



In vitro regeneration with callus development of *Asparagus racemosus* by epicotyledonary node

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Abstract

Asparagus racemosus Wild (family Asparagaceae; Liliaceae), commonly known as Satawari (Hindi) is a perennial shrub, with a tuberous root-stock, stems covered with recurved spines, linear leaves arranged in a tuft, white flowers and sweet-scented appears in October. The plant occurs throughout India up to 1500 meters elevation. It is a popular vegetable consumed in many parts of the world and grows naturally throughout India, Asia, Australia and Africa. It is recommended in Ayurvedic texts for prevention and treatment of gastric ulcers as galatogogue and nervine tonic. It is commonly used for the treatment of diarrhoea, dysentery, rheumatism, nervous breakdown, and is thought to be an aphrodisiac (Nadkarni 1976, Chadha 1985). The root of the plant has also been claimed by traditional healers to possess antidiabetic properties. Studies on the extracts of this plant have revealed a wide range of biological activities. *Asparagus racemosus* seeds were cultured on a basal medium of half strength MS medium with activated charcoal and 3% sucrose, incubated at 25±2°C and under a light intensity of about 3000 Lux. The seeds were germinated and elongated after 4-5 weeks. The seedlings (epicotyledonary node) were used as explants. The explants (epicotyledonary node) showed swelling at nodal region which was followed by the emergence of shoot buds. Multiple shoot formation was achieved from per-existing meristems of nodal region of explant. The time taken for shoot initiation from explant was independent of growth hormones and nutrient media but the number of shoots per explants was dependant on the concentration of growth hormones (Table-1). The epicotyledonary node explants of *Asparagus racemosus* were inoculated on MS medium containing BAP with IAA and KIN with NAA or IAA. The number of shoots produced per explants varied in different concentration of plant growth regulator but the time period required for shoot induction in all the treatment was similar. As the KIN concentration increased from 2.32µM to 13.93µM/lit the numbers of shoot buds were also increased. The maximum number of shoots (7.0±0.31shoots with 12.5±0.70mm length) was observed on KIN (13.93µM/lit) with combination of IAA (5.70µM/lit). As far as the effect of various concentration of BAP, and KIN with IAA and NAA on shoots

regeneration of explants (epicotyledonary node) of *Asparagus racemosus*, it was concluded that KIN with combination of IAA were suitable for induction of maximum multiple shoots but the concentration of KIN higher than 13.93 μ M/lit with IAA 5.70 μ M/lit, the shoots number was reduced. On the medium containing KIN 13.93 μ M/lit and NAA 4.83 μ M/lit the number of shoot was same but lengths of shoots were decreased. The lower concentration of NAA was ineffective for growth of shoots.

Keywords: *Asparagus racemosus*, media, *in vitro*, field evaluation.

List of Abbreviations:

Benzyl amino-purine (BAP) Kinetin (KIN) Indole 3-acetic acid (IAA) Naphthalene acetic acid (NAA) and 2, 4-Dichlorophenoxy acetic acid (2, 4-D).

1. Introduction

Asparagus racemosus Wild (family Asparagaceae; Liliaceae), commonly known as Satawari (Hindi) is a perennial shrub, with a tuberous root-stock, stems covered with recurved spines, linear leaves arranged in a tuft, white flowers and sweet-scented appears in October. The plant occurs throughout India up to 1500 meters elevation. It is a popular vegetable consumed in many parts of the world and grows naturally throughout India, Asia, Australia and Africa. It is recommended in Ayurvedic texts for prevention and treatment of gastric ulcers as galatogogue and nervine tonic. It is commonly used for the treatment of diarrhoea, dysentery, rheumatism, nervous breakdown, and is thought to be an aphrodisiac (Nadkarni 1976, Chadha 1985). The root of the plant has also been claimed by traditional healers to possess antidiabetic properties. Studies on the extracts of this plant have revealed a wide range of biological activities. These include antimutagenic, antitumor, antifungal (Edenharder 1990, Shimoyamada *et al.* 1990, Shao *et al.* 1996), diuretic (Balansard & Rayband 1987) and immunostimulatory effects (Thatte & Dahanukar 1988, Rege *et al.* 1999, Dhuley 1997). It has been considered to be a lactogogue in lactational inadequacy (Sharma *et al.* 1996) and useful to decrease post-operative adhesions (scars; Rege *et al.* 1999). The protective effects of this plant against the myelosuppression with single and multiple doses of cyclo-phosphamide have also been demonstrated (Thatte & Dahanukar 1988). Roots of the plant inhibited the growth of human leukaemia HL-60 cells (Shao *et al.* 1996) and more recently it has been shown to exert antioxidant properties in rat liver mitochondrial membranes (Kamat *et al.* 2000). The compounds so far reported include flavonoids, oligosaccharides, amino acids, sulphur-containing acids and steroidal saponins (Shao *et al.* 1996). Various reports suggest that polysaccharides derived from the plant exhibit antioxidant as well as radio protective properties (Gang *et al.* 1997, Liu *et al.* 1997a,b, Zeng *et al.* 1997).

