JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

CALLUS INDUCTION FROM AXILLARY BUD EXPLANTS OF *Gmelina asatica (L)* A Medicinal important plant.

Mandaloju Venkateshwarlu
Department of Botany, Kakatiya University, Warangal-506 009.
Telangana, India.
Email: drvenkat6666@gmail.com.

ABSTRACT

In vitro axillary bud segments and shoot induction was achieved in one of the important medicinal plants. Shoot induction was monitored after 4-6 weeks of inoculation by counting the number of shoots induced from each explants. The advantage with micro propagation is most of the *in vitro* propagated plants of many important medicinal species were found to be uniform, showing less variation in the secondary metabolic content than their wild counter parts (Annaj and Andrzej S 2005) Shoot induction form axillary bud segments were surface sterilized and inoculated into MS media supplemented with various combinations of BAP L-Glutamil acid and KN. Tissue culture techniques are now becoming popular as alternative means of vegetative propagation. Micropropagation involves multiplication of genetically identical individual by asexual reproduction within a short span of time with tremendous potential for the production of high quality plant based medicines (Murch *et al.*, 2000). The effect of benzyl amino purine in inducing shoot induction was already reported in some of the important medicinal plants (Komalavalli and Rao, 2000In Vitro propogation of Damaskrose Tabesh et al 2013 & Gago J 2014). The Axillary bud explants were inoculated on MS basal medium supplemented with various cytokinins i.e., BAP and NAA. Coconut water also had a role in triggering the formation of multiple shoots. Addition of BAP at 3.0 mg/l concentration or NAA at 3.0 mg/l to the MS basal medium, induced regeneration from the Tendril explants. Multiple shoot induction was achieved in one of the impartant medicinal plant of cucurbitacae family, *Gmelina asatica (L)*. MS medium supplemented with 1.0 mg/l BAP + 2.0 mg/l NAA and 2.0 mg/l L-Glutamicacid was found to be optimum to induce shoots. The present study established reliable and reproducible protocol for rapid multiple shoot induction from axilary bud explants of *Gmelina asatica (L)* using different concentrations and combinations of cytokinins.

Key words: Multiple shoots, Axillary bud explants, BAP, L-Glutamic acid, NAA.

INTRODUCTION

In the present paper, a simple and reproducible procedure was devised to obtain multiple shoots, from axillary bud explants of *Gmelina asatica (L).*on MS medium fortified with plant growth regulators along with coconut milk and amino acids. Axillary buds from pumpkin were reported by Jelaska (1972). In comparison to lactose for different straw lactose 3% of lemon grass straw (580 gram) was proved to be less effective to others. The similar findings were also reported by Singh (2005). Growth of *in vitro* propagated plants is often stronger than in those cloned *in vivo.* This is mainly due to rejuvenation and the fact that they are disease free. Propagation is carried out in aseptic conditions, free from pathogens. The plants of Cucurbitaceae suffer from several diseases including the water melon mosoic virus (Gonbad RA 2014 and Bala M. *et al* 2013), Cucumber

green mottle mosoic virus (Nijsden, 1984) and *Gmelina asatica (L)*.also suffers from downey and powdery mildews which seriously limits the crop production. (Cog bill *et al* 2010, Jha R 2004) first demonstrated that virus free plants can be recovered from infected plants through shoot tip cultures. For instance there was no evidence of transpiration in the adaxial surface of the E. Milli and yet Cumulatively it has a higheerate of transaction in plant species studied (Abdul Rahaman *et al* 2017, Jo Shi *et al* 2014).

MATERIALS AND METHODS

The axillary buds were harvested and dipped in water utilized. The effect of MS media composition on multiple shoot induction (axillary bud explants) was studied using at least a single microshoot was scored as responding explants.The Axillary bud segments of 2.0 – 3.0 cm long were cultured and surface sterilized with 0.1% $HgCl_2$ for 5-7 minutes and rinsed with sterile distilled water. They were cultured on MS medium containing 2.5% sucrose and 0.8% Agar-Agar and different concentrations of BAP, NAA and L-Glutamic acid (Table 1). The pH of the medium was adjusted to 5.8 and later was autoclaved at 120^0C for 17 minutes. Cultures were incubated under 16 hrs, illumination (250 lux) at 25 ± 2^0C temperature. Raising the level of BAP (0.5 to 2.0 mg/l) resulted in the an increase in the number of shoots from hypocotyls and cotyledon explants of Niger (Nikam and Shitole, 1993).Induced highest number of multiple shoots over MS medium with 1.0 mgl. BAP (Warhade and Badrere 2017) Sridher *et al* 2014). The results from this study has shown that BAP induced the activation of totipotency at the axillary bud explants, which resulted in the formation of multiple shoots. The elongation of microshoot was attempted using various ways. It gives an estimate of the number of shoots expected to regenerate.

