Content list available at http://epubs.icar.org.in, www.kiran.nic.in; ISSN: 0970-6429

Indian Journal of Hill Farming

June 2018, Volume 31, Issue 1, Page 35-40

In Vitro Propagation of Wild *Asparagus adscendens* Roxb. using Nodal Explants

A. K. Sharma[1*] . J. Pandit[2] . S. V. Bhardwaj[2]

[1]Department of Biotechnology, P P Savani University, Kosamba, Surat, Gujarat- 394125

[2]Department of Biotechnology, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan- 173230

ARTICLE INFO

Article history:
Received 26 July2017
Revision Received 14 January 2018
Accepted 22 February 2018

Key words:
Asparagus adscendens Roxb., Direct Regeneration, Nodal segments, Shoot multiplication, *In vitro* Rooting, Acclimatization

ABSTRACT

A rapid and highly effective method for direct regeneration from nodal segments explants was established for *Asparagus adscendens* Roxb. Nodal segments were inoculated on Murashige and Skoog (MS) medium containing 0.6% agar, 3% sucrose and different combinations of benzylaminopurine (BAP) and kinetin (Kn). Maximum establishment of cultures from nodal explants were obtained on MS medium supplemented with 0.2 mg l^{-1} BAP and 0.2 mg l^{-1} Kn. The nodal segments with shoot buds produced maximum number of shoots (5.50 per explant) on MS medium supplemented with 0.05 mg l^{-1} naphthalene acetic acid (NAA) and 0.3 mg l^{-1} Kn. The multiplied adventitious shoots were successfully rooted (*in vitro*) using half strength MS supplemented with 1.0 mg l^{-1} IBA and 0.3 mg l^{-1} ancymidol, acclimatized and finally transferred to natural conditions.

1. Introduction

Asparagus adscendens Roxb. is a liliaceous plant commonly called as "Dholi Musali" and "Shatawar Misri". It is a member of the genus *Asparagus* originally assigned to a separate family Asparagaceae (Brumitt 1992). It grows in the Western Himalayas, Himachal Pradesh, Punjab, Gujarat Maharashtra, and parts of Central India up to an altitude of 1500m (Mehta and Subramanian 2005). This plant is a sub-erect spinous shrub with branching, heavily crowded whitish cladophylls and internally whitesh tuberous roots (Sharma and Sharma 2013; Mehta and Subramanian 2005). Tuberous root extract of asparagus contain major active medicinal constituents *viz.* steroidal saponins, triterpenoids, glycosides, essential oil and phytoecdysteroids (Tandon and Shukla 1995; Dinan et al. 2001). Various plant parts such as stem, cladodes, rhizomes and seeds have been used in Indian traditional systems of medicine such as Ayurveda and Unani for years.

Asparagus species have been found effective to treat spermatorrhoea, chronic leucorrhoea, diarrohea, dysentery, asthma and general debility (Mehta and Subramanian 2005; Sharma et al. 2016). It is useful as nutritive, tonic, galactagogue and demulcent, in addition possessing anti-filarial activity. Fruit and roots extract of *A. adscendens* Roxb. is helpful in eliminating carnation latent virus from carnations (Mangal et al. 2003 and Bhardwaj et al. 2009). These plant virus elimination properties are likely to increase the demand for this herb manifold in the near future. Significant medicinal properties of this herb has led to its inclusion in the list of threatened plant species as the plant is not commercially grown and it has been overexploited in natural habitats. The plant is normally propagated through seeds and rhizomes. It can also be propagated vegetatively, but this is very slow and laborious process that results in smaller number of new propagules (Tewari 2000). Micropropagation technique can be employed to obtain superior and uniform genetic planting. A dependable protocol for *in vitro* propagation of *A. adscendens* has been developed for promulgation of this endangered medicinal plant.

*Corresponding author: janish.sharma28@gmail.com

2. Material and Methods

1) Collection of plant material

Asparagus adscendens Roxb. plants were collected from the wild and maintained in the glasshouse of Department of Biotechnology, Dr Y S Parmar University, Nauni, India.

