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## *In vitro* Plant Regeneration from Axillary Buds of *Asparagus racemosus* Wild, a Medicinal Plant

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### Abstract

An efficient method was developed for regeneration of *Asparagus racemosus* Wild, from axillary explants. MS media supplemented with different concentrations of cytokinin (BAP) alone or in combination of different concentrations of auxin (NAA or IBA) was tested for their efficiency in multiple shoot induction. High frequency of multiple shoot regeneration was achieved on MS medium supplemented with BAP (0.1mg/l) and NAA (0.05 mg/l). When shoots were well developed they were dissected and subcultured on the same medium to promote more multiple shoots. Eighteen to twenty shoots were obtained on this medium. The shoots were rooted best on half MS medium supplemented with 0.05 mg/l BAP and 1.0 mg/l IBA. Among the five levels of pH tested, 5.7 was the best for multiple shoot proliferation. The result presented here proved to be suitable for the rapid propagation system of *A. racemosus*.

**Key words:** *Asparagus racemosus*, Medicinal plant, Rapid proliferation, Multiple shoots, Auxins and Cytokinins

### Introduction

Over 150 different species of *Asparagus*, an herbaceous perennial, are known to occur in the world (Drost, 1997). *Asparagus racemosus* Wild is belonging to the family Asparagaceae (formerly known as Liliaceae). This plant is commonly known as "Shatamuli". It grows wild in forests and is planted in gardens. Some of the European species to be mentioned are *A. officinalis*, *A. sprengeri* and *A. acutifolius*. *A. officinalis* is reported to be popular vegetable consumed in many parts of the world (Shao *et al.*, 1997). The *Asparagus rom.* is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant (Oketch-Rabah, 1998). All parts such as leaves, flowers, fruits, tubers, roots and bark of this species contains diosgenin, glycosides, sterols and their glycosidase and are very important for the treatment of diarrhoea, dysentery, diabetes, jaundis and other urinary disorders (Ghani, 1998). *A. racemosus* has a low multiplication rate using conventional methods (Ellison, 1986). It is commonly propagated through seeds and rhizomes. Since different parts of the plant are harvested extensively for their therapeutic use, the plant has been over exploited. So, there is a need for mass propagation of the selected clones. Tissue culture techniques have advantages that may provide methods for both large-scale propagation and improvement of plants

in a comparatively short time compared to *in vivo*. Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries demand, in part, on plants for the production of pharmaceutical compounds (Chand *et al.*, 1997). *In vitro* culture of different *Asparagus* species was reported by many researchers (Yang, 1977; Kar and Sen, 1985; Ghosh and Sen, 1994a; 1994b; 1996; Benmoussa *et al.*, 1996; Nayak and Sen, 1998; Stajner *et al.*, 2002; Mehta and Subramanian, 2005). Unlike other extensively studied *Asparagus* sp. there is a little information of *in vitro* propagation of *Asparagus racemosus* and also different factors like the effect of pH on the growth of explants *in vitro*. Therefore, the present study was undertaken to determine the optimum doses of auxins and cytokinins and pH level of the shooting medium for multiple shoot proliferation of *Asparagus racemosus* and makes micropropagation method economically viable and technically feasible.

### Materials and Methods

The experiment was conducted at Biological Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. Young shoots of

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healthy growing plants of *A. racemosus* were collected from the medicinal plant garden of BCSIR, Dhaka and used as primary explants. Explants were washed under running tap water for 10-15 min and then surface-sterilized with 0.1% HgCl<sub>2</sub> for 10 min followed by five to six rinses with sterilized distilled water. Different combinations and concentrations of growth regulators viz., BAP (0.5-0.1 mg/l), BAP+NAA (0.1+0.05, 0.5+0.25 mg/l), BAP+IBA (0.1+0.05, 0.5+0.25 mg/l) were tested for their shoot induction and proliferation. After surface sterilization the nodal explants (2-4 mm) were excised from the shoot segments and inoculated in 150 × 25 mm glass tube containing MS (Murashige and Skoog, 1962) supplemented with above mentioned various combinations of auxins and cytokinins. Before inoculation, the media were solidified with agar (0.7%), sucrose (3%), pH was adjusted to 5.7 and autoclaved at 15 lbs/sq inch and 121°C for 20 minutes. For root induction, in vitro microshoots with fully expanded leaves were excised and transferred to half strength MS medium supplemented with BAP+IBA (0.05+1.0, 0.1+1.0, 0.1+2.0 mg/l), IBA (0.1, 0.3, 0.5, 1.0, 2.0 mg/l). Another phase of experiment was to determine the effect of different pH level on shoot proliferation and multiplication. For this experiment, microshoots (more than 1.0 cm long) were cultured on MS medium adjusted to five different levels of pH viz, 4.0, 4.5, 5.0, 5.7 and 6.0 and supplemented with only BAP 0.1 mg/l and NAA 0.05 mg/l. The tubes containing explants were incubated at 25 ± 0.5°C under white fluorescent tube light with 16 h photoperiod. For each treatment 25 explants were used and all the treatments were repeated thrice. The new shoots induced from the *in vitro* cultures were further used as explants for adventitious shoot regeneration and inoculated into 250 ml

