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# Callus induction and axillary shoot formation in *Asparagus racemosus* Willd.

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## ABSTRACT

An experiment was performed to establish a regeneration protocol for an important medicinal plant, *Asparagus racemosus*. In the present investigation, nodal and internodal explants were employed for callus induction and axillary shoot formation. Maximum callus induction frequency was found on MS medium fortified with 2,4-D (1.0 mg/L) along with NAA (1.0 mg/L) and BAP (0.5 mg/L). However, individual effects of 2,4-D or NAA with BAP showed least callus induction. The higher concentrations of 2,4-D and BAP decreased the response of explants. However, maximum axillary shoot formation was observed on MS medium adjuvanted with BAP (2.0 mg/L) and NAA (0.5 mg/L).

**KEYWORDS:** 2,4-D, *Asparagus*, BAP, callus induction, NAA, shoot formation

## INTRODUCTION

Plants have been used invariably by humans since ages for food, medicines and various other day to day needs. However, increasing population and incessant human needs have put enormous pressure on these bio-resources and lead to their unprecedented depletion from nature. To address this problem, the tissue culture techniques have come to the rescue of depleting plants [1]. Plant tissue culture has been extensively used to boost the large scale micro-propagation of threatened medicinal plants [2]. This not only helps in large scale production of true-to-type plantlets but also helps to conserve rare and threatened plants.

*Asparagus racemosus* (Asparagaceae), commonly known as shatavari, is an important medicinal plant which is recognized as 'vulnerable' mainly due to its destructive harvesting [3]. Being a woody climber, it grows in tropical and sub-tropical areas in India. It can be seen at an altitude of 1500 m. It reaches up to a height of 1-2 m, commonly along rocky and gravelly soils. Its botany consists of green leaves called phylloclades. The white flowers are transformed into blackish purple berries. The fruit berries contain seeds. These seeds are the main propagules of *A. racemosus*. However, vegetative reproduction is slow process in case of *A. racemosus*. It has large medicinal value especially concerned with female medication including phytoestrogen properties and is extensively used in combating menopausal

symptoms and to increase milk production [4,5]. The abundant active compounds found in *A. racemosus* are Shatavarin (I-IV), Shatavaroside A and Shatavaroside B, Filliasparoside C which are steroidal saponins. Asparagamine A is an alkaloid found in roots of *A. racemosus*. Some flavonoids and essential fatty acids have also been reported in *A. racemosus*. In Ayurveda, *A. racemosus* is referred to as queen of herbs because it is used in more than 60 formulations of ayurvedic medicines, mostly the medicines are concerned with female medication. In natural habitat this plant is declared as vulnerable because of destructive harvesting for active compounds.

It mostly proliferates through seeds but can also propagate through vegetative methods, but this process is slow. So, the *in vitro* techniques provide a better way for propagation and conservation of *A. racemosus*, a medicinally important and vulnerable plant. So, the present investigation was aimed to design an enhanced protocol for callus induction and axillary shoot formation in nodal and internodal explants of *A. racemosus*.

## MATERIALS AND METHODS

Nodal and internodal explants of *Asparagus racemosus* Willd. were taken from herbal garden of Jamia Hamdard and were used as experimental material for callus induction and axillary shoot formation. These explants were collected in self-sealing polybags.

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## Explant Sterilization

The nodal and internodal segments of *A. racemosus* were placed in a beaker under running tap water for about 30 min. Two to three pinches of cetrinide were added to beaker containing explants to clean the explants properly. Now the explants were washed properly to remove any traces of detergent. Then the explants were shifted to laminar hood and the explants were treated with 70% alcohol for about 30 min. and then washed thrice with distilled water under sterile conditions. The tissue was now submerged in a beaker containing 0.15% mercuric chloride ( $\text{HgCl}_2$ ) for 1 min. After that, the  $\text{HgCl}_2$  was removed by washing (3 times) with distilled water under aseptic conditions.

## Medium and Culture

The media used in this experiment was Murashige and Skoog [6] media. The medium contains inorganic nutrients, organic, vitamins and sucrose. Various plant growth regulators (PGR's) like 2,4-Dichlorophenoxyacetic acid (2,4-D), 1-Napthalenacetic acid (NAA) and 6-Benzylaminopurine (BAP) were used in this experiment using different concentrations.

