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HIGH FREQUENCY *IN VITRO* CLONAL PROPAGATION OF *SOLANUM SURATTENSE* BURM. F.

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ABSTRACT

Multiple shoots were induced in cotyledon and leaf explants of *Solanum surattense* Burm. f., a medicinally important Solanaceae member. Cotyledon and leaf explants isolated from axenic seedlings (3-5 weeks old) were cultured on the Murashige and Skoog medium supplemented with different concentrations of Thidiazuron (TDZ) (2.27, 2.72, 3.17, 3.63, 4.08 & 4.54 µM) alone or in combination with Indole 3- acetic acid (IAA) (0.28 µM). Maximum number of shoots (72.5±1.70 & 68.1±1.12) were induced directly (without callus) and maximum shoot length (8.25± 0.31 & 7.03 ± 0.53) was observed in cotyledon and leaf explants, respectively. Microshoots obtained in the above treatments were rooted on MS + Indole -3- butyric acid (IBA) (4.92 µM). Complete plantlets (2n=24) were transferred to research field and 80-90 % of these plantlets survived. Plantlets regenerated from cotyledon and leaf explants did not show any morphological and cytological variations and they flowered normally and set fruit.

KEY WORDS: Murashige and Skoog medium, Cotyledon, Thidiazuron, Direct organogenesis.

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INTRODUCTION

Solanum surattense Burm. f. is an important medicinal herb belonging to Solanaceae family. It is a close relative of cultivated brinjal (*Solanum melongena* L.). It is an ethnomedicinal plant used for curing Tuberculosis (TB), Asthma, Lung diseases and Kidney disorders. Recently it has been reported that solasodine and glycosides present in this plant possess anti-cancer properties[1] and solasodine serves as a key intermediate compound in synthesis of steroidal hormones[2]. The plant extract has been used as repellent against agricultural pest, as molluscicide in public health and also to control vectors of filaria[3] malaria and dengue/DHF[4]. *S. surattense* also possess hypoglycemic[5], immunoprotective[6], antispermatozoic[7], antifertility[8], antibacterial[9], antifungal[10], antiviral[11], antifilarial[3] and antinematodal[12] properties. In order to genetically improve this ethnomedicinally important plant *S. surattense* by *Agrobacterium tumefaciens and Agrobacterium rhizogenes*, the basic prerequisite is the development of protocol for large scale *in vitro* clonal propagation. Multiple shoot bud induction from Internodes[13], Nodal[14, 15], Leaf [16, 17, 18], Stem[18] and Shoot tip[16, 18] explants has been reported in *Solanum surattense*, however, the maximum number of shoots produced from the above explants was 36. The present paper reports induction of 72.5 ± 1.70 and 68.1±1.12 shoots from cotyledon and leaf explants, respectively on MS+TDZ (3.17µM) + IAA (0.28µM).

MATERIAL AND METHODS

Seed Collection and Media Preparation: *Solanum surattense* Burm F., seeds collected from plants growing in Kakatiya University Campus, Warangal, A.P, India, were treated with 5% Tween- 20 for 5 min and rinsed in several changes of sterile distilled water. The surface sterilized seeds were inoculated on 200 ml screw cap bottles containing 50 ml Murashige and Skoog[19] (MS) medium for seed germination. Cotyledon and leaf explants admeasuring 1 cm (ca) were obtained from (3-5 weeks old) axenic seedlings and inoculated in 25x150 mm culture tubes containing 15 ml MS medium supplemented with 3% Sucrose (w/v) and Thidiazuron (TDZ) (2.27, 2.74, 3.17, 3.63, 4.08 and 4.54µM) alone or in combination with Indole-3-acetic acid (IAA) (0.28 µM), the pH of the medium was adjusted to 5.8, solidified with 0.8% agar (w/v) and autoclaved at 15 lbs/15 min. The cultures were incubated at 25±2^{0}C under white fluorescent light with 16 hours photoperiod (light intensity was 40 watts from white fluorescent lamp). After 3 weeks of cultures on MS medium supplemented with TDZ +IAA, cotyledon and leaf explants with multiple shoots were transferred to MS basal medium for shoot elongation. After 3 weeks, microshoots (4-5 cm) with 2-3 leaves were rooted on MS medium fortified with Indole -3-butyric acid (IBA) (4.92 µM). Plantlets were removed from culture tubes and transferred to clay pots containing autoclaved soil and irrigated with diluted MS basal medium without sucrose. The potted plantlets were hardened in polyhouse and then transferred to research field.

