

# In Vitro Callus Induction in *Solanum virginianum* L.

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**Abstract:** *Solanum virginianum* L. is an important medicinal plant belongs to family Solanaceae. It is effective on Gonorrhea, Bronchial asthma, Tympanitis, Misperistalsis, Piles, Dysuria and for Rejuvenation in ayurveda. During the present investigation efforts have been made to evaluate standard protocol for induction of callus in *Solanum virginianum*. Explants were taken from elite plant and aseptically inoculated on MS medium supplemented with various concentrations of 2, 4-D alone and in combination with KIN/BAP 2,4-D. Best results were obtained on 0.5 and 1.0 mg/L of 2,4-D with stem, node as explant for induction of callus and its maintenances.

**Keywords:** *In vitro*, *Solanum virginianum*, callus, regeneration.

## 1. Introduction

*Solanum virginianum* L. is an important medicinal plant in ayurvedic medicines belongs to family Solanaceae. It is commonly known as yellow berried nightshade, and in Marathi bhuiringani or Ran Wangi. The genus *Solanum* is comprised of about 1500 species and well represented all over the world.

### Description and distribution of plant:

It is native to Asia (Saudi Arabia, Yemen, Afghanistan, Iran, China, Bangladesh, India, Nepal, Pakistan, Sri Lanka, Myanmar, Thailand, Vietnam, Indonesia and Malaysia) and is adventives in Egypt. In India it is recorded in tropical, subtropical and all four geographical regions. Frequently it has been considered as weed plant but in Ayurveda and folklore medicine since time immemorial there are meager reports in literature about its other potentials (Madhavi *et al.*, 2014). Morphologically *Solanum virginianum* is prickly diffuse bright green perennial herb, somewhat woody at the base while stem is zigzag, branches are numerous. The younger ones clothed with dense stellate tomentum.

### Medicinal and chemical properties:

In ancient Ayurveda, plant is described as pungent, bitter, digestive, alternative astringent. Stems, flowers, fruits are bitter and contains carminative properties. Root decoction used as febrifuge, effective diuretic and expectorant. Charaka and Sushruta used the extract of entire plant and fruits in internal prescription for bronchial asthma, tympanitis, misperistalsis, piles and dysuria and for rejuvenation. Kantkari Ghrita of Charakais was specific for cough and asthma. The whole plant is used traditionally for curing various ailments (Atul *et al.*, 2013). Decoction of the plant is used in gonorrhea; paste of leaves is applied to relieve pains. Seeds act as expectorant in cough and asthma and roots are expectorant and diuretic. They are useful in the treatment of catarrhal fever, coughs, asthma and chest. *Solanum virginianum* is a well-known medicinal plant in traditional medicinal system and recent scientific studies have emphasized the possible use of *Solanum virginianum* in modern medicine (Reddy *et al.*, 2014). Chemically Okram and Thokchom (2010) reported it is a valuable source of alkaloids, sterols, saponins, flavonoids and their glycosides and also carbohydrates, fatty acids, amino acids (Gnana *et al.*, 2013).

*al.*, 2013).

## 2. Materials and Methods

### Surface sterilization of explant:

Explants viz. leaves and stem node were collected from different localities of University campus of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. All explants were washed with tap water twice in laboratory, followed by 70% ethanol for 30 seconds and then surface sterilized with HgCl<sub>2</sub>. Surface sterilization of explant was carried out in laminar air flow. Explants were rinsed with sterile distilled water followed by 0.3% Mercuric chloride (HgCl<sub>2</sub>). Finally all these explants were dissected into small pieces and inoculated on MS medium aseptically.

### Culture media:

For induction of callus Murashige and Skoog media (MS) (1962) was used for stem node and leaf explants of *S. virginianum*. Stem node and leaf were inoculated on MS medium supplemented with 2,4-D, BAP and IAA for callus induction. MS medium fortified with 3% sucrose and gelled with 3 gm/L clarigar and the pH was adjusted to 5.8. The media was sterilized in an autoclave under 15 psi and 121°C.

### Culture condition:

After inoculation culture bottles were transferred to culture room under a 16 h photoperiod supplied by cool white fluorescent cool tubes light and temperature 25± 2°C. Maximum humidity was adjusted with air conditioner. Each experiment set in three sets, five of each.