The polysaccharide kreskin also has been shown to have inhibitory effects on the oxidation of low density lipoprotein (LDL; Liu *et al.* 1997a, b). However, most studies evaluate mixtures of constituents and the fresh root juice of the plant has been found to be effective in dyspepsia, being associated with anti-ulcerogenic activity (De *et al.* 1997, Sairam *et al.* 2003). It has been reported that asparagus decreases gastric emptying time (Dalvi *et al.* 1990). Other studies have shown that the methanolic extracts of asparagus root reduced intestinal propulsive movement, castor oil-induced diarrhoea and intestinal fluid accumulation (Rege *et al.* 1999, Nwafor *et al.* 2000). As well as claims by traditional healers that the roots of the plant have antidiabetic properties, studies have reported reduced blood glucose level in rats and rabbits (Akhtar & Shah 1993, Rana *et al.* 1994).

Asparagus racemosus is usually propagated by planting the separated tuberous roots along with shoot apex. Since roots are the organs used for medicinal purpose there has been a practice of using seeds for plant propagation; but there are following technical problems involved in seed derived multiplication (1). The germination % of seeds is low (Approx. 20%). (2) Seed propagated plants are slow grower, and (3) Existence of heterozygosity in seed germinated plants. Hence it was decided to develop a protocol for large scale clonal propagation of this plant by *in vitro* technique using epicotyledonary node explants. Since the cultivation of *Asparagus* has not been scaled up to a commercial level, most of the requirements of the Indian industry are met through the wild resources from the forest. Because of its significant medicinal properties, it has been over exploited; which in turn has led to its inclusion in the list of threatened plant species. Unlike other extensively studied. In this paper, we report an efficient and reliable protocol for micropropagation and callus development of *Asparagus racemosus* as a tool for mass propagation of this important endangered medicinal plant. However, there are still major opportunities to produce and distribute high quality medicinal plant. The main advantage of tissue culture technology lies in the production of high quality planting material that can be multiplied round the season basis under disease-free conditions.

2. Material and Methods

Preparation of plant materials

The germplasm (seeds) was collected from various sites. The collected seeds were first washed with a solution of Tween-80 which followed by several washes with distilled water. Surface sterilized seeds of *Asparagus racemosus* were cultured on half strength MS basal medium with activated charcoal (100mg/lit) and 3% sucrose, incubated at

25±2 °C and under 1500-2000 lux intensity of yellow and white light. The seeds were germinated and elongated after 4-5 weeks. The seedlings were used as explants. The explants (epicotyledonary node) showed swelling at nodal region which was followed by the emergence of shoot buds.

Chemicals: Pure or analytical grade chemicals of E. / C. Merck, British Drug Houses (BDH), Sigma chemical company, USA, Koch-light Laboratories, England, C.H. Boehringer Sohn Ingelheim Am Rhien, Germany, Reachim, U.S.S.R., Hi-media Laboratories, India, Qualigens Fine chemicals, India, and Loba chemical company, India were used throughout the period of study.

Preparation of Culture media

The composition of plant tissue culture media is inorganic salts, vitamins, amino acids, plant growth regulators, carbohydrates and the medium matrix. All the components were soluble in distilled water. Only Ms-basal media (Murashige and Skoog-1962) were used in present study. For preparation of the medium, after mixing the salts in distilled water the Agar-Agar was added to the medium. The amount of Agar-Agar varied from 0.5 – 0.8% and that of sucrose 3 to 4% which was used as a source of carbohydrates and Plant growth hormones like Benzylamino-purine (BAP) Kinetin (KIN) Indole 3-acetic acid (IAA) Naphthalene acetic acid (NAA) and 2,4-Dichlorophenoxy acetic acid (2,4-D) were used. The pH was adjusted to 5.8 ±0.2 with 0.5 N KOH and 0.1N HCl solutions before sterilization. 15-20 ml and 35-40 ml medium was poured in tubes, conical flasks and 40-50 ml medium was poured in culture bottles respectively finally the medium was sterilized by autoclaving it at 15 pound per square inch (Psi) pressure for 15 minutes.