2) Surface Sterilization of explants

Nodal segments containing axillary buds were excised from shoots and roots respectively. Explants were washed with tap water and then placed for 5 mins in a sterile beaker with few drops of detergent. Beaker containing explants was shaken vigorously and washed with running tap water for 30 mins to remove any traces of detergent. The excised explants were then washed thoroughly with distilled water followed by washing in 2 % teepol. The explants were further surface sterilized with different combinations and concentrations of sodium hypochlorite (NaOCl), bavistin, mancozeb and mercuric chloride ($HgCl_2$) inside a laminar air flow chamber.

3) Establishment of explants

Sterilized nodal segments were cultured on MS medium supplemented with different concentrations of BAP (0.1–0.3 mg l^{-1}) alone and in combination with Kn (0.1–0.3 mg l^{-1}). The pH of the medium was adjusted to 5.6 before addition of 0.6% agar. The cultures were incubated under 16 hours photoperiod provided by cool white fluorescent tubes and temperature of 25±2°C. The nodal segments exhibiting vigorous bud growth were selected and transferred to fresh medium.

4) Shoot multiplication in nodal explants

After the establishment of cultures, nodal explants were transferred to shoot multiplication medium. MS medium fortified with different concentrations of Kn (0.0-0.3 mg l^{-1}) in combination with NAA (0.05 - 0.1 mg l^{-1}) was used for shoot multiplication. Best concentration of growth regulators for shoot growth was found out by recording the rate of shoot multiplication, length of shoots and number of shoots per explant after 5 weeks. Serial sub-culturing in fresh medium was performed regularly after 5 weeks

5) *In vitro* Rooting

Healthy micro-shoots with 4 to 5 cm in length were transferred onto rooting medium**.** Half strength MS medium supplemented with IBA was used for root induction. Effects of anti gibberellins compound (ancymidol) on root induction was also studied. The plantlets with roots were washed with distilled water and treated with bavistin (an antifungal agent) to prevent fungal infection before transferring to a mixture of autoclaved cocopeat and 1.0 % organic manure in small polycups for further development and acclimation.

7) Statistical Analysis

For each treatment, 20 culture tubes/flasks were inoculated with the desired explant and experiments were repeated thrice. The experiments were conducted in a completely randomized design. The data recorded pertaining to different parameters was subjected to analysis of variance (ANOVA) using CRD (Gomez and Gomez 1984).

3. Results and Discussion

During present studies an efficient protocol for multiple shoot regeneration followed by *in vitro* rooting in *Asparagus adscendens* Roxb. has been standardized. The results show superiority of nodal segments with a bud from juvenile explants obtained from mature plants. As the plant becomes older, its regeneration capacity often decreases and therefore juvenile parts of plants were preferred for shoot multiplication over mature parts, especially in case of trees and shrubs. There is a large body of literature available on the use of different explants (nodal segments, rhizomes, spears) as a source for *in vitro* culture of different *Asparagus* species

Table 1. Effect of sterilants on surface sterilization of Nodal segments

Treatment	NaOCl (0.525%v/v)	Bavistin (0.2%w/v) +	$HgCl_2$ (0.1% w/v)	Mean Survival Rate (%)
T_1	5 mins	5 mins	30 sec	43.33
T_2	10 mins	10 mins	1 mins	63.33
T_3	15 mins	15 mins	1.5 mins	91.33
T_4	20 mins	20 mins	2 mins	86.66
$CD_{0.05}$				5.43
SE				2.36

