American Journal of Plant Sciences, 2016, 7, 525-532
Published Online March 2016 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2016.73046

Scientific Research Publishing

Influence of Ethylene Inhibitor Silver Nitrate on Direct Shoot Regeneration from *in Vitro* Raised Shoot Tip Explants of *Sphaeranthus indicus* Linn.—An Important Antijaundice Medicinal Plant

K. Harathi, C. V. Naidu*

Department of Biotechnology, Dravidian University, Kuppam, India
Email: *challagundlav@yahoo.co.in, harathi.k10@gmail.com

Received 23 November 2015; accepted 20 March 2016; published 23 March 2016

Abstract

In the present investigation, an attempt has been made to study the influence of ethylene inhibitor silver nitrate on direct shoot regeneration in *Sphaeranthus indicus*, an important antijaundice medicinal plant, by using *in vitro* raised shoot tip explants. The effect of various concentrations of kinetin, BAP (0.5 - 3.0 mg/l), and NAA (0.1 - 0.5 mg/l) along with $AgNO_3$ (0.1 - 1.0 mg/l) was studied. Among the combinations tested MS medium augmented with kinetin (1.0 mg/l), NAA (0.1 mg/l) and $AgNO_3$ (0.4 mg/l) was found to be optimum for production of multiple shoots (34.3 ± 0.36). Addition of $AgNO_3$ to the media not only increases shoot number in all the concentrations tested but also shoot length. $AgNO_3$ at the concentration of 0.4 mg/l produced 35% more number of multiple shoots when compared to multiple shoots (10.8 ± 0.12) produced in control. In the present study by the addition of ethylene inhibitor silver nitrate and growth regulators, more number of multiple shoots (three folds) and shoot length was observed compared to control. These *in vitro* raised shoots were transferred to the rooting medium containing different concentrations of auxins such as NAA and IAA along with $AgNO_3$ (0.1 - 0.6 mg/l). Better rooting response (21.6) was observed on NAA (2.0 mg/l) and $AgNO_3$ (0.4 mg/l) containing media. The healthy rooted plantlets were transferred to polybags containing soil and vermiculate in 1:1 ratio for hardening. Finally the hardened plants were transferred to field environment for utmost survivability.

Keywords

***Sphaeranthus indicus*, *In Vitro* Regeneration, Shoot Tip Explants, Silver Nitrate, Ethylene Inhibitor**

*Corresponding author.

How to cite this paper: Harathi, K. and Naidu, C.V. (2016) Influence of Ethylene Inhibitor Silver Nitrate on Direct Shoot Regeneration from *in Vitro* Raised Shoot Tip Explants of *Sphaeranthus indicus* Linn.—An Important Antijaundice Medicinal Plant. *American Journal of Plant Sciences*, **7**, 525-532. http://dx.doi.org/10.4236/ajps.2016.73046

1. Introduction

Sphaeranthus indicus Linn., belonging to the family Asteraceae, is a herbaceous aromatic medicinal plant distributed in India, Srilanka, Australia, Malaysia, China and Africa. It is commonly known as "Boddasoram" in Telugu and "East Indian globe thistle" in English. The plant is much branched, aromatic and grows up to 30 - 60 cm height. All parts of the plant are employed for treating jaundice, diseases of spleen, pain in uterus and vagina, dysentery and hemicranias. The powdered seeds and roots are given as an anthelmentic. The bark powder mixed with whey is a valuable remedy for piles. Flowers are credited with alternative, depurative and tonic properties [1]. The natural means of propagation of *S. indicus* is via seeds. However, it encounters the problem of low seed set, viability and germination. As the plant is exploited for its medicinal use, immediate need is required to propagate the plant for its conservation. Hence, efforts have been made to study the effect of ethylene inhibitor silver nitrate on *in vitro* propagation of this medicinally important plant.

Ethylene is well-known as an ever-present plant hormone which influences the growth as promoter or inhibitor based on the species used [2]. In tissue culture, closed vessels are used with the purpose of avoiding contamination. However, sometimes this may cause abnormal plant growth due to gas accumulation such as ethylene in tissue culture vessels. Ethylene, as a plant growth regulator, produced by tissue, callus and plantlets, is known to influence *in vitro* morphogenesis [3]. *In vitro* studies have indicated that ethylene can hamper shoot rejuvenation, callus growth and somatic embryogenesis. Addition of ethylene antagonists into the culture media directly affects the level of ACC (1-amino cyclopropane-1-carboxylic acid), thereby affecting the ethylene levels [4]. Various views and experimental evidences have been put forth to explain the silver ions capability in blocking ethylene receptors to make plants insensitivity to ethylene [5]. Thus, in the present investigation an attempt has been made to study the efficacy of silver nitrate on *in vitro* direct shoot regeneration from shoot tip explants for the first time through micropropagation.

