International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

High Frequency Regeneration in Important Medicinal Plant *Uraria picta* Jacq. DC

Avinash Jadhav[1], Rohit Shete[2], Narayan Pandhure[3]

Tissue culture Laboratory, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431001

Abstract: ***Present investigation is aimed to develop the high frequency regeneration in Uraria picta. This plant is overexploited for preparation of the drug Dashmula. Explants used during investigation were axillary buds, segment of leaves and nodal segments which responded well to all growth regulators. High frequency regeneration of shoots was achieved within 21 days on modified Murashige and Skoog's medium supplemented with 0.5 mg/L of Indole-3-acetic acid along with 2.5 mg/L 6-benzylaminopurine. Isolated shoots rooted on MS along with in 0.5 mg/L indole-3-acetic acid. In vitro raised plants after hardening were grew normally in soil after treatment with inorganic salt solution.***

Keywords: *Uraria picta,* Conservation, Multiplication

1. Introduction

Uraria picta is extensively used in the Asian traditional systems of medicine belongs to family Leguminosae. It is a perennial herb grow up to 1 to 2.5 m in height and widely distributed throughout India, Australia, Africa and all most all parts of Asia. In India it is found in dry grasslands and open forests in the sub-Himalayan tract from Kashmir to west Bengal, Assam and wide distribution in Maharashtra, it ranges from Western Ghats, Nanded, Gondia districts of Vidarbha. It is one of the important constituents in ten herb formulation called 'Dashmula, a well-established Ayurvedic drug of the Indian system of medicine. The whole plant are use in medicine Roots are aphrodisiac in nature. They are mainly used for fracture healing properties. Its decoction is useful in cough, chills and fevers. Leaves are antiseptic in nature these are mainly useful in gonorrhea and also for acaricidal properties in human and animal. It is also used for its analgesic, anti-inflammatory, antioxidant activity and its bark contains isoflavanones which have anti-microbial activity. Thus the major bioactive flavonoid rhoifolin has been considered as marker compound for qualitative and quantitative standardization of *Uraria picta* based on liquid chromatographic analysis. Therefore it is need to conservation of this valuable medicinal plant and evaluation standard in vitro protocol of *Uraria picta* for human welfare. In present research work it is proposed to developed the high frequency regeneration of *Uraria picta*

2. Materials and Methods

Plants of *Uraria Picta* was grown in the greenhouse located in Botanical Garden, Dr. Babasaheb Ambedkar Marathwada University Aurangabad and used as experimental material. The temperature in the greenhouse varied from 28 to 32^{0}C and 60% of humidity was maintained with sprinkler. Different explants were used for establishing cultures from leaves, shoot tips, nodal segment etc. Leaves and nodal segments were taken from 1 year-old plants and inoculated after surface sterilization on MS fortified with different growth regulators.

Surface sterilization and Inoculation

Young green leaves and nodal segments were taken from 1st to 3rd node of lateral branches and young shoot tips nodal segments, and washed twice with tap water. Leaves were surface-sterilized with 0.01% (w/v) mercuric chloride for 3 min, followed by 3–4 times washed with sterile double distilled water and inoculated on agar-solidified MS (Murashige and Skoog, 1962) medium supplemented with different concentrations of 2,4-D and KIN, either alone or in combination. The pH of the medium was adjusted to 5.8 before sterilization. Cultures were maintained at 25±1ºC with a 16-h photoperiod with 40 mol m^2/ s provided by cool white fluorescent tubes. Callus was subcultured after 25 days on the original callus-inducing medium. Established callus were transfer on medium containing different concentration of IAA and BAP for multiple shooting.

3. Results and Discussion

Initiation of Callus

After 15 days of inoculation callus was found proliferated from cut edges of explants viz. leaf, stem etc. Table 1 revealed that, induction of callus or shoot regeneration from axillary leaves and nodal segments explant as well high frequency regeneration of *Uraria picta*. Callus induction was archived on MS medium supplemented with various concentrations of 2, 4 D and IAA along with BAP using leaves and nodal segment as an explant. Both combinations were good for the induction of callus from both explants at less or more frequency. Maximum callus was recorded on IAA, 3.0 mg/L along with BAP, 3.0 mg/L with 73.33 % callus induction frequency. From axillary node and massive callus along with shooting using nodal segment explants. Lower concentrations were induced poor or average type of callus, however, higher concentration revealed average and massive callus induction. Induction of callus was also achieved on MS media supplemented with various concentration of 2, 4 D along with 0.5 mg/L of BAP. It was recorded that, all concentrations of 2, 4 D and BAP were found best for induction of callus. Maximum induction of callus was achieved on 3.0 mg/L of 2, 4 D using axillary leaves explant whereas, 4.0 mg/L using nodal segment of explant.