Cytological Preparations

Root tips of 25 plantlets were randomly fied in ethanol and acetic acid (3:1) at 10:00 am. They were transferred to watch glass and treated with 1N HCl and 2% aceto-orcein (1:9) and heated until effervescence. The root tips were squashed with a drop of 45% acetic acid and covered with a cover slip and observed under a Magnus MLX compound microscope at 60 X magnifications.

RESULTS

In our study morphogenic response of cotyledon explants (75%) was higher than leaf explants (68%). The cotyledon explants

produced maximum number of shoots (72.5±1.70) with mean shoot length (8.25±0.35) (Table.1), and leaf explants produced maximum number of shoots (68.1±1.12) with mean shoot length (7.03±0.53) on medium containing MS+TDZ (3.17 µM) + IAA (0.28µM), respectively (Table. 2). Shoot induction was observed from cut ends after 10 days of inoculation, without intervening callus. The shoot regeneration was low on MS medium supplemented with TDZ (3.17 µM) alone compared with TDZ (3.17 µM) + IAA (0.28µM). Microshoots were rooted on MS+IBA (4.92µM). Plantlets regenerated from cotyledon and leaf explants did not show any morphological and cytological variations (Fig.1 A & F).

Table 1.

Effect of TDZ or TDZ+IAA on multiple shoot induction from cotyledon explants of Solanum surattense.

Hormone	Concentration (µM/L)	% Morphogenic Response	Mean number of shoots/explant ±S.E.	Mean length of Shoot ±S.E
TDZ	2.27	47	36.75±2.66	4.18±0.55
	2.74	61	41.40±2.73	5.29±0.63
	3.17	74	66.95±2.46	7.20±0.81
	3.63	68	59.30±1.51	6.64±0.64
	4.08	61	51.00±1.32	5.88±0.38
	4.54	54	42.60±1.73	5.58±0.64
TDZ+IAA	2.27+0.28	55	44.60±0.77	4.73±0.52
	2.74+0.28	62	49.00±2.23	6.02±0.37
	3.17+0.28	75	72.55±1.70	8.25±0.35
	3.63+0.28	70	61.90±1.24	7.21±0.45
	4.08+0.28	64	53.90±1.08	6.11±0.33
	4.54+0.28	60	46.05±1.33	5.86±0.55

Values are mean of 20 explants ± S.E. and each experiment was repeated twice

Table 2.

Effect of TDZ or TDZ+IAA on multiple shoot induction from leaf explants of Solanum surattense.

Hormone	Concentration (µM/L)	% Morphogenic Response	Mean number of shoots/explant ±S.E.	Mean length of Shoot ±S.E
TDZ	2.27	35	34.60 ± 1.69	3.40 ± 0.16
	2.74	42	41.45 ± 1.86	4.22 ± 0.17
	3.17	62	62.80 ± 1.56	6.65 ± 0.40
	3.63	53	55.95 ± 1.41	5.65 ± 0.13
	4.08	48	46.40 ± 0.96	5.03 ± 0.31
	4.54	44	39.35 ± 1.35	4.47 ± 0.30
TDZ+IAA	2.27+0.28	48	39.05 ± 1.24	4.03 ± 0.43
	2.74+0.28	56	47.50 ± 1.87	5.07 ± 0.40
	3.17+0.28	68	68.10 ± 1.12	7.03 ± 0.53
	3.63+0.28	62	59.75 ± 1.16	6.41 ± 0.68
	4.08+0.28	55	50.05 ± 1.18	5.75 ± 0.41
	4.54+0.28	51	43.20 ± 1.56	5.47 ± 0.67