2. Materials and Methods

2.1. Source of Plant Material

The plant material of *Sphaeranthus indicus* (Linn.) for the experiment was collected from the Herbal garden of Dravidian University, Kuppam, Andhra Pradesh, India, and also from the fields in the surroundings of the Dravidian University campus.

2.2. Preparation and Sterilization of Plant Material

Actively growing shoots with 2 - 3 nodes were excised from 2 - 3 months old field grown mature plants raised from the seeds. After leaf excision, the shoot tips were dissected from the shoots and washed in running tap water for 10 - 15 mins, then in 5% (v/v) Tween-20, a liquid detergent for 5 mins, followed by continuous washing in distilled water until all the traces of detergent was removed. Then the explants were soaked in 0.4% (w/v) bavistine for 10 mins and finally surface decontamination of the explant was performed by passing through a solution of 0.1% $HgCl_2$ (w/v) for 2 mins. The optimal exposure time and optimal concentration of the surface sterilent was determined after several initial trials. Surface sterilization was followed by 4 - 5 rinses in sterile distilled water. The cut ends of the explants were further trimmed and shoot tips of length (1.0 - 1.5 cm) were prepared. Then the explants were blotted on sterile filter paper discs to absorb excess of water before planting them vertically on agar gelled MS media in culture vessels.

2.3. Selection of Medium

Surface sterilized explants were inoculated on different media such as MS, B_5, SH and KS supplemented with Kn (2.0 mg/l), NAA (0.5 mg/l) and $AgNO_3$ (0.1 - 1.0 mg/l). It was found that MS medium showed maximum response for shoot induction, regeneration and proliferation from field grown plants. Further experiments were continued with the MS medium.

2.4. Culture Medium and Culture Conditions

The shoots formed *in vitro* in different media were separated aseptically and shoot tips excised from the regenerated shoots were used as explants as a prerequisite for the present study. The excised shoot tips were inocu-

lated on MS medium [6] containing 3% (w/v) sucrose, various concentrations of cytokinins (BAP and Kn), auxins (NAA and IAA) and $AgNO_3$. pH of the medium was adjusted to 5.8 with 0.1N Hcl and 0.1N NaOH and it was made to a known volume. Before dispensing the media into the containers (15 ml for 25 × 150 mm test tubes) 0.8% (w/v) agar was added to the media and melted. Culture vessels containing media were sterilized at 15 lbs. Pressure in an autoclave at 121˚C for 15 - 20 min. After the completion of sterilization, the tubes were removed from the autoclave and placed in a slanting position to get more surface area to inoculate the explants.

All cultures of *Sphaeranthus indicus* were maintained in a culture room at temperature of 24˚C ± 2˚C and 55% - 65% RH with 16 h/8h photoperiod at a photon flux density of 3000 lux or 50 - 70 $Em^{-2}{\cdot}s^{-1}$ provided by cool white fluorescent tubes. Sub culturing was carried out at regular intervals of thirty days. Visual observations of the cultures were taken for every transfer and the effects of different treatments were quantified on the basis of percentage of cultures showing response.

2.5. Data Collection and Statistical Analysis

Visual observations were recorded on the frequency in terms of number of cultures responding for axillary shoot proliferation, shoot development, number of shoots per explant, average length of the regenerated shoots, number of roots per shoot and average root length.

Despite scarcity and limitations encountered with the plant material, for most of the treatment a minimum of 10 replicates were used. All the experiments were repeated at least twice/thrice and the cultures were observed at regular intervals. The qualitative data were subjected to statistical analysis by using standard error (SE±) for shoot length, rate of shoot multiplication and then number of roots per shoot.