(Sharma et al. 2016; Kumar and Vijay 2009; Mehta and Subramanian 2005; Stajner et al. 2002; Ghosh and Sen 1994). The nodal explants were surface sterilized for establishment of uncontaminated cultures. Nodal segments with a bud, taken from juvenile plants, the treatment of 0.525% NaOCl for 15 min followed by 0.2% bavistin for 15 min along with 0.1 %$HgCl_2$ for 1.5 min was found efficacious and resulted in 91.33% uncontaminated cultures (Table 1). NaOCl has also been used by various workers in combination with $HgCl_2$ to achieve significant results in surface sterilization of explants of *Asparagus* (Sharma et al. 2016, Behera and Sahoo 2009; Ahmed and Kumar 2009; Bopana and Saxena 2008). Different response of media-explant interaction was observed during direct shoot regeneration from *A. adscendens* Roxb. Murashige and Skoog (1962) medium supplemented with vitamins, 3% sucrose as a carbon source and various growth regulators was used for micropropagation. Medium was gelled with 6% agar. Literature is full of reports on usage of basic MS medium sufficing the requirement for culturing *Asparagus* species (Kumar and Vijay 2009; Bojnauth et al. 2003; Kar and Sen 1985). It is well established that cytokinins and auxins are indispensable for the growth and organ differentiation in plants (Skoog and Miller 1957). *Asparagus* explants also had an absolute requirement of cytokinins (BAP, Kn) and auxin (NAA) for the establishment and multiplication. Sharma et al. 2016 reported combination of two cytokinins -BAP and Kn was best for establishment of rhizome explants of *Asparagus adscendens* and our findings also corresponds to their as

MS medium with 0.2 mg/l BAP and 0.2 mg l^{-1} Kn resulted in high establishment of nodal segments (Table 2). Nodal segments were multiplied by method of enhanced release of axillary buds (Murashige 1974). Explants showing vigorous growth were transferred to shoot multiplication medium after 4-5 weeks. The medium for multiplication was MS medium supplemented with 0.3 mg l^{-1} Kn and 0.05 mg l^{-1} NAA giving a multiplication rate of 5.50 in case of nodal segments (Table 3). Multiplication was achieved by sub-culturing the shoots at an interval of 4 weeks on the shoot multiplication medium. Researchers had previously reported that use cytokinin along with auxin in lower concentrations to be useful in shoot multiplication in *Asparagus* species. Mehta and Subramanian in 2005 reported use of 0.27 µM NAA and 0.46 µM Kn in MS medium for shoot multiplication of *A. adscendens*. Pant and Joshi (2009) establish that both shoot and bud initiation was promoted by BAP and combination of BAP along with NAA resulted in shoot, bud, callus and root initiation in *A. racemosus*. Similarly, BAP and NAA were used by Afroz et al. (2010) for the successful *in vitro* multiplication of *A. racemosus*. MS medium containing 0.5 mg l^{-1} of BA and 0.2 mg l^{-1} of NAA resulted in high frequency of shoot multiplication in *A. densiflorus* (Toma and Rasheed 2012). After scrutinizing literature carefully it was observed that the major bottle neck of *in vitro* micropropagation of *Asparagus* was not the culture establishment or shoot multiplication but root induction. Rooting was not observed in individual shoots upon their transfer to hormone free half strength and full strength MS basal medium supplemented with 3%

Table 2. Establishment of nodal segme

Treatment	**BAP (mg l^{-1})**	**Kn (mg l^{-1})**	**Mean no. of explants inducing shoots**	**Mean no. of leafy shoots per explant**
T_1	0.0	0.0	0.00	0.00
T_2	0.1	0.0	0.00	0.00
T_3	0.2	0.0	2.66	1.33
T_4	0.3	0.0	2.33	1.67
T_5	0.1	0.1	3.33	1.67
T_6	0.1	0.2	4.33	2.33
T_7	0.1	0.3	3.67	2.00
T_8	0.2	0.1	4.33	2.67
T_9	0.2	0.2	9.00	4.67
T_{10}	0.2	0.3	7.33	4.33
T_{11}	0.3	0.1	8.33	3.67
T_{12}	0.3	0.2	8.33	4.33
T_{13}	0.3	0.3	7.33	4.00
$CD_{0.05}$			1.07	0.81
SE			0.52	0.39

