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STUDY OF PHYTOHORMONES EFFECT ON MICROPROPAGATION AND SHOOT INDUCTION IN ASPARAGUS RACEMOSUS

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ABSTRACT

The present investigation was undertaken to study the effect of different concentrations of BAP, Kin, Ads on shoot induction in *Asparagus Racemosus*. There was shoots induction after 3 weeks when Nodal explants segments were cultured on basal medium supplemented with BAP, Kin and Ads. Shoots induction on MS basal medium supplemented with BAP (1 mg/l) + kinetin (1 mg/l) were transferred to MS basal medium containing BAP (1.5mg/l) + Ads (100mg/l) and BAP (1.5mg/l), Kin (1 mg/l) respectively to the Proliferation shoots. Stem segments cultured on MS basal medium supplemented with various concentrations of BAP (0.75, 1.5, 1.0 mg/l), AdS (50, 100 mg/l) and Kin (0.75, 1.0, 1.5 mg/l) induce shoot, But a combination of 1 mg/l BAP + 1 mg/l Kin and 1.5 mg/l BAP + 100 mg/l Ads showed highest percentage of shoot proliferation after 7-14 days.

Key words : *Asparagus Racemosus*, shoot induction, BAP, Kin and Ads

INTRODUCTION

Asparagus racemosus Wild is a multidimensional medicinal plant. Family Liliaceae English name Asparagus, Indian name shatmul, Satavari, (Sanskrit) Satavar, satavari, (Hindi) , Its distribution Tropical and subtropical India. It is straggling or scandent, much branched, spinous shrub. The plant is a climber growing to 1-2m in length. The leaves are like pine needles, small and uniform. The inflorescence has tiny white flowers, in small spikes. The roots are finger like and clustered (Purohit and Vyas, 2004). It has thorny branches the ripen fruit is small, round and red. Seeds are black. Stem woody, whitish gray or brown armed with strong, straight or reduced spines, 5-13mm long; cladodes more or less acicular, 2-6 cm long, falcate; finely acuminate; leaves reduced to sub-erect or sub-recurred spine, fragrant, small profuse in simple or branched racemes up to 7m long; scarlet, tri-lobed, 4-6 in diameter (Dutta, 2007). Its phytoestrogen properties are extensively used in combating

menopausal symptoms and increasing lactation (Sabin *et al.*, 1968). It also has antioxidants (Kamat *et al.*, 2000) and properties (Rage *et al.*, 1989) and is widely used in Ayurveda for treating dyspepsia (Dalvi *et al.*, 1990). The major active medicinal constituents are steroidal saponins shatavarins I-IV that is present in the roots of the plant (Sairam, 2003).

It has been reported in the Indian and British Pharmacopoeias and in traditional systems of medicine such as Ayurveda, Unani, and Siddha. The multiple uses of this species have increased its commercial demand, resulting in over-exploitation. Because of destructive harvesting, the natural population of it racemosus is rapidly disappearing, and it is recognized as vulnerable (Bopana and Saxena 2001). The development of an efficient micropropagation protocol will play significant role in meeting the requirements for commercial cultivation, thereby conserving the species in its natural habitat.

According to ayurveda, asparagus is much useful

in case of reproductive disease. Plant is reach source of plant derived estrogens. It is good source of folic acid, potassium, dietary fiber. It is cardio tonic, hypoglycemic, antioxytocis to uterine contraction, diuretic, antioxidant, insulin secreting an improving potency, many formulation based on Asparagus are in commerce used for bleeding disorders, gout, low sperm count (Goyal *et al.*, 2003). The methanol extracts of roots of Asparagus wild is reported to show considerable *in vitro* antibacterial activity against various common pathogens (Mandal *et al.*, 2007). There is nothing to hoist in speaking that *A. racemosus* is a doctor. It is suggested in nervous disorders, dyspepsia, diarrhoea, dysentery, neuropathy, cough, certain infectious disease (Goyal *et al.*, 2003). Root paste which stimulates milk secretion, is used as an invigorating tonic to lactating women and live stock. It is taken to treat high fever. Root tubers are fed to get relief from milking disorder of cattle, which is regarded as appetizer. They are also useful in dysentery, tumors, inflammations, disease of blood and eyes, throat complains, tuberculosis leprosy, epilepsy and high blindness. Roots are also used in disease of Kidney and liver, scalding urine, gleet. Fruits are eaten to treat pimple (Dutta, 2007; Rajbhandari, 2001). The whole plant is used for treatment of diarrhoea, Diabetes, and Rheumatism. Seed are also used for blood purification.

MATERIALS AND METHODS

Preparation of culture media: Double distilled water was used for the preparation of medium. The amounts of macro and micro nutrients, organic salts, vitamins, growth regulators and sucrose were added to the double distilled water kept in distilled water. The final volume was made in a graduated cylinder/breaker by adding double distilled water. The pH of the solution was adjusted to 5.7-5.8 using either 0.1N HCl or 0.1N NaOH. For solidification of the medium, agar powder (Tissue culture grade, agar-agar type) @

0.8% w/v was added to warm solution and then, boiled for proper dissolving and melting of agar powder. Then the medium was poured in glass vessel (i.e. culture tube or culture bottle). After that the vessel was covered by lid or with aluminium foil. Basal nutrient media used during this investigation are given in table was BM1-BM7 modification of Murashige and Skoog medium (1962) was used in present project.

