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IN VITRO MULTIPLE SHOOTING AND CALLUS CULTURE OF *SOLANUM XANTHOCARPUM* BY DIFFERENT CONCENTRATION OF PLANT GROWTH REGULATORS

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Abstract

The study aims to investigate the effect of various concentrations and combinations of plant growth regulators on callus induction, plant regeneration from callus, root regeneration and multiple shoots using intermodal and nodal explants in *Solanum xanthocarpum.* Direct regeneration of nodal explants and their multiplication have been optimized using cytokinin BAP (3.5 mgL^{-1}) and combination of 0.1 mgL^{-1} NAA+ 2.0 mgL^{-1} BAP respectively. The best growth of callus was observed in the MS medium supplemented with 3mgL^{-1} NAA + 0.5mgL^{-1}BAP and 3mgL^{-1} NAA + 3mgL^{-1}BAP at 4 weeks. Thus, reproducible protocol was established for induction of multiple shooting and callus formation via direct and indirect organogenesis. Moreover, 2, 4-D, BAP and combination of NAA and BAP was reported to be necessary for callus induction and shoot formation. Best callus formation and growth rate was observed in the medium containing 3.0 mgL^{-1} 2, 4-D and 3mgL^{-1} NAA in combination with 3 or 0.5mgL^{-1} BAP. Calluses obtained were harder and conspicuous as well as light green in color which later on turns into light brown color.

Index terms- *Solanum xanthocarpum*, Callus, Explant, Plant growth regulator.

I. INTRODUCTION

Solanum xanthocarpum is commonly known as Indian night shade or yellow berried night-shade plant (Schrad and Wendl) belongs to family solanaceae. *Solanum xanthocarpum* is found throughout the India; mainly grown in various states viz. Bihar, Uttar Pradesh, Punjab, Uttaranchal, West Bengal, Assam and other North-Eastern States .They grows as weed on all kinds of soil but does well on dry places on road side and waste lands (Anonymous., 1998). In ancient ayurveda ,it was suggested as bitter, antihelmintic and also suggested to possess many activities such as anti-fertility, antipyretic, anti-allergy, anti-inflammatory, anti-histaminic, hypoglycemic, antiasthmatic, anti-tussive, anti oxidant, anti-bacterial, anti-fungal, anti-helmintic activities (Lohar DR., 2007),(Smith, 1991),(WHO, 2002). All plant parts were used for medicinal purpose. Leaves are used in relieving pains. Roots are useful in treatment of coughs, asthma, chest pain and catarrhal fever. They are diuretic and expectorant. Stem, flowers and fruits are prescribed for treating burning sensation in the feet accompanied by vesicular eruptions and also used for treating cough, fever and heart diseases. (Joy, P. P. et al., 2001). Fruits and seeds contain sterol in the form of carpesterol and are used in treatment of asthma. Plant was rich in many chemical constituents but active principles of this plant are steroids and alkaloids *viz.* solasonine, solamargine and solasodine. In pharmaceutical industries solasodine was used for production of steroidal drugs which have antispermatogenic, antidiabetic, (Johnson, N. C., Graham, J. H., & Smith, 1997) dental analgesic, useful in infantile atopical dermatitis and has anticancerous

activities. It is also reported as an antinsecticidal, (Hunter IR et al., 1976) antiaccelerator cardiac activities (Crabbe PG, 1980). Many herbal formulations made from this nontoxic plant (Roshy Joseph C IR, 2012). There is an increase demand of such formulations, because they are safe for human use, cheaper, easily available with no side effects. Hence, they considered to be the first line of defence for curing various ailments mostly in rural and tribal communities (Dwivedi et al., 2008). Due to these reasons they are become exploited for the application by human beings, since an ancient time has led to exploitation of their resources and are in danger of extinction. In vitro multiplication through tissue culture technique is an alternative method for conservation of the same. Even very limited studies have been reported for micropropagation of *S. xanthocarpum* (AN and Jawahar, 2011)(Saxena et al., 1982)(Rahman MM et al., 2011). The present study aims to investigate the effect of plant growth regulators which aids in plant growth enhancement and multiple shoot induction in *S. xanthocarpum* for mass propagation.