Many workers had reported increased *in vitro* rooting in *Asparagus* species using ancymidol which has anti GA_3 activity (Desjardin et al. 1987; Kunachak et al. 1987; Chin 1982 and Stajner et al. 2002 and Stajner 2012). Excellent rooting response was recorded by Mehta and Subramanian (2005) in *A. adscendens* on MS medium fortified with 1.48 µM IBA, 3.90 µM ancymidol and 3% sucrose. In the present study, the highest number (3.93) of roots per shoot with root length (4.58 cm) was observed on 1/2 strength MS medium supplemented with 1.0 mg l^{-1} IBA + 0.3 mg l^{-1} ancymidol. On this medium, 69.16% shoots developed roots. Ulukapi et al. 2014 also reported root regeneration in *A. stipularis* Forssk on half-strength MS supplemented with 3 g activated charcoal and 1.0 mg l^{-1} IBA. Our results clearly indicate that a reduction in MS salt concentration along with ancymidol is the pre-dominant reason for the improved rooting of *in vitro* shoots. The plants with well developed root system were successfully hardened in potting mixture of coco-peat and 1% organic manure with 71.50% percent survival rate (Fig1.h).

Conclusion

The present study established micropropagation protocol for *Asparagus adscendens* using nodal explants and the procedure can be fruitfully exploited for large scale production of cloned plants for their rehabilitation in natural habitat, germplasm conservation and sustainable utilization of this valuable endangered medicinal plant.

Table 3. Shoot multiplication rate of nodal explants

Treatment	NAA (mg l-1)	Kn (mg l-1)	Shoot multiplication rate (%)
T1	0.0	0.0	0.00
T2	0.0	0.1	1.43
T3	0.0	0.2	1.73
T4	0.0	0.3	2.35
T5	0.05	0.1	3.42
T6	0.05	0.2	4.47
T7	0.05	0.3	5.50
T8	0.1	0.1	4.33
T9	0.1	0.2	4.73
T10	0.1	0.3	5.15
CD0.05			0.10
SE	0.04		

Table 4. *In* vitro root regeneration on half strength MS medium supplemented with IBA and Ancymidol

Medium composition	Percent rooting (%)	No. of roots	Root length (cm)
Full Strength MS	0.00	0.00	0.00
1/2MS	0.00	0.00	0.00
1/2MS+0.1 mg l^{-1}IBA+	0.00	0.00	0.00
1/2MS+0.2 mg l^{-1}IBA	0.00	0.00	0.00
1/2MS+0.3 mg l^{-1}IBA	0.00	0.00	0.00
1/2MS+0.4 mg l^{-1}IBA	0.00	0.00	0.00
1/2MS+0.5 mg l^{-1}IBA	16.16	3.13	3.40
1/2MS+0.6mg l^{-1} IBA	26.20	3.20	3.58
1/2MS+0.7 mg l^{-1}IBA	35.40	3.26	3.64
1/2MS+0.8 mg l^{-1}IBA	47.26	3.40	3.66
1/2MS+0.9 mg l-^{1}IBA	59.60	3.73	3.82
1/2MS+1.0 mg l^{-1}IBA	63.03	3.76	4.06
1/2MS+2.0 mg l^{-1}IBA	65.80	3.86	4.10
1/2MS+3.0 mg l^{-1}IBA	61.70	3.78	4.06
1/2MS+1.0 mg l^{-1}IBA+0.1 mg l^{-1} Ancymidol	63.80	3.80	4.34
1/2MS+1.0 mg l^{-1}IBA+0.3 mg l^{-1} Ancymidol	69.16	3.93	4.58
1/2MS+1.0 mg l^{-1}IBA+0.5 mg l^{-1} Ancymidol	67.26	3.84	4.40
CD (p=0.05)	6.52	0.10	0.15

Acknowledgement

Work was done in Department of Molecular Biology and Biotechnology, Dr Y S Parmar University of Horticulture & Forestry, Solan, India and we are thankful to Dean, College of Horticulture & Dr S V Bhardwaj, (ex-HOD), Molecular Biology and Biotechnology department, Dr Y S Parmar University of Horticulture & Forestry, Solan, India for encouragement and financial support.

References

Ahmed BA, Kumar A (2009). *In vitro* propagation of monocot (*Costus pictus* D.Don) - An antidiabetic medicinal plant. J Agril Tech 5(2): 361-369.

