Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2017.134.141

Research Article
Seaweed Extract Promotes High-Frequency *in vitro* Regeneration of *Solanum surattense* Burm.f:A Valuable Medicinal Plant

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Abstract

Background and Objective: *Solanum surattense* (*S. surattense*) is an important medicinal plant used for several medicinal purposes. Seaweed extract is being used as a biostimulant due to the presence of plant growth regulators. Hence in this study, seaweed extract was used in combination with cytokinins to develop an efficient protocol for *in vitro* regeneration of *Solanum surattense*. **Methodology:** The explants were excised from 7 days old seedling and inoculated in MS medium supplemented with different concentrations and combinations of cytokinins and seaweed extract. Data were analyzed using SPSS software version 17 by one-way ANOVA. **Results:** The best number of shoots (8.5) with 100% of response was observed in cotyledonary node at 1.5 mg L^{-1} 6-Benzylaminopurine (BAP)+30% *Turbinaria decurrens* extract (TDE) and 80% of explants have responded on 1.5 mg L^{-1} of BAP alone. The excised shoots from the multiple shoots were cultured on MS medium supplemented with Indole-3-butyric acid (IBA) at 0.4 mg L^{-1} of concentration produced roots (5.6) with 89.6% of response was observed. The excised shoots were cultured on MS media fortified with the combination of IBA 0.4 mg L^{-1} and 20% of *Turbinaria* extract produced maximum number of roots (6.2)/shoot with 95.6% of response was found. **Conclusion:** The seaweed extract and plant growth regulators are important to promote the *Solanum surattense* plant regeneration and also noted that the cotyledonary node explant is the suitable material for the production of more number of multiple shoots.

Key words: Seaweed, *Solanum surattense*, *Turbinaria decurrens*, cotyledonary node, N^6-benzyladenine

Citation: Packiaraj Gurusaravanan, Sadasivam Vinoth, Ganesan Prem Kumar and Pandiselvi, 2017. Seaweed extract promotes high-frequency *in vitro* regeneration of *Solanum surattense* Burm.f: A valuable medicinal plant. Res. J. Med. Plants, 11: 134-141.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus *Solanum* of family *Solanaceae* is well known for the presence of valuable medicinal compounds like solasodine, alkaloids, phenolics, flavanoids, sterols, saponins and their glycosides[1]. To date, several studies have been carried out in this genus because of their high therapeutic properties[2]. *Solanum surattense* is an important potent medicinal plant in family *Solanaceae* and are most commonly used in Indian traditional medicine systems. *S. surattense* has multipurpose roles as antibacterial[3], antinociceptive[4], larvicidal[5] and anti-plasmodial[6]. The medicinal plants with novel compounds reach the status of threatened, vulnerable, endangered, or extinct because of its exploitation due to human activities. Therefore, intensive research priority has to be given to conserve such pharmacologically important plants using *in vitro* techniques.

Micropropagation technique is an alternative approach for conservation of precious medicinal plants. Marine seaweeds are often regarded as an underutilized bio-resource. Many have been used in as a source of food, as industrial raw materials and in therapeutic and botanical applications for centuries[7]. Seaweed extracts have been applied as foliar spray and used inorganic farming[8]. They are commercially used as biostimulant due to the presence of growth regulators such as auxins and cytokinins[9]. Presence of plant growth regulators and micronutrients in seaweed extracts has been confirmed by chromatographic techniques[10].

Furthermore, the wide range of growth responses induced by seaweed extracts implies the presence of more than one group of plant growth-promoting substances or hormones[9-11]. *In vitro* plant regeneration is influenced by several factors, such as culture conditions, nature of explants and growth regulators. Explants such as hypocotyls, cotyledonary node, apical meristem, stems petioles, leaves, anthers and inflorescences have been widely used for shoot induction in *Solanum* species[12,13]. Seaweed extracts have been shown to induce several growth responses in plants due to the presence of plant growth promoting substances. *In vitro* regeneration of plants is determined by various factors such as genotype, medium composition, growth regulators, gelling agent, culture vessel etc and shoot regeneration in *Solanum* species.

Two types of explant (hypocotyl and cotyledon) were tested in *Brassica oleracea* in which hypocotyl explants were found to be more suitable for callus induction and organogenesis than cotyledon explants for all cultivars tested[14].

