www.pharmaerudition.org

ISSN: 2249-3875

International Journal of Pharmaceutical Erudition

Research for Present and Next Generation

AUG. 2017

Vol: 07 Issue:02

(25-39)

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

Research Paper

IN VITRO CONSERVATION AND EVALUATION OF DIFFERENT PARTS OF MEDICINAL PLANT (ASPARAGUS RACEMOSUS)

Rajesh Iamror[1], B. L Jat[2], B L Yadav[3]

[1]Research Scholar, Mewar University, Gangrar
[2]Assistant professor, Bhagwant University, Ajmer.
[3]Professor, Mewar University, Gangrar.

Development of somatic embryos in the in vitro cultures of zygotic embryos and hypocotyl seedlings of *Asparagus racemosus* has been demonstrated, which is a first report for this species. The simple sterilization procedure resulted in recovering 100% sterile cultures and was adopted from an earlier report on garlic (Bhojwani 1980). Somatic embryo formation has been extensively studied in *A. officinalis* (Kunitake and Mii 1998). In *A. officinalis* the most favoured explant for somatic embryogenesis has been stems and cladodes (Li and Wolyn 1995, 1997; Limenton-Grevet and Julien 2000; Delbriel et al. 1994) though hypocotyls (Willmar et al. 1968), stems (Reuther 1977), buds (Levi and Sink 1990), shoot apices (Dupire et al. 1999) and cell suspension cultures (Levi and Sink 1992) have also been used.

Key Words : Stems, Shoot apices, Cell suspension cultures.

INTRODUCTION

Preparation of plant tissue for tissue culture is performed under aseptic conditions under HEPA filtered air provided by a laminar flow cabinet. Thereafter, the tissue is grown in sterile containers, such as petri dishes or flasks in a growth room with controlled temperature and light intensity. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so their surfaces are sterilized in chemical solutions (usually alcohol and sodium or calcium hypochlorite)[1] before suitable samples (known as explants) are taken. The sterile explants are then usually placed on the surface of a sterile solid culture medium, but are sometimes placed directly into a sterile liquid medium, particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar.

The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots. A balance of both auxin and cytokinin will often produce an unorganised growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition. As cultures grow, pieces are typically sliced off and subcultured onto new media to allow for growth or to alter the morphology of the culture. The skill and experience of the tissue culturist are important in judging which pieces to culture and which to discard. As shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants.[2]

REGENRATION PATHWAY:

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, axillary 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.

Shoot regeneration efficiency in tissue culture is usually a quantitative trait that often varies between plant species and within a plant species among subspecies, varieties, cultivars, or ecotypes. Therefore, tissue culture regeneration can become complicated especially when many regeneration procedures have to be developed for different genotypes within the same species.

The three common pathways of plant tissue culture regeneration are propagation from preexisting meristems (shoot culture or nodal culture), organogenesis and non-zygotic embryogenesis.

Aim &Objectives Of Work

1. To develop efficient, reproducible and commercially viable tissue culture protocol for high frequency regeneration of *Asparagus racemosus.*

2. Development of technology for induction Multiplication and regeneration of somatic embryo directly from explants and through callus culture

And encapsulation of somatic embryo to produce artificial seeds.

3. To evaluate phytochemicals from different parts of *Asparagus racemosus* using chemoprofiling tools like HPLC, GC, MS etc.

4. Isolation, Identification and purification of secondary metabolites and aromatic compounds present in culture of the proposed plant and their comparison with naturally growing plants using molecular markers.

5. *In vitro* studies using cell suspension culture system will function as continuous and reliable source for the production of medicinally important compounds in large scale.

6. Analysis using combination of various biotechnological tools will an approach towards conservation of *Asparagus racemosus*.

Plan of work:

Development of reproducible and commercially viable tissue culture protocol for clonal mass micro-propagations of proposed plant species.

(a). Survey and collection of explants.

(b). Surface sterilization.

(c). Culture initiation, establishment & Multiplication.

(d). Callus culture.

(e). Somatic Embryogenesis.

(f). Root induction.

(g). Suspension culture.

(h). Hardening and acclimatization of *in vitro* plantlets.

(i). Field trials.

Biochemical Analysis:-

Extracted biochemical's will be analyzed by TLC (thin layer chromatography), GC (gas chromatography), HPLC and MS (mass spectrometry) to identity various secondary metabolites and other useful bio-chemicals. RAPD molecular marker technique will be used to find out genetic variability of *Asparagus racemosus.*

MATERIALS & METHOD

Evaluation of Phytochemical from different parts of *Asparagus racemosus:*

Alkaloid was extracted from the cultures as per the previously published methods (Mathur et al., 1994). Briefly, cells were extracted separately with methanol (1:2) overnight and procedure was repeated 4 times. All the extracts were pooled and concentrated at 60°C on a rotary evaporator to dryness. The residue was redissolved in 10 ml of H_2O and further extracted with n- butanol. The n- butanol fraction was finally concentrated to dryness on a rotary evaporator under reduced pressure and redissolved in 5 ml of methanol and stored and analyzed using standard Shatavarin IV (procured from Regional Research Laboratory, Jammu, India) as the marker. These extracts were also compared with standard alkaloids (Sigma chemicals), which is a precursor of Shatavarins. For the preparation of standard solutions, Shatavarin IV was dissolved in methanol and was diluted to get a final concentration range of 100 to 500 gml^{-1}The solutions were filtered through a 0.2 µmacrodisc. Evaluation of each point was repeated 3 times at 220 nm and the calibration curve was fitted by linear regression. This calibration curve was utilized for the estimation of total Shatavarin present in the methanolic extracts from the wild type plant and *in vitro* extracts.

RP- HPLC analysis of the alkaloid samples were carried out using a Knauer smart line manager- 5000 system (Germany) fitted with a C18 column (4 um, 150 mm x 3.9 mm I.D.), UV detector and 20_L injection loop. Acetonitrile and 30% aqueous methanol were used as the mobile phase with gradients from 8 to 100% of Acetonitrile in 60 min. The volume of sample injection was 20 _l in all the cases. The peaks of Shatavarin were identified by comparing the retention time of the peaks with those of the reference compounds eluted under same conditions.

