e- ISSN: 2394-5532
p- ISSN: 2394-823X

Scientific Journal Impact Factor: 3.762

International Journal of Applied And Pure Science and Agriculture

www.ijapsa.com

Effect of Some Plant Growth Regulators on Regenerative Potential of Nodal Explants of *Uraria picta* (Jacq.) DC.

Shivani Verma[1] and B. D. Vashistha[2]

[1]Research Scholar, [2]Professor

Department of Botany, Kurukshetra University, Kurukshetra -136119, Haryana, India

Abstract

Effect of some plant growth regulators have been studied on the regenerative potential of nodal explants of Uraria picta (Jacq.) DC. under in vitro conditions. Multiple shoots were induced on Murashige and Skoog's medium supplemented with varying concentrations of auxins (IAA, NAA, 2, 4-D, IBA) and cytokinins (BAP, KN) individually as well as in various combinations. The maximum number of shoots were induced on MS medium fortified with BAP (1.0 mg/l) and IAA (1.0 mg/l) in combination. The maximum average shoot length (3.5cm) was observed on BAP (1.0 mg/l). Root induction on in vitro generated shoots was achieved on half-strength MS medium supplemented with IBA (1.0mg/l). The in vitro raised plantlets were acclimatized successfully to pots containing a mixture of autoclaved soil, sand and compost in 2:1:1 ratio with 70% survival.

Abbreviations: BAP, 6-Benzyl aminopurine; KN, Kinetin, IAA, Indole-3-acetic acid; IBA, Indole-3-butyric acid; NAA, Naphthalene acetic acid; 2, 4-D, 2, 4-Dichlorophenoxy acetic acid.

Keywords: Prashniparni, Uraria picta, Fabaceae, Multiple shoots, Auxins, Cytokinins.

I. INTRODUCTION

Uraria picta (Jacq.) DC. (family-Fabaceae) is a tall, suffruticose herb well known for its medicinal properties. It is commonly known as Prsniparni or Chitraparni (Sanskrit) and Pithvan (Hindi). It is commonly found in dry grasslands producing poorly viable seeds [1]. The whole plant is medicinally important. Roots are aphrodisiac in nature. The fruits and pods are effective against oral sores in children and the root decoction is prescribed against cough and fever [2] [1]. It is a major ingredient of an Ayurvedic medicine Dasamula which is used to cure the patients of primary neurological disorders [3]. The herb is also used in preparation of drug Abana, for the treatment of tachycardia, hypertension and angina [4]. Pulverized leaves of this plant are used for curing gonorrhea. Prsniparni is also known for healing factures as it has the property of accumulation of phosphorus and deposition of calcium. Traditionally the plant is used as an antidote to the venom of a dangerous Indian snake, *Echis carinata* [2]. This species is increasingly becoming rare due to over exploitation by various pharmaceutical industries as well as local tribes for medicines. It is naturally propagated by seeds. Seed set and seed viability is poor and percentage of germination is very low [5]. Since conventional methods of propagation are very slow and inadequate to provide the planting material required for large scale cultivation. Therefore, there is an urgent need to develop alternative means for the propagation of genetically stable and true-to-type progeny of this species. Plant propagation by means of tissue culture represents one of the possible approaches to overcome these problems. So, the present study has been designed to develop a suitable *in vitro* technique for rapid and large scale multiplication of this important endangered, medicinal plant species.

II. MATERIALS AND METHODS

A. Plant material

The young plants were collected from "Dr. Sushila Tiwari Herbal Garden" Rishikesh (Uttarakhand) India. These were planted in the botanical garden of Kurukshetra University, Kurukshetra to provide explant source. Nodal explants (1.0-1.5cm) were excised from actively growing shoots and then washed in running tap water to remove dust particles. All the explants were washed with liquid detergent Teepol solution for 5 min. and again explants were washed several times with tap water followed by washing with distilled water to remove all the traces of detergent. Surface sterilization was carried out in Laminar Air Flow chamber by treating these explants with 0.1% (w/v) mercuric chloride solution for 4-5 minutes and subsequently washed with sterilized double distilled water to remove all the traces of mercuric chloride. The explants were trimmed aseptically to smaller segments and used. The surface sterilized nodal explants were inoculated on MS medium.

