IN VITRO PROPAGATION OF AN ENDANGERED MEDICINAL PLANT ASPARAGUS RACEMOSUS

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ABSTRACT

Asparagus racemosus Wild (Asparagaceae) is an endangered medicinal plant of tropical and subtropical India. Its medicinal usage has been reported in the Indian and British Pharmacopoeias and in traditional systems of medicine such as Ayurveda, Unani and Siddha. Over exploitation coupled with inadequate cultivation and unsatisfactory attempts for its replacement, the wild stock of this species has been markedly exhausted and now it is listed as threatened species. Hence, there is an urgent necessity to safeguard this germplasm, and plant tissue culture has proved helpful in conserving threatened plant species. In this direction, in the present study, an efficient protocol was developed for callus induction and plantlet regeneration from nodal explants of Asparagus racemosus. Well-organized plant regeneration was achieved on MS medium containing different concentrations and combinations of growth regulators. MS medium supplemented with BAP 1.5 mg/l and KN 1.5 mg/l was effective for regeneration response.

Key words: *Asparagus racemosus, conservation, endangered plant, MS medium,BAP, KN.*

INTRODUCTION

The World Health Organization (2003) has estimated that 80% of the population of developing countries being unable to afford pharmaceutical drugs relies on traditional medicines, mainly plant based, to sustain their primary health care needs. India is one of the most medico-culturally diverse countries in the world where the medicinal plant sector is part of a timehonoured tradition that is respected even today which include Ayurveda, Unani and Siddha. The earliest mention of the use of plants in medicine is found even in the Rigveda which was written between 4500 and 1600 BC.

Asparagus racemosus is an important medicinal plant of tropical and subtropical India. Its medicinal usage has been reported in the Indian and British Pharmacopoeias and in indigenous systems of medicine. The genus *Asparagus* includes about 300 species around the world. The genus is considered to be medicinally important because of the presence of steroidal saponins and sapogenins in various parts of the plant. Out of the 22 species of *Asparagus* recorded in India; *Asparagus racemosus* is the one most commonly used in traditional medicine [1].

Asparagus racemosus Willd. of family Liliaceae, is commonly called Satavari, Satawar or Satmuli in Hindi; Satavari in Sanskrit; Shatamuli in Bengali; Shatavari or Shatmuli in Marathi; Satawari in Gujarati; Toala-gaddalu or Pilli-gaddalu in Telegu; Shimaishadavari or Inli-chedi in Tamil; Chatavali in Malayalam; Majjigegadde or Aheruballi in Kannada; Kairuwa in Kumaon; Narbodh or Satmooli in Madhya Pradesh; and Norkanto or Satawar in Rajasthan [2].

The plant grows throughout the tropical and subtropical parts of India up to an altitude of 1500 m. The plant is a spinous under-shrub, with tuberous, short rootstock bearing numerous succulent tuberous roots (30–100 cm long and 1–2 cm thick) that are silvery white or ash coloured externally and white internally. The roots are the part that finds use in various medicinal preparations. The stem is woody, climbing, whitish grey or brown coloured with small spines. The plant

flower during February–March leaving a mild fragrance in its surrounding and by the end of April, fruits can be seen with attractive red berries [3].

It is widely used for multiple purposes and its medicinal importance has been recognized by Ayurveda for centuries. Although almost all parts of this plant have some medicinal properties, roots and young shoots are of higher significance. Young spears are consumed as vegetable or salad and are considered as a balanced health food with many essential nutrients. Traditionally the roots are used mainly to promote milk secretion and as a demulcent, diuretic, aphrodisiac, tonic, alterative, antiseptic, antidiarrheal, glalctogogue and antispasmodic [4]. It is also used to treat debility, especially in women and infertility, impotence, menopause, stomach ulcers, dehydration, lung abscess, haematemesis, cough, herpes, leucorrhoea and chronic fevers, delay ageing process and form health food ingredients in several Ayurvedic formulations [5].

Using the modern scientific tools many active compounds like several steroidal saponins [6], aglycones, alkaloids like *asparagin*-an anticancer agent [7] and many other active pharmacologically important compounds have already been isolated from the roots of this species. Leaves contain rutin, diosgenin and a flavonoid glycoside identified as quercetin-3-glucuronide. Flowers contain quercetin hyperoside and rutin. Fruits contain glycosides of quercetin, rutin and hyperoside and steroidal saponins [8], while fully ripe fruits contain cyanidin-3-galactoside and cyanidin-3-glucorhamnoside.

Only a small percentage of medicinal plants, used in the industry are cultivated. Most of them are collected from the wild, very often in a destructive and unsustainable manner. Keeping the above facts in mind, namely the gradual decline of this endangered species, the present study was undertaken to develop a suitable protocol for its rapid multiplication.

MATERIALS AND METHODS

Plant material and explant source

Healthy explants of nodal segments were collected from Namakkal district , were washed under running tap water for at least 30 min, and followed by soaking in 5% (v/v) Teepol for 5 min. After thorough washing in sterilized distilled water, the explants were surface sterilized with freshly prepared 0.1% (w/v) $HgCl_2$ for 3 min. Following repeated washing with sterile distilled water, the explants were inoculated onto culture media.