conical flasks. The cultures were regularly subcultured on fresh medium at four weeks intervals and observations were recorded. Data were analyzed statistically according to Mian and Mian (1984). The healthy plantlets were taken out from the culture tubes, washed to make free from agar gel with running tap water and transplanted to plastic pots containing soil, sand and cow dung (1:1:1) for hardening. The plantlets were kept in a polychamber at 80% relative humidity, 32 ± 2°C under 12 h photoperiod for acclimation. Established plantlets were transplanted in earthen pots under natural conditions and the survival rate was recorded.

### Results and Discussion

Among the different combination, best results were observed on the medium supplemented with 0.1 mg/l BAP and 0.05 mg/l NAA after 15-18 days of culture. Shoot induction was not found in MS basal medium (control) even after four weeks of culture (Table I). Proliferation of axillary shoot from the nodal segments of the mature plants and *in vitro* raised shoot origin was remarkably influenced by types and concentrations of cytokinin and auxin. The nodal explants showed slight swelling prior to the emergence of shoot buds developing from the pre existing material within 5-8 days after inoculation. In the same medium, 90% explants produced 20.8 ± 1.0 shoots (Fig. 2g and 2h) per culture and the average length of the shoot was 2.0 ± 0.15 cm (Table. I). On the other hand, a combination of BAP (0.1 mg/l) and IBA (0.05 mg/l), 70% explants produced 6.8 ± 1.0 shoots per culture and the average length of the shoot was 1.2 ± 0.55 cm. Comparatively a lower number of adventitious shoots were observed in the medium containing BAP alone. On the medium containing 0.1 mg/l BAP, 33% explants produced 3.0 ±

**Table I. Effect of different concentrations of auxins and cytokinins with their combination on multiple shoot formation of *A. racemosus***

Growth regulators (mg/l)	Multiple shoot induction (%)	No. of shoots/explant	Average shoot length (cm)
BAP			
0.05	-	-	-
0.10	33	3.0 ± 0.15	1.0 ± 0.25
BAP + NAA			
0.1 + 0.05	90	20.8 ± 1.0	2.0 ± 0.15
0.5 + 0.25	75	8.0 ± 0.15	1.5 ± 0.45
BAP + IBA			
0.1 + 0.05	70	6.8 ± 0.10	1.2 ± 0.55
0.5 + 0.25	60	4.5 ± 0.15	1.0 ± 0.35
0 (Control)	-	-	-

Each treatment consists 25 replicates

0.15 shoots per culture and the average length of the shoot was  $1.0 \pm 0.25$  cm and medium containing 0.05 mg/l BAP failed to produce any axillary shoot (Table. I). When the medium containing higher concentrations of 0.5 mg/l BAP with 0.25 mg/l NAA, the percentage of explant responded but the number of shoot per culture and the average length of the shoot decreased gradually with the higher concentration of growth regulators. The present study demonstrated that the nodal explant of *A. racemosus* has the ability to differentiate readily into shoots on media supplemented with lower concentrations of BAP in combination with NAA (Table. I). The results are in agreement with Yang, (1977). These results confirmed that plant species have some endogenous hormones which generally does not require any exogenous growth regulators for regeneration (Hussey, 1982). Stajner *et al.* (2002) reported that shoot initiation of *A. maritimus* could be achieved with a combination of NAA, Kinetin and BAP with or without ancymidol, except in combination with Kinetin with NAA. On the other hand, in *A. adscendens*, the best shoot multiplication medium reported by Mehta and Subramanian (2005) is MS supplemented with NAA and Kn. This might be due to genotypic variations of explants reinforced by the cultural and environmental conditions. Repeated subcultures of explants on fresh shoot proliferation medium helped to achieve continuous production of healthy shoot buds.