II. MATERIAL AND METHODS

2.1 Collection of plant materials

The plant samples of wild species *Solanum xanthocarpum* were collected from the premises of Government science college Vankal campus. Different organs of *S. xanthocarpum* were *viz*., stem (nodes and internodes), axillary buds selected as an explants. Selected healthy organs were cut with sterile scalpel and stored in sterile polythene bag at 4^0 C till they were processed.

2.2 Sterilization of explants

Healthy explants without any symptoms were taken as explants and went for sterilization treatments. Different sterilization agents were used for sterilization of selected organs; explant segments were treated with 5% Bavistin, 70% Ethanol, 4% Sodium hypochlorite, 0.1% Mercury chloride and finally with sterile distilled water. Evaluation of sterilization treatment was done by impression method.

2.3 Media preparation and culture conditions

Sterilized explants were inoculated on MS (Murashige T, 1962) medium containing 3% sucrose as carbon source and 0.8% agar as solidifying agent. The pH of medium was adjusted to 5.8 prior to autoclaving at 121˚ C and 15 psi for 20 minutes. The Inoculated culture tube and bottles were incubated and maintained at 25 ± 1 °C, 55±5% relative humidity under a 16-h light and 8 h dark photoperiod supplemented with cool white fluorescent light with intensity of 2500 lux.

2.4 Culture establishment

Three different experiments were performed including direct shoot regeneration from nodal shoot segments, callus induction from node explants, and finally shoot regeneration from the node-derived calli. Nodal explants and axillary buds with the length of about 0.5-1 cm were inoculated in MS medium supplemented with different concentration of plant growth regulators such as auxin (NAA, 2, 4-D) and cytokinin (BAP and KIN) alone or in combination were used for callus induction and shoot formation.

III. RESULTS AND DISCUSSION

Internodal and nodal segments were used as an explants for callus and shoot induction. A particular combination of NAA (1 mgL^{-1}) and BAP (2 mgL^{-1}) were effectively induced multiple shoots. The results showed that the treatment containing NAA (0.1 mgL^{-1}) and BAP (2 mgL^{-1}) in combination was found to be the best one for shoot regeneration from nodal segments. Moreover, BAP (3.5 mgL^{-1}) alone and combination of NAA (0.1 mgL^{-1}) and BAP (2.0 mgL^{-1}) and combination of NAA (1.0 mgL^{-1}) and BAP (2.0 mgL^{-1}) and NAA(1.0 mgL^{-1}) combination with BAP (2.0 mgL^{-1}) were found to be the efficient treatments for shoot regeneration from callus. At the initial stage shoot premodia were observed which subsequently converted to shoots with several leaves (Fig 2- A & B). The auxin, 2, 4-D results hard and compact callus in compared to combination of NAA and BAP which gives green friable callus. It seems that relatively more concentrations of both auxin and cytokinin were necessary for callus formation. The results of callus formation and shoot regeneration from different treatments have been shown in Table 1.

Table 1: Effect of different concentrations of various PGRs on callus induction and shoot regeneration

Plant growth regulators and their combinations	Concentration (mgL^{-1})	Degree of growth	Morphological response
2,4-D	2.0	++	White hard callus
2,4-D	2.0	+	Light green callus
2,4-D	3.0	+	Green hard callus
BAP	2.5	++	Whitish green hard callus
BAP	2.5	+++	Multiple shoot formation
BAP	2.0	++	Whitish green hard callus
BAP	3.0	+	Green callus
BAP	3.5	+++	Light green Callus+ shoot with 3 leaves
NAA	1.5	+	Brown hard callus
NAA + BAP	3.0+3.0	+++	Light green compact/friable callus
NAA + BAP	1.5+1.5	+	Light brown callus with shoot initiation
NAA + BAP	2.0+3.0	+++	Callus+shoot
NAA + BAP	2.0+4.0	+++	Light green callus
NAA + BAP	3.0+4.0	++	Light green hard callus
NAA + BAP	3.0+4.0	+++	Multiple Shoot+brown Callus
NAA + BAP	1.0+1.0	+++	White + light green callus
NAA + BAP	1.0+2.0	+++	Multiple shoot formation
NAA + BAP	1.0+2.0	+++	Multiple shoot formation
NAA + BAP	1.0+2.0	+++	White light green callus
NAA + BAP	1.5+4.5	++	Callus+shoot
NAA + BAP	2.0+0.5	++	White callus
NAA + BAP	2.0+3.0	+++	Light Brown+ green Friable callus +shoot initiation
NAA + BAP	0.1+2.0	+++	Brownish Callus+shoot with 3 green leaves
NAA + BAP	0.1+2.0	+	Light green callus
NAA + BAP	0.5+0.5	+++	White callus
NAA + BAP	0.5+1.5	++	Light green callus
NAA + BAP	1.5+3.0	+++	White light green callus
NAA + BAP	1.5+0.5	+	White callus
NAA + BAP	1.5+4.5	+++	White callus
NAA + BAP	3.0+0.5	+++	Light green compact friable callus