Procedure

The medium was prepared in sterile flasks. Required quantities of stock solutions 50 ml of stock 1, 5ml of stock 2, 10ml of stock 3, , 10ml of stock 4, 10ml of stock 5 was added. The pH of the solution was adjusted 5.8 by adding NaOH and HCl drop by drop. Final volume of medium was made to 1 litter by adding distilled water. Adding of 8% agar, which was dissolved in warm water followed by constant stirring. The medium thus prepared was dispensed into different culture tubes or culture bottles each having 20 to 30 ml of the media. The medium was autoclaved at 15 lbs pressure and 121°C temperature for 20 minutes. The medium was finally allowed to cool and solidify.

The tissue culture technique involves the isolation, sterilization, inoculation and cultivation of plant cells, tissues and organs under aseptic conditions in culture vials, containing nutrient medium. In tissue culture, the organized structures like shoot tips, root tip etc are culture in-vitro to obtain their development as organized structure under controlled environmental conditions (temperature, light, humidity, aeration) which effectively controls the expansion of any genotype or phenotypic potential in explants.

Isolation of Explant

Source of explants: The explant parts such as nodal segments shoot tip, and In vivo germinated seeds were taken from plants of *Asparagus racemosus* Wild in herbal garden of SINGHANIA UNIVERSITY. Pacheri

Bari, Jhunjhunu.(Rajasthan) during February - March seasons and from in vitro germinated seeds. The specific differences in the regeneration potential of different organs and explants have various explanations. The significant factors include differences in the stage of the cells in the cell cycle, the availability of or ability to transport endogenous growth regulators, and the metabolic capabilities of the cells. The most commonly used tissue explants are the meristematic ends of the plants like the stem tip, auxiliary bud tip and root tip. These tissues have high rates of cell division and either concentrate or produce required growth regulating substances including auxins and cytokinins.

METHOD

Sterilization of explants: Young stem segments, young shoot tip and seeds were surface sterilized, by first washing them in Extran detergent in running tap water for 10-15 min to remove soil particles and debris, after that were wash with Bavestin (100mg/ml) 2-3 drop in 100 ml water then wash were with distilled water, dipping in absolute alcohol for 2 min and also dipping in 20% (v/v) commercial sodium hypochlorite solution for 5-6 min. wash with sterilized distilled water for one times , followed immediately, immersing them in 0.1% (w/v) HgCl₂ solution for 5 min treatment under Laminar Air Flow Unit, they were thoroughly washed 5-6 times in sterile double distilled water. This entire treatment was carried out at low temperature to prevent damage to the shoots.

Inoculation

Before inoculation, inside the laminar air flow chamber the ultraviolet lamp was put on carefully to avoid contamination for at least 20 minutes, the working table of laminar air flow chamber was wiped thoroughly with 70% ethanol before

use. The material required for inoculation was steam sterilized. The hands were cleaned with 70% ethanol. Then, the individual explants were inoculated in individual culture tubes or culture bottles having solidified culture medium. Forceps and scalpels were flame sterilized before each inoculation. The explants were then, cultured on MS medium supplemented with different concentration of BAP, Kn, AdS and NAA.

Table:1. Different Combination of BAP, Kin and Ads used for Shoot proliferation

S, No.	MS-Medium (Lit)	BAP (ml/L)	Kin (ml/L)	AdS (mg/L)
BM I	1	1.5	-	-
BM2	1	-	1.5	-
BM3	1	1	1	-
BM4	1	1.5	-	100
BM5	1	0.75		
BM6	1	-	0.75	-
BM7	1	0.5	0.5	-

Incubation of Culture

After inoculation cultures were kept in the incubation room where the temperature and light period was maintained at $25 \pm 2^{\circ}\text{C}$ and 16 hr. photoperiod with approximate 1500 lux intensity of light respectively. Source of the light was from the four florescent tube lights each of four feet and 40 W (Philips make) a total of 22 culture bottles for each treatment has been kept on the culture racks. After three week period callus developed in the medium or shoots/roots develop from explants.

RESULTS AND DISCUSSION

Shoot Initiation: Nodal explants were cultured on MS medium supplemented with different concentrations of BAP, Kin and Ads. The nodal segments get swollen after 3 days and the small originating shoot segment were observed as shown in table 2. When the hormone concentration is given as 1.5ml BAP(fig.4) ,1.5ml

Kn(fig.1) and 1.5mlBAP and 100mg Ads in 1l MS medium then 60% response was observed. When the hormone concentration is given as 1ml BAP and 1ml Kn in 1l MS medium then 80% (maximum) response was observed (fig.3). When the hormone concentration is given as respectively 0.75ml BAP (fig.2) and 0.75ml Kn(fig.5) in 1l MS medium then 40% response

was observed. When the hormone concentration is given as respectively 0.5ml BAP and 0.5ml Kn in 1l MS medium then 20% response was observed (fig.6). Good response was observed in fig.7 and fig.8 when transferred to same medium. The explants showed shoot initiation after three weeks.