B. Culture media and culture conditions

Culture medium contains MS media salts, 3% sucrose and gelled with 0.8% (w/v) agar [Himedia laboratories, India]. The pH of the medium was adjusted to 5.8 by 1N NaOH and 1N HCl. The media were sterilized in an autoclave at 121^0C temperature and 15 psi for 20 min. The cultures were incubated at a temperature of 25±2°C and a photoperiod of 16 hours (light intensity 3500 lux) and 8 hours of dark period.

C. Shoot initiation and multiplication

MS basal medium supplemented with various concentrations (0.5-4.0 mg/l) of auxins (IAA, NAA, 2, 4-D and IBA) and cytokinins (BAP, KN) alone and in combinations were used for shoot initiation and multiplication. All the cultures were transferred to fresh medium after 2-3 weeks to maximize shoot multiplication.

D. Root formation

The *in vitro* raised shoots (3.0-4.0cm long) were aseptically excised and implanted on the half strength MS medium supplemented with NAA, IAA and IBA (0.5-2.0 mg/l) individually for root development. Data on the number of roots were recorded after 4 weeks.

E. Hardening and acclimatization

Complete regenerated plantlets with sufficient roots were taken out from the medium and washed in sterile distilled water to remove the traces of agar. The plants were then transferred to small plastic cups (6cm diameter) containing sterilized soil, sand and vermiculite mixture (2:1:1). The cups were covered with polythene bags with small holes to maintain high humidity (80-90%) and kept in the culture room (Fig. 1F). The plantlets were irrigated with MS ¼ strength salt solution on alternate days. These plants were taken out from the culture room after 2 weeks and polythene bags were removed for 3-4 hours daily to expose the plants to natural conditions for acclimatization. After about one month, the surviving plants were shifted to the pots containing sterilized garden soil, sand and compost (2:1:1) and maintained in glasshouse to get acclimatized (Fig. 1G). After 4 weeks, the plants were transferred to the soil under natural conditions.

