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Direct *In vitro* Propagation of *Asparagus adscendens* Roxb.

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Abstract

A very high rate of multiple shoots was obtained from nodal explants of *Asparagus adscendens* Roxb. on MS supplemented with 0.27 µM NAA, 0.46 µM Kn and 0.6% agar. Good rooting response was observed when individual regenerated shoots were inoculated on to MS with 1.48 µM IBA, 3.90 µM ancymidol and 3% sucrose. Such plantlets were successfully transferred to soil after hardening with a high rate of survival. After six months, rhizomes of both *in vivo* mother plants and their *in vitro* regenerated counterparts were collected followed by their comparative phytochemical fingerprinting studies through HPTLC. The analysis did not reveal any phytochemical variation among the regenerants.

Introduction

Asparagus adscendens Roxb. is a member of the genus *Asparagus* belonging to the family Liliaceae. This Liliaceous plant is commonly known as "Dholi Musali" and "Shatawar Misri". It is normally found and propagated in sandy loamy soil of northern plains of the Western Himalaya and the Punjab at higher altitudes up to 5,300 ft., Gujarat, parts of Maharashtra, Rohilkhand and Central Mussori hills. It is a sub-erect prickly shrub having tall, much branched, ascending branchlets and white tuberous roots. Roots are 30 to 100 cm long, 0.7 to 1.0 cm thick, white, odourless and sweetish in taste. All parts such as stem, cladodes, seeds and rhizomes of this species are very important in Indian and Unani traditional medicinal remedies for treatment of spermatorrhoea, chronic leucorrhoea, diarrhoea, dysentery, general debility, senile pruritus, asthma and fatigue. The rhizome powder is given as a nutritive tonic with milk in case of seminal weakness and impotence (Kapoor 2001). The rhizome extract**s** contain a large number of very important steroids, triterpenoids, glycosides, saponins, essential oil and phytoecdysteroids that are analogues of invertebrate steroid hormones (Tandon and Shukla 1995; Rao et al. 1952; Dinan et al. 2001). It has also been identified as one of the drug*s* to control the symptoms of AIDS (Trivedi and Upadhyay 1993).

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A. adscendens is normally propagated through seeds and rhizomes; but conventional methods yield a low rate of germination. 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 *Asparagus* spp., there are no reports of *in vitro* micropropagation of *A. adscendens*. In this paper, we report an efficient and reliable protocol for micropropagation of *A. adscendens* as a tool for mass propagation of this important endangered medicinal plant.

Materials and Methods

Asparagus adscendens Roxb. (Liliaceae) plants were collected from the Medicinal Plants Section, Gujarat Agriculture University, Anand, Gujarat during the monsoon season (Fig. 1a). These plants were maintained and grown in the Botanical Garden, Sardar Patel University, Vallabh Vidyanagar. From one-year-old mother plant**s**, newly emerging shoots were collected and washed in running tap water for 20 - 30 min to remove soil particles and debris followed by a number of distilled water wash. The explants were then treated for 15 min with a 0.525% (v/v) sodium hypochlorite (Qualigens, Mumbai) solution to which two drops per 100 ml Tween-20 (Himedia, Mumbai) were added. This was followed by 0.1% (w/v) mercury chloride (Qualigens, Mumbai) treatment under Laminar Air Flow Unit (LAFU). The explants were thoroughly rinsed with sterile distilled water six to seven times. This entire treatment was carried out at a low temperature to prevent damage to the shoots. Shoots were blotted on to a sterile blotting paper and nodal explants were excised from ~~the~~ shoot segments. The cladodes were cut off carefully one by one from the nodal explants and inoculated on to MS supplemented with MS vitamins, 3.0% (w/v) sucrose (Himedia, Mumbai), 0.6% (w/v) agar (Himedia, Mumbai), 0.5% (w/v) Em-Carb – a fungicide (12% carbendazime and 63% Mancozeb, Sabero Organics, Gujarat) and plant growth regulators like IAA, IBA, NAA, BA, Kn, 2,4-D and ancymidol as required alone or in combination. Media were adjusted to pH 5.8 ± 0.05. Borosil glass tubes (25 × 150 mm) each containing 20 ml of the culture medium and capped with plugs of non-absorbent cotton were autoclaved at 121°C at 1.05 kg^{-2} for 20 min. Cultures were maintained under 16 h photoperiod at 25 ± 0.5 °C under cool white fluorescent lights (light intensity 50 µmol m^{-2} s^{-1}) and 55 – 60% relative humidity. Each experiment was repeated thrice with 15 replicates per treatment.

