6 & 7. Root initiation in shootlets.
Figure 8. Complete plant with cladode, crown and root system.
Figure 9. Potted plant.

Lateral roots required 30–40 days to develop. Rooted plantlets were then transferred to hormone free liquid half strength MS basal medium. Complete plants with well established root system, eladode and crown, from which future shoots may arise, developed in one month (Figure 8). The plants obtained were morphologically uniform in their size of internode and number of cladodes in tuft.

Transfer to soil

Prior to transfer to the potted soil, all plantlets were maintained in half strength liquid MS basal medium with 1% sucrose. After 2–3 weeks, the plants were transferred to the same basal medium without sucrose. Two weeks later, the plants were washed with water, planted into pots containing sandy soil and humus (3:1) and kept in growth chamber (Temp. 25 °C, Humidity 55%, Light 30×10^8 μmoles sec^{-1} m^{-2} for 16/8 h light/dark photoperiod). After 4 weeks the pots were transferred to the field and 70% plants survived (Figure 9).

Steps in propagation of A. racemosus

1. Callus formed in shoot sections in the presence of 2, 4-D (1 mgl^{-1}) + Kinetin (1 mgl^{-1}).
2. Shoots regenerated from callus using IAA (0.5 mgl^{-1}) + BAP (1.0 mgl^{-1}).
3. Excised shoots of 2–3 cm from apical region of growing regenerated shoots recultured in presence of IAA (0.1 mgl^{-1}) + BAP (1.0 mgl^{-1}) for multiplication.
4. Rooting induced on excised shoots grown in half strength MS basal medium + IBA (0.5 mgl^{-1}).
5. Rooted plantlets grown in half strength MS basal medium for one month.
6. Plants with well established root system, crown and cladode maintained in half strength MS basal medium with 1% sucrose for 2–3 weeks and then transferred to the same basal medium without sucrose.
7. After two weeks planted in pots and kept in growth chamber for four weeks.
8. Finally pots transferred to field and plants survived.

Discussion

The most effective asexual multiplication of shoot apices achieved in *A. officinalis* is through the use of NAA in combination with kinetin [14]. Improved multiplication rate in *A. racemosus* has been obtained in the present investigation using IAA with higher concentration of BAP. Yang [14] recorded rooting of shoots with NAA in a kinetin free medium. In the

present study roots could be induced well on shoot growing on half strength MS basal medium supplemented with IBA probably indicating the role of mineral salt inhibition [5] of the rooting process.

The time required for developing stock plants transferred into soil from in vitro organ culture of *A. racemosus*, calculated from the time of multiplication, was 5–6 months as compared to 6–8 months required by Yang in *A. officinalis* [14]. Approximately 350 plants were obtained from one stem discs after eight months using the present protocol. Unsatisfactory survival rates of plantlets after transfer to soil have been overcome by gradual transfer of in vitro grown plants to simpler media and finally to soil.

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