Vol-05 Issue 12, December - 2021 ISSN: 2456-9348
Impact Factor: 5.004

iJETRM
International Journal of Engineering Technology Research & Management

INVITRO PROPAGATION FROM LEAF EXPLANTS CULTURE OF COCCULUS HIRSUTUS (L)

***Mandaloju Venkateshwarlu,**
T. Asheervadam
T. Ugendhar
[*]Department of Botany, Kakatiya University, Warangal Urban-506 009. T.S. INDIA
Department of Botany, Degree and PG College, Mahabubabad T.S. INDIA

ABSTRACT
The alternative and a novel approach for the improvement of this crop is to complement the traditional breeding with Leaf Explants culture techniques to regenerate plants from single cells and organized tissues and to transfer desirable genes from other sources. Applied biotechnolofy molecular Biology Senez. J.C. (1986). Salyers A (1995). And Sasson A (1984). Biotechnology and challenges. The development of protocols for the production of transgenic plants requires several factors to be fulfilled with main focus on finding cells or organized tissue that are competent for both transformation and regeneration (Pounti-Kaerlas, 1993, Christou, 1994). M. Venkateshwarlu (2019,2020). T. Yugender *et al* (2018). Experimental mutagenesis *in vitro* shoots induction from stem node explants and enhancement of Sec. Metabolities in tissue culture. These biotechnological techniques provide an opportunity to produce plant cultivars. Ex. *Vivo* implication of phytochemicals on various responses. Rathore *et al* (2010). R. Prasad & Venkateshwarlu M (2018). Effect of Gamma rays in stem node explants of *Cucurbita maxima* Venkateshwarlu M (2020). Shoottip explants from stem node explants B.R. Prasad *et al* (2019). New varieties that can exhibit disease resistance and improvements in quantity and quality yield in *In vitro* culture methods. Single cell proteins Scrimshaw N.S. (1968). The present review provides an overview of *in vitro* and genetic transformation studies undertaken for the improvement of groundnut and also outlines the future prospective. Methods for *In vitro* culture explants are the same as described for plant cells release of genetically modified organisms. Stewart *et al* (1992).

Keywords:
Invitro propagation, BAP, NAA, L-Gltamic acid, *Cocculus hirsutus* (L)

INTRODUCTION

In addition, as the pongamia is a preferred candidate for eco-restoration programmes in areas where high grassing pressure exist. Due to its significant socio-economic importance and its contributions to sustenance of rural economy. Gene transfer in legumes has, so far, been difficult and challenging because of their recalcitrant nature to regeneration *in vitro* Leaf Explants and competency of regenerative cells for transformation is not efficient (Christou, 1997). In some cases, in spite of successful pollination and fertilization embryo does not develop. As compared with cultivars the wild species have greater resistance to pest and pathogens and produce grains of better quality Sanygin G.A. (1974), plant freezing Trevan MD (1980). Plant Application Biotechnology. None of the regeneration protocols developed, so far, in legumes fulfill the demand of being widely applicable in terms of genotype and species independence, efficient so as to produce large number of regenerated plants, reproducible in all laboratories, fast and simple (Jacobsen, 1992). Therefore, the development of procedure by which plants can be regenerated from single cells and organized tissues and specific gene transferred to plant cells are the pre-requisite for practical genetic engineering for grain legumes improvement.

MATERIALS AND METHODS:

MS supplemented with IBA at 5.0 mg/l, NAA 0.2 mg/l. The cotyledons excised from water-soaked seeds on BAP enriched medium developed only a limited number of shoot buds. This appears that the presence of the embryonal axis stimulated the production of buds on the cotyledons. Callus proliferation medium supplemented the basal medium on different combinations and compositions of (BAP, NAA, Kn) hormones and other growth adjuvants for green callus proliferation and small shoot buds. Leaf Explants regeneration of multiple shoot buds from *Cocculus hirsutus* (L) without an intervening

iJETRM
International Journal of Engineering Technology Research & Management

callus phase has been achieved from entire mature cotyledons (Mehta and Mohan Ram, 1980) or distal half of cotyledons (Mohan and Krishnamurty, 1998) and shoot apices. Model system in plant tissue and protoplast cultures Hassanein (2000), Senthetic seed prospects and limitations. Ara. H (2000) Multiplication, Multiple shoot induction from cotyledonary explants M. Venkateshwarlu (2020). The shoot buds were also developed from the proximal and distal ends of the mature entire cotyledons (George and Eapen, 1994). The specific nature of this stimulus is not known Chsmpestrini (2006). Cloning protocol study of *Aloe vera*. The cotyledonary node explants do not form shoot initials on MS + BAP but continue to form new shoot initials on MS medium containing BAP and supplemented topically with IAA (Prakash *et al.,* 1994). Proliferation of shoot initials from the emplants only in the presence of added IAA suggests that thy cytokinin: auxin ratio is important for this response.