Licensed Under Creative Commons Attribution CC BY

Multiple shoot induction

Multiple shooting were obtained by sub-culturing well developed callus on MS medium supplemented with 0.5 mg/L of IAA in combination with BAP and KIN. Induction of high regeneration of *U. picta* was achieved using various concentration of BAP in combination with 0.5 mg/L IAA, maximum number of shoot induction were recorded from 2.0 and 4.0 mg/L of BAP with 7.8±0.374 and 7.2±0.374 number of shoots per explants, 93.33 and 86.66 % of shoot regeneration using axillary leaves explants respectively.

Various concentrations of KIN along with 0.5 mg/L of IAA also tried for multiple shoot induction in *U. picta.* Maximum numbers of shoots recorded on MS with 2.0 mg/L of KIN with 7.2±0.374 and 86.66 % of shoot regeneration. Lower concentration was not found to be effective in induction of multiple shoots however, 2.0 mg and above concentrations were found best for high frequency regeneration of *U. picta.* High frequency regenerated cultures were transferred on rooting medium to achieve rhizogenesis, 1.5 mg/L of IBA was found to be best concentration for induction of roots. Well rooted plantlets were hardened by transferring into coco pit for 1 week and later on in 1:1 ratio of FYM and soil mixture.

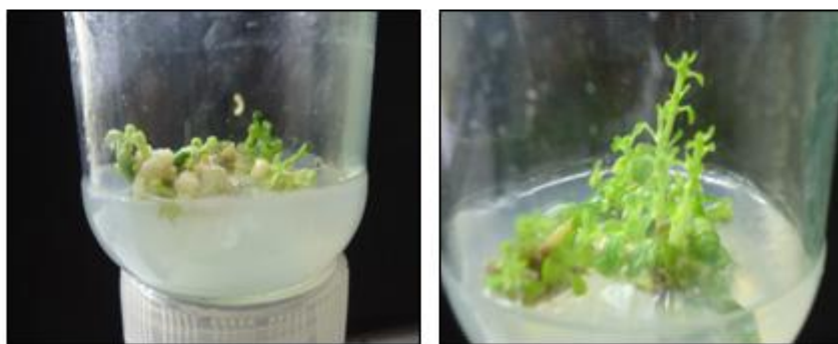

Figure 1(a): Induction of callus with Multiple shoot

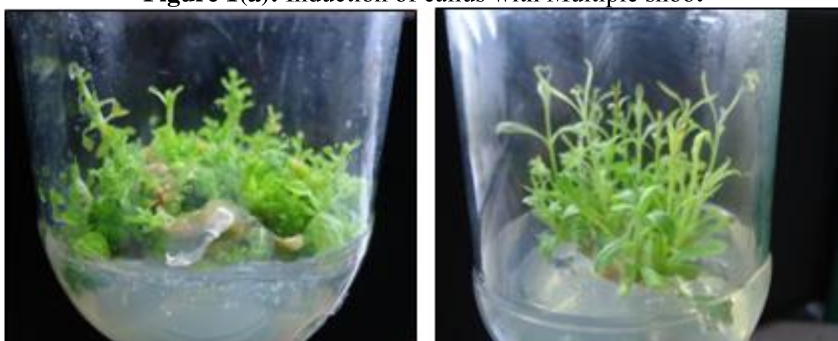

Figure 1(b): High frequency regeneration on MS

Rai *et al* (2010) was developed an efficient *in vitro* rapid production of cloned plants of *Uraria picta*. Result revealed that regeneration of shoots on modified Murashige and Skoog's medium supplemented with 0.25 mg l–1 each of 6-benzylaminopurine and indole-3-acetic acid and 25 mg l-1adenine sulfate and 0.5 mg/L of Gibberellic acid. After two weeks high frequency of regeneration were achieved and plantlets were transfer on rooting media supplied with 0.25 mg/L indole-3-butyric acid. In vitro-raised plants after hardening in inorganic salt solution grew normally in soil.