Among different levels of pH, the pH from 5.0-5.7 levels was optimum for multiple shoot proliferation and at 4.0 -4.5 levels showed poor quality and produced less number of shoots (Fig. 1). Results obtained in this study are in

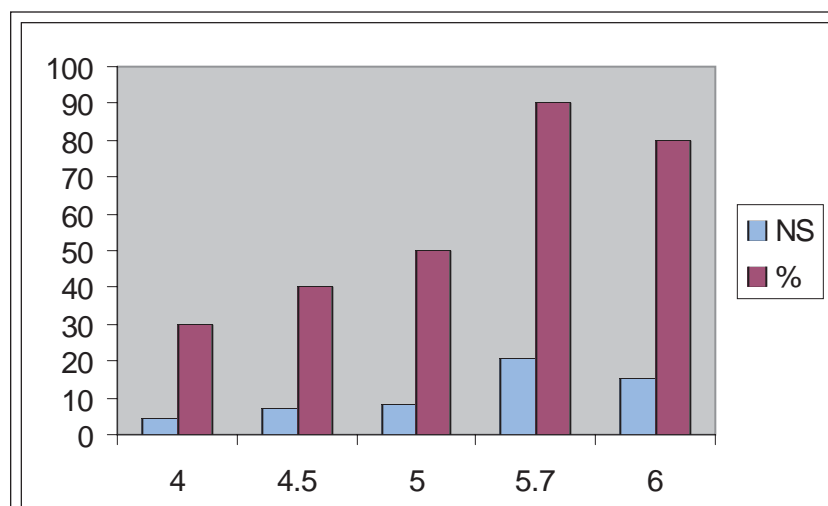
agreement with Wan *et al.*, (1994); Gautam *et al.*, (1993); Barna and Walklu, 1988. In vitro shoot proliferation and multiplication were found to be maximum when the pH was adjusted at 5.7 before autoclaving. It may be due to the comparatively higher (4.0 - 4.5) or less acidic pH (6.0) gave harder gel which might have adverse effects on proliferation and shoot multiplication. The present report showed that BAP (0.1mg/l) and NAA (0.05 mg/l) and pH 5.7 are proved more effective on shoot proliferation from nodal explants of *A. racemosus*.

Induction of roots in regenerated shoots was observed in 85% shoots (2-3 roots/shoots) when cultured on half MS with 0.05 mg/l BAP and 1.0 mg/l IBA for three weeks (Table II). IBA was

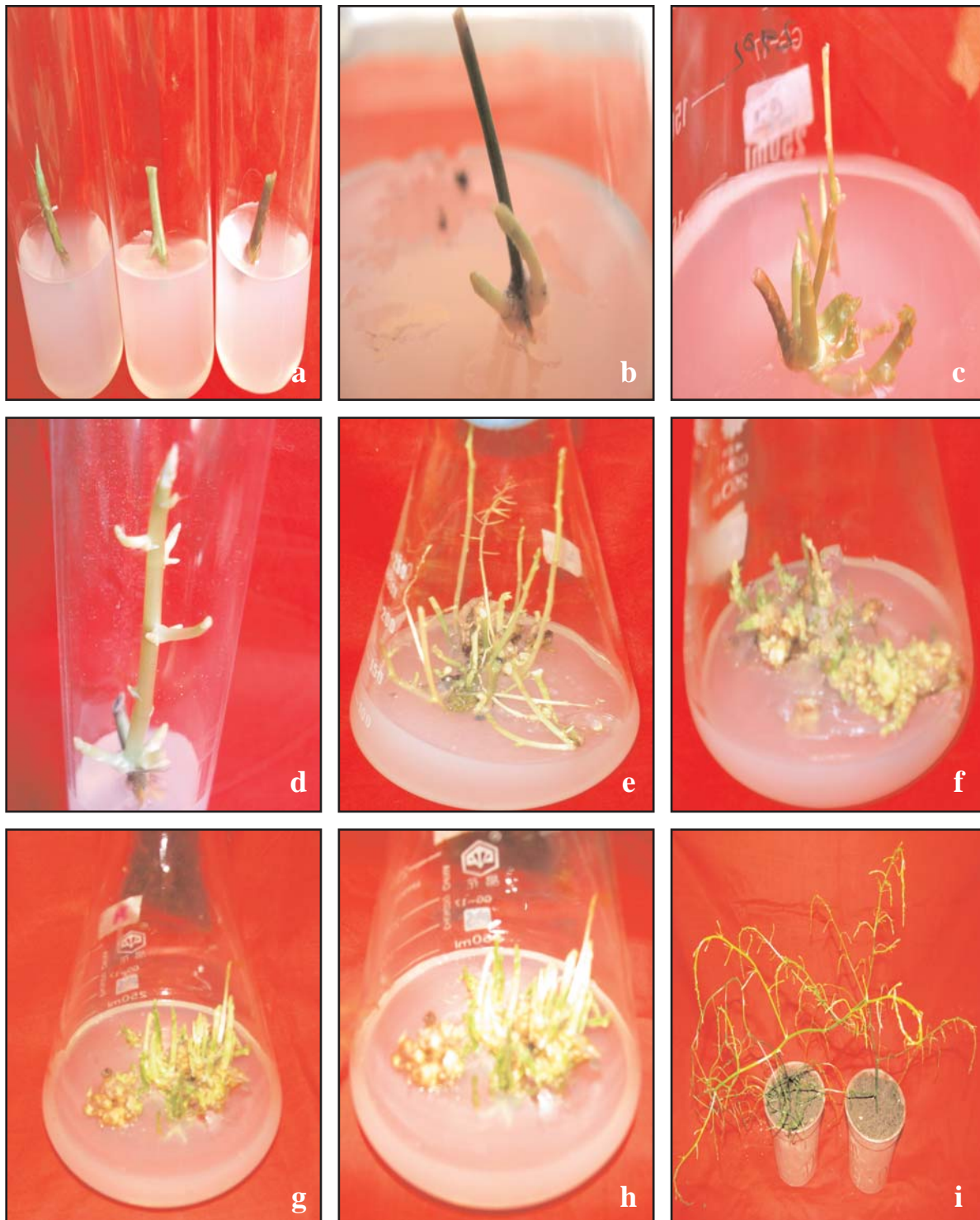
**Table II. Effect of half strength MS with different concentration of auxins and cytokinins with their combinations on *in vitro* grown shoots of *A. racemosus***