Behera KK, Sahoo S (2009). An efficient method of micropropagation of ginger (*Zinziber officinale* Rosc.cv.Suprava and Suruchi) through *in vitro* rhizome bud culture. Indian J Plant Physiology 14(2): 162-168.

Bhardwaj SV, Manisha M, Handa A (2009). An extract of *Asparagus adscendens* and *Asparagus* officinalis, useful for the elimination of carnation latent virus from infected plants by supplementing the tissue culture medium. Indian Patent No.232448.

Bojnauth G, Puchooa S, Bahorun T (2003). *In vitro* regeneration of *Asparagus officinalis*: Preliminary Results. AMAS 2003, Food and Agriculture Research Council, Reduit, Mauritius.

Bopana N, Saxena S (2008). *In vitro* propagation of a high value medicinal plant: *Asparagus racemosus* Wild. In vitro Cell Dev Biol - Plant 44(6): 525- 532.

Brummitt RK (1992). Vascular Plant Families and Genera. A listing of the genera of vascular plants of the world according to their families, as recognized in the Kew Herbarium, with an analysis of relationships of the flowering plant families according to eight system of classification. Royal Botanic Gardens, Kew, p 804

Chang DCN, Peng KH, Nichiols M (1996). Phloroglucinol and tryptone enhance *in vitro* rooting and survival rate of *Asparagus* nodal sections. Acta Horticulturae 415: 411-416

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

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

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

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

Gomez KA, Gomez AA (1984). Statistical Procedures for Agricultural Research. John Wiley and Sons, New York.

Kar DK, Sen S (1985). Propagation of *Asparagus racemosus* through tissue culture. Plant Cell Tissue and Organ Culture 5: 89-95.

Kumar A, Vijay N (2009). *In vitro* plantlets regeneration in *Asparagus racemosus* through shoot bud differentiation of nodal segments.<http://www. science20.com/humbolt_fellow_and_science/blog/vitro _plantlet regeneration_*Asparagus*_ *racemosus* _through _shoot_bud differentiation_ nodal segments> [Accessed in September 8th 2009]

Mangal M, Bhardwaj SV, Mangal AK (2003). *In vitro* use of an inhibitor of plant origin in the production of carnation latent virus (CLV) free plants. Plant Cell Biotech Mol Biol 4(3 & 4): 189-192

Mehta SR, Subramanian RB (2005). Direct *in vitro* propagation of *Asparagus adscendens* Roxb. Plant Tissue Culture 15(1): 25-32

Murashige T (1974). Plant propagation through tissue cultures. Ann Rev Plant Physiol 25: 135-136

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497

Sharma A, Sharma V (2013). A Brief review of medicinal properties of *Asparagus racemosus* (Shatawari). International J Pure Applied Biosci 1(2): 48-52

Sharma AK, Gautam V, Chand H, Pandit J, Bhardwaj SV (2016). Mass Multiplication and Genetic Fidelity Assessment of *Asparagus adscendens* Roxb. Ind J Ecology 43 (Special Issue-1): 224-228

Stajner N, Bohanec B, Jakse M (2002). *In vitro* propagation of *Asparagus maritimus*: a rare mediterranean salt resistant species. Plant Cell Tissue and Organ Culture 70: 269-274.

Stajner N (2012). Micropropagation of *Asparagus* by *in vitro* shoot culture. Protocol for micropropagtion of selected economically-important horticultural plants. Methods in Mol Biol 994: 341-351

Tewari DN (2000). Report of the task force on conservation and sustainable use of medicinal plants. Government of India, Planning Commission, p 90

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

Toma, R S, Rasheed KA (2012). *In vitro* propagation through seed culture and regeneration of *Asparagus densiflorus* L. through callus cultures derived from hypocotyls. International J Pure Appl Sci Technol 9(2): 94-102.

Ulukapi K, Nasircilar A G, Onus A N, Baktir I (2014). *In vitro* propagation and determination of the nutrient content of naturally grown *Asparagus stipularis* Forssk Arch Biol Sci Belgrade. 66 (4): 1333-1338.

