



**PLANTLET REGENERATION VIA CALLUS INDUCTION FROM LEAF EXPLANTS OF
SOLANUM SURATTENSE BURM. (F) A MEDICINALLY IMPORTANT PLANT**

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Article Received on 24/12/2015

Article Revised on 13/01/2016

Article Accepted on 03/02/2016

ABSTRACT

Solanum surattense Burm (F) a fast growing medicinal plant has been successfully plantlet regeneration via callus induction from leaf explants under *in vitro* condition. In this study the Leaf explants of two weeks old seedlings of *S. surattense* were induced for callus induction on MS media containing different combinations and concentrations of growth regulators. Different callusing media containing varying levels of 2,4-D/IAA/NAA (1.0-5.0mg/L) were tested for callus induction response. Maximum (90%) callusing response was obtained from leaf explants on MS medium containing (3.0mg/L) NAA. After 8 weeks of induction, the calluses were transferred on different regenerated media containing varying level of BAP/Kn/TDZ (1.0-5.0mg/L). Maximum shoot bud differentiation from callus culture was achieved on MS Medium fortified with BAP (3.0mg/L). Elongation and further development of shoot buds into shoots were achieved on MS medium supplemented with NAA (0.5mg/L) and BAP (3.0mg/L). This is the first report of *in vitro* callus induction and plantlet regeneration in *S. surattense* Burm (F). The regenerated elongated shoots were transferred to Indole Butyric Acid (IBA) (1.0mg/L–5.0mg/L) for root induction. Rooting was observed within two weeks of culture. Rooted plantlets were successfully hardened under culture conditions and subsequently established in the field conditions. The recorded survival rate of the plants was 86%. Plants looked healthy with no visually detectable phenotypic variations.

KEYWORDS: Callus induction, Regeneration, BAP: 6-Benzylaminopurine; IAA: Indole-3- Acetic Acid; MS: Murashige and Skoog IBA: Indolebutyric acid, Kn:- Kinetin, NAA: Naphthyl acetic acid.

INTRODUCTION

Solanum surattense Burm. (F) Is a medicinal herb belonging to family Solanaceae distributed in arid and semiarid regions of the world, especially in Southeast Asia, Malay, tropical Australia and India. The plant is used as digestive, diuretic and astringent agent and in bronchial asthma.^[1] It is also valued for antispasmodic, antitumor, cardiotoxic, hypotensive and anaphylactic activity. *S. surattense* produces glycolalkaloids in all parts of the plant body which on hydrolysis and removal of sugar residues yield steroidal alkaloids solanine, solamargine and solasodine. Solasodine is considered as a potential alternative to diosgenin for commercial steroid drug synthesis like progesterone and cortisone.^[2] *S. surattense* is propagated only by seeds, but this method is beset with difficulties such as: (I) The seeds show a low level of germination under normal conditions (ii) the seeds lose their viability on storage and (iii) seed derived progenies are not true-to-type due to cross

pollination. Due to over exploitation for high medicinal values and destruction of the habitat, this plant is becoming endangered^[3], hence there is need for *ex situ* conservation through tissue culture technique.^[4]

A number of regeneration studies from leaf^[5], root^[6], anther^[7], nodal and shoot tip explants^[8 and 9] were reported. A protocol on plantlet regeneration through somatic embryogenesis from cotyledon and leaf explants and streptomycin resistant plantlets using *in vitro* mutagenesis was also developed in *S. surattense*.^[10,11] *Agrobacterium* mediated genetic transformation using leaf explants of *S. surattense* was also reported by.^[12] However, there is no report on *in vitro* micropropagation using the seeds as an explant. We report in this communication on multiple shoots formation and plantlet establishment in *S. surattense* for the first time via callus induction from leaf explants.

METHODOLOGY

Plant material

Seeds of *S.surattense* were soaked in Distilled Water for 24 hours. These seeds were surface sterilized with 0.1% (W/V) Mercuric chloride (HgCl₂) solution for 3-5 minutes followed by three rinses with sterile distilled water. These seeds were germinated aseptically on MS basal medium^[13] solidified with 0.8% (W/V) difco bacto agar at pH 5.6 ± 2.0 in 250 ml Ehrlen meyer flasks (50ml medium/flask).