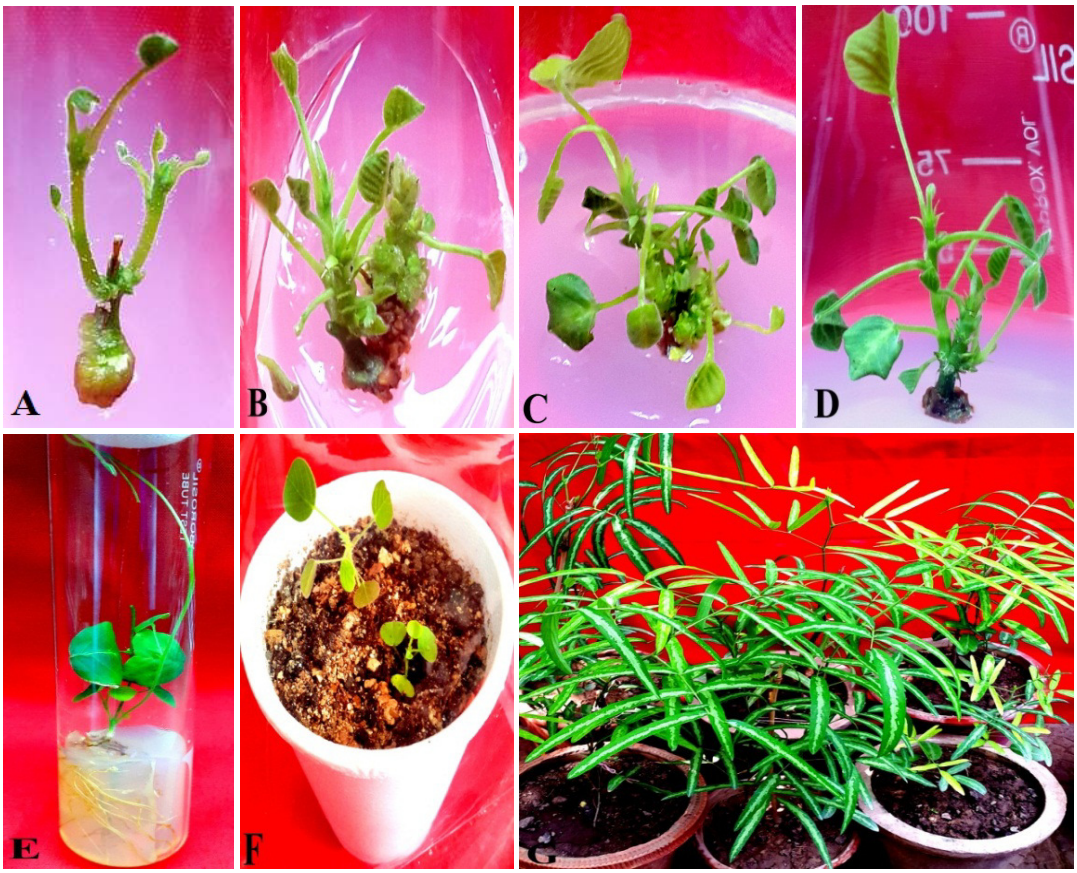

Fig. 1: Direct regeneration of shoots from nodal explants on MS medium fortified with auxins and cytokinins individually and in combinations.

(A) Shoot regeneration on MS+IAA 1.0 mg/l. (B) Shoot regeneration on MS+BAP 1.0 mg/l. (C) Shoot multiplication on MS+BAP 1 .0mg/l+IAA 1.0 mg/l. (D) Shoot regeneration on MS+KN 1.0 mg/l+ IAA 0.5 mg/l. (E) In vitro regenerated shoots with well-developed roots on MS half strength+IBA 1.0 mg /l. (F) Establishment and acclimatization of in vitro regenerated plantlets. (G) Hardening of plantlets in pots.

F. Statistical analysis

Each experiment was repeated three times and each treatment had 10 replicates. The number of explants exhibiting regeneration was identified and the number of shoots, shoot length, number of roots and root length per explant was determined. The data were analysed using one-way analysis of variance (ANOVA) and means were compared using Duncan's Multiple Range Test at 5% level of significance ($p<0.05$).

III. RESULTS AND DISCUSSION

Nodal explants cultured on MS medium devoid of growth regulators (control) failed to stimulate bud break even when the cultures were maintained beyond the 4 weeks period which is probably due to insufficient level of endogenous growth regulators to induce bud break and it requires an exogenous supply. Medium supplemented individually with auxins and cytokinins induced formation of shoots and among the various concentrations, BAP (1.0 mg/l) was proved to be optimum for initiation of shoots in which about 4.5 shoots were produced with maximum 94.4% response and maximum average shoot length of 3.5cm

(Table 1, Fig. 1B). As the concentration of BAP increases or decreases from 1.0 mg/l, rate of shoot regeneration decreases.

Table 1. Effect of auxins and cytokinins on nodal explants of Uraria picta cultured on MS medium.