Total phenolic contents were determined by Folin Ciocalteu reagent (McDonald et al., 2001). A dilute extract of each crude extracts (0.5 ml of 1:10g ml –l) or garlic acid (standard phenolic compound) was mixed with FolinCiocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ml solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g-l of dry mass), which is a common reference compound.

Determination of Total flavonoids Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al., 2002). Each extracts (0.5ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solution at concentrations 12.5 to 100g ml -1 in methanol.

Antioxidant activity

Free Radical scavenging activity of the sample extracts were measured by colorimetric assay using 2,2-diphenyl picrylhydrazyl radical (DPPH, a stable free radical)) as a source of free radical in accordance with

the method of Blois (1958). One ml of the test extract solution (2.5-1000 kept the reaction mixture for 35 min at room temperature in dark and absorbance was measured at 517nm using a UV-visible spectrophotometer. L-Ascorbic acid was used as the 74 positive control. A mixture of 1ml DPPH solution and 1ml methanol was used as blank. The DPPH radical scavenging activity was calculated using the following formula:

% Inhibition = [(A0 As/A0) × 100]

A0: absorbance of the control,

As: absorbance of test solution or the standard sample.

EC50 value i. e., the concentration of extract or standard to scavenge 50% of the initial DPPH was obtained. (Lesser the EC50 value denotes higher the activity of the extract).

RESULT & DISCUSSION

Fig 1:Plant of *Asparagus racemosus*

Somatic Embryo Induction: For somatic embryo induction, ancymidol at threeconcentrations was introduced into MS with 0.1 mg L^{-1} NAA and 0.5 mg L^{-1} kinetin. Various concentrations of NAA and kinetin were also investigated in combination with ancymidol or without ancymidol and revealed that interaction of NAA and kinetin displayed a significant variation in somatic embryo production (Table 1; Fig. 2). In the presence of ancymidol only the compact and nodular callus differentiated into somatic embryos, whereas the friable

Table 1: Effect of NAA and kinetin on somatic embryo induction in zygotic embryo CEC cultures of *Asparagus racemosus*

S. No.	Medium	Embryos	White Hair
1	0.05 NAA + 0.5 KN	4.83±0.39^{a*}	10.71±1.43^{de}
2	0.05 NAA + 1.0 KN	3.88±0.64^{a}	12.29±1.49^{e}
3	0.1 NAA + 0.5 KN	2.94±0.76^{a}	8.12±0.68^{bc}
4	0.1 NAA + 1.0 KN	2.71±0.78^{a}	8.53±0.66^{cd}
5	0.05NAA + 0.5KN+0.75 AC	11.07±1.14^{b}	3.47±0.38^{a}
6	0.05 NAA + 1.0 KN + 0.75 AC	9.67±1.03 ^{b}	4.53±0.70^{ab}

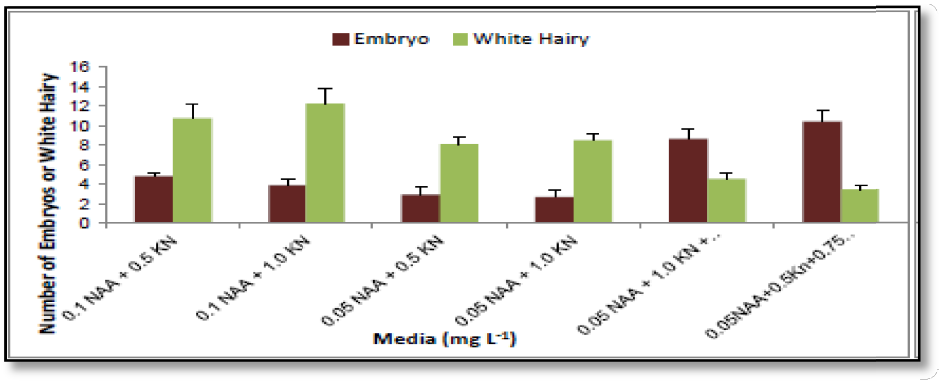


Fig. 2: Effect of NAA and kinetin on embryo production in zygotic embryo cultures

callus did not show any response. Moreover, no malformed embryos were formed when ancymidol was introduced and there was a significant reduction of white hairy structures and therefore resulted into a gradual increase of somatic embryo production. After six additional weeks asynchronous globular and bipolar embryos with or without a green tip were observed in all ancymidol combinations (Fig. 3). Number of both globular and bipolar embryos increased as ancymidol concentration increased from 0.5 to 0.75 mg L^{-1} and any further increase in ancymidol concentration to 1.0 mg L^{-1} both globular and bipolar embryo numbers significantly decreased (Table 2). The most effective ancymidol concentration was 0.75 mg L^{-1} on which highest number of globular and bipolar embryos differentiated (Fig. 3).

Table 2: Effect of different ancymidol concentrations on somatic embryo (SE) development in *Asparagusracemosus*.Ancymidol was supplemented to MS + 0.1 mg L^{-1}NAA + 0.5 mg L^{-1}Kinetin.

S. No.	Ancymidol concentration (mg L^{-1})	3weeks		6weeks	
		Mean No. of Globular Embryos	Mean No. of Bipolar Embryos	Mean No. of Globular Embryos	Mean No. of Bipolar Embryos
1.	0.5	6.00±0.99 a	2.4±0.60 a	13±1.70 a	7.5±1.29ab
2.	0.75	8.66±1.0 a	3.1±0.65 a	14±1.44 a	10.8±1.46b
3.	1.0	7.23 ±0.93 a	1.6±0.53 a	11±1.47a	5.4±0.86a

*Data in columns followed by the same letter did not differ significantly at 0.5% level.

