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Micropropagation of *Asparagus racemosus* (Shatavari)

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ABSTRACT

Asparagus racemosus (Shatavari) is a plant with immense medicinal values. It is reported to be recalcitrant to tissue culture practices. Sterile cultures were obtained when the explants were treated with (0.5%) Bavestine, Indophyl and 8HQC for 7 min and $HgCl_2$ for 5 min.The present study on micropropagation of Asparagus racemosus through nodal explants showed better result in BAP (2 mg/l) along with IAA (0.2 mg/l) combination. Treatment of 0.5 % Bavestine, indophyl and 8HQc for 7 min along with 0.1% $HgCl_2$ for 5 min gave 80% contamination free culture.Combination of BAP (2 mg/l) along with IAA (0.2 mg/l) was the best for shoot proliferation and gave 5 shoots on the 20th day of inoculation.Maximum rooting was obtained in MS basal media incubated in the dark.

Key words: *Asparagus racemosus*, Nodal explants, BAP, IAA

INTRODUCTION

Tissue culture technique has been widely accepted as a tool of biotechnology for vegetative propagation of plants of agricultural, horticultural and forestry importance [Dave *et al*., 2003]. *In vitro* propagation enables a broad range of species to be cloned under highly controlled conditions. The technique has been successfully used in propagation of wide range of herb and shrub species [Murashige, 1974]. In conventional cultivation many plants do not germinate, flower and produce seed under certain climatic conditions or have long periods of growth and multiplication. Micropropagation insures a good regular supply of medicinal plants, using minimum space and time [Prakash and Staden, 2007]. *Asparagus racemosus* Willd is multiplied through vegetative propagation in nature. There is always problem of heterogeneity in obtaining uniform seed stock. Studies using seeds as means of propagation indicated that plants developed through seeds are poor in vigour. Seeds have been reported to have a low viability and they have a slow rate of growth [Gupta *et al.*, 2002].The present investigation is carried out to standardize protocols for rapid multiplication of the plant through tissue culture of nodal explants.

MATERIALS AND METHODS

Plant material: Healthy and disease free *Asparagus racemosus*plants were collected from the Department of Horticulture, SHIATS. Tender shoots were used as explants.

Explant preparation: Nodal explants of 1.0-1.5cm size were excised and used as explant source for all the experiments.

Washing of glassware and appliance: The glassware was washed thoroughly with a detergent solution. They were then cleaned with a jet flow of tap water, further rinsed with distilled water and oven dried.

Media preparation: The culture medium used in the present study was Murashige and Skoog [MS, 1962**]** media. Sucrose was added to MS medium with growth regulators to promote multiple shooting. After adding growth regulators, pH of the medium was adjusted to 5.8± 0.1 followed by gelling with 0.8% agar. The media was autoclaved at 121^0C and 15 lbs pressure for 15 min. Qualligens and Merck make chemicals and Sigma make hormones were used in all tissue culture experiments.

Preparation of Stock Solution: Macronutrients, micronutrients, iron chelate, vitamins and amino acid stocks were prepared by weighing required quantity of chemicals as listed in Table 3.1 and dissolved in double distilled water. They were stored in refrigerators at 4 ± 1^0C and were used within 1-2 months from the date of preparation. The iron chelate stock was prepared by individually dissolving $FeSO_4.7H_2O$ and EDTA and made to a final volume by mixing both solutions. Auxin (IAA) was dissolved in 1ml absolute alcohol and the final volume (10ml) was made by addition of double distilled water. Cytokinin (BAP) was dissolved in 1 ml of 1 N NaOH and then made up to a final volume using double distilled water.

Preparation of Nutrient Medium

Specific quantities of stock solutions and hormones were pipetted into a conical flask. 0.3% (w/v) sucrose was used as carbon source. Sucrose and 0.1 % (w/v) myoinositol were added fresh and dissolved. The pH of the medium was adjusted to 5.8 with 0.1 N NaOH or 0.1N HCl using a pH meter.Agar (0.8% w/v) was dissolved in the medium by boiling.

Surface sterilization of explants

Explants used for experiments included nodal segments (~1-1.2cm). The explants were initially washed thoroughly with liquid detergent (Extran) for 5 min followed by continuous washing under running tap water for 1 hour until all traces of the detergent were removed. After rinsing thoroughly in tap water explants were surface sterilized with a solution of bavestin along with 8HQC and indophyl for upto 7 min . They were further surface sterilized with $HgCl_2$ (0.1% w/v) for 5-7 min in the laminar air flow chamber. The surface sterilized plant materials were then rinsed in sterile distilled water several times till all the traces of sterilants were removed.

Inoculation

All aseptic transfers were performed in a laminar air flow chamber having horizontal work flow over the working area kept in a dust free room.

Maintenance of cultures

The temperature of the culture room was maintained at 25 +2^0C and relative humidity at 50 to 60%. A twelve hour photoperiod with a light intensity of 3000 lux was provided using cool white fluorescent tubes. Responding cultures wee periodically sub cultured.

Auxiliary Bud Proliferation

Explants were designed for the selection of the most desirable plant parts and optimum concentration of growth regulator and medium. MS medium enriched with different concentrations of BAP (1-2.5mg/l) in combination with IAA (0.2-0.5 mg/l) were used for culturing nodal segments.

Root initiation

Rooting of microshoots was initiated on ½ strength and full strength MS basal media or MS media fortified with IBA or NAA.

RESULTS AND DISCUSSION

Successful establishment of the explant in culture is limited by several factors. The selection of an explant giving maximum response is an important step for the success of any tissue culture programme. Best results were achieved when the explants are harvested during the active phase of growth [Torres, 1989]. Hence for the present study, explants were collected from actively growing field grown plants.