Inoculation: The inoculation of plant materials were carried out in a laminar air flow cabinet under sterile conditions. All the appliances were sterilization and the explants after surface sterilization were inoculated vertically or horizontally on the surface of media. The inoculated culture room under controlled conditions.

Culture conditions: The cultures were maintained at the temperature of 28±2 °C with 14 hrs illumination of light of intensity 2000 to 2500 lux and relative humidity (RH) 60-70%. The source of light was cool fluorescent tubes and incandescent bulbs. The temperature, light and humidity were varied according to the experiments.

Sub-culture and multiplication of propagules: After establishment of explants aseptically in culture media. The sub-culturing was carried out in some cultures for 5-6 times to multiply them for further experiments. The tissues were subcultured regularly after an interval of 4-5 weeks. The growth hormones were used either individually or in combinations of different concentrations.

Callus Development: Epicotyledonary node explants (length 26.5mm) from sterile *in vitro* seedling were cultured on MS medium containing different concentration of 2,4-D and IAA + NAA for 4 weeks.

3. Results and Discussion

Results

***In vitro* regeneration from seedlings of *Asparagus racemosus* (Table-1)**

Asparagus racemosus seeds were cultured on a basal medium of half strength MS medium with activated charcoal and 3% sucrose, incubated at 25±2 °C and under a light intensity of about 3000 Lux. The seeds were germinated and elongated after 4-5 weeks. The seedlings (epicotyledonary node) were used as explants. The explants (epicotyledonary node) showed swelling at nodal region which was followed by the emergence of shoot buds. Multiple shoot formation was achieved from per-existing meristems of nodal region of explant. The time taken for shoot initiation from explant was independent of growth hormones and nutrient media but the number of shoots per explants was dependant on the concentration of growth hormones (Table-1). The epicotyledonary node explants of *Asparagus racemosus* were inoculated on MS medium containing BAP with IAA and KIN with NAA or IAA.

The number of shoots produced per explants varied in different concentration of plant growth regulator but the time period required for shoot induction in all the treatment was similar. As the KIN concentration increased from 2.32µM to 13.93µM/lit the numbers of shoot buds were also increased. The maximum number of shoots (7.0±0.31shoots with 12.5±0.70mm length) was observed on KIN (13.93µM/lit) with combination of IAA (5.70µM/lit). As far as the effect of various concentration of BAP, and KIN with IAA and NAA on shoots regeneration of explants (epicotyledonary node) of *Asparagus racemosus*, it was concluded that KIN with combination of IAA were suitable for induction of maximum multiple shoots but the concentration of KIN higher than 13.93µM/lit with IAA 5.70 µM/lit, the shoots number was reduced. On the medium containing KIN 13.93µM/lit and NAA 4.83µM/lit the number of shoot was same but lengths of shoots were decreased. The lower concentration of NAA was ineffective for growth of shoots.

Combined effect of BAP+IAA

The shoot buds regenerated from node of explant, however grew poorly. In order to enhance the growth and vitality of such buds several attempts were made by incorporating different auxins. On BAP and IAA supplemented medium the number of shoots produced was increased on IAA (5.70µM/lit) supplemented with BAP (13.31µM/lit) 4 shoot buds were produced from the explant. As concentration of IAA increased the number of shoot buds decreased and

the explant showed callusing. These experiments proved that for induction of healthy multiple shoot buds the explants should be cultured on MS medium supplemented with BAP (13.31 μ M/lit) and IAA (5.70 μ M/lit).

Effect of KIN +IAA/ NAA

Results obtained are presented in these prove that the BAP in combination with IAA was not found as effective as KIN for induction as well as for growth of shoots from subcultured shoot buds. The KIN (2.32 μ M to 23.23 μ M/lit) was incorporated in the medium. On lower concentration of KIN up to 2.32 μ M/lit with NAA 0.53 μ M/lit only 2 \pm 0.09 shoots (4.5 \pm 0.19mm length) were produced. As the concentration of KIN increased to 13.93 μ M/lit with increased concentration of NAA 4.83 μ M/lit the maximum shoots (7 \pm 0.29 shoots with 8.5 \pm 0.41mm length) were produced. The length of shoots on higher concentration of KIN (18.58 μ M/lit) and NAA (6.71 μ M/lit) was reduced. But results found that the KIN (13.93 μ M/lit) in combination with IAA (5.70 μ M/lit) was more effective for maximum shoots regeneration and length (7 \pm 0.31shoots with 12.5 \pm 0.70mm length).