Medium and conc. of growth regulators (mg/l)	Days required for bud initiation (mean value)	% Response*	No. of shoots per explant*	Shoot length* (cm)	Callus growth
MS Medium	0.00	0.00±0.00	0.00 ± 0.00^{k}	0.00 ± 0.00^{j}	-
MS+IAA(0.5)	7.0	90.2±1.93	1.6 ± 0.11^{fghi}	2.1 ± 0.10^{e}	+
(1.0)	6.4	88.8±1.93	1.9 ± 0.20^{fg}	2.5 ± 0.15^{d}	+
(2.0)	6.0	91.6±0.25	1.7 ± 0.14^{fghi}	2.0 ± 0.05^{ef}	+
(4.0)	6.6	90.2±1.93	1.8 ± 0.10^{fgh}	1.2 ± 0.05^{hi}	+
MS+NAA(0.5)	9.0	70.0±0.26	1.4 ± 0.30^{ghi}	1.3 ± 0.07^{hi}	++
(1.0)	8.6	77.7±1.93	1.5 ± 0.25^{ghi}	1.5 ± 0.07^{gh}	++
(2.0)	8.0	80.5±1.97	1.2 ± 0.21^{hi}	1.7 ± 0.06^{fg}	++
(4.0)	8.3	72.2±1.97	1.1 ± 0.23^{ij}	1.0 ± 0.00^{i}	++
MS+2,4D(0.5)	0.00	0.00±0.00	0.00 ± 0.00^{k}	0.00 ± 0.00^{j}	-
(1.0)	15.0	53.3±1.95	0.55 ± 0.04^{jk}	1.7 ± 0.20^{fg}	+
(2.0)	14.0	66.7±2.90	0.62 ± 0.05^{j}	1.4 ± 0.25^{gh}	+
(4.0)	0.00	0.00±0.00	0.00 ± 0.00^{k}	0.00 ± 0.00^{j}	-
MS+BAP(0.5)	7.5	90.2±1.93	2.8 ± 0.52^{cd}	3.2 ± 0.10^{ab}	++
(1.0)	6.6	94.4±1.97	4.5 ± 0.20^{a}	3.5 ± 0.11^{a}	++
(2.0)	5.5	91.6±2.28	3.9 ± 0.24^{b}	2.1 ± 0.06^{e}	++
(4.0)	6.2	83.3±1.97	2.6 ± 0.09^{de}	1.2 ± 0.18^{hi}	++
MS+KN (0.5)	6.6	84.7±1.97	2.5 ± 0.12^{de}	2.6 ± 0.12^{d}	+ +
(1.0)	6.0	86.4±2.20	3.2 ± 0.15^{c}	2.7 ± 0.14^{cd}	+ +
(2.0)	5.5	91.2±1.93	3.8 ± 0.18^{b}	3.0 ± 0.12^{bc}	+ +
(4.0)	5.5	90.6±1.93	2.2 ± 0.12^{ef}	2.0 ± 0.10^{ef}	+ +
LSD (P<0.05)			0.54	0.31	
ANOVA			$F_{20,42}$ =45.96	$F_{20,42}$ =80.78	

- *Values are Means ±S.E of three independent experiments, each consisted of 10 replicates per treatment. Data were recorded after 10 weeks of culture.
- No Response, + Poor growth, + + Moderate growth, + + + Good growth.
- Means values within a column sharing the same superscript are not significantly different at P<0.05 according to Duncan's Multiple Range Test.

The addition of BAP and KN with various concentrations of IAA significantly increases the frequency of shoot formation as compared to BAP and IAA alone, as maximum number of shoot buds was initiated in the BAP and IAA combinations (Table 2). Among all the tested growth regulator combinations, BAP (1.0 mg/l) with IAA (1.0 mg/l) was found to be best which promoted maximum number of shoots (6.5) with an average shoot length of 2.4cm (Table 2, Fig. 1C). Explants cultured on KN (1.0 mg/l) with IAA (0.5 mg/l) supplemented medium produced 3.5 average shoots with an average shoot length of

2.4cm (Table 2, Fig. 1D). As the concentration of IAA increases or decreases from 1.0 mg/l in combination with BAP (1.0 mg/l), average number of shoots decreases (Table 2).

Table 2. Effect of auxins and cytokinins in combination on nodal explants of Uraria picta cultured on MS medium.

