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RESEARCH ARTICLE

In-vitro clonal propagation of *Asparagus racemosus* by nodal explants

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ABSTRACT

Asparagus one of the most important medicinal plants, found in India, China and other parts of the world, known to produce steroidal saponins called Shatavarins, which is used in many Ayurvedic and Homeopathic drugs. It is recommended in Ayurvedic texts for prevention and treatment of gastric ulcers as galatogogue and nervine tonic. An experiment was conducted to optimize the concentration of phytohormone for multiple shoot induction from different explants of *Asparagus racemosus*. Sterile culture were obtained when the explants were treated with (0.1%) bavestine, for 5-10 min, (70 %) alcohol for 2 min, (20%) sodium hypochlorite 5 min, (0.1%) $HgCl_2$ for 3 min, washed with sterile distilled water for 6 times. After proper treatment of explants with antimicrobial agent, explants were transferred to MS medium supplement with different combinations and concentration of auxins (2, 4-D and NAA) and cytokinins (BAP and Kinetin). Among the different combinations tested best shooting was found in MS medium supplemented with 2.0 mg/lit. Kn followed by 4.0 mg/l BAP.

Key words : Asparagus, *In-vitro*, Shooting, Auxin, Cytokinin, 2, 4-D: 2, 4-Dichlorophenoxy acetic acid, NAA: Naphthalene acetic Acid, Kn: kinetin

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INTRODUCTION

Asparagus racemosus wild (family Asparagaceae; Liliaceae), commonly known as Satawari (Hindi) is a perennial shrub, with a tuberous root-stock, stems covered with recurved spines, linear leaves arranged in a tuft, white flowers and sweet-scented appears in October. The plant occurs throughout India up to 1500 meters elevation. It is a popular vegetable consumed in many parts of the world and grows naturally throughout India, Asia, Australia and Africa. It is recommended in Ayurvedic texts for prevention and treatment of gastric ulcers as galatogogue and nervine tonic. It is commonly used for the treatment of diarrhoea, dysentery, rheumatism, nervous breakdown, and is thought to be an aphrodisiac (Nadkarni, 1976 and Chadha, 1985). The root of the plant has also been claimed by traditional healers to possess antidiabetic properties.

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Studies on the extracts of this plant have revealed a wide range of biological activities. These include antimutagenic, antitumor, antifungal (Edenharder, 1990; Shimoyamada *et al.*,1990 and Shao *et al.*, 1996), diuretic (Balansard and Rayband, 1987) and immunostimulatory effects (Thatte and Dahanukar, 1988; Rege *et al.*, 1989 and 1999 and Dhuley, 1997). It has been considered to be a lactogogue in lactational inadequacy (Sharma *et al.*, 1996) and useful to decrease post-operative adhesions (scars; Rege *et al.*, 1999). Roots of the plant inhibited the growth of human leukaemia HL-60 cells (Shao *et al.*, 1996) and more recently it has been shown to exert antioxidant properties in rat liver mitochondrial membranes (Kamat *et al.*, 2000). The compounds so far reported include flavonoids, oligosaccharides, amino acids, sulphur-containing acids and steroidal saponins (Shao *et al.*, 1996). Various reports suggest that polysaccharides derived fromthe plant exhibit antioxidant as well as radio protective properties (Gang *et al.*, 1997; Liu *et al.*, 1997a and b and Zeng *et al.*, 1997).

Within the Asparagus genus, micro propagation protocols have been extensively studied in *A. officinalis* (Murashige *et al*., 1972) and other species used mainly as ornamental or medicinal plants using media supplemented with MS (Murashige and Skoog, 1962) medium and various concentrations of auxins and cytokinins. Several methods of *in vitro* regeneration of Asparagus have been established namely: direct organogenesis (Murashige *et al*., 1972), indirect organogenesis (Reuther, 1984) and somatic embryogenesis (Reuther, 1977). Among the existing pathways of Asparagus *in vitro* regeneration, none of them are used on a large commercial scale for propagation, as regenerated plant lets have poor survival rate either at hardening or at field level (Sarabi and Almasi, 2010). In view of this an experiment was designed to induce multiple shoots from different explants of *Asparagus racemosus* for clonal propagation in less time.

MATERIAL AND METHODS

Plant materials :

Healthy plants of *Asparagus racemosus* (Shatavari) was procured from nursery and planted in the pots in a polyhouse of the Department of Molecular and Cellular Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad.

The different parts of *Asparagus racemosus viz.*, nodal segment, leaf, root and stem were used as explants for shoot induction. The explants were washed with detergent solution and distilled water for 30 min. These plant parts were surface sterilized inside a laminar hood firstly with 0.1 per cent $HgCl_2$ for 3 min and then with 70 per cent ethanol 2 min followed by 5 times washing with sterile distilled water. These plant parts were then dried and cut into small pieces to be used as explants for the inoculations.

