Biospectra : Vol.9(2), September, 2014. pp.83-86

An International Biannual Refereed Journal of Life Sciences

Plant Sciences

ISSN : 0973-7057

Callus induction in medicinal plant *Solanum torvum* Sw. from leaf explants

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Received , 22nd June, 2014; Revised: 30th July, 2014

Abstract: To promote *in vitro* callus induction, from leaf explants of *Solanum torvum* Sw. were inoculated on MS Medium with different concentration of 2,4-D alone and different combination with NAA, BAP, IAA, IBA. The explants were maintained at (25° C ± 1° C) and 16h light cycle. The best calluses develop with IBA (1.0 ppm) in combination with BAP (1.0 ppm). On this combination of hormones light green and soft compact callus was observed. The responses of callus induction with other hormonal combination were negative. The present paper depicts the callus induction in *Solanum torvum* Sw. in *in vitro* conditions that provide a novel broad spectrum among researchers as a protocol for further research and investigation in tissue culture of medicinal plant *Solanum torvum* Sw.

Key words: *Solanum torvum* Sw., 2, 4-D, BAP, in vitro, Callus

INTRODUCTION

The family Solanaceae represent one of the most economically and medicinally important families of angiosperms.

The genus *Solanum* is a hyper-diverse taxon of this family. There are about 2000 species of *Solanum* in the world that are mainly distributed in the tropical and sub-tropical areas, with a small number in the temperate areas (Jennifer *et al.,* 1997). About 21species and one variety in this genus are used as herbal medicines (Hu *et al.,* 1999). *Solanum torvum* Sw. is a small solanaceous shrub, distributed widely in Pakistan, India, Malaya, China, Philippines, and tropical America (Nasir, 1985). For many decades, different ethnic groups have used the dried stem and root of this plant for treatment of various ailments. Among the major chemical constituents of *S. torvum* are steroids, steroid saponins, steroid alkaloids, and phenols.

Pharmacological studies indicate that the stem and root of *S.torvum* have anti- tumour, anti-bacterial, anti-viral, anti-inflammatory, and other medicinally important effects.

Traditional Medicinal Uses

Solanum torvum Sw. is a pharmacologically important species of the family Solanaceae. Traditional medicinal uses of S.torvum, have been highlighted in the Ayurveda and Chinese pharmacopsida.

Taxonomical Classification

Kingdom : Plantae, Plants; Subkingdom: Tracheobionta, vascular plants; Super division: Spermatophyta, Seeds plants;

Division: Angiosperma; Class: Dicotyledons; Order: Tubiflorae; Family: Solanaceae; Genus; *Solanum*; Species: *torvum*

In last few decades, due to rapid decrease in natural vegetation there is limited supply of medicinal plants, which has prompted new methods development for their supply. *In vitro* propagation of plant is the best method for production of plant based medicines. Present paper describes callus development in leaf explants of *Solanum torvum* Sw with different combination of NAA, IBA, BAP and IAA for micro propagation.

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MATERIALS & METHODS

Solanum torvum Sw (Solanaceae) palnt has been collected from college campus. The fresh new and soft leaves, nodes, internodes and also dried seeds were collected and washed 5 times with running tap water and then with distilled water. Then explants were kept in 100 ml of autoclaved distilled water containing 2 drops of savlon for 10 minutes. After that explants were washed 3 times by autoclaved distilled water then surface sterilization was done by dipping explants in 70% Ethanol for 1 min. and finally washed with autoclaved distilled water for 2-3 times. The explants were sterilized by 0.1% Mercuric Chloride ($Hgcl_2$) solution for 2-3 min. and continuously shaking was done. The explants were again washed 3 times with autoclaved distilled water in laminar air flow cabinet. The explants were dried using sterilized blotting paper and inoculated into culture jars and flasks with MS medium (Murashige and Skoog, 1962) containing 3% Sucrose solidified with 0.8% agar. The phytohormones were first dissolved in Ethanol and then autoclaved distilled water was added. The aqueous solutions of phytohormones Indole-3-Butyric acid (IBA), 6- Benzyl amino purine (BAP), 2,4-Dichlorophenoxy acetic (2,4-D) and Naphthaline-1-acetic acid (NAA) were added in required concentrations. The PH of the medium adjusts to 5.8 followed by autoclaving at 121° C and 1.06 kg/m^2?for 20 min in autoclave. Before starting the inoculation, all the required equipments/materials were transferred to laminar air flow chamber. Then the UV light was switched on for 20 min. After that the equipments were sterilized by dipping in 70% Ethanol followed by flaming and cooling. The sterilized explants were placed on the medium at the centre of culture tubes by the help of Sterilized forceps. The culture was incubated at 25°C ± 1° C with 16 h light and 8 h dark cycles. Each experiment was repeated three times.