Rooted plants were washed with distilled water and treated with a 0.1% Em-Carb to prevent the fungal infection. These treated plantlets were transferred to

the greenhouse in small polycups containing cocopeat and a 1.0% farmyard organic manure; covered with a transparent plastic bag with holes to maintain humidity. Subsequently, they were transferred to the botanical garden and planted in the field after one month. During the acclimation period, plantlets were provided with MS basal medium without sucrose (as a carbon source) and plant growth regulators.

For statistical analysis, means are based on 15 replicates for each treatment. Standard error is given to indicate the variation among means of three experiments repeated in time. Data regarding shoots and roots were collected after four and five weeks, respectively after inoculation.

For phytochemical fingerprinting and/or analysis, 5 g of *in vivo* and *in vitro* dried rhizome powder were cold extracted with 50 ml of methanol (in 1 : 10) (Qualigens, Mumbai) for 24 h. The extract was filtered through Whatman No.1 filter paper and the filtrate was concentrated to 5 ml using Rotavapour Apparatus (Buchi, Switzerland). This methanolic extract was partitioned with an equal volume of water-saturated n-butanol (Qualigens, Mumbai) several times. The organic phase was separated, concentrated to about 1 ml, and used for HPTLC analysis. HPTLC was performed using 10 cm × 10 cm silica gel 60 F_{254} precoated TLC plates (Merck, Germany). 20 µl of both n-butanolic fractions were applied on plates with the help of Camag Linomat – 5 Applicator (Camag, Switzerland) fitted with 100 µl Hamilton micro-syringe. Chromatograms were developed at room temperature in a 10 cm × 10 cm chamber using chloroform : glacial acetic acid : methanol : water [7 : 3.5 : 2.5 : 1.5 (v/v)] with a drop of formic acid as solvent system and derivatized with 10% H_2SO_4 followed by heating at 110°C for 10 min (Wagner and Bladt 1996). After development, the chromatogram was evaluated and scanned under λ = 254, 366 and 546 nm using Camag TLC Scanner-3 (Camag, Switzerland).

Results and Discussion

Yang (1977) established a very good tissue culture technique for micropropagation of *Asparagus* using spears as explants. However, this protocol was lengthy and took 20 weeks. Using different explants as a source, several workers have developed protocols for *in vitro* culture of different *Asparagus* species (Štajner et al. 2002; Nayak and Sen 1998; Ghosh and Sen 1994a,b 1996). To our best knowledge this is the first report for multiplication of *Asparagus adscendens* from nodal explants.

Use of a**e**rial plant parts as a source of explants reduces the chances of contamination. So, the nodal explants were inoculated on to MS containing various concentrations of auxins and cytokinins for multiple shoot induction. Among different concentrations NAA and Kn solidified with a 0.6% agar proved

best for initiation of multiple shoots visible within a week of inoculation (Fig. 1b). Furthermore, none of the tubes showed contamination. The maximum number of multiple shoots was obtained in MS supplemented with 0.27 μM NAA and 0.46 μM Kn after four weeks of incubation (Fig. 1c). Further increase in the concentration of NAA and Kn did not show any improvement (Table 1). This preliminary study proved that nodal explants responded better at a lower level of NAA and Kn. When IAA and IBA were used in place of NAA with Kn, all explants died. These results confirmed that some plant species have enough levels of endogenous hormones and do not require a high level of exogenous

Table1. Effect of various concentrations of NAA and Kn on multiple shoot formation of *A. adscendens* nodal explants.