Node explant culture is a widely method for micropropagation of plants. There are reports available for regeneration like organogenesis and embryogenesis directly from explants. Direct organogenesis of shoot from cotyledonary nodes in black gram and tomato was reported[15]. Auxin supplementation was not necessary either for bud primordium differentiation in cotyledonary explants or proliferation of regeneration of shoots in *Garcinia mangostana*. Plant regeneration was achieved by using hypocotyls, leaf and cotyledonary segments. Single node explants elicited more number of multiple shoots when compared to shoot tip explants in medium containing BAP and Kinetin. The fast growing shoots were achieved by the media supplemented with either N^6-benzyladenine (BA) or thidiazuron (TDZ) in combination with 1-Naphthalene acetic acid (NAA)[16]. Seaweeds are green, brown and red marine macroalgae which nearly constitute 10,000 species distributed worldwide[17]. Among them, brown seaweeds are extensively used for commercial production of extracts for applications in agriculture. In general, seaweed extracts are capable of inducing an array of physiological plant responses, such as the promotion of plant growth, improvement of flowering and yield and enhancing nutritional quality of edible products as well as shelf life, even at low concentrations[18]. The seaweed, *Turbinaria decurrens* is a genus of brown algae (*Phaeophyceae*) found in tropical marine waters. It acts as growth promoting substances because it contains many bioactive compounds[19]. Seaweed and seaweed-derived products have been widely used as amendments in crop production systems due to the presence of a number of plant growth-stimulating compounds. Moreover, the adventitious shoot formation is based on the type of explants used and plays a major role in plant growth regulation. The effect of various seaweed extracts (SE) were examined on *in vitro* plant tissue culture. Combination of seaweed extract (SE) and plant growth regulators with MS medium increased adventitious shoot regeneration on various types of explants[20]. The seaweed extracts act as biostimulants of plant growth and development. The plant regeneration protocol with seaweed extract could be suitable for a wide range of medicinal plant cultivars and the efficient method of protocol for mass propagation has very low cost-effective method by using seaweed extract for biostimulants instead of synthetic chemicals. This experiment will make an advance to utilize seaweed extracts as potent biostimulants for *in vitro* culture of valuable medicinal plants.

The purpose of study is to give experimental evidence to the positive role of seaweed extract *in vitro* regeneration of valuable medicinal plants. Moreover the present study is the

first report on evaluating the effect of *Turbenaria decurrens* extract in enhancing shoot regeneration in *Solanum surattense* culture. Further significant finding of this study can be utilized to other crops as well.

MATERIALS AND METHODS

The seaweed, *Turbinaria decurrens* was collected from the Ramanathapuram District, Tamil Nadu, India in the year 2016. The samples were cleaned with seawater 4-5 time to remove sand particles and other unwanted impurities before transport to the Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu. The seaweed was then again washed with tap water for 5 times and shade dried and finely chopped into fine powder. In order to prepare the seaweed extracts, about 500 g of each sample was boiled in a conical flask containing 500 mL distilled water. Then the extracts were cooled at room temperature and filtered through Whatman No. 41 filter paper for 2 times. The seaweed extract was applied in tissue culture media for studies and remaining seaweed extract was stored at -20°C for further uses.

Multiple shoot induction: Plants of *Solanum surattense* were collected from wild area and maintained in Experimental Garden, Department of Botany, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, Virudhunagar District, Tamil Nadu, India. Cotyledonary node explants were excised from 7 day old and surface sterilized *in vitro* condition using 70% (v/v) ethanol (Analytical grade) for 1 min, followed by three washes with sterile distilled water for 2 min each. The explants were then treated with 0.1% of mercuric chloride ($HgCl_2$–Analytical grade) for 5 min followed by five washes with sterile distilled water for 2 min each. The explants were cultured on Murashige and Skoog (MS) medium[21] containing 1% (w/v) sucrose and 0.8% agar (w/v), pH 5.8 supplemented with different concentrations and combinations of plant growth regulators (0.5-2.5 mg L^{-1} 6-Benzyl amino purine (BAP), 0.5-2.5 mg L^{-1} Kinetin (Kin); 10-50% of *Turbenaria decurrens* (TDE) extract. All cultures were maintained in culture room under 16-h photoperiod (~3000 lx) at 23±2°C. Explants were subcultured regularly at 4 week intervals.

The shoot induction and multiplication from explants were observed after 4 week of culture, while time requirement for the process initiation and growth characteristics were observed on every week. After 6 week of culture, single shoots were excised from multiples shoots and transferred to rooting medium consisting of MS salts, 1% sucrose and 0.7% agar (pH 5.7), supplemented with NAA at 0.2-0.8 mg L^{-1} and IBA at 0.2-0.8 mg L^{-1} concentration were tested. In another set, combination of IBA and TDE was tested for root induction. The formation of roots from single shoots was calculated after 4 week of culture.

Statistical analysis: Each treatment included 5 replicates and experiments were repeated 3 times. The data were subjected to one-way analysis of variance (ANOVA) and the differences among means were compared by Tukey's test ($p<0.05$).

RESULTS

The seeds of *S. surattense* were germinated on earthen pots on Department Experimental Garden, Ayya Nadar Janaki Ammal College, Sivakasi. The cotyledonary node used as explants were excised from 7 days old seedlings with 3-4 cm long for *in vitro* culture.

Effect of BAP on shoot induction: The present study showed that nominal *in vitro* plant regeneration was observed at optimum concentration of cytokinins (Table 1). In another set of experiment, the extract of *Turbinaria decurrens* (TDE) was used as plant growth regulator (PGR) for shoot regeneration.