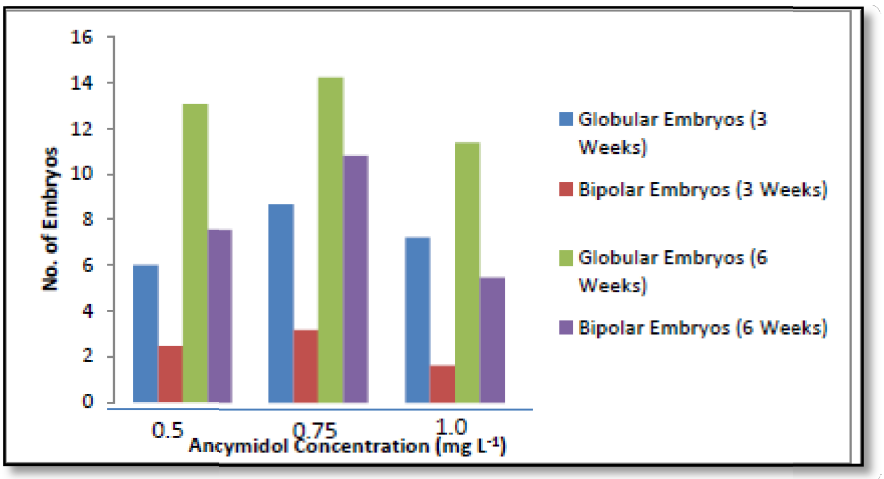


Fig. 3: Globular and bipolar embryos formed in cultures of*A. racemosus*at the end of third and sixth week asaffected by different concentration of ancymidol supplemented to MS with 0.1 mg L^{-1} NAA and 0.5 mg L^{-1} kinetin. Globular embryos cultured for three and six weeks were compared separately. Similarly, bipolar embryos cultured for three and six weeks were compared separately.

Somatic Embryo Germination:

Embryos were cultured for six weeks on the SEI mediumand transferred to somatic embryo germination medium (0.015 mg L^{-1} NAA+0.1 mg L^{-1} BAP), with two different Sucrose concentration i.e. 3 and 5 % Sucrose, respectively. No significant germination was observed in either media but a dramatic increase in number of root hairs was observed (Table 3; Fig. 4). Bipolar and globular embryos cultured on SEI medium for a period of six weeks were transferred to the germination medium supplemented with ancymidol. Within two weeks of the transfer the Somatic embryo germination was found to be as high as 65 %. The MS medium composition was as follows: MS + 0.1 mg L^{-1} NAA + 0.5 mg L^{-1} kinetin 0.75 mg L^{-1}ancymidol + 600 mg L^{-1} glutamine + 400 mg L^{-1} casein hydrolysate and 3 % sucrose. In a SEG medium with 5 % sucrose, an increase in the glutamine and casein hydrolysate concentration resulted in less percentage of embryos with green primordial.

Table 3: Effect of NAA and BAP on somatic embryo germination in hypcotyl cultures with 3% and 6% sucrose (M1= 0.015 mg L-1NAA+0.1 mg L-1BAP+3 % sucrose; M2=0.015 mg L-1NAA + 0.1 mg L-1BAP + 6 % sucrose)

	Zygotic Embryos			
	M1		M2	
	No. of Embryos	No. of White Hair	No. of Embryos	No. of White Hair
3rd Week	3.81±0.65^{ab}	3.63±0.76^{a}	6.09±0.90^{b}	4.72±0.58^{a}
6th Week	3.14±0.56^{a}	10.71±0.82^{b}	3.57±0.42^{a}	11.14±1.12^{b}

*n=15. One way ANOVA with Duncan's Multiple range test was applied. *Data in columns followed by the same letter did not differ significantly at 0.05 % level

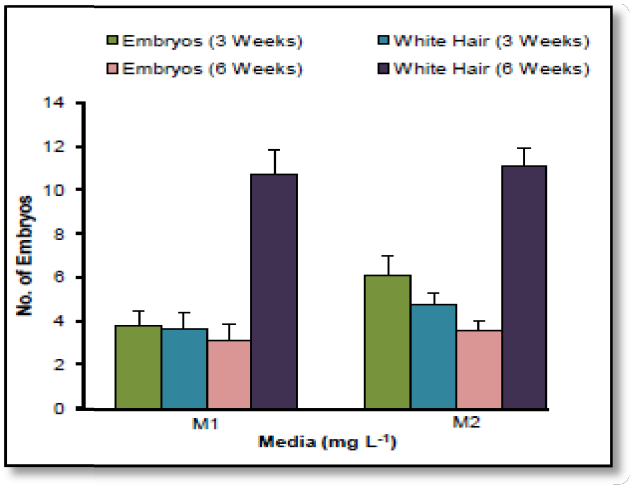


Fig. 4: Effect of Effect of NAA and BAP on somatic embryo germination in zygotic embryo cultures with 3% and 6% sucrose (M1= 0.015 mg L-1NAA + 0.1 mg L-1BAP +3 % sucrose; M2=0.015 mg L-1NAA + 0.1 mg L-1BAP + 6 % sucrose)

Table 4: Mean per cent germination of somatic embryos of *Asparagus racemosus* as effected by concentration of sucrose, glutamine and casein hydrolysate supplemented to MS + 0.1 mg L^{-1} NAA + 0.5 mg L^{-1} kinetin + 0.75 mg L^{-} ancymidol.

