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In vitro plant regeneration from leaf and stem explants of S*olanum xanthocarpum* Schrad & Wendl. – an important medicinal herb

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Induction of callus and multiple shoots using leaf and stem explants of *Solanum xanthocarpum* was achieved. Leaf and stem explants were cultured on MS (Murashige and Skoog) medium supplemented with different concentrations of IAA, NAA, BAP and Kn alone. The frequency of callus induction increased with increasing concentrations of all growth regulators used. The optimal levels were formed to be 11.4 μML^{-1} of IAA / 10.7 μML^{-1} of NAA and 8.8 μML^{-1} BAP/9.3 μML^{-1} Kn. Among the different growth regulators tried, auxins IAA and NAA alone induced whitish or brownish friable callus, where as BAP and Kn induced greenish friable and green compact callus. Leaf was found to be more efficient in producing callus and multiple shoots. Shoots were induced from green compact callus cultures of these explants on the same medium. Maximum number of multiple shoots was obtained from leaf derived callus. The regenerated shoots were rooted on MS medium without growth regulators, and were successfully hardened and transferred to the field.

Key words: *Solanum xanthocarpum*, Leaf, Stem, Callus, Regeneration, IAA – Indole acetic acid, NAA – Napthalene acetic acid and BAP- 6- benzyl aminopurine

Introduction

Solanum xanthocarpum Schrad & Wendl. is a medicinally important member of Solanaceae. All the parts of *S. xanthocarpum* were used as traditional Ayurvedic medicinal herb in India. It was used for TB, fever, asthma, lung diseases and kidney disorders. Pharmacological studies on this herb have shown that aqueous and alcoholic extracts of the plant possess hypotensive effect, which is partly inhibited by atropine. The species contains steroidal alkaloids, solasonine and solamargine. Solasodine serves as an important intermediate in synthesis of steroidal hormones (Butcher, 1977) and

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is a potential alternative to diosgenin as a precursor in synthesis of steroidal hormones (Macek, 1989). Most of the medicinal plants are even today collected from the wild sources. The pressure on the natural population of medicinal herbs is so serious that several precious medicinal plants are likely to be designated as threatened, vulnerable, endangered, extinct etc. Tissue culture techniques are also being developed for the mass propagation of medicinal plants are of prime importance for mass production and conservation of the particular species or endangered species because of their medicinal properties. There is a need to employ biotechnological methods to improve the yield of metabolites for their medicinal purposes.

Only limited success has been reported for *in vitro* organogenesis and regeneration in *Solanum* species (Bhatt and Sussex 1979; Macek 1989; Baburaj and Thamizhchelan, 1991; Bhalsingh and Maheswari, 1997).

Present communication reports a method of multiple shoots induction from leaf and stem explants of *S. xanthocarpum*.

Materials and methods

Leaf and stem explants were collected from the college herbal garden. The explants were washed with running tap water for 30 minutes and surface sterilized, initially in 70% ethanol for 2 min, followed by 0.1% (w/v) $HgCl_2$ for 1-2 min. and finally rinsed in sterile distilled water for 2-3 times. MS medium (Murashige & Skoog, 1962) with 3% sucrose (w/v) adjusted to pH 5.7 and solidified with 0.8% agar (w/v) and autoclaved at 1.05 kg cm^{-2}, was used as a culture medium.

For organogenesis leaf and stem explants were cultured on MS medium fortified with various concentrations of IAA, NAA, BAP and Kn. All the cultures were maintained under white fluorescent light (80 μ $EM^{-2}S^{-1}$) at the temperature 25±2°C with 16hrs photoperiod.

The *in vitro* regenerated shoots (5-7 cm long) were transferred to MS medium for rooting. The *in vitro* regenerated plants were transferred to plastic cups containing the sterile soil mix red soil: sand: compost (1:1:1) and kept in green house. At least 20-24 explants were cultured in each treatment, and all the experiments were repeated three times.

Results and discussion

Callus induction

S. xanthocarpum leaf and stem explants were cultured on MS medium supplemented with different concentrations of IAA (0.6 – 28.5 μM), NAA (0.5

