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RESEARCH ARTICLE

IN VITRO STUDIES IN *Solanum xanthocarpum* TO COMPARE THE POTENTIAL OF DIFFERENT EXPLANTS TOWARDS CALLUS INDUCTION AND SHOOT FORMATION

***Dr. Sanjay Kumar, Dr. Pooja Gulati and Dr. Rajeev Kumar Kapoor**

Department of Microbiology, Maharshi Dayanand University, Rohtak- 124001, Haryana, India

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ABSTRACT

Solanum xanthocarpum, a medicinal herb is of great economic importance due to its immense therapeutic and pesticidal properties. The present study was carried out to develop guidelines for *in vitro* multiplication of *Solanum xanthocarpum.* Explants like stem nodes, leaves and axillary buds were cultured on MS media supplemented with phytoharmones *viz.* BAP, 2,4 D, Kinetin, NAA, in various combinations and their response towards callus induction and shoot formation was observed. While leaves and axillary bud explants exhibited callus formation only, stem node responded to yield both callus and shoot formation. Nodal explants responded best to cytokinins and can be used for regeneration studies of this plant.

INTRODUCTION

Solanum xanthocarpum, a wild medicinal herb found widespread in India and Nepal has enormous therapeutic potential and is known to treat various chronic ailments like skin diseases, hypertension, asthma, bronchitis, haemorrhoids, asthama, and even cancers (Gupta *et al*., 2012; Dewangan *et al*., 2012; Patel *et al*., 2012). It's common name in Hindi is 'Kantkari' and belongs to family Solanaceae, members of which synthesize many desirable chemical compounds like alkaloids, sterols, saponins, flavonoids and their glycosides (Shanker, *et al*., 2011; Chiang *et al*., 2007; Tupkari *et al*., 1972; Beisler and Sato, 1971). Several researchers have worked on *S. xanthopcarpum* in context of its various important chemical constituents *viz.* solasodine and steroidal alkaloid (Goswami and Boissya, 1986). Solasodine from *S. xantocarpum* possess antispermatogenic activity and has been found to affect morphology, motility, and glycolytic enzymes of spermatozoa (Purohi,. 1992; Dixit and Gupta, 1982). Solasodine has also been able to bring about the changes in testicular cell population in Rhesus monkey (Dixit and Gupta, 1989). Some chemical constituents of the plant like Lupeol, apigenin, Diosgenin and solamargine exhibit anticancer property (Bhutani. *et al*., 2010; Chaturved *et al*., 2008; Siddiqui *et al*., 2008; Mazzio and Soliman, 2009). The plant possesses anti-allergic property and it has been examined for its clinical efficiency in asthma (Choi *et al*., 1999; Govidan, *et al*., 1999, 2004). *S. xanthocarpum* along with some other herbs has also been shown to possess important hypoglycemic activity along with certain side effects (Kar *et al*., 2006). In addition, the plant has also been reported to exhibit fungicidal, insecticidal and pesticidal properties (Kumar *et al*., 2012; Mohan *et al*., 2007; Fewell *et al*., 1994). Despite of its various demonstrated desirable properties, not much work has been performed regarding its micropropagation (Jaggi, and Bhatnagar, 1987). As *in vitro* micropropagation through plant tissue culture serves as powerful technique to multiply such species of economic importance, we therefore took this study to prepare guidelines for micropropagation of this plant by evaluating the potential of explants like stem node, bud and leaves towards callus induction and shoot formation in response to various phytoharmones.

Corresponding author: sanjaykumarkadam@gmail.com, sanjaykumarkadam@yahoo.com

MATERIALS AND METHODS

Preparation of Medium: Murashige and Skoogs (MS) (Murashige and Skoog, 1962) medium was composed in distilled water by adding all the stocks of mineral salts in appropriate concentrations along with 3% sucrose. Later 0.8 % agar was added and pH was adjusted to 5.8. 40 ml of homogenous medium was then dispensed into 100 ml flasks and sterilized at a pressure of 15lb/in^2 for 20 minutes. After autoclaving various phytoharmones like Cytokinins i.e. 6-Benzylaminopurine (BAP), Kinetin and Auxins i.e. 2,4-dichlorophénoxyacetique (2,4 D) , 1-Naphthaleneacetic acid (NAA) were added in appropriate concentrations (Table 1). Preparation of Explants and inoculation: Explants were collected from the plants growing in ridge area of University of Delhi, north campus, New Delhi, India. A young twig of plant was cut and shoot tip, nodes and leaves were excised with size 2-3cm and used as explants. Explants were washed with detergent and then surface sterilized with 0.1% $HgCl_2$ solution for 1 min. and rinsed with distilled water.

