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IN VITRO BUD INDUCTION FROM THE NODES OF ASPARAGUS RACEMOSUS WILLD AND THEIR GERMINATION BEHAVIORS

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Abstract- Asparagus racemosus Willd. locally known as "Kurilo" or "Shatavari" is one of the most important medicinal plants of Nepal under threat. In order to conserve this species, in vitro bud induction can be one of the promising and rapid methods without any genetic degradation. We observed different kinds of buds in vitro and maximum number was recorded to be 11.666±6.009 when the node explants were cultured on NAA 0.1+ BAP 2.0 mg/l. In general, low concentration of auxin preferably IBA or NAA and relatively higher concentration of cytokinin mainly BAP in combinations were found to be more effective in bud induction. These buds germinated in vitro producing normal roots and shoots.

Keywords: Asparagus racemosus, buds, in vitro, plantlets, hormones, conservation.

1. INTRODUCTION

Asparagus racemosus Willd. locally known as "Kurilo" or "Shatavari" belonging to the family Liliaceae is an undershrub with highly branched woody stems, growing up to 2m in height. This plant can be found growing naturally in the tropical and sub-tropical forests throughout Nepal up to 1500 m above sea level [1]. Eight species of this plant have been reported from Nepal including A.racemosus. Locally, the young shoots are consumed as vegetable or salad and are considered as a balanced health food with many essential nutrients [2]. Besides vegetable this plant is widely used as medicine and has also been recognized by Ayurveda for centuries. Traditionally and in Ayurveda the tuberous roots are used in the treatment of different diseases [3] [4] mainly to promote milk secretion and disorders of female genitourinary tract. Recent researches have revealed that the different parts of this plant contain several active compounds which can be used in treatment of various diseases [5, 6, 7, 8, 9 and 10]. Recent scientific investigations have confirmed and increased importance of this species in various medicines. Hence this species can be considered wholly a medicine having potential to cure different diseases. These studies have further strengthened the traditional medical knowledge with scientific bases [11].

This species is in high trade from the forests of Nepal to the international markets. A. racemosus from Nepal is highly traded in India because the ayurvedic physicians prefer the roots of A. racemosus from Nepal (pale brown slightly resinous) as it is more effective than the Indian ones and have also been confirmed that the main source of A. racemosus in India is Nepal [7 and 12]. To meet the local as well as international demand, a large volume of this species is collected annually from different wild areas of the country and sold in both local as well as international markets by local as well as international traders. The exact data however are not available. Due to massive collection, habitat loss and no proper conservation strategy, this species is facing a severe threat in the wild. To overcome this threat of conservation, a reliable and rapid method of mass multiplication of this species is necessary.

Very few works on this species have been previously done [13] probably because it is less known to the western world. Various works on the same genus but different species have been done by various researchers focusing mainly on shoot multiplication through nodal bud. Some examples include: A. densiflorus on factors influencing regeneration from protoplast [14]; plant regeneration in A. verticillatus [15]; direct multiple shoot induction in A. adscendens [16]; and some publications on somatic embryogenesis from the callus cultures of A. officinalis [17, 18, 19, 20, 21 and 22]. In the present study, we are trying to work on the wild Asparagus racemosus which has high medicinal importance.

2. MATERIALS AND METHOD

The seeds of *Asparagus racemosus* were collected from Sim Gaun, Kirtipur, Kathmandu (approx. 1400 m asl). Sterile explants for the experiments were prepared by selecting the healthy seeds. They were treated with liquid detergent for 15 minutes and were washed under running tap water for 45 minutes and again they were treated with 90% ethyl alcohol for 5 minutes and washed with distilled water. Finally, they were treated with 0.2% mercuric chloride for another 5 minutes and washed with sterile water four times under the laminar air flow hood before inoculation in the hormone free (MS) medium [23]. The nodes of *in vitro* germinated seedlings from the seeds on Hormone free MS media were used as explants. Nodes were excised and pieces of about 0.5-1cm were inoculated

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on the MS medium containing MS basal salts, 3% sucrose, 100mg myo-inositol, 0.8% agar and different concentrations of *a*-naphthaleneacetic acid (NAA) and 6- benzylaminopurine (BAP) and NAA and Kinetin (Kin) either singly or in combinations for various responses in glass tubes (150mm × 25mm containing approx. 12 ml media) and jam bottles (approx. 16.5 ml media) inside the laminar air flow cabinet.
The concentration ranges for all the hormones used singly in the media were 0.1, 0.5, 1.0 and 2.0 mg-l., similarly, in combinations; NAA+BAP, NAA+Kin, IAA+BAP, IAA+Kin, IBA+BAP, IBA+Kin auxin concentrations were limited to 1.0 mg-l. whereas BAP and Kin. up to 2.0 mg-l. The media were adjusted to pH 5.8 with 0.1 N KOH/HCl and autoclaved at 121°C and 15lb pressure for 20 minutes. They were cultured under illuminated condition of 16-hour photoperiod using cool white fluorescent lamps at 25^{o}C ±1^{o}C. The shoot buds induced from the first culture were sub cultured in the same media for further response after 6-12 weeks depending upon the response period. In each case a total of 6 replications were used for each treatment and the experimental trials were repeated three times.
The responses of each media on nodes were recorded at an interval of 1 week up to 12 weeks. The individual shoot buds were separated and cultured on various media for germination, further growth or any other change. All the physical conditions were kept constant throughout the experiment. The shoot bud induction period, vigor and their germination responses were recorded calculated and analyzed.

