International Archive of Applied Sciences and Technology
Int. Arch. App. Sci. Technol; Vol 9 [1] March 2018: 13-18
© 2018 Society of Education, India
[ISO9001: 2008 Certified Organization]
www.soeagra.com/iaast.html

IAAST
ONLINE ISSN 2277- 1565
PRINT ISSN 0976 - 4828

CODEN: IAASCA ORIGINAL ARTICLE

DOI: .10.15515/iaast.0976-4828.9.1.1318

In Vitro Conservation of *Ipomoea mauritiana* Jacq.

Bindu.T.K[1], Sheema Dharmapal P [1], P.S.Udayan[1], Rini Vijayan [2], Raghu A.V.[2]
[1] PG Department of Botany and Research Centre, Sree Krishna College, Guruvayur, Ariyannur P. O, Thrissur Dist, Kerala, India. 680102.
[2] Kerala Forest Research Institute, (KFRI), Peechi, Thrissur 680653, Kerala, India.
Corresponding Author:bindutksahadevan@gmail.com

ABSTRACT
The industrial use of medicinal plants is dramatically increasing with the resurgent public concern in plant based medicine. Ipomoea mauritiana of the family Convolvulaceae is a commonly used Ayurvedic medicinal plant of India. The effective protocol developed in this study provides an efficient method of micropropagation of this tuberous medicinal plant. Surface sterilized nodal explants collected from six months old disease free plants were inoculated on Murashige and Skoog (MS) media supplemented with different concentrations and combinations of BAP and KN. Maximum percentage of shoot organogenesis was obtained on MS basal media containing 2 mg L^{-1} BAP and 1 mg L^{-1} KN. The shoot proliferation rate and the number of days for shoot induction studied indicate that the optimum treatment was the combination of 2 mg L^{-1} BAP and 1 mg L^{-1} KN. The highest shoot length was observed on MS media supplemented with 2 mg L^{-1} KN. Rooting was increased on half MS augmented with 0.5 mg L^{-1} IAA. Micropropagated plants were successfully acclimatized to ex vitro conditions and the survival rate was 85%. Plants transferred to a greenhouse showed normal growth and development.
***Key words**: Nodal explants, Convolvulaceae, In vitro, Ipomoea mauritiana.*

Received 24/11/2017 Revised 24/12/2017 Accepted 18/02/2018

Citation of this article
Bindu.T.K, Sheema Dharmapal P, P.S.Udayan[1], Rini Vijayan, Raghu A.V.*In Vitro* Conservation of *Ipomoea mauritiana* Jacq. Int. Arch. App. Sci. Technol; Vol 9 [1] March 2018. 13-18.

INTRODUCTION

Ipomoea mauritana is a branched glabrous twinning shrub with lobed, simple leaves and purple, bisexual flowers. The species is distributed in tropical parts of the world and in evergreen, deciduous forests of India. *I. mauritiana* has ethanomedical importance and tubers are taken orally to increase milk in nursing mothers by the Garo tribal community, Bangladesh [1]. It is one of the source plant of '*Vidari*', an ingredient of important Ayurvedic formulations. '*Vidari*' is used as demulcent, aphrodisiac and galactogogue [2]. The sweet, stimulant and carminative tuberous roots are used in emaciation, spermatorrhea and enteric fever [3].

Leaves, stem and roots of *I. mauritiana* are used in Folk Medicine to treat infrequent urination, excessive bile secretion and pain in bone [4]. The root tubers are important source of phytoconstituents such as scopoletin, β-sitosterol, taxaxerol and taraxerol acetate [5]. Dietary antioxidants lower the risk of age related degenerative diseases induced by oxidative damage [6]. The antioxidants in different fractions of *I. mauritiana* exhibited radical scavenging potential and high therapeutic action [2]. Studies indicated that callus extract of *I. mauritiana* show analgesic and antihyperglycemic potential [1].

Exploitation of medicinal plants for pharmaceutical industry coupled with slow reproduction, habitat loss and destruction of underground parts are the crucial factors in meeting the goal of sustainability [7 & 8]. As a result of urban development and unscientific collection, many important plant species like *I. mauritiana* are disappearing at an alarming rate. The present study achieves an efficient *in-vitro* propagation of *I. mauritiana* using

nodal explants from field-grown plants. Micropropagation is an alternate method to propagate medicinal plants that are sources of important therapeutic aid in disease treatment.