RESULTS AND DISCUSSION

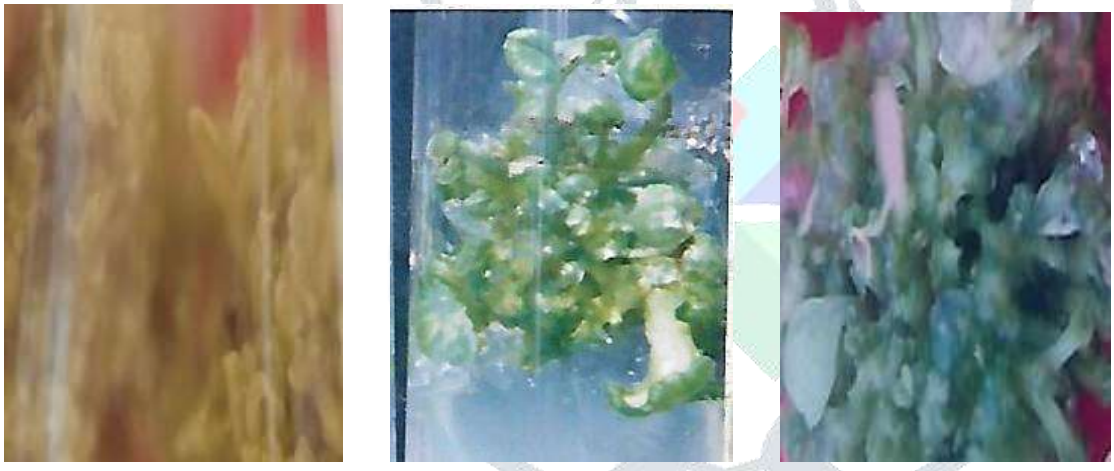
The present investigation, we attempted to elongate microshoots by entire inclusion of higher concentration of BAP 2.0mgl and NSA 3.0mgl culturing the microshoot for an extended duration over the MS medium. There may be because of the increased availability of Cytokinins other nutrients (CM) coconut milk aeration of the culture and dilution of any exudates from the explants. Among all explants used axillary bud segments were the best for multiple shoot induction. With an increase in the level of BAP 2.0 – 3.0 mg/l the percentage of explants producing shoots also increased. The number of shoots developed on the explants ranged from 1-4 to 2-3 by the addition of BAP at a concentration of 2.0 mg/l or NAA at 3.0 mg/l. Among three concentrations used i.e, 10, 15 and 20%, 15% of coconut milk along with 1.0 mg/l BAP had proved to be ideal for multiple shoot induction. MS medium fortified with 1.0 mg/l BAP or 2.0 mg/l L-Glutamic acid also induced shoot buds on axillary bud explants. The mean number of shoots developed on the explants ranged from 1-4 to 2-3 by the addition of different concentrations of BAP and NAA. The axillary bud segments cuttings were inoculated on MS basal medium fortified with various cytokinins i.e., BAP and NAA. Coconut water also had a role in triggering the formation of multiple shoots. Raising the level of BAP (3 mg/l to 4 mg/l) resulted in an increase in the percentage of shoots developed from axillary bud segments cuttings. There was no significant increase in the number of shoots on NAA+ Kn at low and high concentration (Plate 1). MS medium supplemented with 10, 15, 20% of coconut milk also triggered the induction of many multiple shoots. Low concentration of L-glutamic acid (0.5 – 1.0 mg/l, along with BAP (1.0 mg/l, has produced significant mean number of multiple shoots that ranged from 2-3 to 5-6 in both the explants. Addition of NAA failed to produce many shoots but enlarged the axillary bud segments. Lower levels of coconut milk (6, 12%) induced callus formation. The results from study have shown the initiation of shoot buds and formation of multiple shoots of *Gmelina asiatica (L)*

Table-I **Callus induction from Axillary bud explants of *Gmelina asiatica (L)***

Growth Regulators	Tendril	
	% frequency of shoots	Mean No. of shoots
MS + 0.5 mg/l BAP + 0.5 L-Glutamic acid+Kn 0.5mgl/L	40	Callus
MS + 1.0 mg/l BAP + 1.0 L-Glutamic acid+ Kn 1.0 gm/L	35	Callus
MS + 2.0 mg/l BAP + 2.0 L-Glutamic acid+ Kn 1.5 mg/L	30	shoots (2-4)
MS + 3.0 mg/l BAP + 3.0 L-Glutamic acid+2.0mgl/L	25	shoots (2-6)
MS + 0.5 mg/l NAA + CM	35	Callus
MS + 1.0 mg/l NAA + CM	30	Callus
MS + 2.0 mg/l NAA + CM	25	shoots (2-4)
MS + 3.0 Mg/l NAA + CM	20	shoots (2-4)
MS + 4.0 mg/l NAA + CM	10	shoots (2-4)