Culture media and environment

Murashige and Skoog (MS) medium [9] containing 2% (w/v) sucrose and 0.8% (w/v) agar were used in all the experiments. Plant growth regulators and their combinations were added to the medium as specified below. The pH of the media were adjusted to 5.8 prior to autoclaving using 1N HCl and 1N NaOH. For the preparation of semi solid medium, 0.8% agar was added as solidifying agent and the media were heated to boiling for proper mixing. Later, the media plugged with non-absorbent cotton plugs and were autoclaved for 15 lb pressure at 121°C for 20 minutes.

Shoot induction

For shoot induction and proliferation, MS medium supplemented with 0.5, 1.0, 1.5, and 2.0 mg/l concentrations of 6-benzyladenine (BAP) and 1.5mg/l Kinetin (KN). After 7 days of culture, the frequency of shoots and number of shoots per explant were recorded.

In vitro Environment

Established cultures were regularly sub cultured to new medium, inside a laminar flow hood disinfested with 70% ethyl alcohol. Cultures are in general maintained on open shelves in climate controlled laboratories. The cultures were incubated in the culture room at 25±2ºC temperature under 16hrs photoperiod and with a light intensity of 1000 Lux (white fluorescent light) emitted by 40W Philips tubes.

Rooting

Rooting was attempted *in vitro* and ex vitro. *In vitro* root induction was carried out on full-strength hormone-free MS medium or half-strength MS medium. For ex vitro rooting, the basal ends of the healthy shoots were dipped in 0.5 mg/l IBA for 30 min and then planted in small plastic pots containing sterile vermiculite and covered with transparent polyethylene membrane to ensure high humidity. The polyethylene membranes were opened after 2 week in order to acclimatize plants to field conditions. After 1 month plants were transferred to earthen pots filled with 3:1 mixture of soil and organic manure and maintained in a greenhouse under normal day-length conditions.

RESULTS AND DISCUSSION

Shoot initiation and establishment from *Asparagus racemosus* nodal explants cultured on MS basal and MS medium supplemented with various combinations of growth regulators i.e. BAP in combination with KN is described in Table 1. Most of the other research studies for other medicinal plant species have shown the use of cytokinin alone or in combination with other in different concentrations. For example for *Paederia foetida* and *Centella asiatica* multiple shoots were obtained in MS medium supplemented with BAP 1.0 mg /litre [10], and in *Rauwolfia serpentina* on MS medium supplemented with benzyladenine and NAA [11], whereas for *Bacopa*, optimum shoot proliferation was achieved in different combination of hormones in different concentrations.

During initial week after inoculation, bud initiation was very low. However, bud initiation was found to be started in most of the cultures initiated from 9-10 days by showing a small newly sprouted bud, which proliferate into shoot buds with leaves during 21-25 days which were placed in the culture room under the standard conditions of temperature (25 ± 2°C). All the experiments were performed thrice with 3 replicates per treatment. Shrivastava and Rajani, [12] also reported that shoot bud initiation was observed visually on the ninth day of incubation in all replicates in the media having different concentrations of BAP and Kn.

Nodal multiplication in *Asparagus racemosus* was previously reported [13] they used 22.2 µ IBA + 2.68 µM NAA, and reported 4-5 shoots per node. Paramageetham *et al*. [14] reported plant regeneration with higher concentrations of BAP (4.44 and 8.87µM) or kinetin (4.65 and 9.29µM) in combination either with IAA (0.57 and 2.85 µM) or NAA (0.54 and 2.69 µM). Raghu *et al*. [15] reported multiple shoot induction using 0.5mg/l BA+ 0.5 mg/l of KN in the regeneration medium

Shoot lengths were taken at the end of 5^{th} week of the regenerated shoots and maximum growth was observed on MS + 1.5 mg/L BAP + 1.5 mg/l KN . The percentage of explants producing shoots, number of shoots per explants and average shoot lengths were given the table 1.

In vitro Rooting

We achieved an efficient rooting when, 2 to 4 cm long green shoots are pounding in the dark for 4 to 6 days with the basal end set 0.5 cm deep into 0.6% agar-solidified with 1mg/l NAA medium. We have observed that minor changes in the NAA concentration can markedly alter the number of adventitious roots produced. Mohapatra *et al*. [16] reported rooting of *in vitro* raised shoots was best induced on half strength MS supplemented with 0.5 mg dm-3 indole-3-butyric acid (IBA). Matured plantlets with prominent rooting were finally transformed to the soil through a gradual acclimatization process. The rooted plants were potted in washed sand and covered with sealed plastic vinyl bags to keep full humidity at 25°C in light condition . As the plants grew vigorous, the bags were poked with chopsticks to allow air enter inside the bags until to the plants self-supported.

CONCLUSION

In the present investigation, efficient *in vitro* regeneration method was developed for rapid propagation of *Asparagus racemosus* using nodal segments as explants. The tissue culture techniques developed in this study can be useful

for propagation and also for the conservation of the germplasm of this medicinally important plant which can enhance the rate of multiplication and reduce the time period and cost of production.