MATERIALS AND METHODS

Preparation of explants

Young, tender nodal segments were collected from six months old plants regenerated from root tubers. These explants were put through a series of sterilization procedures to minimize the risk of bacterial and fungal contaminations. The initial step involved washing of explants in soap water to remove surface contaminants followed by washing thoroughly in distilled water. The explants were then immersed in Bavistin (15 g L^{-1}), Cefotaxime (200 mg L^{-1}) and Tetracycline (200 mg L^{-1}) solution for 30 minutes followed by distilled water wash. Then these nodal segments were treated with 0.1% (w/v) $HgCl_2$ for 4 minutes under aseptic conditions. These were then rinsed five times with sterile distilled water to remove the sterilants. These explants were then trimmed to an average length of 1.5 - 2.0 cm and inoculated on culture medium taken in borosilicate test tubes.

Culture media and conditions

The nodal segments were cultured on semi solid MS media [9] supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. After the addition of growth regulators the pH of the media was adjusted 5.8 prior to autoclaving at 121°C for 15 minutes. The experiment was carried out at a temperature of 25± 2°C less than 16 hours light and eight hours dark photoperiod regimes maintained under fluorescent light having 2500-3000 lux light intensity with 70 to 80% relative humidity of the growth chamber.

Shoot proliferation and elongation

For *in vitro* shoot induction from nodal explants, various concentrations of 6-Benzylaminopurine - BAP (0.25, 2.00, 2.50, 3.00, 3.50 mg.L^{-1}) and Kinetin - KN (0.25, 0.10, 2.50, 3.00, 3.50 mg.L^{-1}) either alone or in combinations were experimented. MS without growth regulators was kept as control. Data on percent response, number of days for shoot induction and number of multiple shoots were recorded after two weeks of culture. Subcultures were done every 15 days interval into fresh media. Single *in vitro* shoots were inoculated in to similar media taken in jam bottles for proliferation. Healthy 2 cm long microshoots were transferred into elongation media supplemented with KN (0.25, 0.5, 1.0, 1.5, 2.0 mg.L^{-1}). The shoot length and the number of leaves produced were observed and recorded.

In vitro rooting and acclimatization

The elongated *in vitro* shoots were transferred to full strength MS and half strength MS alone and to media supplemented with 0.5 mg.L^{-1}IAA for *in vitro* rooting. To experiment the most appropriate rooting medium, the following characteristics were observed and recorded: number of days for root induction, mean number of roots per plant and the root length after two weeks of culture. Two to three weeks old rooted plantlets were gently picked from culture vessels and then rinsed with distilled water to remove adhering agar and transferred to polycups containing soil and vermiculate in the ratio 1:2. The acclimatized plantlets from the mist chamber were gradually exposed to natural conditions, each day few hours. After 8 weeks, the acclimatized plants were transferred to greenhouse.

Statistical Analysis

All experiments were conducted with three replications, having 30 samples each. The effect of various treatments on selected growth parameters was measured quantitatively and statistically tested using analysis of variance (ANOVA) using "R-statistics package" version 11.0. The significance of the mean values of different treatments was assessed by Duncan's New Multiple Range Test (DMRT) at $p < 0.05$.

RESULTS AND DISCUSSIONS

Effect of BAP and KN on multiple shoot induction and shoot proliferation from nodal explants

Plant growth regulators are the most important factors for successful plant regeneration. Cytokinins have a major role on plant development, such as the regulation of shoot induction and multiplication and the promotion of cell division and cell expansion [10].Various concentrations and combinations of BAP and KN were experimented on

percent response of explants and on the number of shoots per explants. A significant enhancement of the morphogenetic response was evident at the combination of 2 mg.L^{-1} BAP and 1 mg.L^{-1} KN (Fig 1).With BAP (0.25, 2.0, 2.5 mg.L^{-1}) in combination with three levels of KN (2.5, 1.0, 0.25 mg.L^{-1}) showed 60 % to 87% shoot induction within 7 to 9 days. No shoots were developed in explants grown on control medium. After 35 days of culture, highest number of shoots per explants (16.60 ± 0.26) was initiated on MS medium containing 2.0 mg.L^{-1} BAP and 1.0 mg.L^{-1} KN which was the optimum treatment for shoot proliferation. These two cytokinins were effective in terms of shoot induction from nodal explants of *Stereospermum suaveolens* (G. Don) DC. [11], *Costus speciosus* (Koenig) J.E. Smith were analysed [12]. In the present study synergistic effect of the combination BAP with KN was found to be stimulatory for shoot multiplication. The combined effect of BAP and KN showed high rate of shoot multiplication in other crops like *Bambusa glaucescens* [13] and *Lagenaria siceraria* [14].