RESULTS AND DISCUSSION:

Reduction of MS inorganic salts to half has favoured regeneration of complete plantlets from mature somatic embryos (Sreenivasu *et al.,* 1998). In another study, out of the different basal media tried, the induction of maximum number of shoot buds from distal half of cotyledons and calli was observed on EC6 medium and on Blaydes medium (Kumar *et al.,* 1984) and Sreenivasulu *et al* (1998). Various aspects for plant improvement through *In vitro* cultures with plant tissue in laboratory their technique has been referred by some researches as botanical laser whose hormones uses are yet to be fully understood. Organogenesis and Leaf Explants in *Cocculus hirsutus*. Direct organogenesis was obtained from diverse explant cultured on cytokinin containing MS medium. Out of different cytokinins, BAP was found to be the most effective. However, TDZ at low concentrations have been optimal for the induction of multiple shoots in the seed cultures of *Cocculus hirsutus*. Addition of low concentration of IAA to BAP medium showed development of shoot buds from calli derived from mature cotyledons, leaf and root segments. In most plant species presence of high auxin concentration followed by gradual withdrawal from culture media has been reported to be a necessary for somatic embryogenesis. In contrast to this, only cytokinins, BAP, kinetin and adenine sulphate in combination induced somatic embryogenesis (Patel *et al.,* 1994). TDZ alone also induced somatic embryos directly or through embryogenic calli and subsequent withdrawal of TDZ from the MS medium resulted in the maturation and growth of the embryos into plantlets. The somatic hybrid plant inherited many characteristics colour prolongated flowering large and fertile pollen grains. In addition to auxin and induction of organogenesis. *In vitro* produced plants in any crops system have to finally reach to the field wher in vivo conditions. In case of *Coccullus hirsutus* the maximum number of shoot lets obtains on MS medium fortified with the combination of BAP, NAA and L-Glutamic acid. Regenerated shoots are transferred to a root inducing medium. (Plate 1 Fig.1 Leaf Explamnt Fig.2 Callus induction Fig.3 Green Callus with Plantlets) In many cases auxin alone or in combination with a low level of cytokinin will enhance root primordial formation.

Table 1- Invitro propagation Leaf Explants from Coccullus hirsutus (L)

Growth Regulators (mg/l)	Leaf Explants	
	Growth response	
NAA+1.0 L-Glutamic acid+BAP	40	Green Callus
NAA+2.0 L- Glutamic acid+BAP	35	Callus with buds
NAA+3.0 L- Glutamic acid+BAP	25	Green Callus shoots
NAA+4.0 L- Glutamic acid+BAP	20	Small shoot (2-4)
NAA+5.0 L- Glutamic acid+BAP	15	Small shoot bud (4-6)
IAA+1.0 BAP+1.0+IBA+ KN	30	Green Callus
IAA+2.0 BAP+1.0+IBA+ KN	25	Callus + Small buds
IAA+3.0 BAP+1.0+IBA+ KN	20	Shoot buds (2-4)
IAA+4.0 BAP+1.0+IBA+ KN	15	Shoot buds (1-5)
IAA+5.0 BAP+1.0+IBA+ KN	10	Plantlets

iJETRM
International Journal of Engineering Technology Research & Management

Plate 1- Invitro propagation Leaf Explants from Coccullus hirsutus (L)

Fig.1

Fig.2

Fig.3

CONCLUSION:

Coccullus hirsutus plants in several genotypes have been regenerated through direct or indirect organogenesis or embryogenesis using embryo culture explants. However, the regeneration frequency is very low, inefficient, genotype specific and also depends on type of explants, media composition and culture conditions. An optimization of various factors affecting regeneration and gene transfer will help in developing an efficient protocol for the production of transgenic plants. very few studies have been undertaken for the transformation of this crop probably due to the want of the efficient, fast, and reproducible regeneration protocol.