For callus induction the explants viz, Leaf (0.8–1.0 cm²) explants from 8-week old seedlings were excised, these explants were inoculated to MS medium supplemented with various concentrations of (1.0- 5.0 mg/L) of auxins such as 2,4-Dichlorophenoxy acetic acid (2,4-D), Indole 3-acetic acid (IAA), Naphthalene acetic acid (NAA) all the explant growth regulators were used as auxine alone in culture media. All media were adjusted to pH 5.8 before addition of 0.8% agar agar and autoclaved at 121°C and 103 K pa for 20 minutes cultures in 25 x 150 mm cultures tubes. For present investigation, the calli obtained on MS medium supplemented with (3.0 mg/L) NAA for leaf explants were used after 6 weeks of culture. This callus was regularly sub cultured for three passages on them same fresh medium. The callus induced after 4th passages was used for the present investigations.

Culture Media and Culture Conditions

The calli pieces approximately 0.5-10 cm² from leaf derived fresh friable callus cultures were transferred on to regeneration medium containing MS basal salts

supplemented with different concentrations of BAP/Kn/TDZ and also in combination with (0.5 mg/L) NAA. The pH of the media was adjusted to 5.8 either with 0.1 N HCL or 0.1N Na OH, solidified with 0.8% Difco-bacto agar and autoclaved at 121°C under 15 psi for 15-20 minutes. All the cultures were incubated at 25°C with 16h photo period under white fluorescent light of 40-60 mol m⁻² s⁻¹ intensity.

Data analysis

At least 15 replicates were maintained for each treatment and the data were recorded after six weeks of culture.

The Leaf explants showed 90% callus formation after six weeks of culture when placed in media supplemented with NAA (3.0 mg/L) singly. However, the response of the Leaf explants decreased (75%) with increase in concentration of NAA in the medium. Sub culturing of callus onto fresh medium containing the same concentrations of growth regulators resulted in the emergence of callus. Multiple shoot buds were initiated on the callus cultured in MS medium supplemented with cytokinins (BAP/Kn/TDZ) and auxin (NAA) singly and in combination (Table-1). (Fig-a).

Effect of BAP/Kn/TDZ

Callus-mediated shoot regeneration is an indirect method for plant regeneration.^[14,15] To probe into the formation of adventitious shoot from callus, 35-day-old calli were transferred to plant regeneration medium. At 45 days of plant regeneration, the number of adventitious shoots per callus and the rate of adventitious shoot induction were counted as shown in (Table-1).

Table – 1: Morphogenetic response of Leaf explants of *S.surattense* on MS medium supplemented with different concentrations of 2, 4-D, IAA and NAA.

Hormone conc (mg/L)	% of cultures responding	Morphology	Callusing response
2,4 – D			
1.0	70	White compact	+++
2.0	75	White compact	+++
3.0	80	White compact	+++
4.0	60	Cream-friable	++
5.0	50	Cream-friable	+
IAA			
1.0	60	White friable	++
2.0	58	Green nodular	++
3.0	50	Green nodular	++
4.0	45	White friable	+
5.0	40	White friable	+
NAA			
1.0	65	White friable	+++
2.0	80	White friable	+++
3.0	90	Green- friable*	+++
4.0	70	Green-friable	+++
5.0	75	Green- friable	++

Relative amount of Callus formation

- = No, + = low, ++ = moderate, +++ = high *Embryogeniccallus.

The media containing BAP/Kn/TDZ could induce the formation of adventitious shoots the rate of adventitious shoot induction showed that a trend declined with the increased concentration of (1.0-5.0 mg/L) BAP/Kn/TDZ. During the present investigation, variation combinations of (1.0-5.0, mg/L) were tried (Table-2).

The number of shoot buds included increased when callus was sub cultured on MS- medium containing BAP/Kn/TDZ. The entire calluses became compact and later shoot buds differentiated within 4-6 weeks. Maximum number of shoot buds proliferated (14.0 ± 0.23) at 3.0mg/L BAP. At the concentration of (3.0mg/L) Kn and TDZ induced (11.6 ± 0.34) and (10.0 ± 0.45) of shoot buds were obtained. Lower concentrations (1.0-2.0mg.L) and higher concentrations (4.0-5.0mg.L) of BAP/Kn/TDZ decreased the number of shoot buds. (Fig- b & c).