S. No.	Sucrose	600 mgL^{-1} glutamine +400 mgL^{-1} Casein hydrolysate	800 mgL^{-1} glutamine + 500 mgL^{-1} casein hydrolysate
1.	3%	65	53
2.	5%	47	42

*Recorded three weeks after transferring

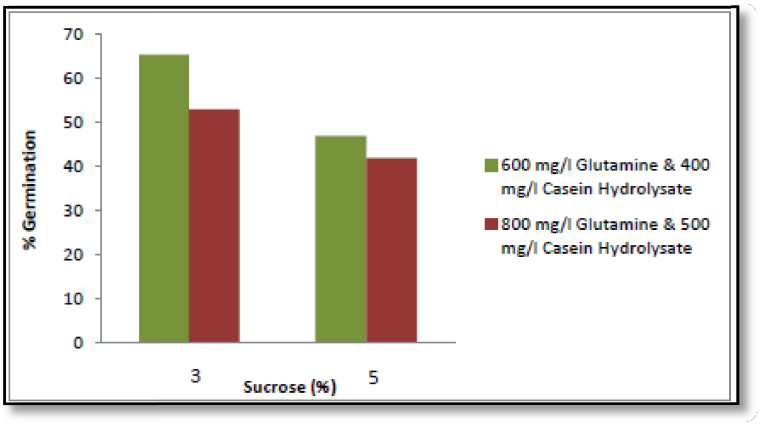


Fig. 5: *In vitro* germination of somatic embryos of *A. racemosus* as influenced by sucrose concentration. The somatic embryo germination medium consisted of MS with Glutamine (600 or 800 mg L^{-1}), Casein Hydrolysate (400 or 500 mg L^{-1}) with 3 or 5% sucrose

Histology: Observations using light microscope showed that embryogenic callus was formed in the presence of 2,4-D and kinetin. Histological studies confirmed the structure of somatic embryos in the present work. A longitudinal section of the bipolar embryo showed a broad dome-like coleoptile enclosing the scutellum with a distinct narrow radicular end. Well-developed meristematic tissues containing small uniform, actively dividing meristematic cells lacking intercellular spaces could be observed.

Scanning Electron Microscopy: SEM of 5-7 week-old embryogenic callus revealed surface structural changes during somatic embryogenesis. Each embryo structure had its own characteristic epidermal layer. SEM revealed that the globular embryos were spherical and smooth surfaced, while the elongated bipolar embryos

Table 5: Effect of BAP concentration on shoot number and shoot length

S. No.	BAP Concentration (mg L^{-1})	Mean No. of Shoots	Mean Length of Shoots (cm)
1	0.002	1.70 ± 0.26^{a}	8.00 ± 0.47^{d}
2	0.01	1.90 ± 0.31^{ab}	8.10 ± 0.53^{ad}
3	0.02	2.36 ± 0.20^{abc}	4.91 ± 0.39^{c}
4	0.04	2.45 ± 0.28^{abc}	3.55 ± 0.53^{b}
5	0.06	2.64 ± 0.31^{bc}	2.59 ± 0.43^{b}
6	0.08	3.00 ± 0.19^{c}	3.09 ± 0.49^{c}
7	1.0	4.36 ± 0.28^{d}	1.24 ± 0.43^{ac}

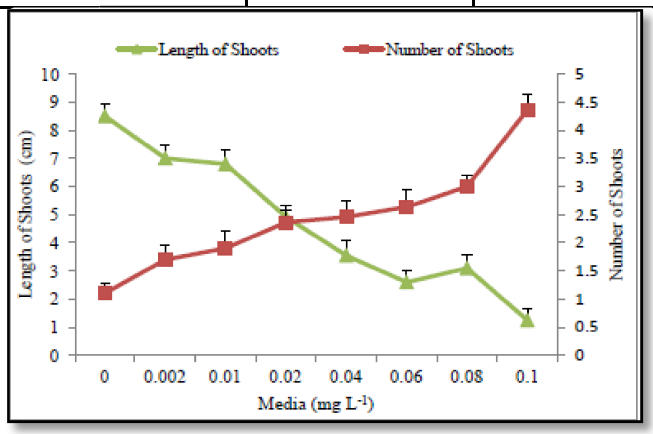


Fig. 6: Effect of BAP concentration on number and length of shoots

had an outer mesh-like epidermis. During the progress of globular embryos into bipolar structures the outer epidermal cells elongate giving a mesh-like appearance.

Nodal Cultures for Axillary Proliferation

Culture Initiation

The surface sterilization protocol resulted in 90 % aseptic cultures. Nodal explants were excised aseptically from the field grown plants and cultured on various concentrations of BAP (0.002, 0.01, 0.02, 0.04, 0.06, 0.08 and 1.0 mg $^{L-1}$). Axillary bud break was observed after two weeks in cultured nodal explants. Among the media tried for the nodal explants, MS media supplemented with 0.1 mg L^{-1} BAP elicited the highest number of (4.36) shoots per node with an average length of 1.2 cm. Least number of shoots per explant (1.76) were formed in the presence of 0.002 mg L^{-1} BAP though the length of shoots was maximum (8 cm). There was a higher correlation between BAP concentration and number of shoots formed per explant. With increase in BAP concentration there was a steady increase in the number of multiple shoots buds formed (Table 5). However, concentration of BAP inversely affected the length of shoots with increase in BAP concentration, the shoots length decreased (Fig. 6). Higher concentrations of BAP (1 mg L^{-1}) also resulted in callus formation at the base and hyperhydricity. In an attempt to improve rate of bud break, IAA at 0.001 mg L-1 and 0.01 mg L-1 was used with 0.02 mg L-1 BAP. However no improvement in number of shoot buds formed was observed (Table 6)

Table 6: Effect of BAP and IAA interaction on number and length of shoots

S. No.	BAP Concentration (mg L^{-1})	Mean No. of Shoots	Mean Length of Shoots (cm)
1	0.02 mg/L BAP+0.001 mg L-1 IAA	2.35±0.4	3.06±0.27
2	0.02 mg/L BAP+0.01 mg L-1 IAA	2.5±0.33	2.76±0.42

