COMPARATIVE STUDY OF *IN VITRO* ROOT AND SHOOT PROLIFERATION FROM THE NODE EXPLANTS OF *Asparagus racemosus* Willd.

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ABSTRACT

Asparagus racemosus Willd. locally known as Kurilo or Shatavari is found throughout the tropical and subtropical regions of Nepal. It is a high value herb because of its medicinal and nutritional values which is leading this species decrease in its natural habitat. Hence, to conserve this species, an *in vitro* tissue culture method of its multiplication has been applied as an effective method of *ex situ* conservation. In the experiment, although most of the treatments induced highly insignificant number of adventitious shoots, low concentration of IBA (0.1-0.5 mg/l) in combination with relatively higher concentrations of Kinetin (1.0-2.0 mg/l) in MS medium were found to be significantly inducing up to 8.33±1.308 shoots/node. Among the treatments where hormones were used singly, IAA 0.5 and Kinetin 1.0 induced 4.83±1.08 and 4.66±1.43 shots/node respectively. Hence, a protocol for the effective multiplication of this species has been developed which can be used accordingly.

Keywords: *Asparagus racemosus*, *in vitro*, tissue culture, phyto-hormones, adventitious shoots.

INTRODUCTION

Asparagus racemosus Willd. locally known as "Kurilo" or "Shatavari" belonging to the family Liliaceae is an undershrub with extensively branched woody stems, growing up to 2m in height. The succulent tuberous roots are 30-100 cm long and 1-2 cm thick in bunch attached at the stem base. The leaves are reduced to small scales or needle-like spines called cladodes. The flowers are small, white, fragrant and in simple or branched racemes. Fruits are globular or obscurely 3-lobed, pulpy berries, that are purplish black when ripe; its seeds have hard and brittle testa. This plant can be found growing naturally in the tropical and sub-tropical forests throughout Nepal up to 1500 m above sea level and Nepal is the main source of this species (Khare, 2007).

This plant is highly important in terms of conservation because its population is rapidly decreasing due to various reasons like over exploitation, habitat loss, ignorance, etc. Hence, the government of Nepal has selected this plant under high priority list for research, conservation and domestication (DPR/GoN, 2004). Mainly, the young shoots of this species are consumed as a nutritious vegetable and its roots are used in different Ayurvedic formulations for centuries. Traditionally and according to Ayurveda the tuberous roots are used mainly to promote milk secretion and disorders of female genitourinary tract; as a styptic and ulcer healing agent, intestinal disinfectant, astringent in diarrhea, nervine tonic, sexual debility for spermatogenesis, gout, puerperal diseases, haematuria, bleeding, hyperacidity, demulcent, diuretic, aphrodisiac, tonic, alterative, antiseptic, glalctogogue, antispasmodic and autoimmune disorder (Goyal *et al.,* 2003 and Kumar *et al.,* 1997). Recent scientific research have proved that the traditional medicinal practices to be true by identifying the higher content of pharmacologically active compounds like steroidal saponins (shatavarins I–IV) (Hayes *et al.,* 2006 a and b; Khare, 2007 and Hayes *et al.,* 2007), *asparagin*- an anti cancerous alkaloid (Joy *et al.,* 1998).

Due to these importance this species is traded in high volumes from the forests of Nepal to the international markets like 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 Puri, (2003). Khare, (2007) have also confirmed that the main source of *A. racemosus* in India is Nepal. To meet the demand, a large volume of this species is collected annually from different parts of the country and sold in both local as well as international markets but the exact data for total collection are not available (Pant and Joshi, 2017). All these reasons put this plant under high risk if immediate control and conservation measures are not taken. Hence, to make a small effort in its *ex situ* conservation, an attempt was made to multiply this species using tissue culture technique using different phytohormones and checked their efficiencies in regeneration of roots and shoots from the node explants.

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 3 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 (Murashige and Skoog, 1962). 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 on the MS medium containing MS basal salts, 3% sucrose, 100mg myo-inositol, 0.8% agar and different concentrations of auxins: Indole butyric acid (IBA) and Indole-3-acetic acid (IAA); and a cytokinin: 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 IAA + Kinetin and IBA + Kinetin, auxin concentrations were limited to 1.0 mg-l. whereas Kinetin 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°C ±1°C. The cultures were sub cultured in the same media for further response after 6-8 weeks depending upon the conditions of the culture and media. 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 for multiple shoot induction were recorded at an interval of 1 week up to 12 weeks. All the physical conditions were kept constant throughout the experiment. The multiple shoot induction responses were recorded calculated and analyzed using SPSS.

RESULTS AND DISCUSSION

Form the table 1, it is clear that Kinetin alone at all concentrations can induce multiple shoots. The maximum induction (4.66 shoots/ explant) was observed at 1mg/l however, in similar experiments Bopana and Saxena, (2008) induced a maximum of 5.89 shoots per node with Kinetin 6.97 µM, whereas Kumar and Vijay (2008) found that Kinetin either at very low (0.1 - 0.5 mg/l) or very high (4-5 mg/l) concentrations retarding the shoot induction similar to our present finding; Pant and Joshi (2009) showed that at higher concentrations Kinetin induced callus instead (Figure 2).

