NOVATEUR PUBLICATIONS
INTERNATIONAL JOURNAL OF INNOVATIONS IN ENGINEERING RESEARCH AND TECHNOLOGY
[IJIERT] ISSN : 2394-3696 Website: ijiert.org
VOLUME 8, ISSUE 10, Oct. -2021

PLANT TISSUE CULTURE AND CALLUS INDUCTION FROM COTYLEDONARY SEGMENTS OF CUCURBITA MAXIMA (L)

Mandaloju Venkateshwarlu
Department of Botany, Kakatiya University, Warangal-506 009. Telangana, India.
Email: drevenkat6666@gmail.com

ABSTRACT

The efficiency of regeneration from Cotyledonary segments was compared by containing the number of shoots MS basal medium with growth regulaters BAP 0.5mgl NAA and Kn, 0.5 mgl/L to 3.0 mgl/L, L-Glutamic acid and CM Coconut milk 3.0mgl/L the regeneration MS contained medium 2.0 mgl/L BAP and 2.0 mgl/L L-Glutamic acid NAA Callus, shoot and plant regeneration in Cucurbita maxima the successful establishment of In Vitro requirements for plant regeneration from Cucurbita maxima Cotyledonary nodal segments. The nodal 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 2.0 mg/l concentration or NAA at 3.0 mg/l to the MS basal medium, induced regeneration from the nodal segments Trichosanthes Cucumeriana. In Vitro seeding derived callus saussurea (Joshi 2003, Wawro Sch 2001). Elimination of viruses fro fruit trees (Knapp et al 1995, Cieslinska 2002) Shoot induction was achieved in one of the important medicinal plant of cucurbitacae family, Cucurbita maxima. 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 Cotyledonary explants of Trichosanthes Cucumeriana using different concentrations and combinations of cytokinins. Tissue culture techniques are now becoming popular as alternative means of vegetative propagation. The effect of benzyl amino purine in inducing shoot induction was already reported in some of the important medicinal plants (Komalavalli and Rao, 2000). An effective and reproductive procedure for the regeneration of shoots and plantlets from callus and in vitro tissue culture technique is essential in studies involving gene transfer. Somewhat greater success in regeneration of bean from organ cultures Malik and Sexena (1991) protoplast Isolation from leaf explants of Solanum Surattense A Ramulu, et al (2014) Phytochemical analysis and biological activates in Solanum Surattense Venakteshwarlu et al (2018)

Key words: Nodal segments, NAA, BAP, Cucurbita maxima (L).

INTRODUCTION

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. Cotyledonary segments of Cucurbita maxima (L) on MS medium fortified with plant growth regulators along with coconut milk CM and amino acids. The plants of Cucurbitaceae suffer from several diseases including the water melon mosoic virus Cucumber green mottle mosoic virus (Nijsden, 1984) and also suffers from downey and powdery mildews which seriously limits the crop production. The similar findings were also reported by Singh (2005). Murch et al (2000) In the present investigation we present the result of our efforts to develop a stem node segments from Cucurbita maxima (L) a medicinally important plant. Regenerated shoots were subsequently tested for rooting ability on behalf strength medium with plant growth regulators for all common bean shoots. Embryogenic callus induction and plantlet proliferation of Solanum nigrum Venkateshwarlu M (2017), K Rajesham et al (2013), plant Bio diversity,

direct plant regeneration Phaseolus vulgaris. Ugendar et al (2011) regeneration in vitro glycoalkaloids reduction and evolution of bioactivity callus extract of S tubarasum. Hanan et al (2010).

MATERIALS AND METHODS

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. 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 (1.0 to 3.0 mg/l) resulted in the increase in the number of shoots from hypocotyls and cotyledon explants of Niger (Nikam and Shitole, 1993). The result from this study has shown that BAP induced the activation of totipotency at the stem node segments, which resulted in the formation of shoots The Tendril segments of 1.0 cm – 2.0 cm long were cultured and surface sterilized with 0.1% $HgCl_2$ for 5-7 minutes and rinsed with sterile distilled water. All the above operations were performed under aseptic conditions in a luminar air flow cabinet.