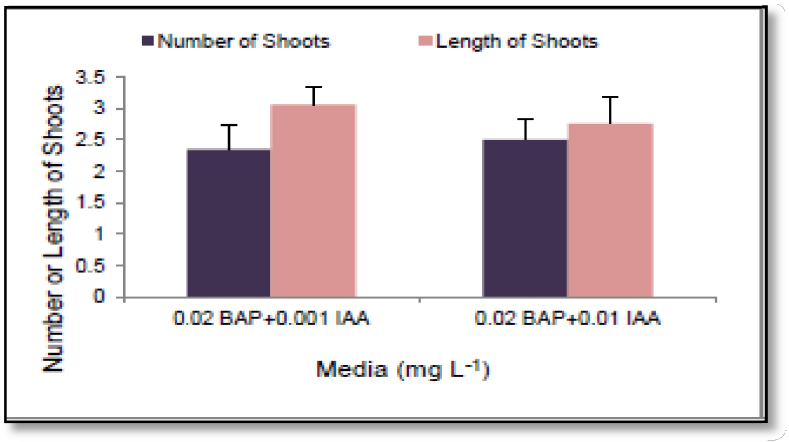


Fig. 7: Effect of BAP and IAA interaction on number and length of shoots

Shoot proliferations

To achieve shoot proliferation, healthy cultures were further subcultured to three concentrations of BAP (0.04, 0.06, 0.08 mg L^{-1}) showing high shoot numbers and with little stunted of shoots. The length of shoots did not show significant difference among the selected concentrations. Despite the fact that the number of shoots was higher in 1.0 mg L^{-1}, the shoots were severely stunted and hence could not be proliferated further. Higher number of shoots (15.87) per cluster was observed in MS basal medium supplemented with 0.08 mg L^{-1} BAP with an average length of 2.93 cm (Table 7). In this medium shoots were thick and healthy.

Shoot Elongation

To promote the shoot length 2-3 cm shoot clusters were transferred to liquid MS basal medium. The results are presented in Table 8 At the end of 2 weeks shoot elongation was significantly greater in MS liquid medium than in the multiplication medium. The shoots attained a length of 6.5 cm in liquid medium. while, in the multiplication medium shoots were only 3 cm. In the liquid medium Increase number of shoot buds was also observed.

Table 7: Effect of BAP on cluster multiplication in *A. racemosus*

S.No.	BAP Concentration (mg L^{-1})	Mean No. of Shoots	Mean Length of Shoots (cm)
1	0.04	11.36±0.51[a]	4.48±0.28[a]
2	0.06	Ab 12.27±0. 65	3.8 A 8± 0.2 9
3	0.08	15.87±0.56[b]	2.93±0.24[b]

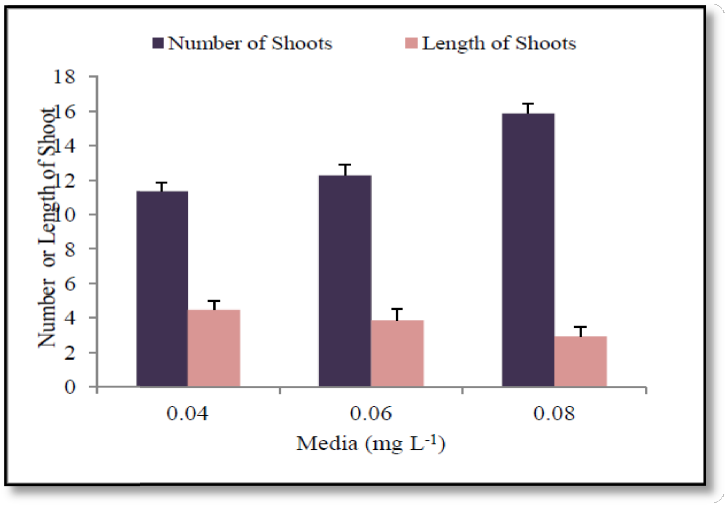


Fig. 8: Effect of BAP concentration in cluster multiplication

Table 8: Influence of liquid medium on length of shoots

S.No.	BAP Concentration (mg L^{-1})	Mean No. of Shoots	Mean Length of Shoots (cm)
1	0.08	14.34±0.82	2.85±0.27
2	Liquid MS	15.35±0.57	6.5±0.75

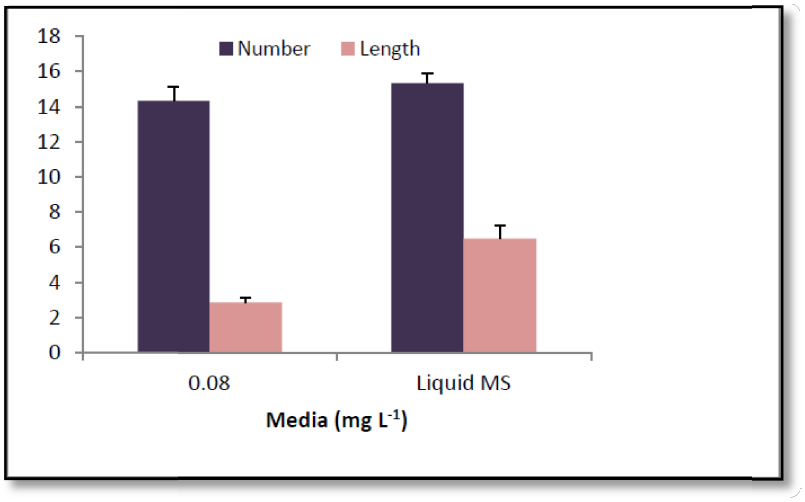


Fig. 9: Effect of MS basal liquid medium on shoot elongation

In Vitro Rooting

Single shoots were transferred to MS basal medium alone or supplemented with No rooting was achieved in any of the media with 1/4 MS, 1/2 MS and full MS (Major salts) and various concentration of IBA. Single shoots were transferred to MS basal medium with fall, half and one-fourth strength of MS major and minor salts. In an additional set up MS full strength salts basal medium was supplemented with IBA at 0.25 and 0.5 mg L^{-1} concentration. In none of the media tried the shoots rooted.