Effect of NAA + BAP/Kn/TDZ

Shoots formation per explants was enhanced in all the concentrations of BAP along with NAA. Maximum number of shoots per explants (20.5 ± 0.62) with 72% of cultures response was observed at (0.5mg/L NAA + 3.0mg/L) BAP in average length of shoot was (4.3 ± 0.72) (Fig I d & e) followed by (2.0 mg/L) BAP. Highest percentage of responding cultures was also found at (0.5mg/L) NAA + (3.0mg/L) BAP. Shoots formation was enhanced in all the concentrations of Kn along with IAA. Maximum number of shoots per explants (14.3 ± 0.35) was observed at (0.5mg/L) NAA + (3.0mg/L) Kn followed by (2.0 mg/L) and (4.0mg/L) Kn. Highest percentage of responding cultures was also found at (0.5mg/L) NAA + (3.0mg/L) Kn.

Table – 2: Effect of BAP, Kn and TDZ on induction of shoots proliferation from Leaf derived callus cultures of *S.surattense*.

Hormone conc (mg/L)	% of cultures responding	Average No. of shoots/explants \pm (SE)*	Average length of shoots (cms) \pm (SE)*
BAP			
1.0	57	9.2 ± 0.05	1.6 ± 0.07
2.0	76	11.3 ± 0.06	2.5 ± 0.35
3.0	81	14.0 ± 0.23	3.0 ± 0.63
4.0	72	12.0 ± 0.22	2.3 ± 0.34
5.0	51	8.0 ± 0.35	1.0 ± 0.32
Kn			
1.0	36	6.4 ± 0.25	1.3 ± 0.34
2.0	48	08 ± 0.33	2.0 ± 0.53
3.0	52	11.6 ± 0.34	2.8 ± 0.42
4.0	36	8.5 ± 0.45	2.2 ± 0.22
5.0	30	6.0 ± 0.45	1.2 ± 0.35
TDZ			
1.0	45	7.2 ± 0.25	1.2 ± 0.42
2.0	53	9.8 ± 0.55	2.4 ± 0.23
3.0	65	10.0 ± 0.45	2.3 ± 0.42
4.0	60	11.8 ± 0.72	1.5 ± 0.32
5.0	57	7.5 ± 0.64	1.5 ± 0.25

*Mean \pm Standard Error.

The different concentrations of TDZ (1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) were added to the MS medium. Lower level of TDZ (1.0mg/L) induced less number of shoots at (3.0 mg/L) TDZ 64% cultures responded and maximum number of shoots/explant (12.5 ± 0.43) were recorded. At the concentration of TDZ was increased to (4.0 and 5.0 mg/L) the percentage of responding.



a)



b)



e)



c)



d)

Fig; I Indirect Plantlet Regeneration from Leaf Explants of *Solanum surattense* Burm. (F) a), Maturation of callus from leaf explants on MS+3.0mg/L NAA, (b) Formation of multiple shoots on MS+BAP (2.0) mg/L from leaf derived callus (c) Proliferation of multiple shoots on MS+BAP (4.0mg/L) from derived callus (d) Formation of multiple shoots on MS+ NAA (0.5mg/L) + BAP (3.0) mg/L from leaf derived callus e) Formation of multiple shoots on MS+NAA (0.5mg/L) TDZ (2.0) mg/L from leaf derived callus cultures reduced to 58 and 46 with maximum number of shoots/explants (7.8 ± 0.34) and (6.4 ± 0.37) respectively (Table-3). After shoot elongated, the healthy regenerated shoots were rooted on 1/2 MS medium supplied with (0.1 mg/L) IBA and rooting rate reached above 90%. Regenerated plants with well developed shoots and roots were successfully acclimated in soil and then transferred to greenhouse for further growth. Moreover, there were no visible detectable variations during the process of growth.

Table – 3: Effect of NAA in combination with BAP/Kn/TDZ On induction of shoots proliferation from Leaf derived callus cultures of *S.surattense*.

Hormone concentration (mg/L)	% of frequency of plantlet production	Average No. of shoots /Explants \pm (SE)*	Average length of shoots (cms) \pm (SE)*
NAA + BAP			
0.5 + 1.0	45	10.9 ± 0.43	2.4 ± 0.28
0.5 + 2.0	59	16.2 ± 0.07	4.4 ± 0.33
0.5 + 3.0	72	20.5 ± 0.62	4.3 ± 0.72
0.5 + 4.0	64	18.3 ± 0.43	2.8 ± 0.32
0.5 + 5.0	57	12.0 ± 0.25	2.6 ± 0.45