Table:2. The explants cultured on MS medium supplemented with different concentration of BAP, Kin and Ads.

S.No	MS-Medium (Lit)	BAP (ml/L)	Kn (ml/L)	AdS (mg/L)	No. of explants Used	Survival Rate	% age Response
BM1	I	1.5	-	-	10	8	***
BM2	1	-	1.5	-	10	8	***
BM3	1	1	1	-	12	10	****
BM4	1	1.5	-	100	12	10	***
BM5	1	0.75	-	-	8	4	**
BM6	I	-	0.75.	-	8	4	**
BM7	1	0.5	0.5	-	10	3	*

%age of response Survival rate / No of explants used * 100 (* = 20%), (** = 40%), (***) = 60%), (**** = 80%).



Fig.1:- Shoot induction from nodal explants of Asparagus on MS Medium supplemented with Kin (1.5ml/L) after 3 weeks.



Fig.2:-Shoot induction from nodal explants of Asparagus on MS Medium supplemented with BAP (0.75ml/L) after 3 weeks.



Fig.3:-Shoot induction from nodal explants of Asparagus on MS Medium supplemented with BAP (1ml/L) + Kin (1ml/L) after 3 weeks.



Fig.6:-Shoot induction from nodal explants of Asparagus on MS Medium supplemented with BAP (0.5ml/L) + Kin (0.5ml/L) after 3 weeks.



Fig.4:-Shoot induction from nodal explants of Asparagus on MS Medium supplemented with BAP (1.5ml/L) after 3 weeks.



Fig.7:- Shoot induction from nodal explants of Asparagus induced on MS Medium supplemented with Kin (0.75ml/L) and transfer to Kin (0.75ml/L) after 40 days.



Fig.5:-Shoot induction from nodal explants of Asparagus on MS Medium supplemented with Kin (0.75ml/L) after 3 weeks.



Fig.8:-Shoot induction from nodal explants of Asparagus induced on MS Medium supplemented with BAP (0.75ml/L) and transfer to BAP (0.75ml/L) after 40 days.

DISCUSSION

Culture Establishment (Stage-1): *Asparagus racemosus* has a variety of usages. The first step on initiating in vitro culture is to successfully control the fungal as well as bacterial contaminations. The general surface sterilization procedure i.e. mercuric chloride for 5 min. failed to control the microbial infection in the explants. Hence, two procedures of surface sterilization were tried in the present study. The treatment of nodal explants with mercuric chloride for 5 min followed by sodium hypo chloride for 5 min. (T-2) was found to be the best in respect of explants survival (90%) and reduced explants contamination over the other treatments. Sodium hypochloride has bactericidal action and is generally used for surface sterilization and also helps in the controlling the microbial infection in the case of shoot proliferation.

Shoots Proliferation (Stage-2): When the nodal explants inoculated on MS medium supplemented with different concentrations of BAP, Ads and Kinetin in different combinations. The best shoot induction was achieved when the cultures were transferred to MS medium supplemented with BAP (1 ml/lit.) + kinetin (1 ml/lit.) and BAP (1.5ml/lit.) + AdS (100 mg/lit.). In other experiment study reported in *Asparagus racemosus* in vitro shoot proliferation was obtained by culturing single node segments in Murashige and Skoog's (MS) medium supplemented with 3.69 μ M 2- isopentyl adenine and 3% sucrose with a multiplication rate of 3.5 (Bopana and Saxena., 2008).

Shoot proliferation (Stage-3): The shoot proliferation medium comprises of different concentration of BAP and Ads in different combinations. The best proliferation with well differentiated micro-shoots was achieved when the small nodal segments were transferred to MS medium supplemented with BAP (1.5 ml/L) and Ads (100mg/lit.). The treatments recorded 15-20 % proliferation after 1 week of first sub-culture. The shoot proliferation in tissue culture is largely

due to the action of BAP. Optimum dose of BAP enhances the multiple shoot proliferation as well as length. In other experiment study reported high rate of multiple shoots was obtained from nodal explants of *Asparagus racemosus* Roxb. On MS supplemented with 0.27 μ M NAA, 0.46 μ M Kn and 0.6 agar (Mehta and Subramanian, 2005).

CONCLUSION

Study was conducted on Micropropagation of Medicinal Plant *Asparagus racemosus* Wild. The following conclusions are:

Nodal segments and apical buds of 20-30 days old *Asparagus racemosus* wild Plants were inoculated on MS medium supplemented with BAP (1ppm/L) + Kinetin (1ppm/L) and BAP (1.5ppm/L) + Ads (100mg/L) and then transferred to same medium was found best shoot induction. Proliferated shoots were separated and transferred to NAA (0.5mg/l) + BAP (1mg/l) + kin(1mg/l) medium was found to best roots formation.

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