RESULTS

The leaf explants of *Solanum torvum* Sw. in MS medium supplemented with different concentrations of 2,4-D (0.5ppm., 1.0 ppm.) gave scanty callus. While there was no callus growth in other phytohormones like IAA & NAA alone with different concentration. However, MS medium supplemented with 2,4-D (1.0 ppm.) + BAP (1.0 ppm.) and IBA (1.0 ppm) + BAP (1.0 ppm) showed callus induction. Initially after seven days hypertrophy was seen on leaf culture and callus formation was started from 15 days onwards. The callus colour was either light green or yellowish brown depending on nature of the phytohormones. The callus was compact and non-embryonic. The maximum growth of callus was on 4th week. There was no callusing was seen on seed explants. There was also less callusing in nodal & intermodal segment. The callusing responses with respect to various concentrations of hormones with leaf explants are presented in Table 1.

Table1 : Callus induction in leaf explants of *Solanum torvum* Sw.

Phytohormones	Conc. (ppm)	No.of culture tubes	Culture tubes with Callus	% Callusing	Morphological Character
2,4-D	0.5	7	4	60	Hypertrophy
	1.0	7	4	20	Scanty callus
	2.0	7	0	0	Callus not develop
2,4-D/BAP	0.5/0.5	7	2	40	Medium callus
	1.0/1.0	7	3	60	Light green friable
	2.0/2.0	7	3	60	Light green friable
IBA/BAP	0.5/0.5	7	5	60	Light green & yellowish brown soft non-embryonic
	1.0/1.0	**7**	**3**	**100**	**Light green & yellowish soft compact non-embryonic**
	2.0/2.0	7	4	80	Light green & yellowish
NAA/BAP	0.5/0.5	7	0	0	Callus not develop
	1.0/1.0	7	0	0	Callus not develop
	2.0/2.0	7	0	0	Callus not develop

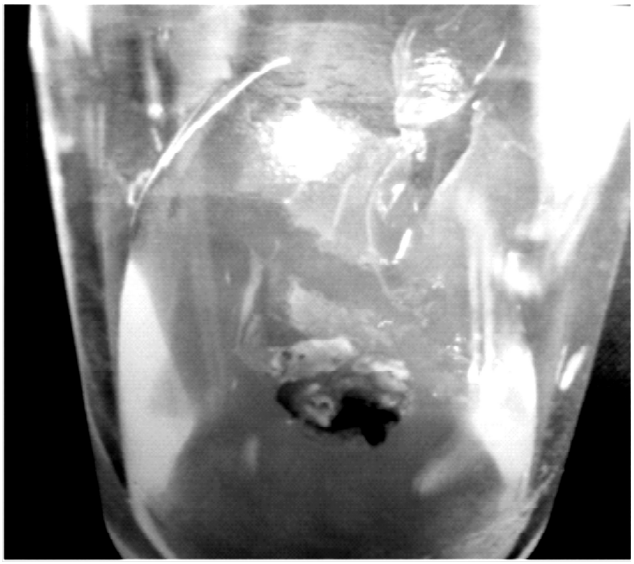

Fig 1: 21 days old callus induction from leaf explants of ***Solanum torvum* Sw.** in a MS+IBA (1ppm) + BAP(1ppm)

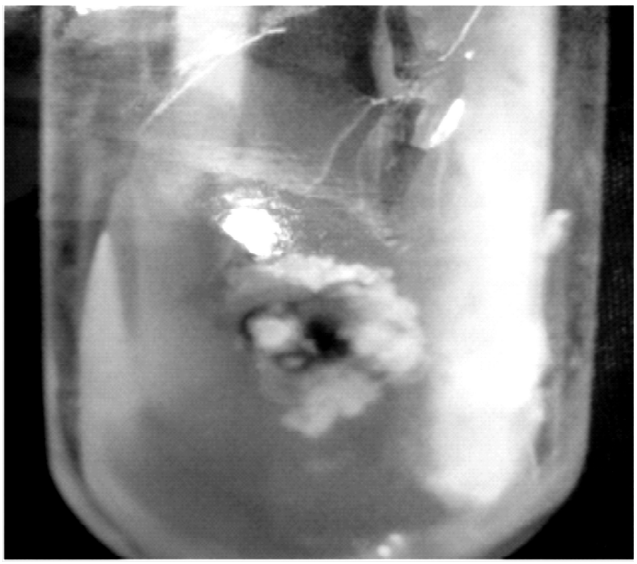

Fig 2: 28 days old callus induction from leaf explants of ***Solanum torvum* Sw.** in a MS+IBA (1ppm) + BAP(1ppm)