Multiplication of propagules in *Asparagus racemosus*

Once the proliferation of shoots is achieved from *in vitro* cultured explants. The next step is to multiply the *in vitro* grown shoots. In *Asparagus racemosus* during present investigation experiments were conducted to further multiply the shoot buds regenerated from epicotyledonary node explants. These shoot buds after 4 weeks of culture, when attained a length of 12.5 \pm 0.70mm were excised from explants and carefully subcultured to the fresh MS medium containing various growth regulators in their combinations were used.

Effect of cytokinins and auxins on shoot multiplication (Table-2)

The shoot buds achieved from epicotyledonary node explants cultured on MS medium containing KIN (13.93 μ M/lit) + IAA (2.85 μ M/lit) were further subcultured on the MS medium with combination of KIN and IAA or NAA.

Effect of KIN + IAA/NAA

Results obtained are presented in these prove that the KIN (13.93 μ M/lit) in combination with IAA (2.85 μ M/lit), same concentration for shoot induction and multiplication were used. The no. of shoots same (13 \pm 0.31 shoots with 12.5 \pm 0.21mm length) but length of shoots increased in same medium.

Effect of 2, 4-D and IAA + NAA on callus induction (Table-3.)

Epicotyledonary node explants (length 12.5 \pm 0.21mm) from sterile *in vitro* seedling were cultured on MS medium containing different concentration of 2,4-D (4.52 μ M to 22.62 μ M/lit) and IAA + NAA (IAA 2.85 μ M to 17.12 μ M/lit and NAA 2.68 μ M to 16.11 μ M/lit) for 4 weeks. The 2,4-D (4.52 μ M/lit) and NAA + IAA (NAA 2.68 μ M/lit, IAA 2.85 μ M/lit) was added in the medium the explants produce 3 shoots with 7mm length and callus induction. As the concentration of 2,4-D increased 18.09 μ M to 22.62 μ M/lit the shoot formation was inhibited and callus formation were achieved. On 2,4-D 22.62 μ M/lit vigorous callus was produced by explants.

Table 1. Effect of different Concentration of growth regulators in MS-Medium on regeneration of axillary shoots from epicotiledonary node explants of *Asparagus racemosus*.

Cytokinins		Auxins		% shoots regeneration (Mean \pm S.D.)	Number of axillary shoots (Mean \pm S.D.)	Length of shoots (MM) (Mean \pm S.D.)
BAP (μ M)	KIN (μ M)	IAA (μ M)	NAA (μ M)			
----	2.32	0.57	----	55.0 \pm 2.02	2.0 \pm 0.11	5.5 \pm 0.26
----	4.64	1.14	----	64.5 \pm 2.41	3.0 \pm 0.16	7.0 \pm 0.33
----	9.29	2.85	----	74.0 \pm 2.81	4.0 \pm 0.19	8.5 \pm 0.43
----	11.61	3.99	----	81.5 \pm 1.83	6.0 \pm 0.21	9.0 \pm 0.46
----	13.93	5.70	----	95.5 \pm 1.41	7.0 \pm 0.31	12.5 \pm 0.70
----	18.58	11.41	----	90.0 \pm 1.85	5.0 \pm 0.41	11.0 \pm 0.81
----	23.23	14.27	----	79.5 \pm 2.13	4.0 \pm 0.37	9.5 \pm 0.83
----	2.32	----	0.53	40.5 \pm 1.07	2.0 \pm 0.09	4.5 \pm 0.19
----	4.64	----	1.07	55.5 \pm 1.06	3.0 \pm 0.21	6.5 \pm 0.13
----	6.96	----	1.61	60.0 \pm 1.41	3.0 \pm 0.19	7.0 \pm 0.29
----	9.29	----	2.68	70.5 \pm 1.70	5.0 \pm 0.17	7.5 \pm 0.41
----	13.93	----	4.83	90.0 \pm 1.22	7.0 \pm 0.29	8.5 \pm 0.41
----	18.58	----	6.71	81.0 \pm 1.85	6.0 \pm 0.19	6.5 \pm 0.07
2.21	----	0.57	----	39.5 \pm 0.92	2.0 \pm 0.19	3.5 \pm 0.41
4.43	----	1.41	----	49.5 \pm 0.81	3.0 \pm 0.23	4.0 \pm 0.19
6.65	----	1.71	----	60.5 \pm 0.79	3.0 \pm 0.27	4.5 \pm 0.21
8.87	----	2.85	----	76.5 \pm 0.67	4.0 \pm 0.17	5.5 \pm 0.07
13.31	----	5.70	----	65.5 \pm 0.56	2.0 \pm 0.06	4.0 \pm 0.09
17.75	----	8.56	----	55.5 \pm 0.58	1.0 \pm 0.09	3.0 \pm 0.03