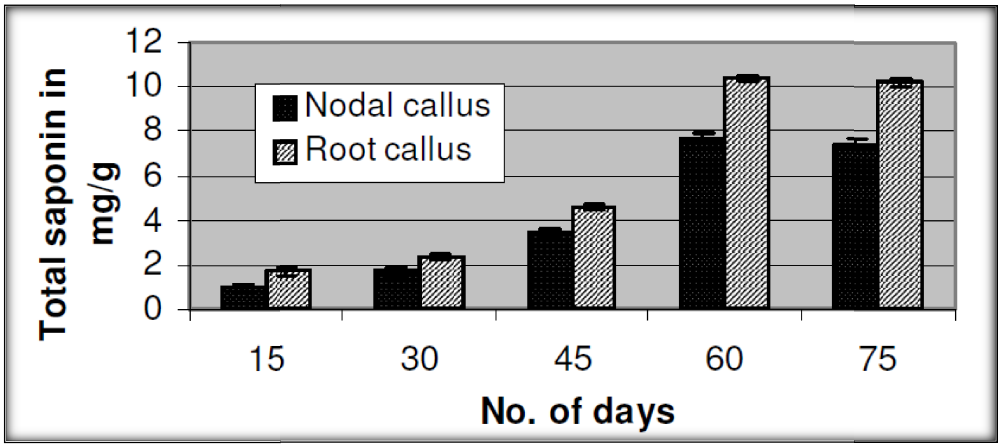
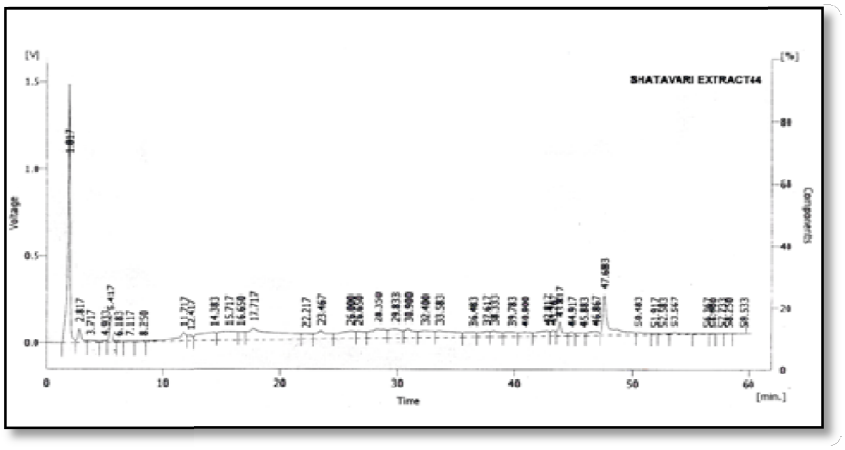
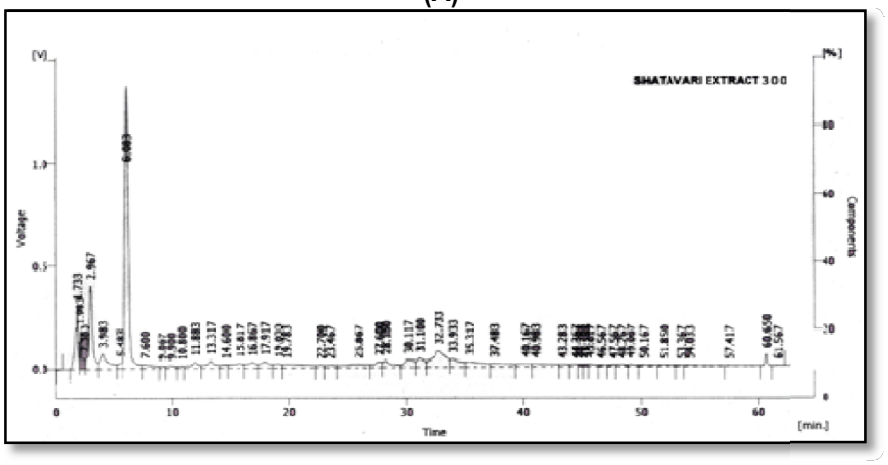


Fig. 10: Total Alkaloid content in callus culture of *Asparagus racemosus*



(A)



(B)

Fig. 11: HPLC profile of (A) Natural root extract (B) Nodal callus extract

Table 9: Total phenolic contents (determined by FolinCiocalteu reagent (McDonald et al., 2001)).

Total phenols content		
Methanol		Aqueous
*N	1214.23 ± 2.42	211.30 ± 1.10
*C	1432.45 ± 2.54	254.32 ± 1.12

Each value in the table was obtained by calculating the average of three analyses ± standard deviation.
*N=natural habitat *C= Callus sample

Table 10: Determination of Total flavonoids (Aluminum chloride colorimetric method)

Total flavonoids content		
Methanol		Aqueous
*N	321.17± 2.10	120.09 ± 0.20
*C	423.12± 3.12	143.19 ± 0.21

Each value in the table was obtained by calculating the average of three analyses ± standard deviation.
*N=natural habitat *C= Callus sample