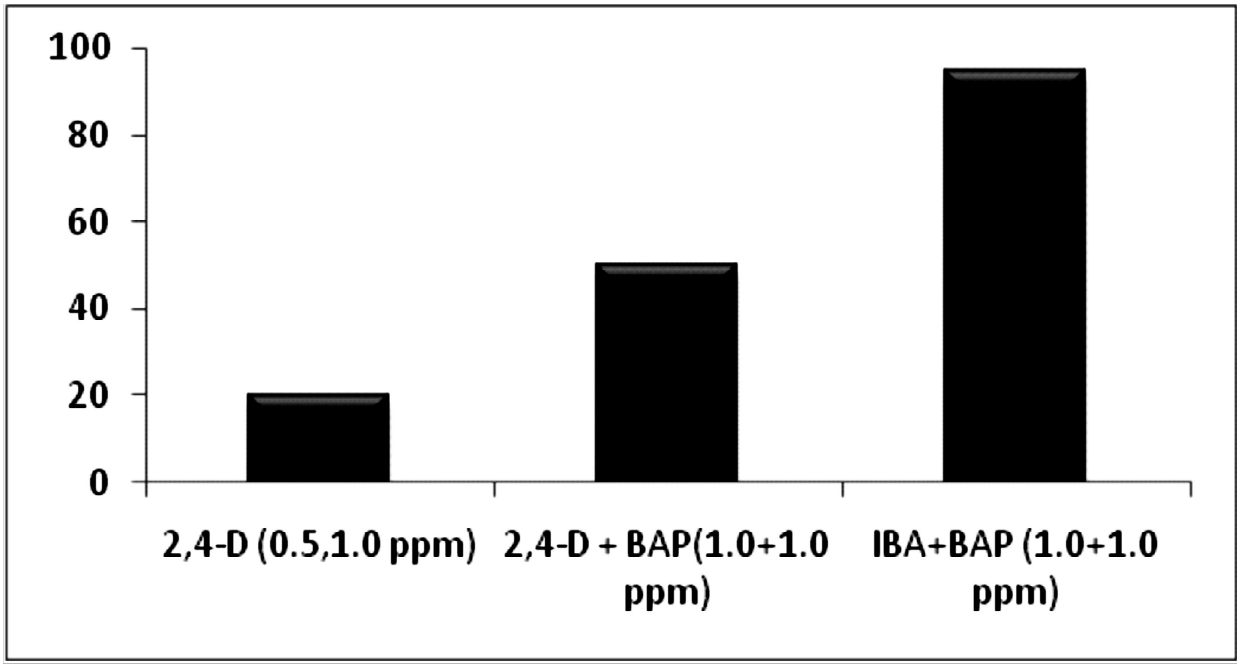

Fig 3: Histograms showing percentage of callus response with respect to phytohormones on leaf explants.

DISCUSSION

In order to carry out any advance studies, callus induction and regeneration are preconditions. *Solanum torvum* Sw. is known to be widely used in different types of diseases. However, reports on tissue culture studies in *Solanum torvum* Sw. is less. In the present investigation callus induction was observed from leaf explants of *Solanum torvum* Sw. by different types of phytohormones supplemented in the MS medium. The percentage of callus induction with different concentration of 2,4-D alone was very less i,e. 20% but callus response with 2,4-D + BAP was recorded to be 60%, and the nature of callus was found to be friable. The callus response with combination of IBA and BAP was 100% and the nature of callus was seen to be compact. The maximum callus growth was in 3-4 week old culture. These findings are in agreement with the result of kayani et.al. (2008) and Wesely et.al.(2010). The timing of surface sterilization was found to be very important in the present investigation. $Hgcl_2$ treatment more than 3 min has lethal effect.

The effect of 2,4-D on callus induction have been already been reported by large number of scientists (Zenk, 1978, wakhlu and Sharma 1998 and De-Saliva et.al. 2003).

In the present investigation best result for callus induction was seen on MS supplemented with IBA (1ppm) + BAP (1ppm).

ACKNOWLEDGEMENT

The authors are very thankful to Dr.R.K.Pandey, Head of the Department, University Department of Botany & Dean Faculty of Science, of Ranchi University, Ranchi for providing the laboratory facilities.

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