The use of BAP and KN alone as growth regulator did not result in high rate of multiple shoot induction compared to its combination treatments. The individual effect of cytokinins like BAP and KN on multiple shoot induction was studied by culturing the nodal explants on MS media with two different concentrations of BAP (3.0, 3.5 mg.L^{-1}) and KN (3.0, 3.5 mg.L^{-1}) alone. The percent response of shoot induction and number of multiple shoots per explants were significantly reduced (Table 1 & 2). Shoot induction delayed for 10 to 12 days at these concentrations of growth regulators. Treatments without BAP showed decreased shoot multiplication rate. Several medicinal plants exhibited increased shoot multiplication on BAP supplemented media [15] which is in conformity with the results of the present study (Table 2).BAP alone was significantly effective on shoot multiplication of *Chlorophytum borivilianum* and the combination treatment of BAP and KN stimulated highest shoot proliferation compared to their individual treatment [16]. In the present investigation, higher concentration of BAP (3, 3.5 mg.L^{-1}) adversely affected the shoot proliferation rate.

Effect of KN on shoot elongation

Excised microshoots showed elongation when inoculated in to MS media fortified with KN at five levels (0.25, 0.5, 1.0, 1.5, 2 mg.L^{-1}). The shoot length differed significantly among the KN concentrations and the length of shoots was enhanced in the presence of all tested concentration of KN compared to the control (Table 3). The lower concentrations cytokinin have been reported to show a crucial role as promoters of cell division and in the induction and development of meristematic tissues leading to the elongation of shoots of sugarcane [17]. Of the different concentrations of KN tested, 2 mg.L^{-1} elicited longer shoots with an average length of 7cm. Similar results were reported in *Matthiola incana* [18] with best shoot length and maximum number of nodes at 2 mg.L^{-1} of KN. It was reported that highest plant length on micropropagation of *Gerbera jamesonii* resulted in MS medium with 2 mg.L^{-1} KN [19]. KN was more effective on shoot elongation in *in vitro* propagation of *C. borivilianum* [16]. In the present experiment, the length of shoots and the number of leaves were observed to increase with increasing concentration of KN. Maximum number of broad, dark green leaves (7.80±0.26) were produced on MS media with 2.0 mg.L^{-1} KN (Fig 1D). The number and size of leaves were significantly reduced with lower concentrations of KN (0.25, 0.5, 1.0 mg.L^{-1}).

Effect of Media and IAA on rooting

Root induction of microshoots is an important step in micropropagation protocol. Rooting was observed from 8 to 12 days of culture on rooting media. Low rate of rooting was observed in the media without IAA. Rooting experiments showed that half MS medium with 0.5 mg.L^{-1} IAA accelerated rooting and it initiated 10.56±0.50 roots (Table 4) from *in vitro* shoots. Healthy roots of 5.86±0.45 cm length initiated within 7.90±0.20 days that were easily established during transfer to green house. Morphogenic response of elongated shoots after two weeks revealed that full strength and half strength MS basal media without growth regulators failed to respond in terms of high rate of root multiplication (Table 4). Weak and elongated roots were observed in this media that failed to acclimatize during hardening (Fig.1H) Moreover, callus growth was observed from the base of *in vitro* shoots on full MS media supplemented with 0.5 mg.L^{-1} IAA. In the present study good response for rooting was resulted in half strength MS media with 0.5 mg.L^{-1} IAA than full strength MS (Fig. 1 F). This rooting treatment resulted in a greater number of shorter and healthy roots

 ©2018 Society of Education, India

per shoots. It has been reported that elongated shoots of *Tinospora formanii* showed optimum response of rooting in half strength MS than full strength MS media [20]. *In vitro* raised *Withania coagulans* shoots were successfully rooted on half strength MS supplemented with NAA while callus formation was observed at the base of *in vitro* raised shoots when inoculated on full strength MS medium supplemented with IAA [21]. The use of comparatively lower concentration of growth regulator is an important factor, as it minimize the risk of producing genetically altered plantlets [22].