Media combination (μM)	% response	Mean No. of shoots (Mean ± SE)	Mean length shoots (mm) (Mean ± SE)
MS + 0.27 NAA + 0.46 Kn*	73.3	8.45 ± 1.49	19.18 ± 1.76
MS + 0.54 NAA + 0.46 Kn*	60.0	6.90 ± 1.46	15.50 ± 2.86
MS + 1.07 NAA + 0.46 Kn*	60.0	6.66 ± 1.97	12.44 ± 2.79
MS + 0.54 NAA + 0.93 Kn	66.7	5.50 ± 1.06	10.00 ± 1.62
MS + 0.54 NAA + 1.39 Kn	46.7	6.71 ± 0.52	10.71 ± 2.06

Observations were made after four weeks. *Media combinations showed some traces of callus.

growth regulators for plant regeneration (Hussey 1982). When nodal explants were inoculated on to MS containing different concentrations of 2,4-D with Kn, dark green, compact, regenerative type of calli developed. Prajapati et al. (2003, 2004) have reported that 2,4-D alone or in combination with other growth regulating hormones is effective for direct organogenesis for *Curculigo orchioides*. In the present study of *A. adscendens* the above combination containing 2, 4-D was ineffective resulting in the production of only creamy pale yellow, friable type of callus.

The major obstacle of *in vitro* micropropagation of *Asparagus* was not the establishment of shoot multiplication but root induction. Rhizogenesis did not occur in individual shoots, upon their transfer to hormone free ¼ MS, ½ MS, full strength of MS basal medium supplemented with 3 and 6% sucrose and White Root Culture medium. Kar and Sen (1985) reported that lower concentrations of IBA added to the medium failed to induce roots in *A. racemosus*. This was true of *A. adscendens*, where IBA did not induce rhizogenesis. Interestingly, the shoots treated with IBA showed elongation and branching (data not shown, Fig.1d). Desjardins et al. (1987) reported that increasing the concentration of sucrose level level in media containing ancymidol (an anti-gibberellin chemical) increased *in vitro* rooting and greatly reduced the time required for production of transplant-

able plantlets. Khunachak et al**.** (1987) and Chin (1982) have also reported that various combinations of auxins and cytokinins did not improve plantlet growth but ancymidol greatly promoted shoots and root development. Individual shoots