– 26.8 µM), BAP (0.4 – 22.2 µM) and Kn (0.5 – 23.2 µM) individually. Both explants were enlarged in size and thickened within a week time. Callus was initiated from surface of the explants after one week. IAA and NAA containing MS medium induced whitish and brownish friable callus, but BAP and Kn induced greenish friable and greenish compact callus (Table 1). Significant callus formation was observed within 10-15 days at the cut ends of the explants. Higher frequency of callus induction was achieved from leaf explants followed by stem explants. Compact green organogenic callus was observed in both explants in cytokinin supplemented medium (Fig 1 a & b). Among the different concentrations of auxins used for callus induction, 11.4 μML^{-1} IAA and 10.7 μML^{-1} NAA were found to be optimum, while increasing the auxin concentrations above this level reduced callus proliferation. Among the different concentrations of cytokinins used for callus induction, 8.8 μML^{-1} BAP and 9.3 μML^{-1} Kn were found to be optimum level for induction of organogenic calli. Baburaj and Thamizhchelvan (1991) observed similar results in *S. surattense*. Muthukumar and Arockiasamy (1998) reported that the 2.0mg/l BAP induced high amount of callus in *Datura metal.* Pawar *et al.* (2002) observed that BAP and Kn were more effective for induction of organogenic callus from leaf explants of *S. surattense*. Thus cytokinins were suitable for induction of organogenic callus compared to auxin used in both explants viz. leaf and stem as reported in literature.

Multiple shoots regeneration

Multiple shoots regeneration occurred through callus from both leaf and stem explants on the same fresh medium within 20-25 days. BAP (8.8µM) and Kn (9.3µM) produced highest shoot regeneration frequency as that of callus induction (Table 2). In the case of BAP leaf callus showed more regeneration capacity (77.6%) than stem callus. But in case of KN, we have observed opposite results, i.e. stem callus produced the highest regeneration frequency (79%). Maximum number of shootlets developed from leaf explants at 8.8 μML^{-1}BAP whereas stem explants at 9.3 μML^{-1} Kn (Fig 1c & d). Similar results were also observed in *S.surattense* by Pawar *et al.* (2002) and Baburaj and Thamizhchelvan (1991). Muthukumar and Arockiasamy (1998) reported that the BAP and Kn were more effective for induction of multiple shoots from leaf explants of *Datura metal*.

Table 1. Effect of different concentrations of IAA, NAA, BAP & Kn on callus induction form leaf and stem explants of *Solanum xanthocarpum.*

Plant growth regulators (μML^{-1})	Leaf	Stem
	% of callus proliferation	% of callus proliferation
IAA		
0.6	22.3±2.5^{v}	21.0±1.7^{w}
2.8	37.6±2.0^{r}	37.0±2.0^{s}
5.7	61.0±3.6^{j}	59.6±3.5^{i}
11.4	80.0±2.0^{c}	79.0±1.0^{b}
17.3	72.3±2.5^{e}	69.0±1.0^{d}
22.8	60.0±5.0^{k}	57.6±2.5^{k}
28.5	46.6±6.1^{o}	45.0± 5.0^{o}
NAA		
0.5	22.3±2.0^{v}	21.6±1.5^{w}
2.7	35.3±3.5^{s}	32.3±2.5^{t}
5.4	60.0±2.6^{k}	58.0±3.6^{k}
10.7	78.3±2.0^{d}	76.3±2.3^{c}
16.1	70.3±1.5^{f}	67.3±2.0^{f}
21.5	57.3±3.0^{l}	55.0±3.0^{l}
26.8	45.0±5.0^{p}	44.3±3.2^{p}
BAP		
0.4	29.6± 2.0^{t}	27.0± 2.6^{uv}
2.2	40.0± 2.0^{q}	38.0± 2.6^{r}
4.4	62.5± 2.5^{i}	61.3±1.5^{h}
8.8	86.3± 2.5^{a}	61.3±1.5^{h}
13.3	70.3±1.5^{f}	68.0±2.6^{e}
17.7	64.3± 2.5^{h}	59.0±1.0^{j}
22.2	52.0± 2.0^{m}	49.0±1.0^{n}
Kn		
0.5	27.3±3.2^{u}	27.6±3.0^{u}
2.3	38.3±2.0^{r}	39.3±2.0^{q}
4.6	61.0±2.6^{j}	62.3±2.5^{g}
9.3	82.0±2.0^{b}	83.6±2.5^{a}
13.9	67.3±2.0^{g}	69.0±1.7^{d}
18.6	59.6±2.0^{k}	61.3±2.5^{h}
23.2	50.0±1.0^{n}	52.6±3.2^{m}

Each value represents the mean ± SD of 20 replicates and each experiment was repeated thrice. Values with the same superscript are not significantly different at the 0.05% probability level according to DMRT.