Table 1: Effect of BAP and KN on multiple shoot induction using nodal explants

Treatments	MS+PGR ($mg.L^{-1}$)	% of explants showing shoot induction	Number of days for shoot induction
T_0	MS basal (control)	00.000^h	00.000^g
T_1	BAP 0.00+ KN 3.50	42.20 ± 0.17^g	12.46 ± 0.25^a
T_2	BAP 0.00+ KN 3.00	48.37 ± 0.41^e	10.00 ± 0.20^d
T_3	BAP 0.25+ KN 2.50	60.50 ± 0.50^c	9.57 ± 0.11^{de}
T_4	BAP 2.00+ KN 1.00	87.37 ± 0.31^a	6.90 ± 0.30^f
T_5	BAP 2.50 + KN 0.25	76.93 ± 0.90^b	9.50 ± 0.36^e
T_6	BAP 3.00 + KN 0.00	52.20 ± 0.26^d	10.76 ± 0.24^c
T_7	BAP 3.50 + KN 0.00	45.33 ± 0.25^f	11.63 ± 0.41^b

Note: Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly ($P>0.05$).

Table 2: Effect of BAP and KN on multiple shoot induction after 35days of culture

Treatments	MS+PGR ($mg.L^{-1}$)	Number of shoots
T_0	MS basal (control)	00.000^h
T_1	BAP 0.00 + KN 3.50	06.43 ± 0.24^g
T_2	BAP 0.00 + KN 3.00	07.50 ± 0.30^f
T_3	BAP 0.25 + KN2.50	11.96 ± 0.20^c
T_4	BAP 2.00 + KN1.00	16.60 ± 0.26^a
T_5	BAP2.50 + KN 0.25	14.00 ± 0.55^b
T_6	BAP3.00 + KN 0.00	10.83 ± 0.32^d
T_7	BAP 3.50 + KN 0.00	09.83 ± 0.35^e

Note: Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly ($P>0.05$).

Table 3: Effect of KN on shoot length and number of leaves

Treatments	MS+PGR($mg.L^{-1}$)	Shoot length (cm)	Number of leaves
T_0	MS basal(control)	0.00^e	00.000^e
T_1	KN 0.25	3.70 ± 0.08^d	4.26 ± 0.20^d
T_2	KN 0.5	4.13 ± 0.30^c	5.06 ± 0.14^c
T_3	KN 1	4.53 ± 0.40^c	5.50 ± 0.40^c
T_4	KN 1.5	5.16 ± 0.14^b	6.66 ± 0.25^b
T_5	KN 2.0	6.87 ± 0.21^a	7.80 ± 0.26^a

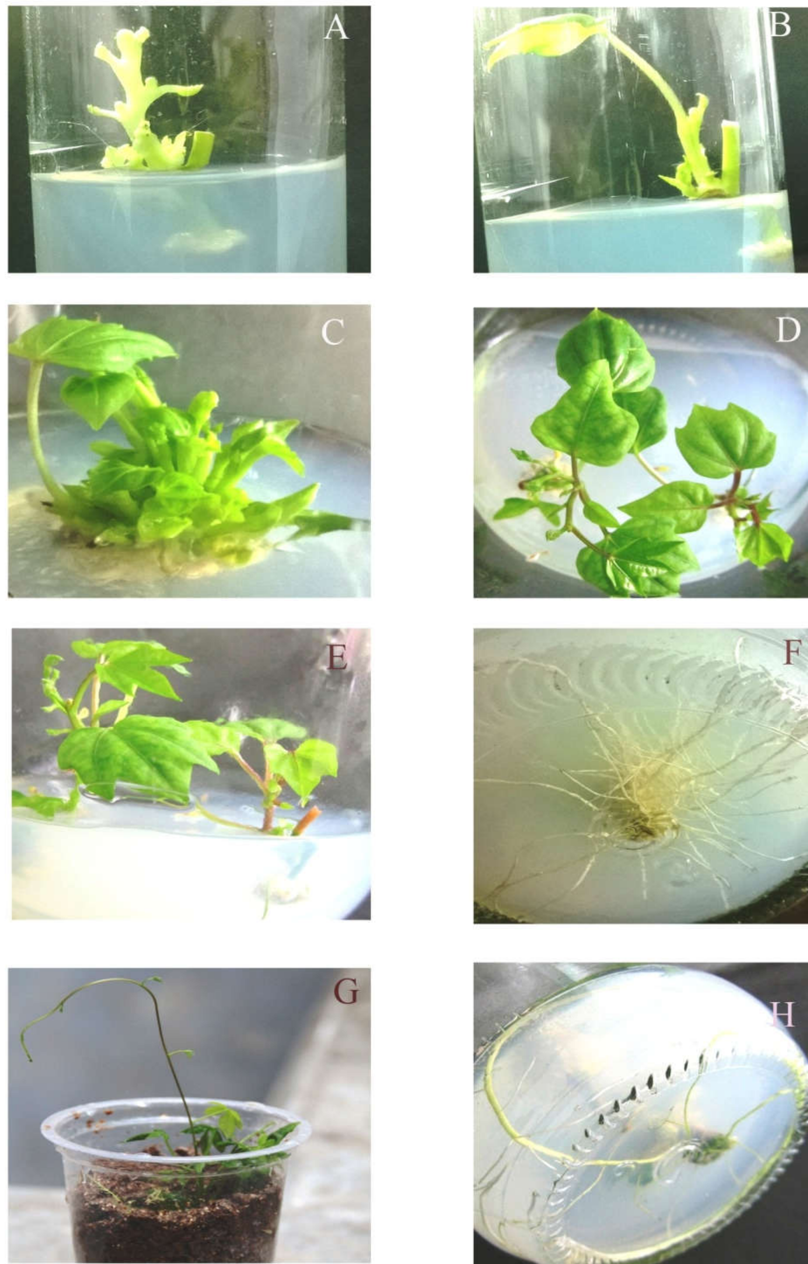
Note: Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly ($P>0.05$).