## Statistical Analysis

Data represents mean  $\pm$  standard deviation (SD). Values are means of three replicates and the presented mean values were analyzed by SPSS software (version 22) for one-way ANOVA. Significant differences were estimated using Duncan's Multiple Range Test (DMRT) at  $p < 0.05$ .

## RESULTS

### Callus Induction

After inoculations of explants, tubes were exposed to a photo period 16h of light and 8h of darkness at  $25 \pm 2^\circ\text{C}$  and sub-cultured at regular intervals of four weeks of incubation in culture room. The cumulative effect of 2,4-D (1.0 mg/L), NAA (1.0 mg/L) and BAP (0.5 mg/L) resulted in maximum callus induction frequency of 40% in nodal explants after 4 weeks of incubation period (Figure 1; Figure 2). The higher the concentrations of 2,4-D and BAP decreased the response of the explants. It was also observed that the individual effects of 2,4-D or NAA with BAP shows least callus induction response of 10% (Table 1).

### Axillary Shoot Formation

The commencement of multiple shoot clumps from nodal explants shows shoot forming response to different combinations and concentrations of growth regulators. Initiation of shoots clumps was observed in cultures grown on various combination of BAP either alone or adjuvanted with NAA within four weeks of inoculation (Figure 3-5). However maximum shoot formation was observed on MS medium supplemented with 2.0 mg/L BAP and 0.5 mg/L NAA resulting in mean no. of 3 shoots per node (Table 2). In the absence of NAA, the induction frequency of BAP decreased, compared to combination. As the BAP

concentration intensified above 2.0 mg/L, the induction ability reduced.

## DISCUSSION

As the *A. racemosus* is medicinally important plant and a vulnerable plant also. Plant tissue culture provides an effective way for conservation and micropropagation of *A. racemosus*.



**Figure 1:** Callus induced on nodal explants of *Asparagus racemosus* inoculated on MS medium supplemented with 2,4-D, BAP, NAA

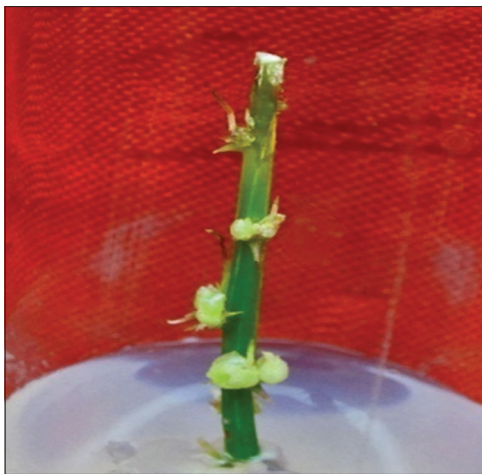


**Figure 2:** Callus induced on nodal segments placed vertically in MS medium having 2,4-D and BAP

**Table 1:** Callus induction frequency of nodal explants of *A. racemosus* when incubated in different concentrations of 2,4-D, NAA and BAP (mg/L), after 4 weeks

2,4-D <sup>w</sup>	NAA <sup>w</sup>	BAP <sup>w</sup>	Callus induction (%)	Fresh weight (g)
1	-	0.5	10	0.10 $\pm$ 0.04 <sup>av</sup>
-	1	0.5	10	0.12 $\pm$ 0.07 <sup>a</sup>
1	1	0.5	40	0.28 $\pm$ 0.03 <sup>c</sup>
2	1	0.5	30	0.23 $\pm$ 0.05 <sup>c</sup>
1	2	0.5	30	0.20 $\pm$ 0.07 <sup>c</sup>
2	2	0.5	20	0.15 $\pm$ 0.06 <sup>b</sup>

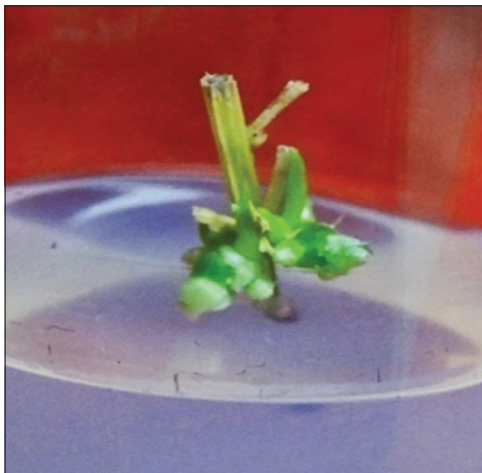
<sup>w</sup> 2,4-D: 2,4-Dichlorophenoxyacetic acid; NAA: 1-Napthalenacetic acid; BAP: 6-Benzylaminopurine <sup>v</sup> Data (mean  $\pm$  SD) followed by dissimilar lowercase letters are significantly different at  $p < 0.05$  by DMRT