Contamination free cultures: Following the selection of explants, the next effort was to initiate maximum disease free cultures, which is usually difficult and problematic, due to high rate of contamination, when the explants are taken from the field grown plants. When $HgCl_2$ was used alone, the rate of contamination was 80%. Since there was fungal contamination even after surface sterilizing with $HgCl_2$ solution, the combination of three fungicides viz. Bavestin, 8 HQC and indophyl at various time durations was found to be effective, when used along with $HgCl_2$. Maximum contamination free cultures were obtained when the explants were disinfected with 0.1% $HgCl_2$ for 5minalong with Bavestin, 8HQC and Indophyl (0.5%) for 7min (Table 1). Though environmentally toxic $HgCl_2$ is a widely accepted surface sterilant in plant tissue culture. Best results are achieved when used judicially and carefully [Zryd, 1988].

Table 1: Effect of various surface sterilants on contamination of explants

Bavestein, 8HQC, Indophyl (0.1%)	**(0.1%) $HgCl_2$**	**% contamination**
-	5 min	80%
5 min	5 min	70%
5 min	7 min	50%
7 min	5 min	40%
Bavestein, 8HQC, Indophyl (0.5%)	**(0.1%) $HgCl_2$**	**% contamination**
5 min	5 min	50%
5 min	7 min	40%
7 min	5 min	30%

Selection of medium

Growth regulators may partly compensate for nutrient imbalances in media [Preece, 1995]. Since growth of the excised tissue *in vitro* is influenced by chemical composition of the medium, attempts are to be made to optimize the medium before the most appropriate growth regulators are selected while experimenting with a new species [George, 1993].

Initially, five combinations of media were used with different combinations of BAP (1-2.5 mg/l) and IAA (0.1-0.5 mg/l). The results obtained are compiled in Table 2. Best growth of the explants were obtained in the media combination A_2 consisting of BAP (2 mg/l) along with IAA (0.2 mg/l), in which 7 shoots were obtained from the nodal explants after 20 days of culture (Figure 2). As the concentration of both BAP and IAA increased it was observed that the number of shoots produced decreased.

Table 2: Effect of various plant growth regulators on shoot formation

Media	**Various hormones used (mg/l)**		**% Growth obtained**	**No. of shoots initiated**
	BAP	**IAA**		
A_1	1	0.1	0	0
A_2	1.5	0.2	90	3
A_3	2	0.2	60	7
A_4	2	0.5	70	3
A_5	2.5	0.5	60	1

Successful rooting of shoots was obtained in MS basal media incubated in dark (Table 3). All the other treatments for rooting was not effective in the present study.

Table 3: Root initiation in various treatments

Code	**Media**	**No. of roots initiated**
A6	MS basal (Light)	2
A7	MS basal (Dark)	5
A8	½ MS basal (Light)	1
A9	½ MS basal (Dark)	3
A10	MS + NAA (0.1 mg/l) (Light)	1
A11	MS + NAA (0.1 mg/l) (Dark)	2
A12	MS + IBA (0.1 mg/l) (Light)	1
A13	MS + IBA (0.1 mg/l) (Dark)	1

Micropropagation enables rapid clonal multiplication of elite mother plants and supply of disease free germplasm. Production of plants from axillary shoots have proved to be the most generally acceptable and reliable method for *in*

vitro propagation. In shoot tip and nodal segment culture, multiplication depends on stimulating precocious axillary shoot growth by overcoming the dominance of shoot apicalmeristem. Cytokinin mediated shoot initiation in tissue culture is extensive and among the cytokinins, BAP is the most widely used one for shoot multiplication [Jain and Nessler, 1996; Norbe, 1996; Tanuwidjaja*et al.*, 1988].

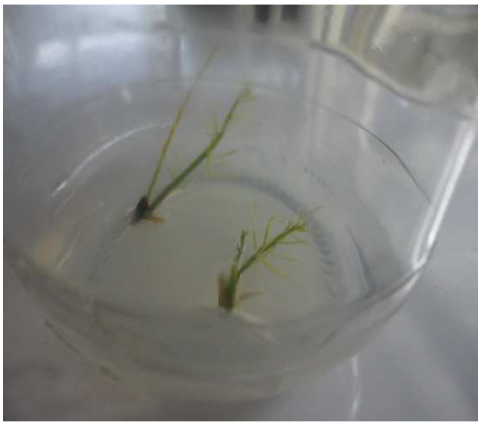

Figure 1: Shoot elongation in MS+ IAA (0.2) (0.2) + BAP (2) after 10 days

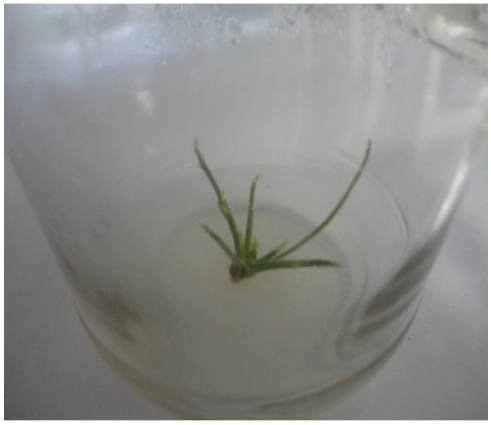

Figure 2: Shoot elongation in MS+ IAA + BAP (2) after 20 days

Root induction in cultures of *Asparagus* sp.was reported to be induced by IBA and IAA by Kar and Sen [1985], Benmoussa *et al*. [1997], Ghosh and Sen [1998] and Mehta and Subramanian [2005] which is in contrast to the present observation of root formation in MS basal media incubated in the dark.

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