3. Results and Discussion

3.1. Effect of Silver Nitrate on Shoot Bud Induction and Multiplication from Shoot Tip Explants

Shoot tip explants derived from *in vitro* raised shoots of *Sphaeranthus indicus* were grown on MS medium supplemented with various concentrations of cytokinins (BAP and Kn) in combination with auxins (NAA and IAA) and ethylene inhibitor silver nitrate. Shoot initiation from cultured explants were observed in all the treatments with varied shoot number and shoot length depending up on the type of plant growth regulators and concentration of $AgNO_3$ used. Shoot buds were emerged from shoot tip explants within 10 - 15 days of inoculation (**Figure 1(a)**). Among the cytokinins and auxins tested, Kn treated explants along with NAA and $AgNO_3$ achieved the highest regeneration frequency, shoot number and shoot length when compared to BAP.

The data obtained revealed that addition of $AgNO_3$ (0.1 - 1.0 mg/l) along with cytokinins such as BAP, Kn (1.0 - 2.0 mg/l) and auxins like NAA, IAA (0.1 mg/l) showed significant enhancement in shoot number and shoot length (**Table 1**). Shoot number increased with increasing the concentrations of $AgNO_3$ up to 0.4 mg/l, but thereafter decreased in growth as the concentration increases. Based on the results, maximum regeneration frequency (100%) and shoot number (34.3 ± 0.36) was observed in the media supplemented with Kn (1.0 mg/l), NAA (0.1 mg/l) and $AgNO_3$ (0.4 mg /l) (**Figure 1(c)**). This treatment ($AgNO_3$ at 0.4 mg/l) produced 35% number of shoots when compared to control. Maximum shoot length (10.5 ± 0.18 cm) was observed in Kn (2.0 mg/l), NAA (0.1 mg/l) and $AgNO_3$ (0.8 mg /l) supplemented media (**Figure 1(d)**). It was found that, silver nitrate at the concentration of 0.4 mg/l showed maximum number of multiple shoots on cultured shoot tips of *S. indicus* compared with the control. Hence, these results clearly emphasised that KN-NAA-$AgNO_3$ supplemented media produced more number of multiple shoots when compared to BAP supplemented media (**Table 2**). The obtained results are in line with the results of sugarcane [7] and date palm [8].

The formation of multiple shoots was detected after 3 weeks (**Figure 1(b)**). Between 82% - 100% of the explants cultured in the MS medium supplemented with all concentrations of $AgNO_3$ (0.1 - 0.4 mg/l) produced multiple shoots ranging from 16.7 - 34.3. There was a significant difference in the number of shoots produced between the control and $AgNO_3$ supplemented medium. This might be due to the fact that when ethylene is produced through cell division during establishment phase under *in vitro* conditions, Ag^+ ions are produced in the medium and an ethylene receptor; ETR1 contains one ethylene binding site per homodimer which is further mediated by a single copper ion (Cu^+) present in the ethylene binding site. The replacement of copper co-factor by silver also serves to lock the receptor into a conformation such that it continuously represses ethylene responses

Figure 1. Direct shoot initiation from *in vitro* grown shoot tip explants of *Sphaeranthus indicus*: (a) Shoot bud initiation from shoot tip explants after 7 days of inoculation on MS medium + kinetin (1.0 mg /l) + NAA (0.1 mg/l) + $AgNO_3$ (0.4 mg/l), (b) Initiation of multiple shoots from shoot tip explants on MS medium + kinetin (1.0 mg/l) + NAA (0.1 mg /l) + $AgNO_3$ (0.4 mg/l), (c) Multiplication of shoots from shoot tip explant on MS medium, (d) Elongated multiple shoots regenerated from shoot tip explant on MS medium + kinetin (2.0 mg/l) + NAA (0.1 mg /l) + $AgNO_3$ (0.8 mg/l), (e) Initiation of roots from the regenerated shoots *in vitro* on MS medium + NAA (2.0 mg/l) + $AgNO_3$ (0.4 mg/l), (f) Plantlet showing elongated root system, (g) Hardened plantlet in polybags containing soil and vermiculite in 1:1 ratio, (h) Plantlet in field condition.

[9]. These findings suggest that silver nitrate plays a more significant role in multiple shoot formation in *S. indicus*.

3.2. Effect of Silver Nitrate on *in Vitro* Rooting

The healthy micro shoots developed *in vitro* were excised from the shoot clumps and transferred to MS basal medium augmented with different concentrations of auxins such as IAA and NAA along with silver nitrate.

Table 1. Effect of different concentrations of Kn, IAA, and NAA in combination with $AgNO_3$ on multiple shoot regeneration from *in vitro* grown shoot tip explants of *Sphaeranthus indicus*. Observation: After 8 weeks, values are mean ± S.E of 10 replicates.