Similarly, the maxim induction among IAA concentrations was recorded to be an average of 4.83 shoots/ node at 0.5mg/l which gradually decreased as the concentration was increased (Figure 4). This shows that IAA at moderate concentration is more effective than at higher concentrations. Similar is the case with IBA (Figure 3), where 0.1mg/l induced a maximum of 2.5 shoots/ explant. From all the data obtained from different hormones tested individually, have an optimum concentration for maximum induction which is generally low to moderate beyond which there is retardation effect.

Table 1: Effects of various concentrations of IAA, IBA and Kinetin individually or in combinations of auxin + cytokinin on nodal explants of *Asparagus racemosus*.

PGR	Shoot number Mean±Std.Er	PGR	Shoot number Mean±Std.Er	PGR	Shoot number Mean±Std. Er
Control	2.000±0.258^{ab}	Control	2.000±0.258^{ab}	Control	2.000±0.258^{ab}
IAA 0.1+Kin. 0.1	1.500±0.428^{a}	IBA 0.1+Kin. 0.1	3.500±1.477^{ab}	IBA 0.1	2.500±0.428^{ab}
IAA 0.1+Kin. 0.5	1.666±0.333^{a}	IBA 0.1+Kin. 0.5	3.000±1.316^{ab}	0.5	2.333±0.557^{ab}
IAA 0.1+Kin. 1.0	2.000±0.577^{a}	IBA 0.1+Kin. 1.0	6.666±0.843*^{d}	1.0	0.833±0.307^{a}
IAA 0.1+Kin. 2.0	5.833±2.056^{b}	IBA 0.1+Kin. 2.0	5.500±0.500^{c}	2.0	1.500±0.341^{a}
IAA 0.5+Kin. 0.1	2.666±0.333^{a}	IBA 0.5+Kin. 0.1	1.333±0.421^{ab}	IAA 0.1	1.666±0.421^{a}
IAA 0.5+Kin. 0.5	1.666±0.333^{a}	IBA 0.5+Kin. 0.5	1.166±0.307^{a}	0.5	4.833±1.077^{b}
IAA 0.5+Kin. 1.0	1.500±0.341^{a}	IBA 0.5+Kin. 1.0	4.500±1.979^{ab}	1.0	1.833±0.307^{a}
IAA 0.5+Kin. 2.0	2.333±0.494^{a}	IBA 0.5+Kin. 2.0	8.33±1.308*^{e}	2.0	1.333±0.333^{a}
IAA 1.0+Kin. 0.1	1.666±0.421^{a}	IBA 1.0+Kin. 0.1	3.500±0.991^{ab}	Kin. 0.1	1.333±0.210^{a}

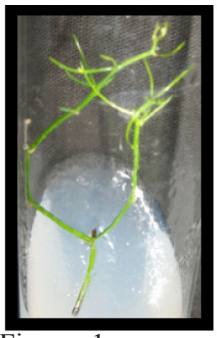
Figure: 1

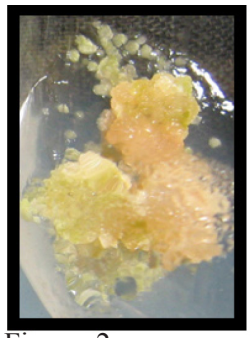
Figure: 2


Figure: 3


Figure: 4

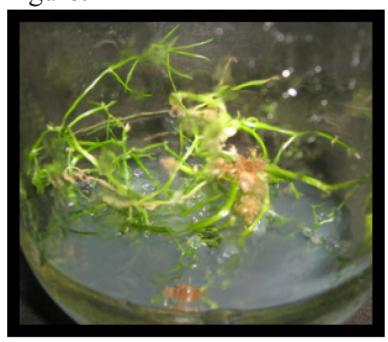
Figure: 5


Figure: 6

Figure 1- Multiple shoot induction from the node after 10 wks of culture on hormone free MS medium.
Figure 2- Callus formation from the shoot on MS + Kn 2.0 mg/l after 12 weeks of culture.
Figure 3- Multiple shoots from the node on MS+IBA 0.5 mg/l after 12 weeks.
Figure 4- Normal multiple shoots from vitrified shoot on MS+IAA 0.5 mg/l in 10 weeks.
Figure 5- Multiple shoots induced from nodes on MS+IBA 0.5+ Kin. 2.0 mg/l after 14 weeks.
Figure 6- Long multiple shoots from the node on IAA 0.1+Kin. 2.0 mg/l after 12 weeks.

CONCLUSION

From the overall data, it can be concluded that auxins at relatively lower concentrations and cytokinins at higher concentrations are effective in multiple shoot induction both in individual and combined treatments. Among both the auxins tested IBA proved to be better than IAA in terms of adventitious shoot proliferation from the nodes of this species. Kinetin alone as expected did not perform well in our experiment. The error or fluctuations in data might be because of the culture conditions like light and temperature fluctuations in the culture room, biological conditions like maturity of nodes etc. and chemical conditions like purity and handling errors.

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