Values are mean of 20 explants ±S.E. and each experiment was repeated twice

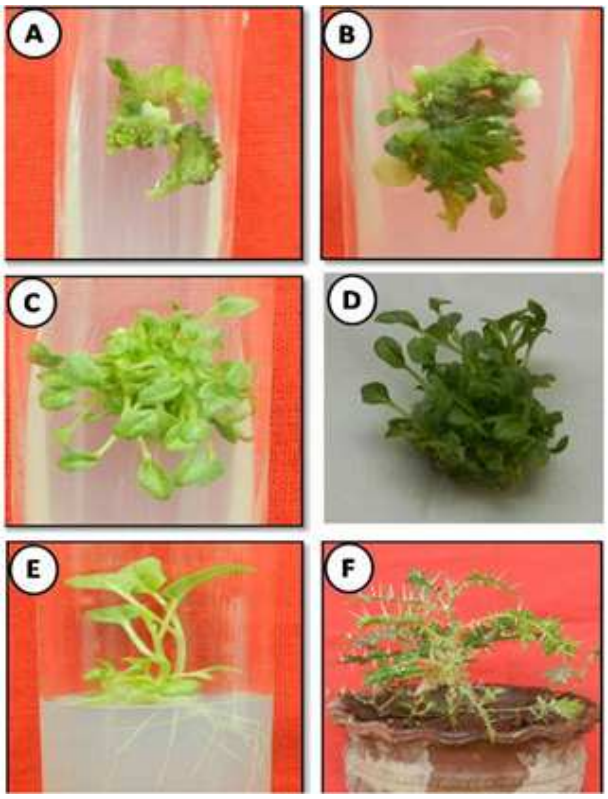

Figure 1

The effect of TDZ or TDZ+IAA on multiple shoot induction in S. surattense. (A) Multiple shoots induced in cotyledon explant on MS medium containing TDZ (3.17 µM) + IAA (0.28 µM). (B) Multiple shoots induced in leaf explant on MS medium containing TDZ (3.17 µM) + IAA (0.28µM). (C&D) Elongation of multiple shoots. (E) Rooting of microshoots on MS+IBA (4.92 µM). (F) Acclimatization of regenerated plantlet.

DISCUSSION

In this study we have developed a procedure for induction of multiple shoots 72.5±1.70 from cotyledon and 68.10 ± 1.12 leaf explants cultured on MS+TDZ (3.17µM) + IAA (0..28µM. Internodes[13] cultured on MS+BAP (0.5 mg/L^{-1}) induced 58.2 shoots per explant. Nodal[14, 15] explant cultured on MS+BAP+NAA (8.88+2.69 µM) produced 25.81 shoots per single node. Leaf[18] explant cultured on MS+BAP+TDZ (1+0.1 mg/L) induced 54.2 shoots per explant. Stem[17] explant cultured on MS+Kn (9.3µM) produced 33 shoots per explant. Shoot tip[18] explant gave 180 shoots on MS+BAP+ Kn medium via callus phase. It is well known that somaclonal variations are common in plantlets regenerated from callus based tissue culture and are not ideal for use in genetic transformations experiments. In conclusion, we developed a procedure for high frequency *in vitro* clonal propagation of *Solanum surattense* Burm F.,which can be used for *Agrobacterium* transformations studies.

REFERENCE

1. Cham B.E., Solasodine rhamnosyl glycosides specifically bind cancer cell receptors and induce apoptosis and necrosis. Treatment for skin and hope for internal cancer. Res. J. Biol. Sci. (2): 503-514, (2007).