CM = Coconut milk water

Plate –I Callus induction from Axillary bud explants of *Gmelina asiatica (L)*



CONCLUSION:

The effectiveness of MS medium in inducing callus regeneration in the axillary bud explants. Although MS Medium supported the regeneration in explants the regeneration was inefficient to trigger regenerative process of the explants in the present investigation. This is considered as one of the methods to increase the response in explants has suggested that repeated transfer of explant on multiplication media containing cytokinins succeeds in activating the plant materials. The method of repeated transfer of explant is considered to be useful for large scale production of plants, as it avoids isolation and culture of new explants.

REFERENCES:

1. Bala M Singh KP Singh SK and Prasad KV (2013) Standardization of an efficient protocol for *in vitro* mass multiplication of Hybrid Tea rose CV Raktima Indian Journal of Horticulturear 1: 404-410.
2. Cogbill S, Faulcon T, Jones G, MC Daniel M, Harmon G, Blackmon R (2010) Advatitious shoot regeneration from Cotyledonary explants of rapid cycling fast plants of *Brassica rapa L* Plant cell tissue and organ culture 101: 127-133.

3. Gago J Martinez-NunezL, Landin M Flexas J and Gallego P (2014) modeling the effects of light and sucrose on *in vitro* propagated plants A multiscale system analysis using artificial intelligence technology PLOS ONE 9:1-11.
4. Tasbesh F, Kermani M J Neknies MK. Monsavi A and Khalighi A (2013) In Vitro propagation of Danask rose (Roja damaskenas) (V-Ispahan) Annals of Bilogical Research 4: 134-138.
5. Joshi Sulekha and Shringhi SK (2014) Floristic diversity with special reference to rare and threatened plants of Jawaharsagar Sanctuary area near Kota, Rajashtan Bilogical form an International J 6(1) 84-91.
6. Abdul Rahaman A and Oladele FA (2017) Leaf size and transpiration rates in Agave Americana and *Aloe vera* phytologia Balcanica 23(1) 95-100.
7. Jha R (2004) Role of In vitro propagation in the improvement of Rose cultivars review Agriculture reviews 25: 100-114.
8. Gonbad RA, Sinniah UR, Aziz MA and Mohammad R (2014) Influence of Cytokinins in combination with GA on shoot multiplication and elongation of Tea clone Iran 100(Camellia Sinensus (L). The Sci. World J. (Doi Org 10.1155/2014. 943054.
9. Warhade M I and Badere R S (2017) Seasonal variation in the shoot regeneration potential of the node explants of *Rosa Setigera* Journal of Indian Botanical Society 96: 199-208.
10. Venkateshwarlu m. Rajendra Prasad B, Odelu G, Sammaiah D and Ugander T (2019) Direct High frequency plantlet Regeneration from leaf explants of *Solanum torvum* (SWARTZ) A medicinal important plant J Indian Bot. Soc. Vol.98 (3&$) 137-145.
11. Annaj and Andrzejs (2005) Mammalian sex hormones in plants folia Histchencia et Cyto biolgica 43: (2) 71-79
12. Halder, T.E. and Gadgil, V.N (1982). Shoot bud differentiation in long term callus cultures of *Momordica* and *Cucumis Ind. J. Expt. Boil*, 20: 80-782.
13. Harris, P. J. C. and Moore, T. H. M. (1989). Nitrogen fixing trees Res Rep 7-12b.
14. Jelaska, S. (1974). Embryogenesis and organogenesis in pumpkin explants. *Physiol. Plant.* 31: 257-261.
15. Komalavalli, N. and Rao, M.N. (2000). *In vitro* micropropagation of *Gymneme sylvestre* a rare multipurpose medicinal plant. *Plant Tissue Organ Cult.* 61(2): 97-105.
16. Murch, S.J. Krishna Raj, S. and Saxena, P.K. (2000). Tryptophan is a precursor for melatonin and serotonin biosynthesis in *in vitro* regenerated St. Jhon's wort (*Hypericum perforatum L.* cv. *Anthos*) Plants, *Plant Cell Rep.*19: 698-704.
17. Nijsden, A.P.M. den (1984). Attempts to introduce resistance from related species into the cucumber. Proceedings of the third meeting on breeding of cucumber and melons 2-5 July 1984 Poldiv Bulgaria.
18. Nikan, T.D. and Shitole, M.E. (1993). Plant Tissue and Organ Culture 32: 345.
19. Singh Rochica (2005). Studies on some larger fungi of faizabad with reference to their eco-physiological characteristics. Ph.D. Thesis, Dr. R.M.L. Avadh. University, Faizabad. PP. 114-115.