AgriCos e-Newsletter
ISSN: 2582-7049
AgriCos e-Newsletter
Open Access Multidisciplinary Monthly Online Magazine
Volume: 04 Issue: 08 August 2023 Article No: 33

Micropropogation of Shatavari (*Asparagus racemosus* L.)

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SUMMARY

The experiment entitled "Micropropagation of Shatavari (*Asparagus racemosus* L.) from nodal explants" was conducted at College of Agricultural Biotechnology,Saralgaon .This study was carried out to standardize sterilizing agent type for sterilization of explants, to standardize Media composition for in vitro culture and to standardize short establishment protocol.Explants exposed to higher concentration of Mercuric chloride may leads to browning in colour, decrease growth percentage and increase the percentage of tissue damage.

INTRODUCTION

Shatavari (*Asparagus racemosus* L.)is an important medicinal plant of tropical and subtropical regions of India. Its medicinal usage has been reported in the Indian and British Pharmacopoeias and in indigenous systems of medicine. The genus Asparagus includes about 500 species around the world,out of these the 22 species of Asparagus recorded in India. It is the one most commonly ‘used in traditional medicine. Shatavari (*Asparagus racemosus* L.) Wild. of family *Liliaceae*, is commonly called Shatavari. Plant tissue culture techniques have become a powerful tool for studying and solving basic and applied problems in plant biotechnology. During the last thirty years, micro propagation and other in Vitro techniques have become more widely used in commercial horticulture and agriculture for the mass propagation of crop plants (George, 1993). In Vitro cell and tissue culture methodology is envisaged as a mean tor germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large-scale production, and for genetic manipulation studies.

Combinations of in vitro propagation techniques and cryopreservation may help in conservation of biodiversity of locally used medicinal plants. In India, the Red Data Book has reports endangered species total 427 in which 124 species are endangered, 1OO Species are rare, 81 species are vulnerable, and 28 are considered extinct, 34 insufficiently known species. There are more than 300 species around the world (Gaur, 1999). It is widely used for multiple purposes and its medicinal importance has been recognized by Ayurveda for centuries. Traditionally the roots we used mainly to promote milk secretion, demulcent, diuretic, aphrodisiac, tonic, alterative, antiseptic, ant diarrheal, and antispasmodic. It is also used to treat debility, especially in women and dehydration, lung abscess, infertility, impotence, menopause, stomach ulcers, delay ageing process and haematemesis, cough, herpes, leucorrhoea and chronic fevers, actions. Using the modern scientific tools many active compounds like several steroidal saponins, aglycones, form health food ingredients in several Ayurvedic formal alkaloids like asparagine-an anticancer agent and many other active pharmacologically important compounds have already been isolated from the root diosgenin and a flavonoid glycoside identified as of this species. The present study was done by using following objectives.1.To Standardization of Surface Sterilization technique of Shatavari (*Asparagus racemosus* L.)2.To standardization of MS Media for protocol in vitro-rapid propagation of Shatavari (*Asparagus racemosus* L.) 3.To study of effect of growth regulator (BAP and NAA) on propagation of Shatavari (Asparagus *racemosus* L.)

Material And Methods

The work was carried out in the College of Agricultural Biotechnology, Saralgaon. The research work is undertaken with aim of Micro propagation of Shatavari (*Asparagus Racemosus* L.) from nodal explant.

A.Materials -

Explants Source: Five healthy Shatavari (*Asparagus Racemosus* L.) plants were collected from nursery of College of Agricultural Biotechnology, Saralgaon.

Chemicals:

A) Chemicals for media preparation: Salts of macro and micro elements of analytical grade,Vitamins and amino acids,Sucrose as a carbon source,Myo-inositol,Agar-agar as a gelling agent,0.1 N HCL and 0.1 N NaOH for pH adjustment.

B) Chemicals for surface sterilization: Mercuric Chloride ,8HQC,Mancozeb 25,Bavistin
C) Plant growth regulators: BAP ,IAA

Glasswares: reagent bottles. conical flask, pipette. culture tube, petri plates, and funnels **Laboratory Equipments:** Autoclave, Magnetic stirrer, Microwave oven, Weighing balance, pH-meter and laminar Air flows were used at the time of project work.

B.Method:

1.Plant Materials: nodal explant of Shatavari (*Asparagus Racemosus* L.)

2.Stock Plants Establishment: Collected plants are grown under observation in college farm Also given pretreatment of fungicides namely carbendazim (0.2%) and mancozeb (0.2%) alternatively.

3.**Preparation of Stock Solutions:** The medium consists of macronutrient, micronutrient, Fe-EDTA, Vitamins, amino acid, and sucrose, agar, and plant growth regulators. All the stock solution and final medium were prepared by following the procedure.