2. Butcher DN, Plant tumor cells in plant tissue and cell culture scientific publ. Oxford, USA: Pp 429-461, (1977)
3. Rajkumar S, Jebanesan A, Repellary of volatile oils from *Moschosma polystachyum* and *Solanum surattense* against filariral vector *Culex quinquefasciatus* say. Trop Biomed (2): 139-142, (2005).
4. Singh K V & Bansal S K, Larvicidal properties of perennial herb *Solanum surattense* against vector of malaria and dengue/ DHF, Curr Sci, (84): 749-751, (2003).
5. Kar DM, Maharana L, Pattnaik S, Dash GK, Studies on hypoglycaemic activity of *Solanum xanthocarpum* Schrad & Wendl fruit extract in rats. J Ethnopharmacol; 108(2): 251-256, (2006)
6. Sultana R, Khanam S, Devi K. Evaluation of immunomodulatory activity of *Solanum surattense* fruits aqueous extract. Der Pharmacia Letter, 3(1): 247-253, (2011)
7. Gupta RS, Dixit VP. Effects of short – term treatment of Solasodine on cauda epididymis in dogs. Indian J Exp Biol; 40(2): 169-173, (2002).
8. Dixit VP, Gupta RS, Gupta S. Antifertility plant products: testicular cell population dynamics following Solasodine ($C_{27}H_{43}O_2N$) administration in rhesus monkey (*Macaca mulatta*). Androologia, 21(6): 542-546, (1989)
9. Patel SJ, Venugopalan N, Pradeep S. Screening for antimicrobial activity of weeds. Internet J Mocrobiol, 4:1, (2007)
10. Guleria A. S, Kumar B. A, Tikua AK, Toxicity of *Solanum surattense* fruit extract against *Alternaria Brassicae*, causal agent of *Alternaria* blight of Indian mustard (*Brassica juncea*) Arch Phytopathol Pl Protection; 43(3): 283-289.(2008).
11. Roychoudary R. Effect of extracts of certain solanaceous plants on plant virus infection. Acta. Bot. Indica: 8(1): 91-94, (1980).
12. Tiyagi S, Ajaz S. Possible utilization of weeds for the management of Plant parasitic nematodes infesting some vegetable crops. Arch Phytopathol Pl Protection; 36(2): 95-202, (2003)
13. Md. Mahababur Rahman, Muhammad Nurul Amin, Md. Zohurul Islam, Rubaiyat Sharmin Sultana. Mass propagation of *Solanum surattense* Burm. Using direct adventitious shoot organogenesis from internode. Acta agriculture Slovenica, 97-1, 11-17 (2011)
14. K Ramar, V Nandagopalan, Rapid *in vitro* propagation of medicinally important plant *Solanum surattense* Burm F. Int. J. of Pharm. & Life Sci., 2(1), 499- 501 (2011).
15. Ramaswamy N, Ugandhar T, Praveen M, Lakshman A, Rambabu M & Venkataiah P, *In vitro* Propagation of medicinally important *Solanum surattense,* Phytomorphology , (54): 281-289, (2004)
16. Sandeep K, Yadav, Sumita Kachhwaha Kotari S. L.. Comparison of *in vitro* regeneration efficiency of leaf explants in response to different cytokinins and assessments of genetic uniformity of regenerated plants of *Solanum surattense* Burm F. Afr. J. Biotechnol. 9(53) pp 8991-8997 (2010).
17. Sundar A. N, Jawahar M, *In vitro* plant regeneration from leaf stem explants of *Solanum xanthocarpum* Schrad & Wendl. – an important medicinal herb. J. of Agricultural Technology, 7(2), 301-306 (2011).`
18. Pawar P K, Pawar C S, Narkhede B A, Teli N P, S R Bhalsing, V L Maheswari, *in vitro* propagation of *Solanum surattense* Burm. F., Indian journal Boitechnol, (1): 201-204, (2002).
19. Murashige T and Skoog T, A revised medium for rapid growth and bioassay with tobacco plant. Plant Physiology.15 475-479,(1962).