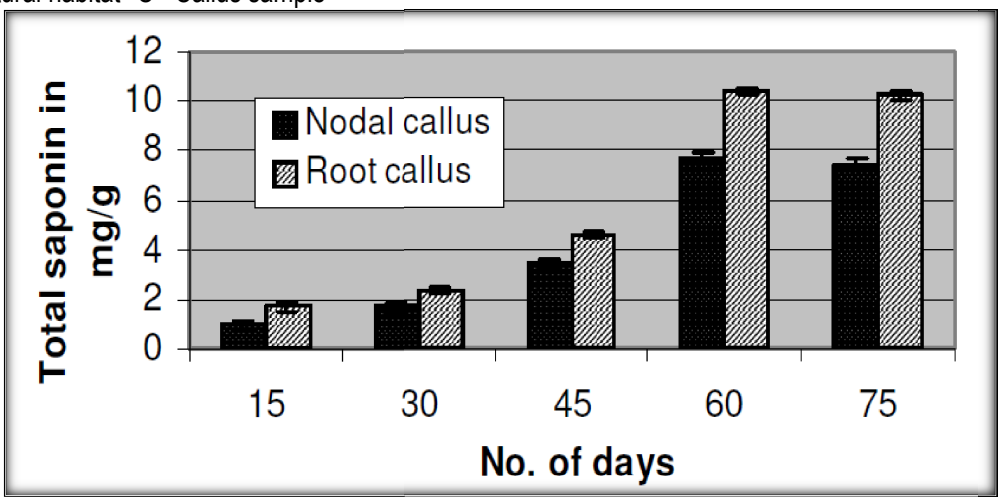
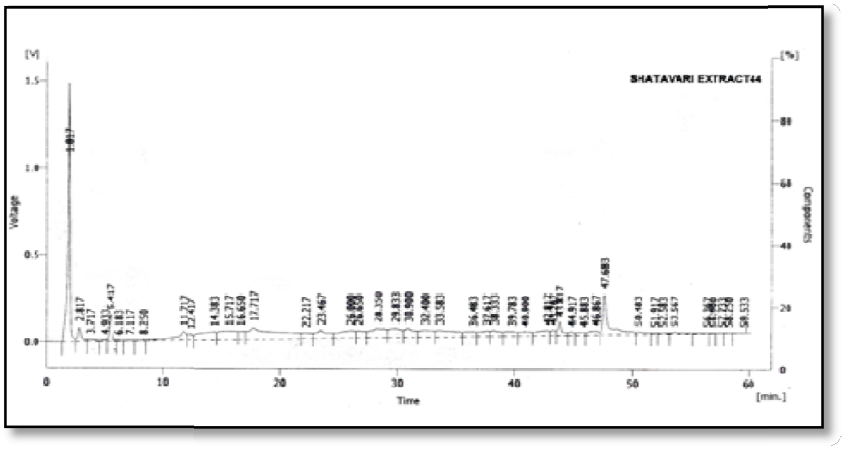
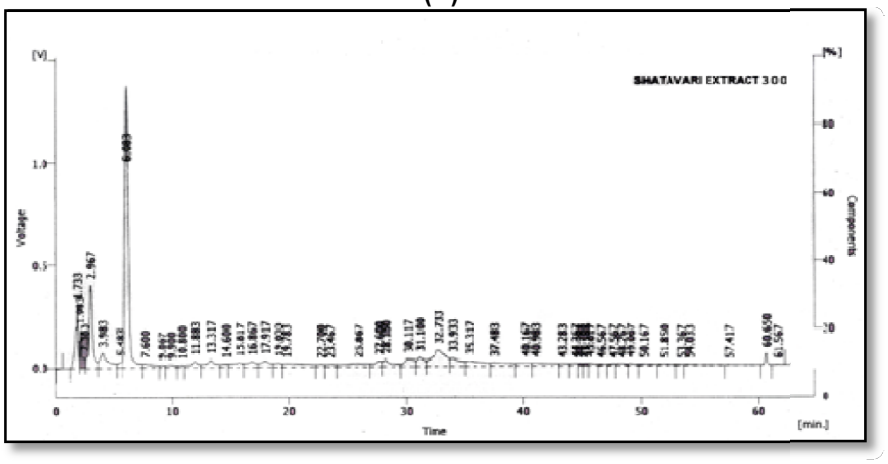


Fig. 12: Total Alkaloid content in callus culture of *Asparagus racemosus*

Free Radical scavenging activity of the sample extracts were measured by colorimetric assay using 2,2-diphenyl picrylhydrazyl radical (DPPH, a stable free radical)) as a source of free radical in accordance with the method of Blois (1958). DPPH EC_{50} was found to be 344.96 ±0.76



(A)



(B)

Fig: 13: HPLC profile of (A) Natural root extract (B) Nodal callus extract

Table 11: Total phenolic contents were determined by FolinCiocalteu reagent (McDonald et al., 2001)

Total phenols content		
	Methanol	**Aqueous**
*N	1214.23 ± 2.42	211.30 ± 1.10
*C	1432.45 ± 2.54	254.32 ± 1.12

Each value in the table was obtained by calculating the average of three analyses ± standard deviation.
*N=natural habitat *C= Callus sample

Table 12: Determination of Total flavonoids Aluminum chloride colorimetric method was used for flavonoids determination

Total flavonoids content		
	Methanol	**Aqueous**
*N	321.17± 2.10	120.09 ± 0.20
*C	423.12± 3.12	143.19 ± 0.21

Each value in the table was obtained by calculating the average of three analyses ± standard deviation.
*N=natural habitat *C= Callus sample

Phytochemical evaluation

Callus cultures derived from the nodal and root explants were screened for the presence and accumulation of alkaloids at various growth phases. The results have been graphically represented in Figure 12. It was found that a callus culture derived from the root explants produces more alkaloids compared to nodal callus cultures and maximum accumulation was found to be 10.38 ± 0.14 mg/g after 60 days of inoculation. Total alkaloids from the nodal calli were found to be 7.69 ± 0.136 mg/g of callus. In the wild type roots Shatavarin IV is generally found to be 0.05 to 0.08% where as in our cultures it was found to be 1.1% which indicates that there is approximately 20 fold increase in the

Table 13: Comparison of antioxidant activity of *Asparagus racemosus* sample and Callus

Asparagus racemosus		**Callus sample**	
Concentration (ppm)	**% Inhibition**	**Concentration (ppm)**	**% Inhibition**
20	82.45	75	60.69
10	55.08	50	47.96
05	31.85	25	28.17
		Growth medium	Not significant
EC50=9.70		EC_{50}=50.41	