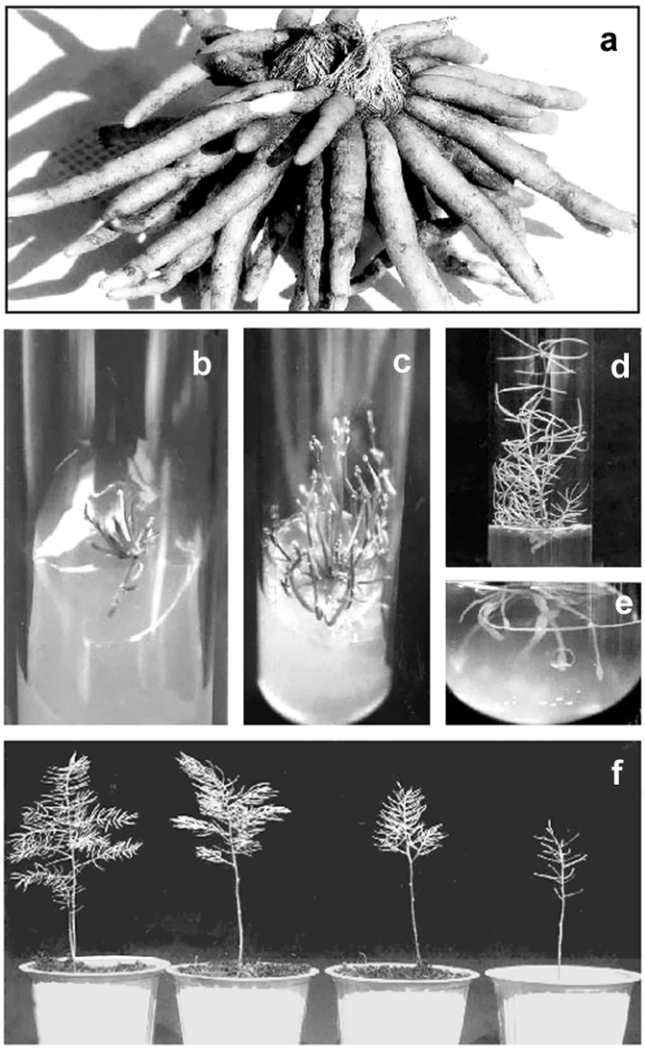

Fig. 1. (a) Rhizome of the *in vivo* mother plant. (b) Initiation of multiple shoots from nodal explants on MS supplemented with 0.27 μM NAA and 0.46 μM Kn after one week. (c) High rate of multiple shoots per explant after four weeks on the same medium composition. (d) Shoot elongation, cladode development and branching on 0.98 μM IBA. (e) Rooting response on MS supplemented with 1.48 μM IBA and 3.90 μM ancymidol after five weeks of subculture. (f) Acclimated plantlets ready for transplantation.

of *A. adscendens* inoculated on to MS with lower concentrations of IBA, ancymidol and 6% sucrose showed root induction with healthy shoots within two weeks of subculture. Present results are in agreement with those reported by

Štajner (2002) and Desjardins et al. (1987). Further incubation for three weeks led to a vigorous root growth recorded on MS with 1.48 µM IBA and 3.90 µM ancymidol (Fig.1e, Table 2). Shoots on ancymidol supplemented medium appeared healthy, thick and rigid compared to those obtained from MS supplemented with auxins and cytokinins.

Table 2. Influence of various concentrations of IBA and ancymidol on *in vitro* rooting response of individual shoots of *A. adscendens*.

Media combination (µM)	% response	Mean No. of roots (Mean ± SE)	Mean length of roots (cm) (Mean ± SE
MS + 0.49 IBA + 0.78 ancymidol#	35.6	2.38 ± 0.33	4.14 ± 0.21
MS + 0.98 IBA + 1.95 ancymidol#	42.2	3.84 ± 0.45	4.27 ± 0.42
MS + 1.48 IBA + 3.90 ancymidol#	68.9	5.68 ± 0.27	5.92 ± 0.24
MS + 0.49 IBA + 4.68 ancymidol#	53.3	3.96 ± 0.33	4.44 ± 0.23
MS + 0.98 IBA + 5.85 ancymidol#	44.4	3.20 ± 0.24	3.25 ± 0.18
MS + 1.48 IBA + 7.80 ancymidol#	37.8	3.00 ± 0.19	2.59 ± 0.13

Observations were made after five weeks. #Media combinations were supplemented with 3 % of sucrose.

The rooted plantlets were washed with distilled water and treated with Em-Carb (an antifungal agent) to prevent fungal infection before transfer to a mixture of autoclaved coco-peat and 1.0 % organic manure in small polycups for further development and acclimation (Fig. 1f). Subsequently they were transferred to the botanical garden in pots filled with autoclaved soil with manure and planted in the field after one month. Acclimated *in vitro* raised