The culture medium used in the present study was Murashige and Skoog (1962) media. After adding growth regulators, pH of the medium was adjusted to 5.8± 0.1 followed by gelling with 0.8 per cent agar. The media was autoclaved at 121^0C and 15 lbs pressure for 15 min.

Macronutrients, micronutrients, iron chelate, vitamins and amino acid stocks were prepared by weighing required quantity of chemicals and dissolved in double distilled water. They were stored in refrigerators at 4 ± 1°C 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 upto a final volume using double distilled water.

The inoculation of plant materials was carried out in a laminar air flow cabinet under sterile conditions. All the appliances were sterilized and the explants after surface sterilization were inoculated vertically or horizontally on the surface of media. The inoculated cultures were kept in culture room under controlled conditions.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Screening of explants of *Asparagus racemosus* node:

In Asparagus the various explants *viz.*, leaf, root, and node were cultured on MS medium supplemented with different phytohormone combinations and concentrations. It was found that only nodal explants has

shown positive response toward various plant hormones combinations and the explants like leaf showed no response and root turned brown or dead in various culture mediums.

According to the results observed Asparagus node showed best shooting response in the phytohormone combinations such as Kn followed by BAP moderate response in BAP+ Kn, NAA+BAP and BAP+IAA and it showed poor response and late response in 2,4-D+ NAA+Ads (Table 1).

Effect of phytohormone combinations and concentration in shoot formation of *Asparagus racemosus* :

The results showed that the nodal explants showed different response in different phytohormone combinations. The best combination for shoot induction from nodal explants was found to be BAP (4 mg/lit.) and Kn (2 mg/lit.) as it gives response in 15 days and least response in BAP (3mg/lit.) + Kn (1 mg/lit.). MS supplemented with NAA (1 mg/lit.) +2, 4-D (3 mg/lit.) +Ads (50mg/lit.) induced shooting in 45 days not callusing. Effect of different combinations on days of response in explants of Asparagus node is shown in (Table 2).

Shooting formation :

Effect of plant growth regulators on shooting :

Shooting was observed after 2-3 weeks when nodal explants were cultured on MS medium supplemented with BAP, Kin, NAA, 2,4-D, IAA, and Ads.

Shooting in *Asparagus racemosus* by the effect of plant hormone combinations at different concentration (Fig. 1 and 2):

In *Asparagus racemosus* different effects of phytohormone combinations were seen and according to

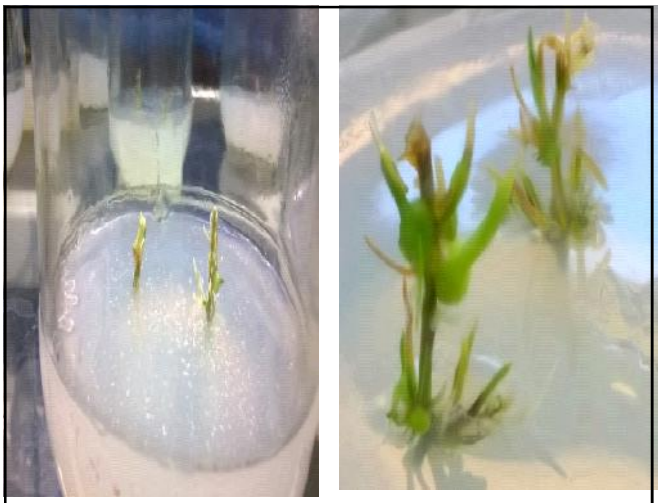
Table 1: Shooting response of nodal explants of *Asparagus racemosus* in various phytohormone combinations

Phytohormone treatment	Shooting response
BAP	+++
BAP+NAA+IAA	-
2,4-D+NAA+BAP	-
BAP + Kn	++
Kn	+++
NAA+BAP	++
2,4-D+ NAA + Ads	+
BAP+IAA	++

- = No response, + = Poor response, ++ = Moderate response, +++ = Excellent

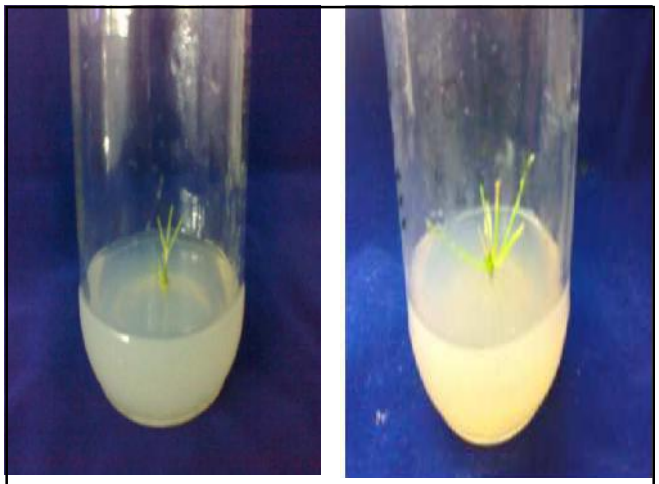
Table 2 : Days of response for shoot formation of nodal explant of Asparagus *racemosus*