4. Conclusion

Looking towards increasing popularity of Ayurvedic medicines at abroad, there is heavy pressure on collection of raw material for preparation of these medicines. Horticultural means of multiplication are not practiced and dependency is increasing on natural resources. Modern techniques for multiplication of medicinal are beneficial for large scale multiplication. Secondly medicines could be extracted from cultures as they are grown in controlled conditions throughout the year.

Table 1: Effect of various PGRs along with MS on various explants

Growth regulators (mg/L)			Induction of callus /shoot %	
			Source of explant	
IAA	BAP	2, 4 D	Axillary leaves	Nodal segment
1	1		40.00 PC	40.00 PC
2	2		46.66 AC	66.66 MC
3	3		73.33 MC	73.33 MC/S
4	4		73.33 MC/S	60.00 AC/S
	0.5	1	53.33	60
		2	60	60
		3	73.33	73.33
		4	60	80
High frequency regeneration				
IAA	BAP	KIN	Number of shoots	Shoot induction %
0.5	1		5.0±0.316	73.33
	2		7.8±0.374	93.33
	3		6.4±0.244	80
	4		7.2±0.374	86.66
0.5		1	4.8±0.374	66.66
		2	7.2±0.374	86.66
		3	6.4±0.244	80
		4	6.1±0.124	73.33

Licensed Under Creative Commons Attribution CC BY

5. Acknowledgements

Authors are thankful to Head, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, for providing all the necessary facilities and encouragement during this endeavor.

References

[1] **Baburaj S. and Gunasekaran K** (1995) *In vitro* differentiation of shoots from leaf callus cultures of *Withania somnifera* (L.) Dunal. J. Ind. Bot. Soc. 74: 323–324

[2] **Chopra RN, Chopra IC, Handa KL & Kapur LD** (1958) Indigenous Drugs of India (pp 436–437). UN Dhur and Sons, Calcutta

[3] **Glotter E, Kirson I, Abraham A & Lavie D** (1973) Constituents of *Withania somnifera* Dun. XIII. The withanolides of chemotype III. Tetrahedron 29: 1353–1364

[4] **Gomes F & Seraj ZI** (1991) An attempt to produce regeneration of multiple shoots from fast growing tree species. *In Vitro* 27: 145A

[5] **Kirson I, Glotter E, Lavie D & Abraham A** (1971) Constituents of *Withania somnifera* Dunal.XII. The withanolides of an Indian chemotype. J. Chem. Soc. 11: 2032–2044

[6] **Mathews H** (1987) Morphogenetic responses from *in vitro* cultured seedling explants of mung bean (*Vigna radiata* L. Wilczek). Plant Cell Tiss. Org. Cult. 11: 233–240.

[7] **Murashige T & Skoog F** (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497.

[8] **Ozias-Akins P & Perera S** (1990) Organogenesis in cultured adventitious root segments and in protoplast-derived callus of sweet potato. Hort. Science 25: 121

[9] **Roja G, Heble MR & Sipahimalani AT** (1991) Tissue cultures of *Withania somnifera*: Morphogenesis and withanolide synthesis. Phytother. Res. 5: 185–187**.**

[10] **Santosh Kumar Rai, Meena Sharma, Madhu Jain, Abhishek Awasthi, Dharmendra Kumar Purshottam, Narayanan Kuttanpillai Nair and Ashok Kumar Sharma (2010).** Rapid In Vitro Production of Cloned Plants of Uraria picta (Jacq.) DC— A Rare Medicinal Herb in Long-Term Culture, *Appl Biochem Biotechnol* 162:1929 -1937.

[11] **Sen J & Sharma AK** (1991) Micropropagation of *Withania somnifera* from germinating seeds and shoot tips. Plant Cell Tiss. Org. Cult. 26: 71–73

[12] **Subhash K & Christopher T** (1988) Direct plantlet formation in cotyledon cultures of *Capsicum frutescens*. Curr. Sci. 57: 99–100

[13] **Suffness M & Douros J** (1982) Current status of the NCI plant and animal product program. J. Nat. Prod. 45: 1–14

[14] **Tyler VE, Brady LR & Robbers JE** (1981) Pharmacognosy. Lea and Febiger, Philadelphia (520 p).

Licensed Under Creative Commons Attribution CC BY