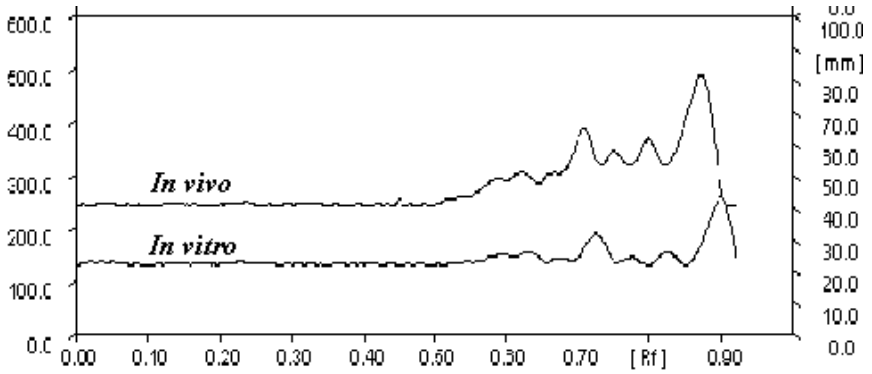

Fig. 2. Showing no variation among *in vivo* and *in vitro* rhizome extract of *Asparagus adscendens* by HPTLC graph pattern scanned at λ = 254 nm.

plants showed a survival rate of 98.21 and 95.78% in the greenhouse and field, respectively. After one year of growth in the experimental field, both *in vitro* raised plants and *in vivo* mother plants were dug out to collect rhizomes. For

comparison dried rhizome powders were extracted and analyzed for phyto-constituents by HPTLC through comparative phytochemical fingerprinting.

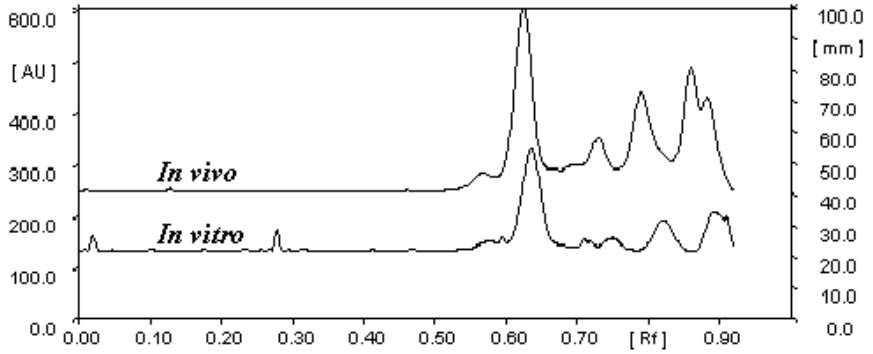

Fig. 3. Showing no variation among *in vivo* and *in vitro* rhizome extract of *Asparagus adscendens* by HPTLC graph pattern scanned at λ = 366 nm.

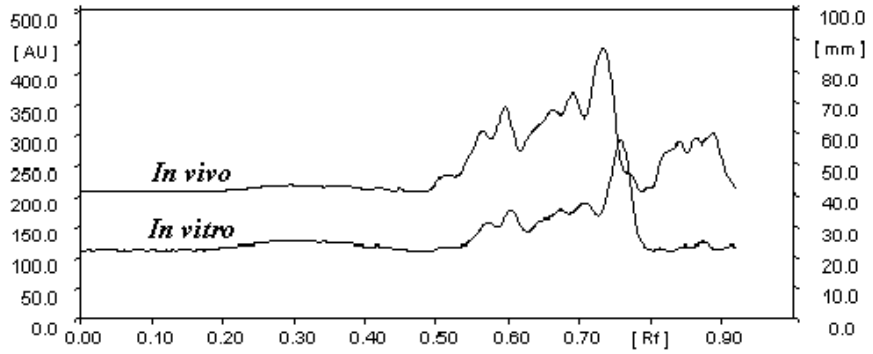

Fig. 4. Showing no variation among *in vivo* and *in vitro* rhizome extract of *Asparagus adscendens* by HPTLC graph pattern scanned at λ = 546 nm.