**Figure 3:** Axillary shoots induction from nodes on BAP (1.0 mg/L) and NAA (0.5 mg/L) added MS medium



**Figure 5:** Axillary shoot obtained on MS medium supplemented with 3.0 mg/L BAP and 0.5 mg/L NAA



**Figure 4:** Multiple shoot clumps emerging out from nodal portion on 2.0 mg/L BAP and 0.5 mg/L NAA

Our main aim was to develop an efficient protocol for callus and axillary shoot formation, so that the *in vitro* techniques provide a way to its propagation and commercial utilization of its active compounds. In present study it was found that combination of auxins and cytokinins showed the best result as compared with either single dose of auxin or cytokinin.

In the present study, callus induction and proliferation from nodal explants of *A. racemosus* was evaluated under the influence of 2,4-D, NAA, BAP amended MS medium. Combinations of auxins and cytokinins have shown high callus induction frequency and growth of callus from nodal explants as compared to either single auxin in high concentration or combination of auxins. Plants are known to behave differently to cultural conditions depending on PGR combinations used and the genotype of the plant species. Previous studies on *A. racemosus* reveal that NAA, and 2,4-D show successful callus induction with low concentrations of BAP in MS medium [7]. Borjian and Arak [8] also observed maximum callus induction in MS medium when lower concentrations of BAP

**Table 2:** Shoot induction frequency from nodal explants on BAP and NAA (mg/L) amended on MS medium after 4 weeks of incubation

BAP <sup>▼</sup>	NAA <sup>▼</sup>	Shoot induction %	No. of shoots induced /node
1	-	-	-
1	0.5	10	1
2	-	30	1
2	0.5	40	3
3	-	10	1
3	0.5	30	2
4	-	10	1
4	0.5	10	1

<sup>▼</sup> BAP: 6-Benzylaminopurine; NAA: 1-Naphthalenacetic acid

was supplemented along with NAA and 2,4 -D combination in *Brassica napus*. Most monocots are showing recalcitrance to tissue culture conditions due to the absence of secondary growth. Monocots show meager response and take long time for initiation of callus [9]. It is reported that in various monocot species such as *Allium sativum*, the induction and proliferation of callus is challenging and sluggish and somehow tough to maintain [10]. For shoot induction 0.1 mg/L NAA and 0.5 mg/L BAP was found to be most effective. In bud formation, the trend was found to be just opposite to callus induction i.e., increase in BAP concentration showed increase in the number of buds per explants until the NAA concentration was limited. Direct shoot regeneration from nodes of *Phalaenopsis* orchids using different concentrations of BAP and NAA was reported by Polonca et al. [11]. The maximum regeneration was obtained in medium supplemented with lower concentration of NAA and higher concentration of BAP. Similar results were obtained by Sharan et al. [12]. Dharmendra et al. [13] revealed that shoot multiplication required BAP and was found to be more effective than kinetin in *Oxystelma secamone*. This synergistic outcome of BAP and NAA has also been established in *Santolina canescens* [14] and in *Bupleurum fruticosom* [15]. Propagation of *A. racemosus* from callus culture on medium containing IAA and BAP has been reported [16], but it was not found to be very effective in the present work. We observed that combination of

BAP and NAA was needed for better axillary shoot induction. Cheetam et al. [17] reported Shoot tip culture of *Asparagus officinalis* on medium containing kinetin and NAA; however, in the present work, kinetin along with NAA was found inefficient for shoot growth in *Asparagus racemosus*. Similarly, BAP plays a part in shoot and bud induction, although combinations of NAA and BAP at different levels were found to be effective in almost all cases.

## CONCLUSION

Different plants behave differently to cultural conditions depending on the plant growth regulators, genotype of the plant. The callus induction was higher at 1.0 mg/L 2,4-D, 1.0 mg/L NAA, 0.5 mg/L BAP with 40% callus formation. Percentage of shoot induction was higher at 2.0 mg/L BAP and 0.5 mg/L NAA in this study of shoot induction in *A. racemosus*. So we can conclude that in case of *A. racemosus* callus induction was best using two different auxins and a cytokinin while as the shoot induction showed its best results with combination of auxin and cytokinin.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to declare.

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