## 3. Results and discussion

One of the important steps in plant tissue culture was surface sterilization of explants. Keeping this point in view different concentrations ranging from 0.1-0.3% of HgCl<sub>2</sub> were tried. Minimum contamination and higher rate of survival rate was achieved on 0.1% of HgCl<sub>2</sub> for leaf explants whereas 0.3% of HgCl<sub>2</sub> for stem node explant. Surface sterilized explants were inoculated on MS medium supplemented with various concentrations of 2, 4-D alone and in combination of KIN and BAP. All combinations of growth regulators were found more or less potent for induction of callus.

It could be revealed that, higher concentration of 2, 4-D has potential to induce profused callus. Callus induced with these concentrations was whitish to greenish colour with friable nature. Maximum rate of callus induction were recorded on 2.0 mg/L of 2, 4-D using leaf as explant. However it was found maximum at 2.0 mg/L and 4.0 mg/L of 2, 4-D using stem node as explant with 93.33% of callus induction frequency.

Leaf, stem and node explants also shown formation of callus on MS medium supplemented with 2, 4-D in combination of BAP. Moderate callus was induced with whitish yellow colour which was friable in nature. Highest frequency of callus induction was recorded on 1.0 mg/L of BAP in

combination with 2.0 mg/L of 2, 4-D with 46.66 and 53.33 % of callus induction frequency by using leaf and stem node as an explant respectively. Callus induction was also tried with 2, 4-D in combination of KIN but poor, yellowish and friable callus was developed. Maximum induction of callus was revealed by 4.0mg/L of 2, 4-D along with 2.0 mg/L of KIN with 33.33 and 40.00 percent of callus induction frequency. However lower concentration of growth regulators were found less effective for induction of callus or poor type of callus induction was achieved. These induced calluses were subcultured on MS medium with different concentrations of hormones to achieved miropropagation.

**Table 1:** Effects of different concentration of PGR's on Callus Induction of *Solanum virginianum* L.

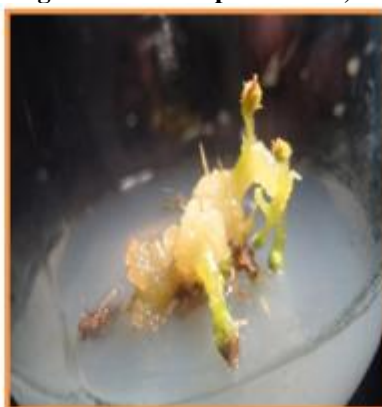
Growth regulators (mg/L)			Leaf explant		Stem node explant	
BAP	2, 4-D	KIN	Colour of callus	frequency of callus induction (%)	Colour of callus	Callus induction frequency (%)
--	0.5	--	Yellowish	26.66	--	--
--	1.0	--	Whitish	33.33	Whitish	33.33
--	1.5	--	Whitish	33.33	Whitish	60.00
--	2.0	--	Greenish	<b>73.33</b>	Greenish	<b>93.33</b>
--	2.5	--	Whitish	53.33	Greenish	80.00
--	3.0	--	Greenish	66.66	Greenish	86.66
--	3.5	--	Whitish	60.00	Greenish	86.66
--	4.0	--	Whitish	53.33	Greenish	<b>93.33</b>
0.5	1.0	--	Yellowish	20.00	Yellowish	26.66
1.0	2.0	--	Whitish	<b>46.66</b>	Whitish	<b>53.33</b>
1.5	3.0	--	Whitish	40.00	Whitish	46.66
2.0	4.0	--	Whitish	33.33	Whitish	33.33
--	1.0	0.5	Yellowish	20.00	Yellowish	20.00
--	2.0	1.0	Yellowish	26.66	Yellowish	20.00
--	3.0	1.5	Yellowish	20.00	Yellowish	26.66
--	4.0	2.0	Whitish	<b>33.33</b>	Whitish	<b>40.00</b>

Percentage response on three separate experiments, each based on a minimum of five replicates.

**1: Photo plate for callus induction by using stems node explant and 2,4-D as a growthregulator**



Greenish



Whitish



Yellowish

**2: Photo plate for callus induction by using leaf Explants and 2, 4-D as a growth regulator**



Greenish



Whitish



Yellowish

#### 4. Conclusion

Medicinal plants are voraciously collected for treatments of many disorders. These plants are bioreactors. If these plants propagated through modern techniques like tissue culture, raw Material could be utilized for therapeutic purpose. Present piece of work is useful for developing callus and secondary metabolites as well.

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