Table 2. Effect of different concentration and combination of plant growth hormones in Ms Medium on multiplication of shoots of *Asparagus racemosus*.

Cytokinins KIN (μM)	Auxins		Number of multiple shoots (Mean \pm S.D.)	Length of shoots (MM) (Mean \pm S.D.)	Callusing
	IAA (μM)	NAA (μM)			
2.32	0.57	----	5.0 \pm 0.21	7.5 \pm 0.37	–
4.64	1.14	----	7.0 \pm 0.29	8.0 \pm 0.31	–
9.29	2.28	----	9.0 \pm 0.07	9.5 \pm 0.41	–
13.93	2.85	----	13.0 \pm 0.31	12.5 \pm 0.21	–
18.58	5.70	----	10.0 \pm 0.43	11.0 \pm 0.44	+
23.23	11.41	----	8.0 \pm 0.07	9.5 \pm 0.09	+
2.32	----	1.34	4.0 \pm 0.19	6.0 \pm 0.07	–
4.64	----	2.68	5.0 \pm 0.11	7.0 \pm 0.13	–
9.29	----	5.37	6.0 \pm 0.19	8.5 \pm 0.27	–
13.93	----	8.05	11.0 \pm 0.17	11.0 \pm 0.39	+
18.58	----	10.74	9.0 \pm 0.29	9.5 \pm 0.21	+
23.23	----	13.42	7.0 \pm 0.27	9.0 \pm 0.19	+

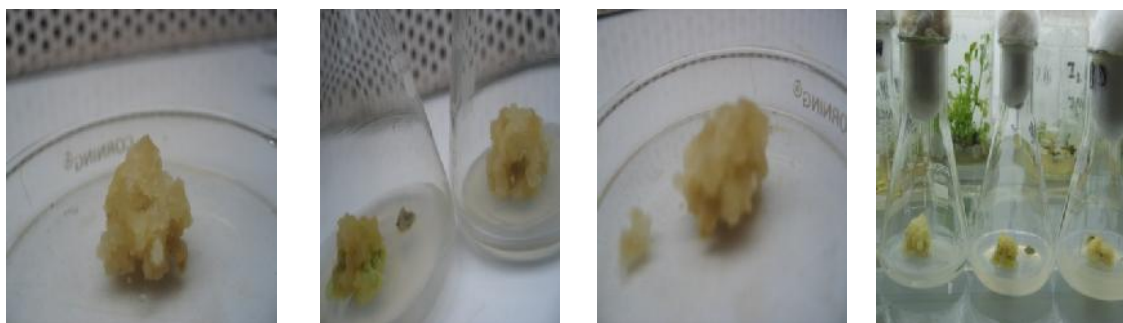


Figure 1. Multiplication of shoots of *Asparagus racemosus*.

Table 3. Effect of auxins on callus formation in different concentration in. *Asparagus racemosus*

2,4-D (μM)	IAA (μM)	NAA (μM)	Response of shoots for callusing
4.52	----	----	–
6.78	----	----	+
9.04	----	----	+
13.57	----	----	++
18.09	----	----	+++
22.62	----	----	+++
----	2.85	2.68	–
----	5.70	5.37	–
----	11.41	10.74	+
----	17.12	16.11	++

– = No callusing, + = Little callusing, ++ = Moderate callusing, +++ = Vigorous callusing.