Table 4: Effect of media and IAA on *in vitro* rooting

Treatments	Media strength	PGR ($mg.L^{-1}$)	Number of roots	Root length(cm)	Number of days for rooting
T_0	Full MS	Basal	1.70 ± 0.53^d	9.70 ± 0.53^a	11.60 ± 0.46^a
T_1	Full MS	0.5 IAA	2.83 ± 0.30^c	8.43 ± 0.28^b	9.73 ± 0.47^b
T_1	Half MS	Basal	3.93 ± 0.35^b	7.30 ± 0.20^c	8.67 ± 0.25^c
T_2	Half MS	0.5 IAA	10.56 ± 0.50^a	5.86 ± 0.45^d	7.90 ± 0.20^d

Note: Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly ($P>0.05$)

 ©2018 Society of Education, India



Fig;1.Micropropagation of *Ipomoea mauritiana*; A&B, Shoot bud initiation on MS supplemented with BAP 2mg.L-1+ KN 1mg.L-1; C, Multiple shoot induction from nodal segments in MS supplemented with BAP 2mg.L-1+ KN 1mg.L-1; D, Shoot elongation in MS supplemented with KN 2 mg.L-1; E, Root initiation; F, Root mul tiplication in half MS supplemented with 0.5 mg.L-1 IAA; G, Acclimatisation, H; Elongated roots in full MS medium, failed to acclimatize.

CONCLUSION

This study aims to develop a standard protocol that can be used for the *ex situ* conservation and propagation of this species which will minimize the pressure on wild population. The protocol can be applied for the mass multiplication of this valuable taxon which will help in the sustainable availability of root tubers and plant products.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. G. Jayakrishnan, Head of the Department of Botany, Sree Krishna College, Guruvayur and Dr. E.M. Muraleedharan, Scientist, Kerala Forest Research Institute, KFRI, Peechi for providing facilities and encouragement during the work.