Table 2. Effect of different concentrations of BAP and Kn on multiple shoots induction form leaf and stem callus of *Solanum xanthocarpum.*

Plant growth regulators(μML^{1})	Leaf			Stem		
	Regeneration (%)	Average no.of Shoots /explant ± SE	Average Shoot length (cm) ±SE	Regeneration (%)	Average no.of Shoot/ explant ±SE	Average Shoot length (cm) ±SE
BAP						
0.4	22.0±2.0^{l}	12.3±2.5^{i}	2.0±1.0^{j}	20.0±1.0^{k}	11.0±1.0^{i}	1.0 ±1.0^{h}
2.2	42.3±2.5^{j}	19.0±1.0^{f}	4.0±1.0^{g}	40.6±2.0^{i}	15.6±2.0^{g}	3.3±0.5^{f}
4.4	62.3±2.5^{e}	22.6±2.0^{d}	7.3±1.5^{c}	60.3±2.5^{e}	20.3±1.5^{f}	6.6±0.5^{c}
8.8	77.6±2.5^{a}	32.0±2.0^{a}	10.0±1.0^{a}	75.0±3.0^{b}	29.6±1.5^{b}	9.6±0.5^{a}
13.3	67.6±2.5^{c}	24.0±2.0^{c}	7.0±1.0^{c}	61.3±2.5^{d}	21.3±1.5^{e}	6.3±1.1^{c}
17.7	56.6±2.0^{g}	17.0±1.0^{g}	5.6±1.1^{e}	52.3±3.2^{g}	13.6±1.5^{h}	5.0±1.7^{d}
22.2	46.3±1.5^{i}	12.0±1.0^{i}	4.3±1.5^{g}	41.0±1.0^{i}	9.6±1.5^{j}	3.6±2.0^{e}
Kn						
0.5	20.6±2.0^{m}	12.0±2.0^{i}	1.6±0.5^{j}	23.0±1.0^{j}	14.0±2.0^{h}	2.0±1.0^{g}
2.3	41.3±1.5^{k}	18.6±0.5^{f}	3.3±0.5h^{i}	45.0± 3.0^{h}	21.0±1.0^{e}	4.3±0.5^{e}
4.6	61.3±1.5^{f}	20.6±2.0^{e}	6.6±0.5^{d}	67.3±2.5^{c}	22.0±2.0^{d}	7.0±1.0^{b}
9.3	76.0±2.6^{b}	30.0±1.0^{b}	8.3±0.5^{b}	79.0±1.0^{a}	33.3±2.0^{a}	10.0±1.0^{a}
13.9	65.0±3.0^{d}	22.6±2.5^{d}	6.6±0.5^{d}	67.3±2.5^{c}	23.0±2.0^{c}	7.3±1.1^{b}
18.6	52.0±2.0^{h}	15.3±2.5^{h}	5.3±0.5^{f}	57.0±2.6^{f}	16.6±1.5^{g}	6.0±1.0^{c}
23.2	42.6±2.0^{j}	9.0±1.0^{j}	3.6±1.5^{h}	44.6±4.1^{h}	10.3±1.5^{j}	5.3±0.5^{d}

Each value represents the mean ± SD of 20 replicates and each experiment was repeated thrice. Values with the same superscript are not significantly different at the 0.05% probability level according to DMRT.

Root induction

Well-developed shoots were cultured on MS medium without growth regulators for *in vitro* rooting. Absolute percentage of shoots from both leaf and stem callus developed roots within a week (Fig1 e). The rooted plantlets were transferred to plastic cups and kept in green house for 8-10 days, and then the plantlets were transferred to the field. Similar results were also reported by Pawar *et al.,* (2002) in *S. surattense*.

Thus, this protocol is uesful for conservation of this important medicinal plant and also for large-scale propagation.

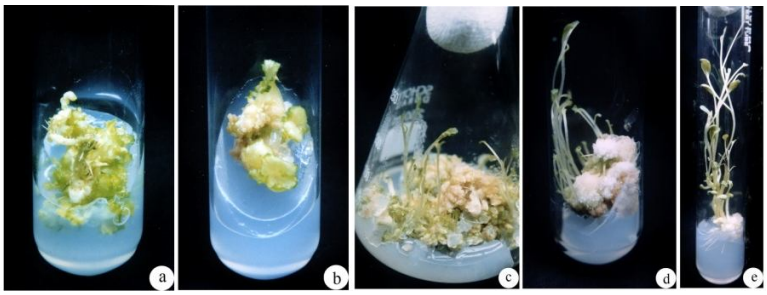

Fig. 1. Callus induction and regeneration from leaf and stem explants of Solanum xanthocarpus. a and b = callus induction from laef and stem on MS + BAP 8.8 uML^{-1}, c and d = multiple shoot regeneration on MS + BAP 8.8 uML^{-1} and MS + Kn 9.3 uML^{-1} leaf and stem callus, respectively, e = root induction on MS without growth regulations.

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