Conc. of plant growth regulators (mg/l)			Concentration of $AgNO_3$ (mg/l)	Regeneration frequency (%)	Mean no. of shoots/explant ±S.E	Mean no. of shoot length ±S.E (cm)
Kn	IAA	NAA				
1.0	0.1	-	-	75	4.5 ± 0.24	4.7 ± 0.15
1.0	0.1	-	0.1	80	7.0 ± 0.19	3.8 ± 0.42
1.0	0.1	-	0.2	85	10.8 ± 0.14	4.7 ± 0.23
1.0	0.1	-	0.4	90	23.3 ± 0.53	3.9 ± 0.04
1.0	0.1	-	0.6	90	9.6 ± 0.15	6.2 ± 0.37
1.0	0.1	-	0.8	86	7.3 ± 0.17	4.2 ± 0.21
1.0	0.1	-	1.0	83	5.2 ± 0.12	4.0 ± 0.35
1.0	-	0.1	-	80	10.8 ± 0.04	3.9 ± 0.12
1.0	-	0.1	0.1	82	16.7 ± 0.20	5.1 ± 0.15
1.0	-	0.1	0.2	90	21.2 ± 0.56	3.4 ± 0.12
1.0	-	0.1	0.4	100	34.3 ± 0.36	5.6 ± 0.09
1.0	-	0.1	0.6	83	18.2 ± 0.19	5.8 ± 0.17
1.0	-	0.1	0.8	80	11.4 ± 0.28	6.2 ± 0.40
1.0	-	0.1	1.0	75	8.3 ± 0.08	7.0 ± 0.12
2.0	0.1	-	-	78	6.7 ± 0.36	5.6 ± 0.15
2.0	0.1	-	0.1	80	7.8 ± 0.72	3.6 ± 0.06
2.0	0.1	-	0.2	87	8.9 ± 0.11	4.0 ± 0.34
2.0	0.1	-	0.4	92	17.6 ± 0.28	3.6 ± 0.46
2.0	0.1	-	0.6	90	9.6 ± 0.03	5.9 ± 0.25
2.0	0.1	-	0.8	85	8.3 ± 0.14	9.7 ± 0.18
2.0	0.1	-	1.0	83	7.1 ± 0.11	8.3 ± 0.06
2.0	-	0.1	-	77	5.7 ± 0.52	4.8 ± 0.21
2.0	-	0.1	0.1	80	7.2 ± 0.35	4.0 ± 0.15
2.0	-	0.1	0.2	85	9.1 ± 0.36	4.8 ± 0.26
2.0	-	0.1	0.4	90	12.8 ± 0.05	3.9 ± 0.37
2.0	-	0.1	0.6	85	8.8 ± 0.29	8.6 ± 0.26
2.0	-	0.1	0.8	85	7.3 ± 0.21	10.5 ± 0.12
2.0	-	0.1	1.0	80	6.7 ± 0.22	9.7 ± 0.18

Among all the combinations tested, maximum number of roots (29.4 ± 0.12) and root length (12.8 ± 0.23) with highest rooting frequency (93%) was observed in NAA (2.0 mg/l) and $AgNO_3$ (0.4 mg/l) supplemented media (**Table 3**).

Among the auxins employed for rhizogenesis, NAA facilitates maximum rooting frequency along with $AgNO_3$ (**Figure 1(e)**). Whereas, the number of roots produced per shoot was less in media containing auxins alone when compared to those produced in the presence of an auxin and silver nitrate. These results emphasized that addition of silver nitrate to the medium along with an auxin significantly augmented the induction of roots. The above results are similar with the previous studies in *Decalepis hamiltonii* [10] and *Vanilla planifolia* [11].

Table 2. Effect of different concentrations of BAP, IAA, and NAA in combination with $AgNO_3$ on multiple shoot regeneration from *in vitro* grown shoot tip explants of *Sphaeranthus indicus*. Observation: After 8 weeks, values are mean ± S.E of 10 replicates.