Medium and conc. of growth regulators (mg/ l)	Days required for response (mean value)	Per cent Response*	No. of shoots per explant*	Shoot length* (cm)	Callus growth
MS+BAP(0.5)+IAA(0.5)	7.0	83.3±3.42	3.6 ± 0.14^{def}	1.1 ± 0.06^{fg}	+
(1.0)	6.6	88.8±1.93	4.1 ± 0.15^{cd}	1.2 ± 0.08^{efg}	+
(2.0)	6.4	84.7±1.97	3.0 ± 0.17^{ghi}	1.2 ± 0.10^{efg}	+
MS+BAP(1.0)+IAA(0.5)	6.2	90.2±1.93	5.1 ± 0.20^{b}	1.6 ± 0.04^{cde}	++
(1.0)	6.0	94.4±1.97	6.5 ± 0.21^{a}	2.4 ± 0.05^{a}	++
(2.0)	6.5	91.6±3.37	4.5 ± 0.18^{c}	1.4 ± 0.09^{defg}	++
MS+BAP(2.0)+IAA(0.5)	6.6	83.3±3.42	2.5 ± 0.13^{ij}	1.0 ± 0.13^{g}	++
(1.0)	6.7	80.5±1.97	3.2 ± 0.17^{fgh}	1.5 ± 0.22^{cdef}	++
(2.0)	6.0	77.7±1.93	3.8 ± 0.19^{de}	1.3 ± 0.11^{defg}	++
MS+KN(0.5)+IAA(0.5)	7.0	63.8±3.90	2.3 ± 0.12^{j}	1.2 ± 0.23^{efg}	+
(1.0)	7.7	66.6±0.00	2.4 ± 0.10^{j}	1.3 ± 0.11^{defg}	+
(2.0)	7.6	69.4±1.97	2.6 ± 0.14^{ij}	1.5 ± 0.08^{cdef}	+
MS+KN(1.0)+IAA(0.5)	6.5	87.4±3.30	3.5 ± 0.05^{efg}	2.4 ± 0.09^{a}	+
(1.0)	6.0	83.3±3.42	3.0 ± 0.18^{ghi}	1.7 ± 0.05^{bcd}	+
(2.0)	6.2	80.5±1.97	2.7 ± 0.23^{hij}	1.4 ± 0.07^{defg}	+
MS+KN(2.0)+IAA(0.5)	6.7	70.8±3.40	2.4 ± 0.22^{j}	1.5 ± 0.21^{cdef}	+
(1.0)	6.5	77.7±1.93	2.6 ± 0.20^{ij}	1.9 ± 0.24^{bc}	+
(2.0)	6.5	72.2±1.97	2.8 ± 0.19^{hij}	2.1 ± 0.19^{ab}	+
LSD (P<0.05)			0.48	0.38	
ANOVA			$F_{17,36}$ =43.56	$F_{17,36}$ =9.68	

- *Values are Means ±S.E of three independent experiments, each consisted of 10 replicates per treatment. Data were recorded after 10 weeks of culture.
- - No Response, + Poor growth, + + Moderate growth, + + + Good growth.
- Mean values within a column sharing the same superscript are not significantly different at P<0.05 according to Duncan's Multiple Range Test.

In the present study, nodal explants of *Uraria picta* did not respond on MS basal medium. This was in agreement with the results of *Tinospora cordifolia* [6]) and *Terminalia bellerica* [7] on MS basal medium. Supplementation of cytokinins induce bud break as well as shoot growth. Similar observations have been reported in *Anogeissus sericea* [8] and *Olea europaea* [9]. Application of exogenous growth regulators in present investigation had variable effects which varied with the type of growth regulator and their concentrations. The number and frequency of shoot length were mainly dependent on the combination of auxin and cytokinin used in the culture medium. The potential for shoot multiplication seems to be more in the presence of BAP with IAA in the culture medium. Some workers also suggest

that formation of multiple shoots *in vitro* is a function of combination of BAP with IAA as in *Withania somnifera* [10] and *Alpinia galanga* [11].

In vitro regenerated shoots rooted best on the medium fortified with IBA in the present study (Table 3, Fig. 1E, 2b). Similar observations have also been reported in *Bacopa monnieri* [12] and *Plectranthus amboinicus* [13].

Table 3. Effect of auxins on root induction of in vitro regenerated shoots of Uraria picta cultured on MS medium.