+++ = Vigorous callusing, ++ = Moderate callusing, + = Little callusing,

Discussion:

The objective of present investigation has been to devise the method of mass propagation by developing efficient, for *Asparagus racemosus*. This plant have also been appreciated and recognized for their aesthetic and ornamental value. The natural propagation of these plants is very poor and their populations are declining with an alarming rate in the fragile ecosystems of Indian desert and Aravali areas. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies. Moreover, indeed the market and public demand has been so great that there is a great risk that many medicinal plants today face either extinction or loss of genetic diversity. *Asparagus racemosus* is commonly used for the treatment of diarrhoea, dysentery, rheumatism, nervous breakdown and is thought to be an aphrodisiac. There might be several factors including genetic and environmental for poor seed viabilities of this medicinal plant species.

Systematic approaches were made in order to achieve multiple shoot induction from seedling segment, (epicotyledonary node) of *Asparagus racemosus*. In *Asparagus racemosus* KIN 13.93 μ M/lit with IAA 5.70 μ M/lit in MS medium were found optimum for induction of multiple shoot buds (in *Asparagus racemosus* 7 shoots with 12.5mm length) from seedling segments. The shoot buds excised from explants after 5 weeks were subcultured on MS medium containing cytokinins with auxins in various concentrations. Incorporation of PVP (100Mg/lit) ascorbic acid (50Mg/lit) and citric acid (10Mg/lit) checked browning of culture. Beneficial effects of these compounds have been reported by various workers (McComb and Newton, 1981; Hu and Wang, 1983). In a period of 4-5 weeks newly produced shoots attained an optimum length and could be transferred for rooting.

Multiplication of propagules and repeated subculturing of explants:

Shoots of *Asparagus racemosus* were produced by repeatedly subculturing of the original explant which remained after excision of developed shoots each time. The same explants could be subcultured on fresh medium for five times and after each subcultured it produced 30-40 shoots. In the original cotyledonary node explant produced (7-9 shoots in *Asparagus racemosus*) and the same explant after excision of regenerated shoots was subcultured to fresh MS medium containing cytokinin and auxins. This explant could be subcultured four times after 30-35 days, on fresh medium. The formation of new shoots from subcultured explant and development of older shoots took place simultaneously.

Statistical Design

Experiments were set up in Randomized Block Design (RBD) and 10 replicates for each treatment were tested for shooting medium and 10 replicates for each treatment were tested for callus development medium. Data from each experimental stage were analyzed separately and mean and SD (Standard Deviation) of each experimental stage were calculated.

4. Conclusion

Based on current research and financial investments, medicinal plants will, seemingly, continue to play an important role as a health aid. Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies. Moreover, indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity. This plant have also been appreciated and recognized for their aesthetic and ornamental value. Such popularity of healthcare plant-derived products has been traced to their increasing acceptance and use in the cosmetic industry as well as to increasing public costs in the daily maintenance of personal health and well being. Examples of such beauty-oriented therapeutics are skin tissue regenerators, anti-wrinkling agents and anti-age creams. Most dermaceuticals are derived from algal extracts that are rich in minerals and the vitamin B group.

Skincare products such as skin creams, skin tonics, etc. derived from medicinal plants are grouped together as dermaceuticals. Also, amongst the poor, cures and drugs, derived from plants, constitute the main source of healthcare products. Medicinal plants are an integral component of ethno veterinary medicine. Farmers and pastoralists in several countries use medicinal plants in the maintenance and conservation of the healthcare of livestock. A whole range of plant-derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating disease are now being described as functional foods, nutraceuticals and nutraceuticals.

***Asparagus racemosus*:**

Only 2 ± 0.63 shoots (5.5 ± 0.26 mm length) shoot were produced from epicotyledonary node of *Asparagus racemosus*. On kinetin (2.32 μ M/lit) + IAA (0.57 μ M/lit) supplemented MS medium. The shoot number increased from 2 to 7 from explants of *Asparagus racemosus*. As the concentration of kinetin (2.32 μ M to 13.93 μ M/lit) + IAA (0.57 μ M to 5.70 μ M/lit). The maximum number of shoots (7 ± 0.31 shoot) was produced on kinetin (13.93 μ M/lit) +

IAA (5.70 μ M/lit) from explants. The higher concentration of kinetin (18.58 μ M/lit) + IAA (11.41 μ M/lit) inhibited the growth of shoots and decreases the number of shoots (4 shoots). The shoots produced on kinetin + IAA supplemented MS medium attained 12.5mm length. The optimum concentration of kinetin (13.93 μ M/lit) + IAA (2.85 μ M/lit) for induction of multiple shoot buds (13 shoot 12.5mm length).

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