Original Research Paper

Volume-9 | Issue-11 | November - 2019 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Botany

STUDIES ON MICROPROPOGATION IN VITRO SHOOT INDUCTION FROM STEM NODE EXPLANTS OF *CUCUMIS SATIVUS L.*

Mandaloju Venkateshwarlu*

Department Of Botany, Kakatiya University, Warangal-506 009. Telangana State, India. * Corresponding Author

ABSTRACT 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). The stem node explants were inoculated on MS medium supplemented with various cytokinins i.e., BAP and NAA. 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 stem node explants. Micro propagation 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). Multiple shoot induction was achieved in one of the important medicinal plant of Cucurbitacae family, *Cucumis Sativus*. MS medium supplemented with 1.0 mg/l BAP + 3.0 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 stem node explants of *Cucumis Sativus* using different concentrations and combinations of Cytokinins.

KEYWORDS : Stem node explants, multiple shoots, NAA BAP, *Cucumis Sativus*

INTRODUCTION

In Vitro Regeneration of plants via Somatic embryo genesis has much potential for plant propagation and gene transfer In Soybean somatic embryo have been obtained from cultured immature cotyledons Soybean from embryogenic callus line derived from the shoot tip explants (Parvathi, Venkateshwarlu M-2018) *In Vitro* regeneration Via somatic embryo genesis has drawn more attention than other methods because it can produce a large number of plants in a relatively short time (walker *et. al* 2001) The number of protoplasts showed increase during shorter treatment time and reaches a peak at 4-5 hours of after this many reports of soyabean somatic embryogeneis were published (Pathak. *et.al* 2014) The development of protoplast systems has increased the plants for use in both Biochemical and genetic research. Ugandhar *et. al* (2011) has been also reported that high amount of Cytokinin and lower amount of Auxins is the best combination for Somatic embryogenesis which is in accordance of our study. Stem node explants of *Cucumis Sativus* on MS medium fortified with plant growth regulators along with coconut milk and Amino acids. The plants of Cucurbitaceae suffer from several diseases including the water melon mosoic virus (Greber, 1978), Cucumber green mottle mosoic virus (Nijsden, 1984) and *Cucumis Sativus* also suffers from downey and powdery mildews which seriously limits the crop production. Auxiliary buds from pumpkin were reported by Jelaska (1972). The similar findings were also reported by Singh (2005). Georges Morel (1952) first demonstrated that virus free plants can be recovered from infected plants through shoot tip cultures.

MATERIALS AND METHODS

MS Basal Medium was supplemented with various plant growth regulators and 3.0% Sucrose. The PH of the media was adjusted to 5.8, solidified with 0.8% Difco-bacto Agar and Autocloved at 103.4 KPa or 121°C for 15-20min. A single explants was placed in each culture tube and incubated (at25± 1°C with a 16h photoperiod under fluorescent light (40-50m^2 s^{-1}). Explants with In Vitro multiple shoots proliferated on TD2-containing media were transferred to MS Medium containing different concentrations (BAP2.0-3.0mgl+) Multiple shoot intonation from shoot tip explants was observed within 20-25days after inoculation. The effect of different five types of growth regulators on direct plantlef regeneration of tamato from shoot tip explants (T Ugandar & M Venkateshwarlu-2018)

The Result from this study has shown that BAP induced the activation of Totipotency at the stem node explants, which resulted in the formation of multiple shoots. The stem node 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. The pH of the medium was adjusted to 5.8 and later was autoclaved at 120^{0}C for 17 minutes. Cultures were incubated under 16 hrs, illumination (250 lux) at 25±2^{0}C temperature. Raising the level of BAP (0.5 to 2.0 mg/l) resulted in the increase in the number of shoots from hypocotyls and cotyledon explants of Niger (Nikam and Shitole, 1993).

RESULTS AND DISCUSSION

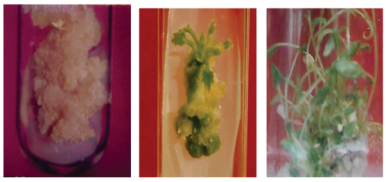
The Stem node cuttings were inoculated on MS basal medium fortified with various cytokinins i.e., BAP and NAA. Coconut water also had a role in triggering the formation of multiple shoots. Raising the level of BAP (3 mg/l to 4 mg/l) resulted in an increase in the percentage of shoots developed from Stem node cuttings. There was no significant increase in the number of shoots on NAA at low and high concentration. 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. 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 number of shoots developed on the explants ranged from 1-4 to 2-3 by the addition of BAP at a concentration of 1.0 mg/l or NAA at 2.0 mg/l. (Plate – I A,B,C). MS medium fortified with 1.0 mg/l BAP or 2.0 mg/l L-Glutamic acid also induced shoot buds on Stem node explants. Addition of NAA failed to produce many shoots but enlarged the stem node segments. The results from study have shown the initiation of shoot buds and formation of multiple shoots from different explants i.e. Stem node cuttings of *Cucumis Sativus.* 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.

TABLE 1: *In Vitro* Shoot induction from Stem node explants of *Cucumis Sativus L.*

Growth Regulators	Stem Node	
	% frequency of Shoots	Mean No. of Shoots
MS + 0.5 mg/l BAP + 0.5 L-Glutamic acid	25	Callus
MS + 1.0 mg/l BAP + 1.0 L-Glutamic acid	30	Callus
MS + 2.0 mg/l BAP + 2.0 L-Glutamic acid	40	shoots (2-4)
MS + 3.0 mg/l BAP + 3.0 L-Glutamic acid	45	shoots (2-6)
MS + 0.5 mg/l NAA + 0.5mg/l	30	Callus
MS + 1.0 mg/l NAA + 2.0mg/l	14-22	Callus
MS + 2.0 mg/l NAA + 2.5mg/l	12-20	shoots (3-4)
MS + 3.0 Mg/l NAA + 3.0mg/l	10-16	shoots (2-4)
MS + 4.0 mg/l NAA + 3.0mg/l	6-10	shoots (4-6)

Submitted : 24th May, 2019 | Revised : 13th July, 2019 | Accepted : 09th September, 2019 | Publication : 01st November, 2019

Plate I. In Vitro shoot Induction from Stem node explants of *Cucumis Sativus L.*



A B C

CONCLUSION

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. 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. The purpose of this work was to study the effect of different concentrations of growth regulators on direct plantlet regeneration of *Cucumis Sativus* from stem node explants. Rooted plantlets were successfully hardened under culture conditions and established in the field conditions.

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