4.Preparation of Medium:- The callus induction of *Asparagus racemosus* is MS medium (Murashige and Skoog (1962). A variety of growth regulators such as 6-Benzyi amino purine (BAP) and indole Acetic acid (LAA) were added to the medium in combinations at various concentration and were used for initiating different experiments, the stock solution of macro, micro, trace Elements and vitamins were prepared with double distilled water and store inside the Refrigerators at 4°C.

Table No. 1- Preparation of culture media

	Components	Concentration
1	Macronutrients	25 ml
2	Micronutrients	5ml
3	Iron stock	5ml
4	Vitamin stock	1ml
5	Amino acid	1ml
6	Myo-inositol	100mg
7	Sucrose	30g
8	Growth regulators	As required
9	Agar (for solid medium)	8 g

The medium was prepared by adding appropriate quantities of the stock solutions and correct volume was made up with the distilled water. The pH was adjusted in all case to 5.8 by using 1N NaOH and 1N HCL and agar 0.8% (w/y) was used for semi solid medium for culture initiation and establishment. They are then capped and labeled properly. These were than autoclave at 121°C for 15 minutes at 15-psi pressure and transferred to the inoculation room they stored under aseptic conditions until their use. Media containing co-autoclavable growth regulators used for culturing in bottle was directly heated in microwave oven to melt agar and pour in bottles and then autoclaved for 20 min at 121^0C

5.Preparation of Stock Solution or Growth Hormones :The growth hormones were dissolved in few drops of the solvent and volume was made up to the required level with double distilled water. Filter sterilized. The stock of growth hormones was prepared in different mg/ml for convenient use at stored at cold condition (4°C).

Table No. 2: Growth regulators stock preparation

Growth regulators	Solution preparation				
	Solvents	Diluents	Powder storage	Liquid storage	Sterilization
BAP	1 N NaOH	distilled water	RT	2-80C	F
IAA	1 N NaOH	distilled water	RT	2-80C	F

RT- Room temperature, F- Filter sterilization

To obtain the final working concentration of 1.0 mg/l of plant growth regulator inculture medium, 1.0 ml of the stock solution was added to 1 lit of medium.

6.Explants Preparation :-

Nodal explants of 1.5 - 2,0 cm size were excised and used as explants source for all the experiment.

7.Surface Sterilization of Explant:-

Explants used for experiments included nodal segments (1.5 - 2.0 cm). 1heexplants were initially washed thoroughly with liquid detergent (Savlon) for 10 min. Followed by continuous washing under running tap water for 30 min. until all traces of Liquid detergents were removed. After rinsed thoroughly in tap water explants were surface Sterilized with a solution of bavistin along with 8HOC and mancozeb M-45 up to 20 n. They were further sterilized with HgCI2 (0.1% w/v) for 10-20 min. in laminar air flow Chamber. The surface sterilized plant materials were then rinsed in sterile distilled water Several times till all the traces of sterilants were removed.

Table No.3 Different combination of explant sterilization

Time	8HQC	Bavistin	M-45	HgCL2	No.of Explants
	10min	20min	15min	15min	8
	15min	15min	10min	15min	8
	20min	10min	20min	15min	8

Explant Initiation:-

The sterilized nodal explants such were inoculated in following MS basal media With various combinations of growth regulator concentrations

Table No.4 Media combination used for shoot formation:-

Treatment	Media Composition various hormone used (mg/l)		No. of explants
	BAP	IAA	
T1	1	0.1	4
T2	1.5	0.2	4
T3	2	0.2	4
T4	2	0.5	4
TT5	2.5	0.5	4

Culture Conditions:-

The temperature of culture room was maintained at 25 + 2^{o}C and relative Humidity at 50 to 60 %. A twelve hour photoperiod with a light intensity of 3000 lux w Provided using cool white fluorescent tubes.

Rooting of Explant: -

After the 25 days of incubation we discard the previous shooting media and prepare media for the root development of explant .

Table No.5 Media combination used for root formation

TREATMENT	MEDIA	NO. OF EXPLANTS
T1	MS basal (light)	4
T2	MS basal (dark)	4
T3	MS+NAA(0.1mg/l) (light)	4
T4	MS+NAA (0.1mg/l) (dark)	4
T5	MS+IBA (0.1mg/l) (dark)	4

Result

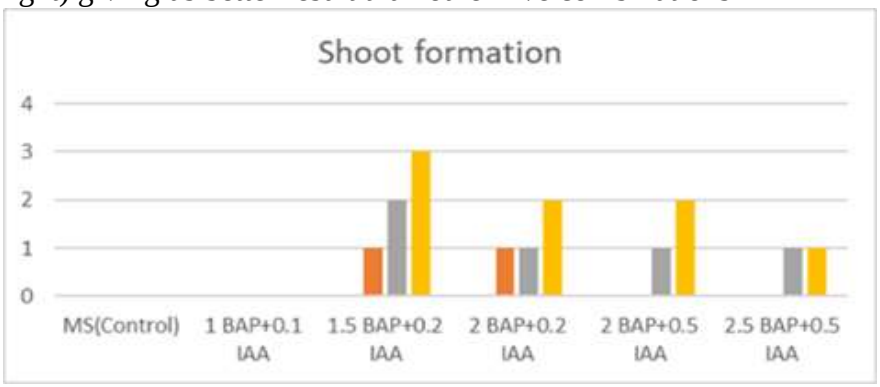
Shoot initiation and establishment from *Asparagus racemosus* nodal explants cultured on MS basal and MS medium supplemented with various combinations of growth regulators i.e. BAP in combination with IAA is described in Table7. Most of the other research studies for other medicinal plant species have shown the use of cytokinin alone or in combination with other in different concentrations.