Growth Regulators Mg/l		% of shoots with roots	No. of roots per shoot	Length of roots(cm)
BAP	IBA			
0.05	1.0	85	$3.2 \pm 0.43$	$4.4 \pm 0.15$
0.10	1.0	68	$2.4 \pm 0.45$	$4.2 \pm 0.35$
0.10	2.0	62	$2.2 \pm 0.30$	$4.3 \pm 0.55$
0.00	0.1	-----	-----	-----
0.00	0.3	-----	-----	-----
0.00	0.5	-----	-----	-----
0.00	1.0	-----	-----	-----
0.00	2.0	-----	-----	-----

Each treatment consists of 25 replicates



**Fig. 1. Effect of pH on growth and shoot proliferation of axillary shoot from nodal segments of *A. racemosus*. Data were recorded after 4 weeks of culture on MS medium containing BAP 0.1 mg/l with NAA 0.05 mg/l**



**Fig. 2.** Different stages of in vitro regeneration from internode explants of mature plant of *A. racemosus* Wild. a. Nodal segment inoculation on MS medium containing different growth regulators; b-d. Shoot initiation on MS medium containing BAP 0.1 mg/l and NAA 0.05 mg/l after two to four weeks of culture; e-h. Multiplication of adventitious shoots on internode-derived base after eight to ten weeks of culture on the same medium; i. Regenerated plantlet on soil after ten weeks of transfer under ex vitro condition

best for rooting of other species such as *Vitex negundo* (Afroz *et al.*, 2008), *Rauvolfia serpentina* (Baksha *et al.*, 2007) *Gloriosa superba* (Hassan and Roy, 2005) and *Syzygium cumini* (Yadav *et al.*, 1990). After four weeks the rooted shoots were transferred to pots. None of the plantlets survived when directly transferred from rooting medium to the pot under natural conditions. About 80% of the transplanted plants of *A. racemosus* survived if the plants in the rooting culture tubes were kept in normal room temperature for seven days before transplantation in pots and reared for three weeks. The plantlets were reared under controlled temperature ( $30 \pm 2^\circ\text{C}$ ) and light (2000 lux) in a chamber with 80 percent humidity. During this period of acclimation shoots elongated, leaves expanded and turned deep green looking healthier (Fig. 2i).

*In vitro* regeneration from nodal explant could be used as an alternative source of raw materials to meet the ever increasing demands of the pharmaceutical industries as clonally propagated plants would also have identical phytochemical profiles (Roja and Heble, 1993). The method developed for rapid shoot multiplication of *Asparagus* is reliable and definitely a promising one for this valuable medicinal plant. The protocol described here may be used for commercial purposes as it reduced time on shoot development at a faster rate in a shorter period.

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