REFERENCES

1. Nishritha B and Sanjay S (2007), "*Asparagus racemosus* -Ethnopharmacological evaluation and conservation needs", Journal of Ethnopharmacology, Vol. 110, pp1-15.

2. Dinan L, Savchenko T, Whiting P, (2001), "Phytoecdysteroids in the genus *Asparagus racemosus* (Asparagaceae)", Phytochemistry Vol.56, pp569–576.

3. Dubey N K, Kumar R and Tripathi P, (2004) "Global promotion of herbal medicine: India's opportunity", Current Science, Vol. 86, pp37–41.

4. Goyal R K, Singh J and Lal H, (2003), "*Asparagus racemosus*-An Update", Indian J. Med. Sci., Vol. 57, No.9, pp 407-414.

5. Hayes P Y, Jahidin A H, Lehmann R, Penman K, Kitching W and De Voss J J, (2006a), "Asparinins, asparosides, curillins, curillosides and shatavarins: structural clarification with the isolation of shatavarin V, a new steroidal saponin from the root of *Asapragus racemosus*", Tetrahedron Letters, Vol. 47, pp 8683-8687.

6. Hayes P Y, Jahidin A H, Lehmann R, Penman K, Kitching W and De Voss J J, (2006b), "Structural revision of shatavarins I and IV, the major components from the roots of *Asparagus racemosus*" , Tetrahedron Lett., Vol. 47, pp 6965-6969.

7. Joy P P, Thomas J, Mathew S and Skaria B P, (1998), Medicinal Plants. Kerala Agriculture University, Aromatic and Medicinal Plants Research Station, India, pp.38.

8. Mandal D, Banerjee S, Mondal N B, Chakravarty A K and Sahu N P, (2006), "Steroidal saponins from the fruits of *Asparagus racemosus*", Phytochem, Vol. 67 pp.1316-1321.

9. Murashige T, and Skoog F, (1962), "A revised medium for rapid growth and bioassays with tobacco tissue cultures", Physiol. Plantarum, Vol.15 pp 473-497.

10. Singh S, Ray B K, Mathew S, Buragohain P, Gogoi J, Gogoi S, Sharma B K and Deka P C, (1999), "Micropropagation of a few important medicinal plants", Annals of Biology (Ludhiana), Vol. 15, No.1 pp. 1-7.

11. Sehrawat A R, Uppal Sanjogta and Punia Anita (2001), "*In vitro* culture and multiplication of *Rauwolfia serpentina* - a threatened medicinal plant", Crop Research (Hisar), Vol.22, No.1, pp. 68-71.
12. Shrivastava N and Rajani M (1999), "Multiple shoot regeneration and tissue culture studies on *Bacopa monnieri* (L.) Pennell". Plant Cell Reports, Vol.18, No.11 pp.919- 923.

13. Tiwari K N, Sharma N C, Tiwari V and Singh B D, (2000), "Micropropagation of *Centella asiatica* (L.), a valuable medicinal herb", Plant Cell, Tissue and Organ Culture, Vol. 63 pp 179-185.

14. Paramageetham C G, Prasad Babu and Rao JVS, (2004), "Ex-situ conservation of centella asiatica (l.) Urban an endangered and threatened species through leaf callus culture", Plant Cell, Tissue and Organ Culture, Vol.7, 9No. 19– 24.

15. Raghu AV, Martin G, Priya V, Geetha SP and Balachandran I (2007), "Low cost alternatives for the micropropagation of *Centella asiatica*". Journal of Plant sciences, Vol.2 No.6, pp 592-599.

16. Mohapatra H, Barik D P, Rath S P, (2008), "*In vitro* regeneration of medicinal plant *Centella asiatica*", Biologia Plantarum, Vol. 52 No.2 pp 339-342.

Table 1. Effect of different concentrations of BAP and KN on shoot regeneration of *Asparagus racemosus*

S. No	Growth regulator concentration in mg/l		No of shoots per explant*	Shoot length (cm)#
	BAP	KN		
1	0.5	0.5	11±0.2	0.7±0.1
2	1.0	0.5	13±0.4	0.8±0.2
3	1.5	0.5	15±0.3	0.9±0.1
4	2.0	0.5	12±0.4	0.7±0.3
5	0.5	1.0	13±0.2	1.1±0.1
6	1.0	1.0	14±0.5	1.3±0.3
7	1.5	1.0	16±0.1	1.5±0.2
8	2.0	1.0	16±0.2	1.4±0.1
9	0.5	1.5	17±0.3	1.5±0.2
10	1.0	1.5	21±0.4	2.4±0.3
11	1.5	1.5	24±0.3	2.8±0.1
12	2.0	1.5	20±0.2	2.3±0.2
13	0.5	2.0	13±0.2	1.6±0.2
14	1.0	2.0	16±0.3	2.1±0.2
15	1.5	2.0	15±0.5	2.2±0.2
16	2.0	2.0	12±0.4	1.7±0.1

*The values represent the mean ± SE, five independent experiments.
#At least 20 cultures were raised for each experiments
Abbreviations
BAP: 6-Benzyl amino purine
KN: Kinetin