REFERENCES:

1. A. Devi, G Odelu, BR Prasad, M Venkateshwarlu, T Ugender (2019). Enhancement of Secondary Metabolities in tissue culture of a medicinal plant *Trigonella foenumgracum* L The journal of Indian Botanical Society 98 (1&2) pp:71-78.
2. B R Prasad, M Venkateshwarlu, G Odelu, A Devi, T Ugender (2019). *In vitro* propagation of Indian Teak (*Tectona grandisis* L) from leaf explants IJ IB Sci. 98 (3&4) pp: 148-156.
3. Christou, P. (1994). The Biotechnology of crop legumes. *Euphytica* **74:** 165-185.
4. Christou, P. (1997). Biotechnology applied to grain legumes. *Field Crops Research* **53:** 83-97.
5. George, L. and Eapen, S.E. (194). Organogenesis and embryogenesis from diverse explants in pigeonpea (*Cajanus cajan* L.) *Plant Cell Rep*. **13:** 417-420.
6. Jacopsen, Hans-Jorg (1992). Biotechnology applied to grain legumes – Current state and prospects. 1st conference Europeeme Sur Les Proteagineux-Angers 99-103.
7. Kumar A.S., Reddy T.P., Reddy, G.M. (1984) Multiple shoots from cultured explants of pigeonpea and *Atylosia* species. SABRAO J. **16:** 101-105.
8. M. Venkateswhwarlu (2019). Studies on micro propagation *in vitro* shoot induction from stem node explants of Cucumis sativus L IJ. Applied Research pp: 9-11
9. Mehta, V., Mohan Ram, H.Y. (1980). Regeneration of plantlets from cotyledons of *Cajanus cajana*, *Indian J. Exp. Biol*. **18:** 800-802.
10. Mohan M.L. and Krishnamurthy, K.V. (1998). Plant regeneration in pigeonpea [(*Cajanus cajan* (L.) Millsp] by organogenesis. *Plant Cell Rep.* **17:** 705-710.
11. Patel, D.B., Barve, D.M., Nagar N. and Mehta, A.R. (1994). Regeneration of pigeonpea, *Cajanus cajan*, through somatic embryogenesis. *Indian J. of Expt. Biol*. Vol. 32, pp.740-744.
12. Pounti-Kaerlas, J. (1993). Methods in grain legume transformation. *Grain Legumes* **2:** 14-15.

iJETRM
International Journal of Engineering Technology Research & Management

13. Prakash, S.N., Pental, D. and Neera Bhalla Sarin (1994). Regeneration of pigeonpea from cotyledonary node via multiple shoot formation *Plant Cell Rep.* **13:** 623-627.
14. Sanygin G.A. (1974). The reasons for plant freezing out (In Russian) Mascow Vol.16, pp: 525-547.
15. Sasson A (1984). Biotechnology challenges and promises. UNESCO-Paris Proc. Soc. Exp. Biol. Vol.134 pp: 112-115.
16. Scrimshaw NS (1968). In single cell proteins MIJ Press Combridge Mass Vol. pp: 3-7.
17. Senez J.C. (1986). In perspectives in Biotechnology and Applied micro-molecular Biology (Eds Alam) vol.33. pp: 163-168.
18. Sreenivasu, K., Milim, S.K., Kumar, P.A. and Sharma, R.P. (1998) Plant regeneration via somatic embryogenesis in pigeonpea (*Cajanus cajan* L. Millsp). Plant Cell Rep. **17:** 294-297.
19. Stewart Jull DES and Sussman M (1992). The release of genetically modified Micro Organisms (REGEM) Plenum press, New York. J. Mol. Biol. Vol. 98. Pp: 603-611.
20. T. Ugender R Prasad M Venkateshwarlu G Odelu (2018). Studies on experimental Mutsagenesis on Chick pea (*Cicer aritinum* L) induced by ultraviolet rays and Ethymethane sulphate EJBPS 5(8) pp: 506-511.
21. Trevan MD (1980). Immobilized Enzymes: An Introduction and Application in Biotechnology, Vol. 117 pp: 599-600.