Medium and conc. of growth regulators (mg/l)		Days required for root induction (mean value)	Per cent Response*	No. of roots per shoot*	Root length* (cm)
MS Medium	-	0.00	0.00±0.00	0.00±0.00^{f}	0.00±0.00^{g}
MS Medium (Half strength)	NAA+ 0.5	21.5	72.2±1.93	11.3±0.31^{c}	4.5±0.19^{d}
	1.0	22.9	63.8±3.41	10.2±0.36^{d}	4.0±0.16^{e}
	2.0	20.0	58.3±0.00	8.6±0.22^{e}	3.3±0.18^{f}
	IAA+ 0.5	0.00	0.00±0.00	0.00±0.00^{f}	0.00±0.00^{g}
	1.0	0.00	0.00±0.00	0.00±0.00^{f}	0.00±0.00^{g}
	2.0	0.00	0.00±0.00	0.00±0.00^{f}	0.00±0.00^{g}
	IBA+ 0.5	17.4	91.7±1.93	17.8±0.53^{a}	5.8±0.18^{c}
	1.0	16.5	94.4±1.93	18.5±0.31^{a}	6.7±0.12^{a}
	2.0	18.3	80.6±1.97	15.8±0.24^{b}	6.2±0.14^{b}
LSD (P<0.05)				0.77	0.36
ANOVA				$F_{9,20}$=860.54	$F_{9,20}$=509.46

- *Values are Means ±S.E of three independent experiments, each consisted of 10 replicates per treatment. Data were recorded after 10 weeks of culture.
- Mean values within a column sharing the same superscript are not significantly different at P<0.05 according to Duncan's Multiple Range Test.

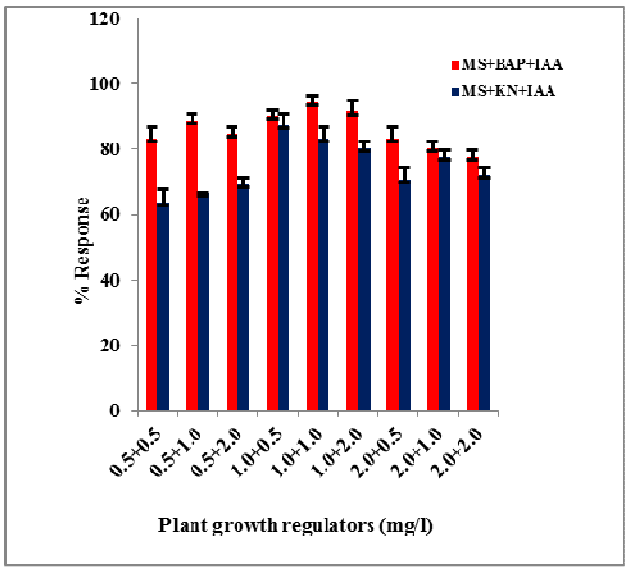

Fig: 2a Effect of different combinations of BAP and KN with IAA fortified with MS medium on per cent shoot multiplication in Uraria picta.

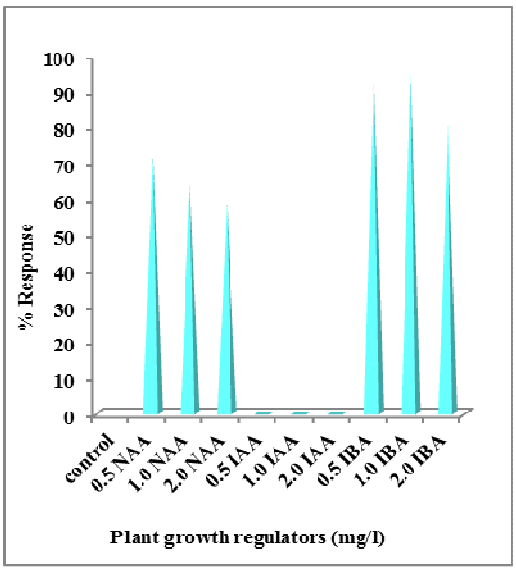

Fig: 2b Effect of different concentrations of plant growth regulators on per cent root induction in Uraria picta.