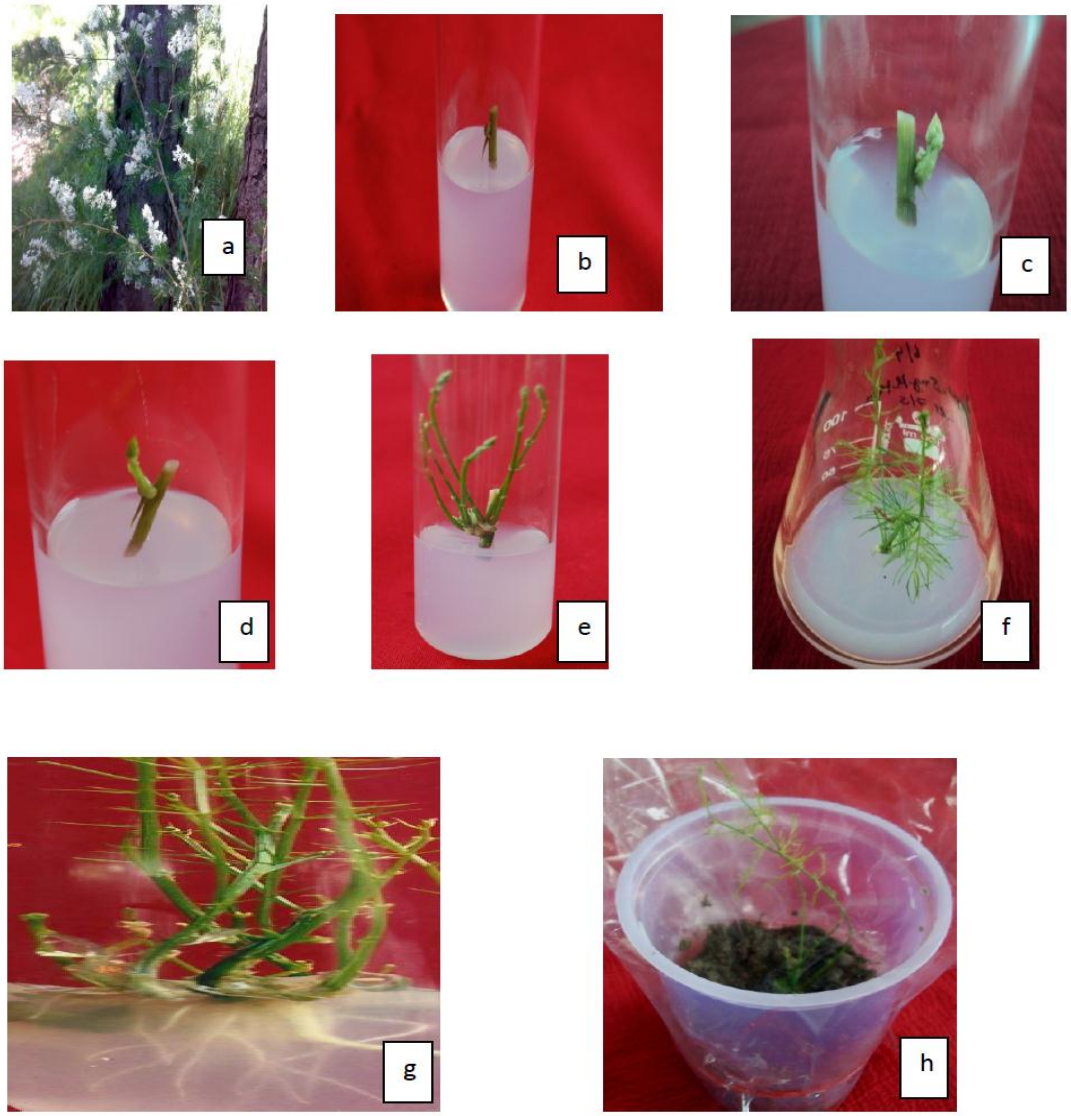

Fig1. a) Flowering in wild *Asparagus adscendens* Roxb.
b) Nodal segment with bud on MS medium for establishment
c) & d) Establishment of nodal explants
e) & f) Shoot multiplication
g) *In vitro* rooting
h) Acclimatization