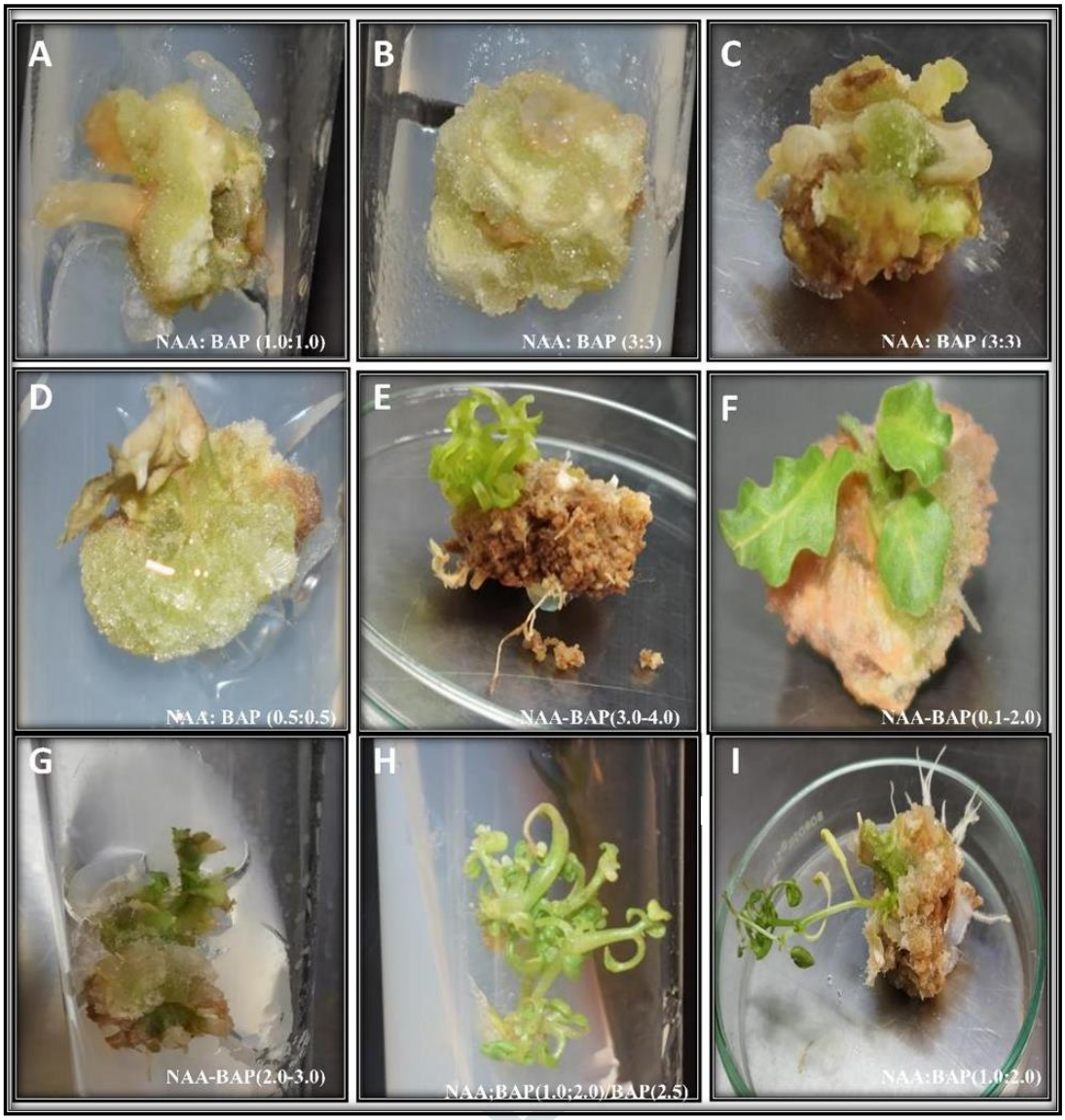

Fig. 2 (A-I) Effects of Plant growth regulators on Callus formation and Shoot formation

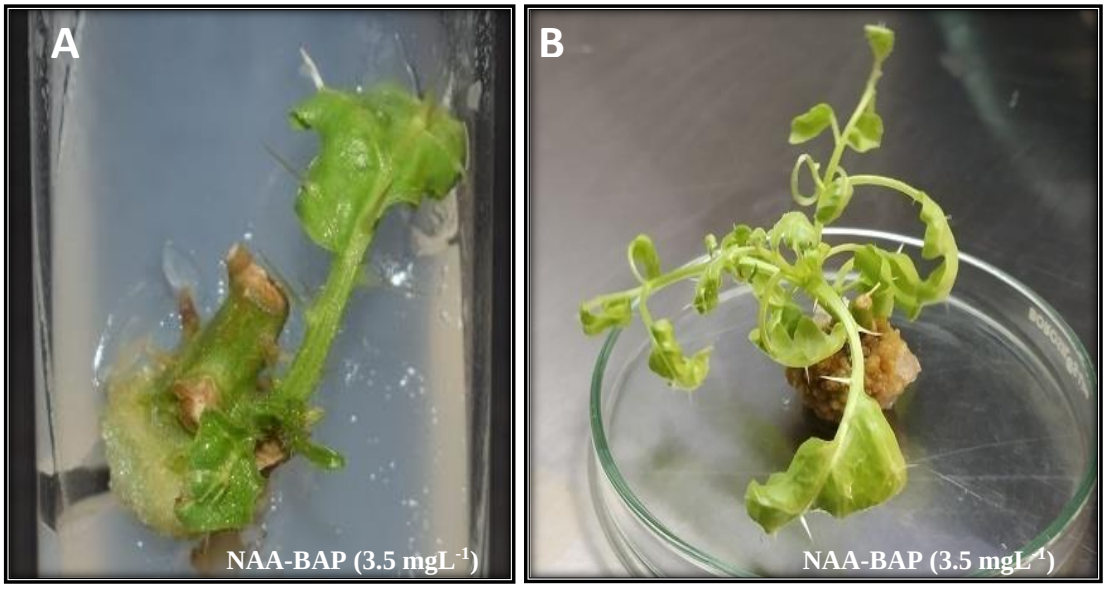

Fig. 2 (A&B) Effect of culture media with fixed concentration of NAA and BAP

Moreover, 2, 4-D, BAP and combination of NAA and BAP was reported to be necessary for callus induction and shoot formation. Best callus formation and growth rate was occurred in the medium containing 3.0 mgL^{-1} 2, 4-D alone and $3mgL^{-1}$ NAA in combination with 3 or $0.5mgL^{-1}$ BAP. Calluses obtained were harder and conspicuous as well as light green in color which later on turns into light brown color. The lowest callus induction response was observed on MS medium supplied with 2, 4-D alone. The similar concentration of NAA and BAP in combination favored good growth of callus formation (Fig.1 A-D). Slight increase in BAP concentration leads to shoot initiation (Fig.1 E-I). The shoots were initiated from after 2 week of callus culture in the similar hormone concentration. The shoots emerged were in the form of dense cluster of leaves in the hormone concentration of NAA and BAP in the ratio of $3mgL^{-1}$ and 4 mgL^{-1}, respectively (Fig.1E).

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