Concentration of plant growth regulators (mg/l)			Concentration of $AgNO_3$ (mg/l)	Regeneration frequency (%)	Mean no. of shoots/explant ±S.E	Mean no. of shoot length ±S.E (cm)
BAP	IAA	NAA				
1.0	0.1	-	-	65	3.15 ± 0.34	2.7 ± 0.04
1.0	0.1	-	0.1	75	3.6 ± 0.12	3.5 ± 0.27
1.0	0.1	-	0.2	80	4.2 ± 0.54	4.3 ± 0.18
1.0	0.1	-	0.4	85	8.1 ± 0.27	4.9 ± 0.05
1.0	0.1	-	0.6	75	5.2 ± 0.06	5.0 ± 0.31
1.0	0.1	-	0.8	70	3.7 ± 0.19	4.54 ± 0.16
1.0	0.1	-	1.0	65	3.0 ± 0.35	5.3 ± 0.21
1.0	-	0.1	-	72	4.7 ± 0.22	4.6 ± 0.34
1.0	-	0.1	0.1	80	5.3 ± 0.56	5.5 ± 0.19
1.0	-	0.1	0.2	85	9.2 ± 0.23	4.2 ± 0.41
1.0	-	0.1	0.4	90	21.6 ± 0.04	3.9 ± 0.56
1.0	-	0.1	0.6	80	10.9 ± 0.17	5.7 ± 0.29
1.0	-	0.1	0.8	75	7.2 ± 0.20	6.3 ± 0.57
1.0	-	0.1	1.0	70	3.6 ± 0.41	4.0 ± 0.33
2.0	0.1	-	-	65	3.4 ± 0.19	2.8 ± 0.14
2.0	0.1	-	0.1	75	3.8 ± 0.37	3.4 ± 0.11
2.0	0.1	-	0.2	78	4.7 ± 0.29	4.6 ± 0.38
2.0	0.1	-	0.4	85	9.2 ± 0.57	5.3 ± 0.43
2.0	0.1	-	0.6	75	6.3 ± 0.43	4.56 ± 0.27
2.0	0.1	-	0.8	70	5.2 ± 0.26	3.1 ± 0.18
2.0	0.1	-	1.0	64	3.3 ± 0.52	5.4 ± 0.53
2.0	-	0.1	-	65	3.8 ± 0.14	2.47 ± 0.47
2.0	-	0.1	0.1	70	4.5 ± 0.31	3.62 ± 0.10
2.0	-	0.1	0.2	78	6.3 ± 0.26	4.4 ± 0.62
2.0	-	0.1	0.4	83	10.15 ± 0.24	3.3 ± 0.16
2.0	-	0.1	0.6	74	7.6 ± 0.42	5.0 ± 0.31
2.0	-	0.1	0.8	70	4.2 ± 0.28	8.8 ± 0.46
2.0	-	0.1	1.0	65	3.1 ± 0.51	6.5 ± 0.28

3.3. Acclimatization and Hardening of *in Vitro* Raised Plantlets

The well rooted plantlets were separated from the culture tubes, washed thoroughly and transferred to polybags containing soil and vermiculate in 1:1 ratio for hardening. Finally the hardened plants were transferred to field condition for maximum survivability.

4. Conclusion

In conclusion, this is the first report in *Sphaeranthus indicus* with protocol for direct organogenesis from shoot tip explants using ethylene inhibitor silver nitrate. A rapid multiplication rate could be obtained through shoot

Table 3. Effect of $AgNO_3$ and different concentrations of NAA and IAA on *in vitro* rooting of *Sphaeranthus indicus* using MS medium. Observation: After 8 weeks, values are mean ± S.E. of 10 independent determinants.

Plant growth regulators (mg/l)		Concentration of $AgNO_3$ (mg/l)	Regeneration frequency (%)	Mean no. of roots/shoot ±S.E	Mean root length (cm) ±S.E
NAA	IAA				
1.0	-	-	65	5.3 ± 0.12	3.4 ± 0.24
1.0	-	0.1	75	8.6 ± 0.23	3.6 ± 0.17
1.0	-	0.2	80	12.2 ± 0.04	5.1 ± 0.10
1.0	-	0.4	85	17.5 ± 0.18	8.8 ± 0.31
1.0	-	0.6	75	8.2 ± 0.13	4.3 ± 0.34
2.0	-	-	70	9.0 ± 0.21	3.6 ± 0.16
2.0	-	0.1	80	11.4 ± 0.11	4.2 ± 0.12
2.0	-	0.2	85	15.6 ± 0.34	5.8 ± 0.06
2.0	-	0.4	93	29.4 ± 0.12	12.8 ± 0.23
2.0	-	0.6	80	21.0 ± 0.15	3.2 ± 0.13
-	1.0	-	60	4.7 ± 0.26	2.8 ± 0.26
-	1.0	0.1	65	7.5 ± 0.15	3.4 ± 0.16
-	1.0	0.2	70	11.8 ± 0.40	3.6 ± 0.30
-	1.0	0.4	78	14.4 ± 0.36	4.4 ± 0.41
-	1.0	0.6	62	10.3 ± 1.22	2.3 ± 0.2
-	2.0	-	65	8.4 ± 0.36	2.0 ± 0.45
-	2.0	0.1	70	10.2 ± 0.21	2.8 ± 0.11
-	2.0	0.2	75	13.3 ± 0.15	3.5 ± 0.26
-	2.0	0.4	80	17.2 ± 0.92	6.2 ± 0.31
-	2.0	0.6	65	11.6 ± 0.34	6.3 ± 0.14