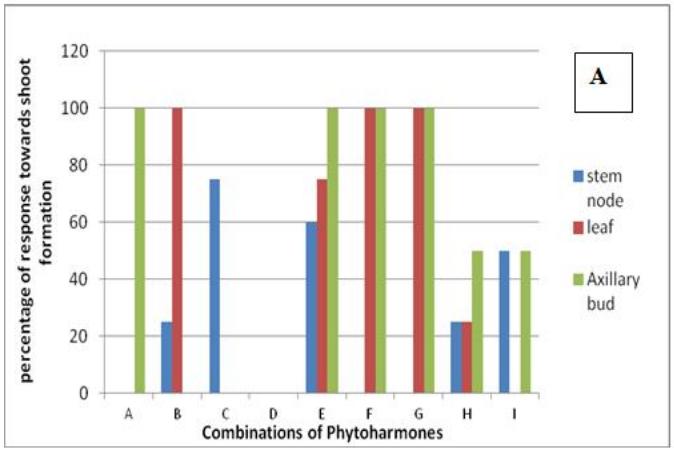
Cultivation of explants

All culture experiments were performed in four separate flasks. The cultures were inoculated in culture chamber at 27± 2°C and light intensity was provided with the help of photoperiodic stimulator to maintain a photoperiod of 16 hours along with 6 hrs dark period. Cultures were observed and changes were monitored, periodically.

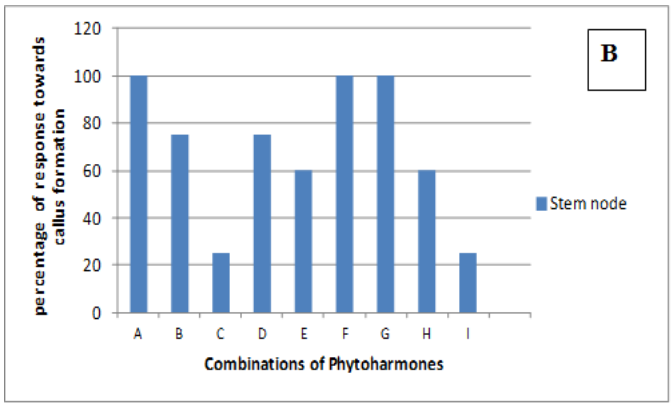
RESULTS AND DISCUSSION

Stem node as explants: Explant nodal segments responded extremely well to the cytokinins by exhibiting callus induction and direct shoot regenerations after 13 days of inoculation. Response of nodal explants towards callus and shoot formation was significantly influenced by varying concentrations of cytokinins and auxins. Maximum frequency of shoot formation was obtained on media supplemented with BAP (0.5mg/l) whether used alone or mixed with Kinetin (0.5 mg/l) or with a mixture of Kinetin (0.5 mg/l) and 2,4 D (0.5 mg/l) (Fig 1A, 1B). Medium supplemented with auxin 2,4 D (3mg/l) suited best for inducing callus in nodal segments with 75 % frequency of response. When the growths were sub-cultured in same

fresh medium, profuse growth was observed in all the cultures (Fig 3A, 3B).



A. Response towards callus formation



B. Response towards stem formation

Figure 1. Percentage of response in explants (stem node, leaf and axillary bud) grown on MS media supplemented with various combinations of plant growth regulators

Leaves as explants: Leaves in general exhibited curling, drying and callus proliferation in response to various phytoharmones but no shoot formation could be seen. Highest frequency of callus induction was reported on medium supplemented with high concentration of BAP (3mg/l) either alone or in combination with other cytokinins and auxins excluding NAA (0.5mg/l) which reduced the response percentage in leaf explants (Table.1). Leaves responded relatively fast towards callus which appeared after 10 days of inoculation. 2,4D could not induce any response in leaves when used alone. Interestingly, leaf explants displayed size enhancement on a medium supplemented with BAP (3.0 mg/l), Kinetin (0.5 mg/l) and NAA (0.5 mg/l) (Fig 2D).