REFERENCES

1. Saiful, I., Md Rasel, A., Rahat A.M., Shahnaz, R., Safiul Azam, F.M., Rownak, J., & Mohammed, R. (2015). Callus extract of *Ipomoea mauritiana* show analgesic and antihyperglycemic activity in Swiss albino mice., J. Appl. Pharm. Sci., 5 (10): 044-047.
2. Pandey, G. (2004). DravyagunaVijnana, Part III, Chowkhamba Krishnadas Academy, Varanasi, (Reprint) 852.
3. Rahmatullah, M., Nuruzzaman, M., Hossan, M.S., Khatun, M.A., Rahman M.M., Jamal, F., Harun-Or-Rashid, M., Nasrin. D., Seraj, S. & Jahan, R. (2010). An ethnomedicinal survey of folk medicinal practitioners of Shitol Para village, Jhalokati district, Bangladesh. Adv Nat Appl Sci., 4: 85-92.
4. Khan, M. S., Nema, N., Kharya, M. D. & Khanam, S. (2009). Chromatographic estimation of maturity based phytochemical profiling of *Ipomea mauritiana.* International J of Phytomedicine., 1: 22-30.
5. Atoui, A., Mansouri, A., Boskou, G. & Kefalas, P. (2005). Tea and herbal infusions; their antioxidant activity and phenolic profile. Food Chem., 89: 27-36.
6. Sulaiman, C.T., Geetha, S.P. & Indira, B. (2014). Identification of phenolic antioxidants in Ipomoea mauritiana Jacq. using spectrophotometric and mass spectroscopic studies. Avicenna J Phytomed.., 4(2): 89-96.
7. Ghimire, S., K McKey, D. & Aumeeruddy-Thomas, Y. (2005). Heterogeneity in ethnoecological knowledge and management of medicinal plants in the Himalayas of Nepal: Implication for conservation. Ecol Soc., 9 (3): Art 6.
8. Kala, C.P. (2005). Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. Ethnobiol Ethnomed., 1: 11.
9. Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. J. Plant Physiol., 15: 473-497.
10. Mok, D.W.S. & Mok, M.C. (2001). Cytokinin metabolism and action. Ann. Rev Plant Physiol Plant Mole. Biol., 52: 89–118.
11. Trivedi, D.R. & Joshi, A.G. (2014). *In vitro* Shoot Regeneration of *Stereospermum suaveolens* DC. using cotyledonary node and nodal explants. Plant Tissue Culture & Biotechnology., 24 (2): 235-246.
12. Raghu, A.V., Geetha, S.P., Martin, G., Balachandran, I. & Ravindran, P.N. (2006). Further research on clonal propagation of *Costus speciosus*. Tropical Medicinal Plants., 7 (2): 229-231.
13. Shirin, F. & Rana, P.K. (2007). *In vitro* plantlet regeneration from nodal explants of field grown culms in *Bambusa glaucescens* Willd. Plant Biotechnol Rep., 1 :141–7.
14. Saha S. Mori H. & Hattori K. (2007). Synergistic effect of kinenin and benzyl adenine plays a vital role in high frequency regeneration from cotyledon explants (*Lagenaria siceraria*) in relation to ethylene production. Breeding Science., 57: 197–202.
15. Lakshimi, M. & Mythili, S. (2003). Somatic embryogenesis and regeneration of callus cultures of *Kaempferia galanga* — A medicinal plant. J of Medicinal and Aromatic Plant Science., 25: 947–51.
16. Mehdi, F.A., Maheran, A.A., Nurashikin, K. & Ismanizan, I. (2014). Effect of cytokinin types, concentrations and their interactions on *in vitro* shoot regeneration of *Chlorophytum borivilianum* Sant. & Fernandez. Electron J Biotechn., 17(6): 275–279.
17. Tolera, B., Mulugeta, D. & Derbew, B. (2014). Effects of 6-Benzyl aminopurine and Kinetin on *In vitro* shoot multiplication of Sugarcane (*Saccharum officinarum* L.) Varieties. Adv Crop Sci Tech ., 2(3) :1-5.
18. Afshin, A., Behzad, K., Alireza, T. & Sahar B.Z. (2011). Effect of different concentrations of kinetin on regeneration of ten weeks (*Matthiola incana*). Plant Omics J., 4(5): 236 - 238.
19. Varnousefaderani, M.T., Rabori, N.A. & Nosrati, S.Z. (2010). Optimization of *in vitro* culture for Gerbera cv. Tropic Blend. Seed and Plant Production J., 4: 389-401.
20. Sheema, D.P., Bindu, T.K., Udayan, P.S. and Elyas, K.K. (2017). *In vitro* conservation of *Tinospora formanii* Udayan and Pradeep - A Rare Endemic Plant from Western Ghats IOSR. J of Biotechnology and Biochemistry., 3(3): 65-69.
21. Harsh, J., Sureshkumar, N., Deepak, S., Mafatlal, M.K. & Nataraj, M. (2016). *In vitro* Shoot Multiplication of *Withania coagulans* (Stocks) Dunal, PTC & B., 26(2):187-195.
22. Raghu, A.V., Geetha, S.P., Martin, G., Indira, B., Ravindran, P.N. & Mohanan, K.V. (2007). An improved micropropagation protocol for Bael- A vulnerable medicinal tree. Research J of Botany., 2(4): 186-194.

 ©2018 Society of Education, India