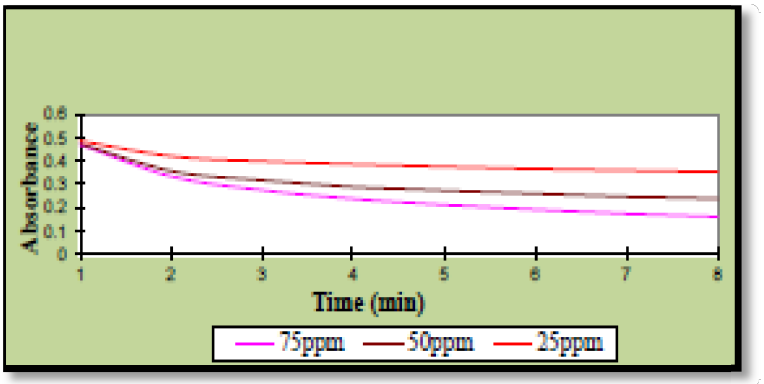


Fig. 14 : Inhibition pattern of *A. racemosus* callus extract

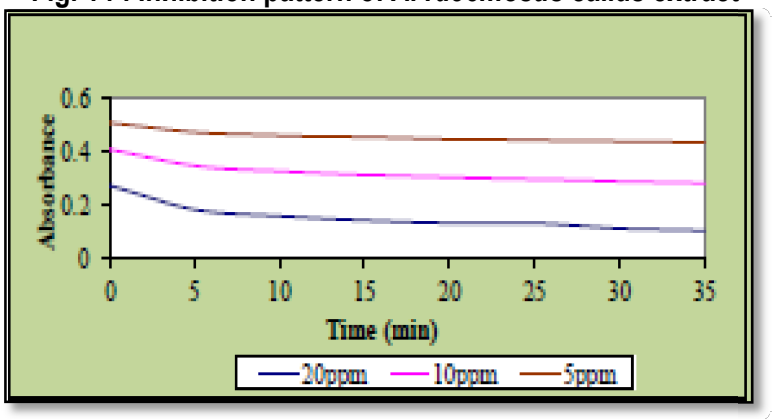


Fig. 15: Inhibition pattern of *A. racemosus* of natural habitat

alkaloids content in the *in vitro* cultures. The high performance liquid chromatography (HPLC) chromatogram of the *in vitro* culture and the natural plant root extract were compared and it was found that all the major peaks were present in the *in vitro* extract and the overall alkaloids profile was similar to the natural root extract (Figures 12 A and B).

SUMMARY AND CONCLUSION

In the present study development of somatic embryos in the in vitro cultures of zygotic embryos and hypocotyl seedlings of *Asparagus racemosus* has been demonstrated, which is a first report for this species. The simple sterilization procedure resulted in recovering 100% sterile cultures and was adopted from an earlier report on garlic (Bhojwani 1980). Somatic embryo formation has been extensively studied in *A. officinalis* (Kunitake and Mii 1998). In *A. officinalis* the most favoured explant for somatic embryogenesis has been stems and cladodes (Li and Wolyn 1995, 1997; Limenton-Grevet and Julien 2000; Delbriel et al. 1994) though hypocotyls (Willmar et al. 1968), stems (Reuther 1977), buds (Levi and Sink 1990), shoot apices (Dupire et al. 1999) and cell suspension cultures (Levi and Sink 1992) have also been used.

In the present study incubation of zygotic embryo and hypocotyl cultures in dark promoted formation of embryogenic callus, while light promoted non-embryogenic callus formation. Incubation in dark was beneficial for somatic embryo formation in *A. officinalis* (Li and Wolyn, 1995, 1996a) and other plants as *Coffea* (Giridhar et al. 2004) and *Echinacea* (Zobayed and Saxena, 2002). It is a very well established fact that 2,4-D is the most preferred auxin for embryogenic callus induction. Further Kn has been also used to complement the action of 2,4-D. However, for induction of somatic embryos, it is essential to remove 2,4-D from the medium. In the present study 2,4-D and Kn do induce formation of compact embryogenic callus but somatic embryo induction was observed even in the presence of 2,4-D. Prolonged exposure of 2,4-D or any other auxin even in the presence of a cytokinin promoted formation of white hairy roots and decreased the number of somatic embryos formed. Ancymidol is a known inhibitor of gibberellic acid biosynthesis and by promoting accumulation of storage protein improves somatic embryo maturation (Li and Wolyn 1996b) as has been in *A. officinalis.*

Inclusion of glutamine and casein hydrolysate in somatic embryo germination (SEG) medium improved both maturation and germination of the somatic embryo in the present study of *A. racemosus*. Glutamine and casein hydrolysate have been shown to improve somatic embryo germination in *A. officinalis* (Li and Wolyn, 1995) while glutamine alone was beneficial in case of wheat (Shrivastava et al. 2001) and chickpea (Patil et al. 2009). Reduced nitrogen is known to improve maturation and germination of somatic embryos (Bhojwani and Razdan 1996). Sucrose concentration is known to influence embryo maturation but in this study either 3 or 5 % sucrose could promote somatic embryo maturation or germination. Howerver, with the inclusion of ancymidol in somatic embryo germination media resulted into more than 50 % germination. Varying sucrose concentrations affected somatic embryogenesis in *A. officinalis* (Li and Wolyn 1997). In the present study, 3% sucrose in the SEG

medium was optimal for normal somatic embryo development and germination. Increasing sucrose to 5% resulted in the production of large number of bipolar embryos but had little or no green shoot primordia. Levi and Sink (1990, 1992) observed that sucrose concentration in SEG medium influenced quality of somatic embryos formed in *A. officinalis*. Sucrose at 2% resulted in small and weak somatic embryos while, 5% and 6% sucrose supported formation of well-developed somatic embryos that germinated optimally and any further increase to 7-10% resulted in the production of large number of abnormal structures. Ancymidol enhances somatic embryogenesis only in combination with certain level of a carbon source and/or osmoticum in the medium (Li and Wolyn 1997).

REFERENCES

1. O.P. Sharma, N. Kumar, B. Singh and T.K. Bhat. An improved method for thin layer chromatographic analysis of saponins. Food Chemistry., Vol. 132, pp. 671-74, 2012.

2. S. Sharma and A. Kumar. Herbal medicinal plants of rajashthan: musli. Life., Vol. 50, pp. 62.

3. M. Irshad, A. Saeed and M. Idrees. Genetic diversity among Asparagus species and cultivars of Asparagus officinalis L. using random amplified polymorphic DNA (RAPD) markers. International Journal of Biodiversity and Conservation., Vol. 6, pp. 392-99, 2014.

4. F.W. Bansode, K.R. Arya, R.K. Singh and T. Narender. Dose-dependent effects of Asparagus adscendens root (AARR) extract on the anabolic, reproductive, and sexual behavioral activity in rats. Pharmaceutical biology, pp. 1-9, 2014 .