Table 1. Different phytoharmone combinations used in study

Combinations	Phytoharmones (mg/l)			
	BAP	2,4 D	Kinetin	NAA
A	0.5	-	-	-
B		-	-	-
C	-	3.0	-	-
D	-	0.5	-	
E	3.0	0.5	-	-
F	3.0	-	0.5	-
G	3.0	0.5	0.5	
H	3.0	-	0.5	0.5
I	3.0	-	-	0.5

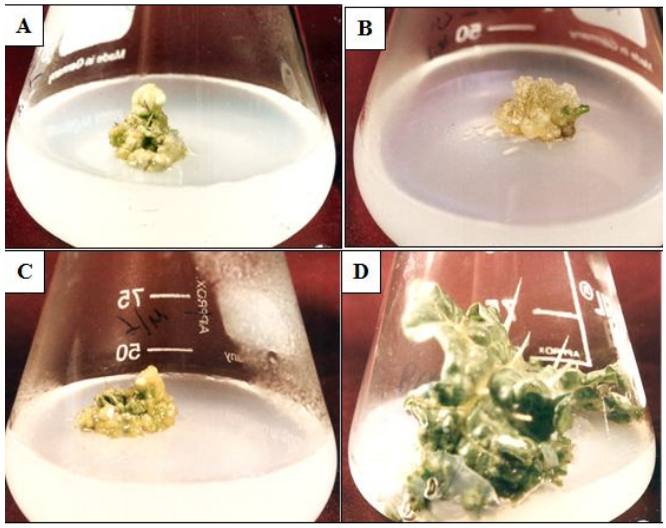


Figure 2

A. An Axillary bud of *Solanum xanthocarpum* showing callus proliferation on MS medium, supplemented with 2,4 D (0.5 mg/l),
B. A nodal explant showing callus proliferation on MS media supplemented with BAP (3.0 mg/l), kinetin (0.5 mg/l) and NAA (0.5mg/l),
C. An axillary bud showing callus proliferation on MS medium supplemented with BAP (3.0 mg/l) & 2, 4 D (0.5 mg/l),
D. A leaf explant showing increase in size supplemented with BAP (3.0 mg/l), kinetin (0.5 mg/l) and NAA (0.5 mg/l).

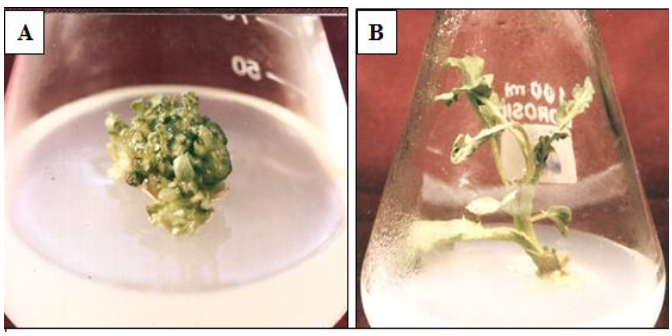


Figure 3

A. A nodal explant showing multiple shoot proliferation on MS media supplemented with BAP (3.0 mg/l).
B. A nodal explant of *Solanum xanthocarpum* showing shoot formation on MS medium supplemented with BAP (0.5 mg/l).

Axillary buds as explants: Axillary buds responded best among all the explants towards callus formation with 100 percent of response for BAP whether alone or in combination with other cytokinins and auxins except NAA (0.5mg/l) for which response remained 50 percent only. However no shoot formation could be obtained for any combination of phytoharmones examined (Fig 2A, 2C). Thus in the present study nodal segments responded best towards shoot formation in response to cytokinins and therefore can be further explored for the regeneration studies whereas leaves and axillary nodes can be taken up for callus induction and phytochemical studies of *S. xanthocarpum.*

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