3. RESULTS AND DISCUSSION

Auxins at all concentrations did not induce the buds significantly (1.000±0.577/ explant) whereas both the cytokinins at moderate concentration (1.0 mg/l) induced buds somewhat better (3.666±0.881/explant) than auxins but still insignificant. This probably is due to apical dominace property of auxin which retards the lateral growth. In auxin-cytokinin combination almost all the combinations were capable of inducing the buds but most of the combinations were insignificant statistically. Only a few combinations among all the treatments showed significant number of buds per explant (Table 1). Detailed data of all the treatments have not been shown; only five best performing treatments have been presented in the Table 3.1.
The buds generally started at the nodes (Fig. 2 and 3) in just after 1-2 weeks of culture but in some cases they started along with the callus mass on the rapidly growing/multiplying shoots as on IBA 0.5 + BAP 0.5 mg/l (Fig. 1). Although they were initiated very early, it took about 7-12 weeks for them to get mature or in other words, they continued to grow until this period. It has also been observed that in the media containing high concentrations of either cytokinins alone or in combinations with auxins induce shoot buds at the nodes. The shape and size of the buds also varied from media to media. The buds grown on the medium containing NAA 0.1 + BAP 2.0 mg/l were the biggest among all (Fig. 4). The germination behaviors of these buds were also different. The MS + IBA 0.5 + BAP 0.5 mg/l grown buds produced 1-2 normal shoots, but the buds induced from NAA 0.1 + BAP 2.0 mg/l were slightly thicker and glassy which might be because of vitrification and could normally grow in media containing low cytokinin concentrations (0-0.5 mg/l). The rest of the media grown buds induced single normal shoots. Under our observation the maximum number of buds/node was observed to be 11.66 when the explants were treated with NAA 0.1+BAP 2.0 mg/l followed by IBA 1.0 + BAP 2.0 mg/l with 10.33. In general, higher concentrations of cytokinin induced shoot buds at all levels. BAP has been found to be more effective than Kn in bud induction in general. In a similar experiment on the same species it has been reported that MS+BA 4.0 mg/l induced buds from the nodes [24]. In combinations of auxins and cytokinins, NAA and IBA induced buds with both of the cytokinins tested whereas IAA with Kn showed less response. Similar to our result, buds from the superficial cells of callus using NAA 1.0 and BAP 1.0 mg/l in the LS medium in A. officinalis have been reported [25].

Table-3.1 Top Five Combinations of Hormones for Bud Induction

S. No.	Hormone Concentrations mg/l	Mean no. of buds± Std.Error
1	Control	0.000±0.000
2	NAA 0.1+BAP 2.0	11.666±6.009*
3	NAA 1.0+BAP 1.0	9.000±6.658*
4	IBA 0.5+BAP 2.0	5.000±0.577*
5	IBA 1.0+BAP 2.0	10.333±3.527*
6	IBA 1.0+Kn 1.0	5.666±1.201*

Those shoot buds as induced on the medium containing IBA 1.0 + BAP 2.0 mg/l slowly mature on the mother plant and finally get easily detached. These buds after detachment from the mother plants behaved as the normal seeds. They germinated producing single to multiple shoots (mainly1-2 shoots) with or without roots. These buds used as explants showed very good response in the initial trials and possibly can be used as one of the good explants for rapid multiplication of A. racemosus (Fig. 1-4). Hence, after further research on their acclimatization capabilities these plantlets can be transplanted into their natural habitats and conserved for future.

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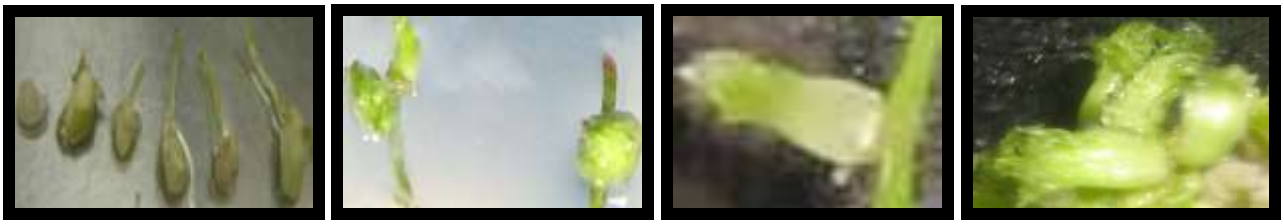

Fig. 3.1 Fig. 3.2 Fig. 3.3 Fig. 3.4

Fig. 3.1-3.4 Bud Induction From the Nodes

Fig. 3.1- Different stages of buds induced from the node induced shoots on the MS medium containing IBA 0.5 + BAP 0.5 mg/l after 11 weeks of culture.

Fig. 3.2- Multiple and different shapes of buds at the nodes on MS+BAP 1.0 mg/l after 8 wks.

Fig. 3.3- A cone shaped bud from the MS + BAP 3.0 mg/l after 8 weeks of culture.

Fig. 3.4- Multiple buds (large number) of buds of different lengths induced from a node on MS + NAA 0.1 + BAP 2.0 mg/l after 12 weeks of culture.

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