Scanning of developed HPTLC plates by TLC Scanner-3 at 254 nm (Fig. 2), 366 nm (Fig. 3) and 546 nm (Fig. 4), revealed no variation among *in vivo* and *in vitro* raised plants confirming that the *in vitro* plants were all true to type. The high rate of direct shoot-root multiplication and their high rate of post-hardening survival indicates that this protocol could be easily adopted for commercial large scale cultivation.

References

Chin CK (1982) Promotion of shoot and root formation in asparagus *in vitro* by ancymidol. HortScience **17**(4) : 590 - 591.

Desjardins Y, Tiessen H and **Harney PM** (1987) The effect of sucrose and ancymidol on the *in vitro* rooting of nodal sections of asparagus. HortScience **22** : 131 - 133.

Dinan L, Savchenko T and **Whiting P** (2001) Phytoecdysteroids in the genus *Asparagus* (Asparagaceae). Phytochemistry **56** : 569 - 576.

Ghosh B and **Sen S** (1994a) Effect of explants, light intensity and growth regulators on stable regeneration of *Asparagus plumosus* Baker. Nucleus (Calcutta) **37** : 24 - 29.

Ghosh B and **Sen S** (1994b) Micropropagation of *Asparagus cooperi* as affected by growth regulators. Biol. Plantarum **36** : 527 - 534.

Ghosh B and **Sen S** (1996) Plant regeneration in *Asparagus verticilatus* L. J. Herbs, Species Med. Plants **4** : 9 - 17.

Hussey G (1982) *In vitro* propagation of monocotyledonous bulbs and corms. Proc. 5th Intl. Cong. Plant Tissue Cell Culture, 677 - 680.

Kapoor LD (2001) Handbook of Ayurvedic medicinal plants. CRC Press, LCC, New York, Washington D.C., pp. 55.

Kar DK and Sen S (1985) Propagation of *Asparagus racemosus* through tissue culture. Plant Cell Tiss. & Org. Cult. **5** : 89 – 95.

Khunachak A, Chin CK, Le T and **Gianfagna** (1987) Promotion of asparagus shoots and root growth by growth retardants. Plant Cell Tiss. & Org. Cult. **11** : 97 - 110.

Nayak S and Sen S (1998) Regeneration of *Asparagus robustus*. Hort. J. Herbs, Species Med. Plants **5** : 43 - 50.

Prajapati HA, Mehta SR, Patel DH and **Subramanian RB** (2003) Direct *in vitro* regeneration of *Curculigo orchioides* Gaertn., an endangered anticarcinogenic herb. Current Science (Banglalore) **84** (6) : 747 - 749.

Prajapati HA, Mehta SR and **Subramanian RB** (2004) *In vitro* regeneration in *Curculigo orchioides* Gaertn., an endangered medicinal herb. Phytomorphology **54** (1&2) : 85 - 95.

Rao PS, Beri RM and **Budhiraja RP** (1952) Studies on tuber mucilage : Part V**.** Mucilage from the tubers of *Asparagus adscendens* Roxb., J. Sci. Industr. Res. **11B** : 127 - 128.

Štajner N, Bohanec B and **Jakše M** (2002) *In vitro* propagation of *Asparagus maritimus* - A rare Mediterranean salt-resistant species. Plant Cell Tiss. & Org. Cult. **70** : 269 - 274.

Tandon M and **Shukla YN** (1995) Phytoconstituents of *Asparagus adscendens, Chlorophytum arundinaceum* and *Curculigo orchioides* : A review**.** J. Med. Aroma. Plant Sci. **17** : 42 - 50.

Trivedi HK and **Upadhyay KK** (1993) AIDS - A serious problem of modern world. Sachitra Ayurved **45** : 821 - 824.

Wagner H and **Bladt S** (1996) Plant Drug Analysis – A Thin Layer Chromatography Atlas, 2nd ed, Springer Verlag, Berlin, pp. 305 - 310.

Yang HJ (1977) Tissue culture technique developed for asparagus propagation. HortScience **12**(2) : 16 - 17.