NAA + Kn			
0.5 + 1.0	48	09.0 ± 0.35	2.5 ± 0.45
0.5 + 2.0	58	10.4 ± 0.45	3.5 ± 0.24
0.5 + 3.0	70	14.3 ± 0.35	4.0 ± 0.13
0.5 + 4.0	60	12.3 ± 0.45	2.4 ± 0.32
0.5 + 5.0	45	11.3 ± 0.45	2.3 ± 0.24
NAA + TDZ			
0.5 + 1.0	43	08.5 ± 0.35	1.8 ± 0.42
0.5 + 2.0	52	09.5 ± 0.35	2.0 ± 0.23
0.5 + 3.0	64	12.5 ± 0.43	2.6 ± 0.32
0.5 + 4.0	58	07.8 ± 0.34	2.0 ± 0.52
0.5 + 5.0	46	06.4 ± 0.37	1.8 ± 0.56

* Mean ± Standard Error.

DISCUSSION

Interaction of auxins and cytokinins plays vital role in cell division, growth, development, differentiation and the formation of plant organs.^[16,17 and 18] The present results showed that different combination of plant hormone could induce the formation of callus in Leaf of *S.surattense*.

The regeneration capacity through indirect shoot organogenesis from leaf derived callus was tested either. Shoots number per explant was influenced strongly by culture medium and application of growth regulators. In our protocol NAA induced organogenic callus from leaf explants. The hormonal supplement was selected because it was optimum for callus formation among many tested combination. The Leaf explants showed 80% callus formation after 6 weeks of culture when placed in media supplemented with auxins NAA (3.0mg/L) singly. However, the response of the axillary buds decreased (75%) with increase in concentration of NAA in the medium.^[19] Had also successfully reported micropropagation of *O. basilicum* using *in vitro* geminated plants. Sub culturing of callus on to fresh medium containing the same concentrations of growth regulators resulted in the emergence of shoot buds. Multiple shoot buds were initiated on the callus cultured in MS medium supplemented with both cytokinins (BAP and Kn) and auxins IAA (0.5mg/L) singly and in combination.

However in control direct multiple shoot buds formed from Leaf cultured in the medium.^[20] Though obtained callus formation on basal MS medium but it degenerated after 8 weeks of culture.^[21] The manipulation of plant growth regulators is essential to optimize the induction of callus. With different combinations of growth regulators in the medium, explants showed 80% response in all the combinations of BAP and Kn. When the callus was cultured in MS medium supplemented singly with 3.0mg/L Kn/BAP the maximum number of shoot buds emergence was (6) above. But the callus showed maximum number of shoot buds (6) emerging in MS medium supplemented with average concentrations of 3.0mg/L BAP in combinations with 0.5mg/L IAA (Table-2).

The manipulation of plant growth regulators is essential to optimize the induction of callus. Such types of callus mediated regeneration were also reported in many plant species including *Triticum aestivum*^[23], *Agave amanuensis*^[24], *Rotula aquatic*.^[25] *Phellodendron amurense*^[26] and *Acmella calva*.^[27]

A procedure of *S.surattense* regeneration via indirect organogenesis and a successful adaptation of plants to greenhouse conditions were developed. NAA being more effective for callus induction than IAA. Plant regeneration via indirect shoot organogenesis was achieved from the leaf-derived callus on MS medium supplemented with (3.0 mg/L) BAP and (0.5 mg/L) NAA. High rate of multiplication (80%), maximum shoot proliferation (20.05 shoots per explants) was observed on MS medium supplemented with 3.0 mg/l BAP and 0.5 mg/L NAA. The optimal medium for rooting was MS containing (0.5 mg/l IBA). The plants were successfully *in vivo* acclimatized. This *in vitro* regeneration system can be used for effective screening and propagation of elite clones of *S.surattense*.

CONCLUSIONS

Ideal medium for callus establishment through Leaf explants was MS-medium supplemented with NAA (3.0 mg/L) callus culture was achieved. Maximum shoot bud differentiation from callus culture was achieved on MS-medium supplemented with BAP/Kn/TDZ (3.0 mg/l) and NAA (0.5 mg/l). Elongation and further development of shoot buds into shoots were achieved on MS-medium fortified with BAP/Kn/TDZ (3.0 mg/L). Therefore, this medium was designated as "Shoot bud differentiation and elongation medium".

ACKNOWLEDGEMENT

We are grateful to **Prof N. Rama Swamy**, Principal University College Kakatiya University & Research Supervisor Department of Biotechnology Kakatiya University for their encouragement. We thank **Sri. P. Nithin** Principal S.R.R. Govt. Arts & Science College Karimngar for their constant encouragement and for providing necessary facilities.

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