RESULTS AND DISCUSSION

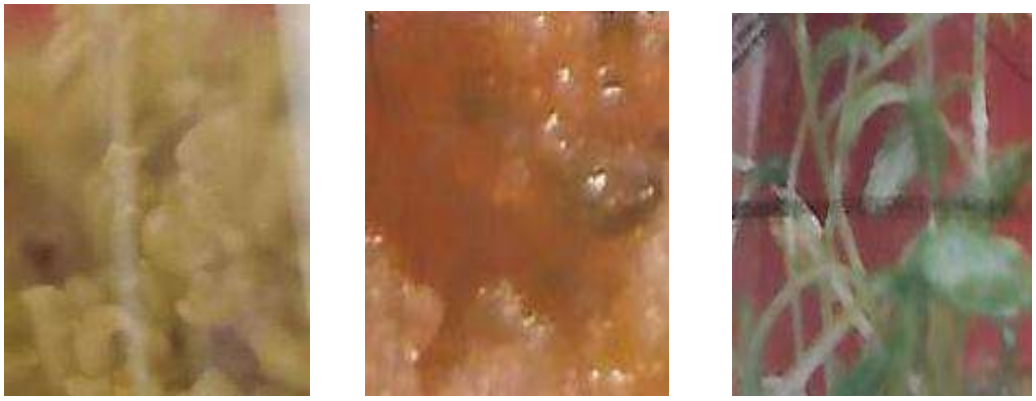
An eminent source of dietary protein constituents from human consumption as a big benefit in a balanced energy and protein diet for those who live in developing countries. In the present investigation we present the result of our efforts to develop a protocol for plantlet regeneration through Cotyledonary segments of Cucurbita maxima young plants of nodal segments collected from outside and grown under shade conditions. 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 – 2.0 mg/l, along with BAP (1.0 mg/l, has produced significant mean number of multiple shoots that ranged from 2-3 to 4-5 in both the explants. (Table–I, Plate-I). The number of shoots developed on the explants ranged from 2-4 to 2-3 by the addition of BAP at a concentration of 1.0 mg/l or NAA at 2.0 mg/l. Among three concentrations used i.e, 10, 15 and 20%, 15% of coconut milk along with 2.0 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 stem node segments. Addition of NAA failed to produce many shoots but enlarged the stem node 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 from different explants i.e. stem Cotyledonary cuttings of Cucurbita maxima. Among all explants used stem node 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 Cotyledonary 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 Cotyledonary segments cuttings. There was no significant increase in the number of shoots on NAA at low and high concentration. The percentage of explants responding was evaluated after 4-6 weeks of cultures. The cultures were transferred to fresh medium after an interval of 3 weeks. Results on nodal segments in Cucurbita are presented in various concentrations developed friable callus after 10-15 days stem node segments were initially cultured with (0.5 mgl/L Kn, NAA & BAP 3.0 mgl/L) an induction frequency of 70% higher value production.

TABLE 1: Plant tissue culture callus induction from Cotyledonary explants of Cucurbita maxima (L)

Growth Regulators	Cotyledonary Segments	
	% frequency of shoots	Mean No. of shoots
MS + 0.5 mg/l BAP + 0.5 L-Glutamic acid+Kn	40	Green Callus
MS + 1.0 mg/l BAP + 1.0 L-Glutamic acid+Kn	35	Green Callus
MS + 2.0 mg/l BAP + 2.0 L-Glutamic acid+Kn	30	Callus+shoots (2-4)
MS + 3.0 mg/l BAP + 3.0 L-Glutamic acid+Kn	25	shoots (2-4)
MS + 0.5 mg/l NAA + CM	20	Callus
MS + 1.0 mg/l NAA + CM	15	Callus
MS + 2.0 mg/l NAA + CM	10	shoots (2-4)
MS + 3.0 Mg/l NAA + CM	05	shoots (1-2)

CM = Coconut milk water

Plate I. Plant tissue culture callus from Cotyledonary explants of Cucurbita maxima (L)



CONCLUSION

MS Medium with BAP, NAA and Kn (0.5-3.0mgl/L) for further development and matuaration of Cotyledonary segment cultures Cucurbita maxima were incubated at 25≠ 2°C under the same condition for callus induction and plant regeneration. The method of repeated transfer of Cucurbita maxima (L) Cotyledonary segments. This is considered as one of the methods to increase the response in explants has suggested that repeated transfer of explants on multiplication media containing cytokinins succeeds in activating the plant materials.

REFERENCES

1) A Ramulu, Venkateshwarlu M, Srilatha T, B Raju, Ugender T (2014) Protoplast Isolation from leaf explants of Solanum Surattence Burm F, A medicinally important plant International Journal of Eng. and Tech. Research (IJETR) vol.2, Issue.10, 2321-0869.
2) Hanan Abd, Al-Hay and Al-Ashaal A (2010) Regeneration In Vitro glycol alkaloids reduction and evolution of bio activity of callus methanolic extract of Solanum tuberesum L Fitoterapia 81 (6) Pp 600-606.

3) Joshi M and Dhar U (2003) In Vitro propagation of Saussurea obvullata (DC) Endgew.an endangered ethnoreligious medicinal herb of Himalaya endangered ethnorelegious plant cell Rep. 21(10) 933-939.
4) 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.
5) K Rajam Narasinga Rao N, Venkateshwarlu m, Sammaiah D, Anitha U and Ugander T (2013) Studies on the medicinal plant Biodiversity in forest Ecosystem of Mahadevpur forest of Karimnagar (AP) India Bioscience Discovery 4(1) Pp : 82-88.
6) Kannap A A Thengave S R and Krishna Murthy KU (1995) New aspects of virus elimination in fruit trees. Acta. Hortic .386: 409-418.
7) Lie Shuska M (2002) Elimination of Apple chlorotic leaf shoot virus (ACLSV) from pear by in vitro thermotherapy and chemotherapy Acta hortic 596: 481-484.
8) 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.
9) 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.
10) 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.
11) T Ugender GPV Shekar, M Venkateshwarlu and K.J. Reddy (2011) Direct plantlet regeneration from seedling explants of Phascolus Vulgaris L Bionano Frontier 131-132.
12) Venkateshwarlu M, Odelu G, Babitha Kumari D, N Raju and Ugander T (2018) Studies in the phytochemical analysis and biological activities of leaves of Solanum Surattense (Burm F). A medicinally important plant . Bioscience Discovery a(1) PP 114-121.
13) Venkateshwarlu M (2017) Studies on photochemical analysis and biological activites in momordica sps. The planning innovation Journal vol.6, Issue 12 pp 437-440.
14) Wawrosch C Malla P.R. and Kopp B 92001) clonal propagation of Lilium nepalense D. Don a threatened medicinal plant of Nepal. Plant cell Rep. 20(4) 285-288.