IV. CONCLUSION

The present micropropagation protocol provides a rapid method for proliferation of shoots and roots from nodal explants over conventional methods for improvement, conservation and large scale production of this medicinally important herb. The plantlets were easily acclimatized to the external environment and undergoing normal physiological development. This protocol ensures a successful and reliable technique which can be used for propagation of *Uraria picta* to minimize the pressure on wild populations and contribute to the conservation of valuable species in India.

V. ACKNOWLEDGEMENTS

Authors are thankful to Dr. Ashok Kumar Sharma, Head of the Non Wood Forest Product (NWFP) Division, Forest Research Institute (FRI) Dehradun, Uttarakhand for helping in procuring the plant material.

BIBLIOGRAPHY

[1] Yusuf, M.; Chowdhury, J. U.; Wahab, M. A. and Begum, J. 1994. Medicinal plants of Bangladesh., BCSIR Laboratories. Chittagong, Bangladesh, Pp. 112–114.

[2] Kirtikar, K. R. and Basu, B. D. 1993. In Indian medicinal plants Vol. 1, Bishen Singh Mahendra Pal Singh, India. Pp. 496–498.

[3] Garg, N.; Garg, M.; Maan, A.; Sandhu, B.; Mittal, S. and Goyal, S. 2012. Phytochemical Studies and anti anxiey activity of *Uraria picta* leaves. *Journal of Pharmaceutical Research and Opinion*, **2:** 39-40.

[4] Khanna, A. K.; Chander, R. and Kapoor, N. K. 1991. Hypolipidaemic activity of abana in rats. *Fitoterapia*, **3**: 27.

[5] Gurav, A. M.; Dhanorkar, V. M.; Dhar, B. P. and Lavekar, G. S. 2008. *In Vitro* propagation of the medicinal plant *Uraria picta* (Jacq.) Desv. ex DC. from cotyledonary node and nodal explants. *Pharmacognosy Magazine,* **4:** 239-245.

[6] Kumar, S.; Narula, A.; Sharma, M. P. and Srivastava, P. S. 2003. Effect of copper and zinc on growth, secondary metabolite content and micropropagation of *Tinospora cordifolia*: a medicinal plant. *Phytomorphology*, **53**: 79-91.

[7] Mehta, J.; Sain, M.; Mathuriya, B. L.; Naruka, R.; Kavia, A. and Sharma, D. R. 2012. Rapid micropropagation and callus induction of *Terminalia bellerica* Roxb. –An endangered plant. *Asian Journal of Plant Science and Research*, **2** (3): 364-368.

[8] Kaur, G.; Singh, R. P.; Rathor, T. S. and Shekhawat, N. S. 1992. *In Vitro* propagation of *Anogeissus sericea*. *Indian Journal of Experimental Biology*, **30**: 788-791.

[9] Peixe, A.; Raposo, A.; Lourenço, R.; Cardoso, H. and Macedo, E. 2007. Coconut water and BAP successfully replaced zeatin in olive (*Olea europaea* L.) micropropagation. *Scientia Horticulturae*, **113**(1): 1-7.

[10] Kannan, P.; Ebenezer, G.; Dayanandan, P.; Abraham, G. C. and Ignacimuthu, S. 2005. Large scale production of *Withania somnifera* (L.) Dunal using *in vitro* techniques. *Phytomorphology*, **55**: 259-266.

[11] Singh, N. M.; Chanu, L. A.; Devi, Y. P.; Singh, W. R. C. and Singh, H. B. 2014. Micropropagation- an *in vitro* technique for the conservation of *Alpinia galanga*. *Advances in Applied Science Research*, **5**(3): 259-263.

[12] Mohapatra, H. P. and Rath, S. P. 2005. *In Vitro* studies of *Bacopa monnieri*-an important medicinal Plant with reference to its biochemical variation. *Indian Journal of Experimental Biology*, **42**: 373-376.

[13] Rahman, Z. A.; Noor, E. S. M.; Ali, M. S. M.; Mirad, R. and Othman, A. N. 2015. *In Vitro* Micropropagation of a Valuable Medicinal Plant, *Plectranthus amboinicus*. *American Journal of Plant Sciences*, **6**: 1091-1097.