During initial week after inoculation, bud initiation was very low. However, bud initiation was found to be started in most of the cultures initiated from 9-10 days by showing a small newly sprouted bud, which proliferate into shoot buds with leaves during 21-25 days which were placed in the culture room under the standard conditions of temperature (25 ± 2°C).

Table No.6-Effect of different media combinations on shoot formation

Treatment	Media Composition	No.of Explants	No. of Shoot Induced
T1	MS(CONTROL)	4	0
T2	MS+1.0mg/lBAP+0.1mg/l IAA	4	0
T3	MS+1.5mg/lBAP+0.2mg/l IAA	4	3
T4	MS+2.0mg/lBAP+0.2mg/l IAA	4	2
T5	MS+2.0mg/lBAP+0.5mg/l IAA	4	2
T6	MS+2.5mg/lBAP+0.5mg/l IAA	4	1

Among the different combination of MS media With growth hormones the table of result shows the T3 combination (MS+1.5mg/1BAP+0.2mg/lIAA) give us better result than all other five combinations. Among the different combinations of MS media with growth hormones for root formation. The treatment no.T5(MS+IBA (0.1mg/l) (light) giving us better result than other five combinations



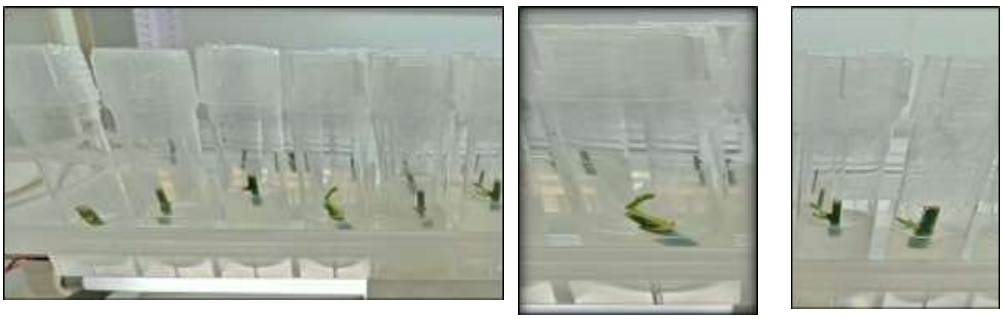


Fig.Shoot Formation

Hardening of Explant: - After successful incubation of for 4 weeks under controlled condition, the explants were ready for hardening. For the hardening we used portrays and filled with the cocopeat for better growth of the explants. The hardening period of the shatavari plant is near by about 5 weeks.

CONCLUSION

From the present investigation, it can be concluded that, Nodal explants showed variable response to different sterilization agents. Treatment involving 0.1 % Mercuric chloride, 3% Tween 20, 1% sodium hypochloride and 70% ethanol shows best growth percentage viz.75%, 75%, 100% and 75%; less tissue damage and no contamination. The explant T3 observed to be best among all other explants for better multiple shoot induction.

REFERENCES

Afroz, F, Jahan, M.A.A., Hassan, A, K, M.and Khatun, R. 2010. In vitro Plant Regeneration from Axillary Buds of Asparagus racemosus Wild. a Medicinal Plant, Bangladesh. J. Sci.ind. Res., 45(3):255-260.

Balakrishnan, V., Latha, M.R., Ravindran, K.C. and Robinson, J. P. 2009. Clonal Propagation of Morus alba L. through nodal and axillary bud explants. Bot. Res. Inter., 2(1):42-49.

Ghosh B. and Sen S. 1994b. Micropropagation of Asparagus cooperi as affected by growth regulators. Biol. Piant., 36: 527-534. Kamat, J. P., Boloor, K. K., Devasagayam T. P. and

Mehta, S. R. and Subramanian, R. B. 2005. Direct In vitro Propagation of Asparagus adscendens Roxb. Plant Tissue Cult., 1s(1): 25-32.

Pise, M., Rudra, J., Bundale, S., Begde, D., Nashikkar, N. and Upadhyay, A. 2011. Shatavarin production from in vitro cultures of Asparagus racemosus Wild. J. Med. Plan. Res., 5(4):507-513.