tip explants by combining the growth regulators with $AgNO_3$. In the present study by the addition of ethylene inhibitor silver nitrate and growth regulators, more number of multiple shoots (three folds) and shoot length was observed compared to control. This protocol has great potential for rapid multiplication and conservation of *S. indicus*.

References

[1] Kirtikar, K.R. and Basu, B.D. (1935) Indian Medicinal Plants, Vol. II. Lalit Mohan Publication, Allahabad, 1347-1348.

[2] Biddington, N.L. (1992) The Influence of Ethylene in Plant Tissue Culture. *Plant Growth Regulation*, **11**, 173-187. http://dx.doi.org/10.1007/BF00024072

[3] Brar, M.S., Moore, M.J., Al-Khayri, J.M., Morelock, T.E. and Anderson, E.J. (1999) Ethylene Inhibitors Promote *in Vitro* Regeneration of Cowpea (*Vigna unguiculata* L.). *In Vitro Cellular and Developmental Biology—Plant*, **35**, 222-225. http://dx.doi.org/10.1007/s11627-999-0082-1

[4] Gong, Y., Gao, F. and Tang, K. (2005) *In Vitro* High Frequency Direct Root and Shoot Regeneration in Sweet Potato Using the Ethylene Inhibitor Silver Nitrate. *South African Journal of Botany*, **71**, 110-113. http://dx.doi.org/10.1016/S0254-6299(15)30159-9

[5] Beyer Jr., E.M. (1976) A Potent Inhibitor of Ethylene Action in Plants. *Plant Physiology*, **58**, 268-271. http://dx.doi.org/10.1104/pp.58.3.268

[6] Murashige, T. and Skoog, F. (1962) A Revised Medium for Rapid Growth and Bioassay with Tobacco Tissue Cultures. *Physiologia Plantarum*, **15**, 473-497. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x

[7] Manchanda, P., kaur, A., Thind, K.S. and Gosal, S.S. (2014) Influence of Ethylene Inhibitor on Shoot Organogensis

and Regeneration in Sugarcane (*Saccharum* spp. Hybrid). *International Journal of Agriculture, Environment and Biotechnology*, **7**, 759-764. http://dx.doi.org/10.5958/2230-732X.2014.01384.9

[8] Bekheet, S. (2013) Direct Organogenesis of Date Palm (*Phoenix dactylifera* L.) for Propagation of True-to-Type Plants. *Scientia Agriculturae*, **4**, 86-92.

[9] Zhao, X.C., Qu, X., Mathews, D.E. and Schaller, G.E. (2002) Effect of Ethylene-Pathway Mutations upon Expression of the Ethylene Receptor ETR1 from *Arabidopsis*. *Plant Physiology*, **130**, 1983-1991. http://dx.doi.org/10.1104/pp.011635

[10] Bais, H.P., Sudha, G., Suresh, B. and Ravishnakar, G.A. (2000) Silver Nitrate Influences *in Vitro* Root Formation in *Decalepis hamiltonii* Wight & Arn. *Current Science*, **79**, 894-898.

[11] Giridhar, P., Reddy, B.O. and Ravishankar, G.A. (2001) Silver Nitrate Influences *in Vitro* Shoot Multiplication and Root Formation in *Vanilia planifolia* Andr. *Current Science*, **81**, 1166-1170.

Abbreviations

BAP 6-benzyl amino purine,
Kn Kinetin,
$AgNO_3$ Silver nitrate,
NAA α-naphthalene acetic acid,
IAA Indole-3-acetic acid,
IBA Indole-3-butyric acid,
MS Murashige and Skoog,
B_5 Gamborg *et al*.,
SH Schenk and Hilderbrandt,
KS Kohlenbach and Schmidt.