Phytohormone combination treatment	Days of response
BAP (3 mg/lit.) + Kn (1 mg/lit.)	30 days
BAP (2 mg/lit.) + Kn (0.5 mg/lit.)	35 days
BAP (3 mg/lit.)	30 days
BAP (4 mg/lit.)	20 days
NAA (1 mg/lit.) + BAP (2 mg/lit.)	38 days
NAA (0.5 mg/lit.) + BAP (2 mg/lit.)	42 days
Kn (2 mg/lit.)	15 days
Kn (3 mg/lit.)	24 days
NAA (0.5 mg/lit.) + 2,4-D (2 mg/lit.) +Ads (50 mg/lit.)	45 days
NAA (1 mg/lit.) + 2,4-D (3 mg/lit.) +Ads (50 mg/lit.)	40 days
BAP (0.2 mg/lit.) + IAA (2 mg/lit.)	45 days
BAP (0.5 mg/lit.) + IAA (mg/lit.)	42 days



(A) (B)

Fig. 1 : Nodal segment of Asparagus inoculated on MS medium supplemented with 2 mg/lit. Kn (A) 5days after culture, (B) shoot proliferation 15 days after culture



(A) (B)

Fig. 2 : Nodal segment of Asparagus inoculated on MS medium supplemented with 4 mg/lit. BAP (A) 5days after culture, (B) shoot proliferation 15 days after culture

Table 3 : Effect of various plant growth regulators on shoot formation of nodal explants of Asparagus *racemosus*

Sr. No.	MS + Phytohormone combination (mg/lit.)						Shooting response
	2, 4-D	NAA	BAP	Kn	IAA	Ads	
1.	-	-	1	-	-	-	-
2.	-	-	2	-	-	-	-
3.	-	-	3	-	-	-	++
4.	-	-	4	-	-	-	+++
5.	-	-	-	1	-	-	-
6.	-	-	-	2	-	-	+++
7.	-	-	-	3	-	-	++
8.	-	-	-	4	-	-	+
9.	-	-	2	0.5	-	-	-
10.	-	-	3	0.5	-	-	-
11.	-	-	3	1	-	-	+
12.	-	-	0.2	-	2	-	++
13.	-	-	0.5	-	2	-	+
14.	-	-	0.5	-	3	-	++
15.	-	1	2	-	-	-	+
16.	-	2	0.5	-	-	-	-
17.	-	0.5	2	-	-	-	++
18.	-	0.5	3	-	-	-	-
19.	3	1	-	-	-	50	+
20.	2	0.5	-	-	-	50	+
21.	0.5	2	-	-	-	40	-
22.	1	3	-	-	-	100	-
23.	0.5	2	-	-	-	-	-

- = No response, + = Poor response, ++ = Moderate response, +++ = Excellent

the results the maximum shooting in node of Asparagus was observed in the Kn (2 mg/lit.) followed by BAP (4 mg/lit.), showed moderate response in BAP (3 mg/lit.) + Kn (1 mg/lit.), NAA (1 mg/) + BAP (2 mg/lit.) and BAP (0.5) + IAA(3) and late response in NAA (1 mg/lit.)+2,4-D (3 mg/lit.) +Ads (50 mg/lit.).

In vitro process is a promising tool for the rapid multiplication of this endangered medicinal species. From the above result it is obvious that NAA at many of the concentrations either singly or in combinations is effective in the induction of callus shoots and roots which might be because of its chemically stable, low mobility in the plant and prolonged action nature (Table 3).

The shoot induction was supported by BAP a low level of NAA (Kar and Sen, 1985). Observed the nodal buds by the use of IAA and BAP in *A. racemosus* similar to our finding where BAP singly is acted as a good shoot bud inducer at higher concentration whereas in multiple shoot induction a lower concentration is favorable. In combination with NAA, BAP promotes shoot bud somatic embryos as well as multiple adventitious shoots at its high concentration and a low NAA level.

Conclusion :

The present investigation concluded that the effect of various shooting combination of 2,4-D, BAP, NAA, Kn, IAA and additive Ads were used to obtained shooting formation. The best combination for shoot induction from nodal explants in *Asparagus racemosus* was found to be Kn (2mg/lit.) followed by BAP (4 mg/lit.).

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