Induction of multiple shoots from cotyledonary node explants varied with the type and concentration of growth regulator (Table 1). BAP at 0.5 mg L^{-1}, induced 4.2 shoots/explant with 45% response. The shoot number slightly increased (4.5) at 1.0 mg L^{-1} with 60% response. With increasing concentrations of BAP, the number of shoots increased along with higher percentage of response upto 1.5 mg L^{-1}. Beyond that concentration shoot number decreased with reduced percentage of response. At 1.5 and 2.0 mg L^{-1} BAP, 5.5 and 3.8 shoots were induced respectively. The highest shoot number (5.5) was recorded on 1.5 mg L^{-1} BAP containing medium, with 80% response.

Effect of Kin on shoot induction: Kin exhibited less shoot-forming potential than BA. At the lowest concentrations (0.5-1.0 mg L^{-1}), it induced 3.0 and 3.5 shoots/explant, whereas 1.5 mg L^{-1} produced 2.6 shoots/explant with 70% response. The shoot number was significantly reduced at 2.0 and 2.5 mg L^{-1}, which produced 2.0 and 1.8 shoots/explant, respectively (Table 1).

Effect of TDE on shoot induction: Shoot induction from cotyledonary node of *S. surattense* varied with different concentrations of TDE (Table 1). Shoot induction percentage

Table 1: Effect of cytokinins and *Turbinaria decurrens* extract (TDE) on multiple shoot induction from node explant of *Solanum surattense* Burm.f.

Plant growth regulators (mg L^{-1})	Response (%)	Number of shoots/explant	Shoot length (cm)
BAP			
0	30±2.5[a]	1.3±0.1[a]	1.5±0.4[c]
0.5	45±1.4[a]	4.2±0.6[a]	1.8±0.3[b]
1.0	60±2.9[d]	4.5±0.2[b]	1.4±0.6[b]
1.5*	80±3.2[d*]	5.5±0.4[d*]	2.2±0.9[a*]
2.0	50±2.2[b]	3.8±0.2[c]	1.6±0.5[d]
2.5	30±1.8[a]	2.6±0.8[b]	1.2±0.4[b]
Kin			
0	40±1.5[a]	2.1±0.1[a]	1.3±0.2[b]
0.5	55±1.8[b]	3.0±0.8[b]	1.1±0.2[c]
1.0*	70±2.2[a*]	3.5±0.5[a*]	1.6±0.5[c*]
1.5	50±2.6[bc]	2.6±0.2[d]	1.6±0.1[d]
2.0	30±1.7[ac]	2.0±0.4[c]	1.2±0.2[b]
2.5	30±1.5[a]	1.8±0.2[a]	1.2±0.5[c]
TDE (%)			
0	20±0.5[b]	2.1±0.3[b]	0.5±0.1[c]
10	35±2.1[d]	2.8±0.2[b]	1.5±0.3[a]
20	50±3.3[a]	3.0±0.9[a]	1.0±0.3[b]
30*	85±3.6[b*]	5.8±1.2[c*]	2.0±0.5[ab*]
40	65±2.7[ab]	4.2±1.6[a]	2.5±0.3[c]
50	40±2.1[d]	3.2±0.8[c]	1.7±0.9[b]

For each growth promoter tested and means followed by the same letter within a column are not significantly different according to Tukey's test ($p<0.05$). *Best results are indicated. Measurements were recorded after 6 week of culture on MSB_5 medium supplemented with cytokinins and TDE. Values represent the mean±SE, BA N^6: Benzyl adenine, Kin: Kinetin

Table 2: Effect of BAP and TDE on multiple shoot induction

Plant growth regulators (mg L^{-1})				
BAP	TDE (%)	Response (%)	Mean number of shoots/explant	Shoot length (cm)
0	0	40±1.2[d]	2.2±0.6[b]	1.3±0.4[b]
0.5	10	60±2.1[ab]	4.8±1.2[b]	1.9±0.2[c]
1.0	20	75±3.0[a]	5.6±1.3[b]	2.6±0.2[a]
1.5*	30*	100±2.8[a*]	8.5±0.7[ac*]	2.5±0.9[c*]
2.0	40	70±2.5[b]	5.8±0.9[b]	1.8±0.4[c]
2.5	50	45±2.0[bc]	2.5±0.1[a]	1.5±0.6[a]

For each growth promoter tested, means followed by the same letter within a column are not significantly different according to Tukey's test ($p<0.05$). *Best results are indicated. Measurements were recorded after 6 week of culture on MSB_5 medium supplemented with BAP and TDE. Values represent the mean±SE

was less on MS medium supplemented with 10% of TDE with 2.8 shoots and with shoot length of 1.5 cm. The shoot number slightly increased (3.0) at 20% of TDE with improved response of 50%. The highest shoot number (5.8) was recorded on 30% TDE containing medium with 85% response which was higher compared to individual treatments of BAP and Kin (Table 1). Beyond that concentration shoot number decreased to 4.2 and 3.2 at 40 and 50% of TDE, with reduced response of 65 and 40%, respectively.

Effect of TDE and BAP combination on shoot induction: As the frequency rate of plant regeneration was inadequate with individual treatments of either BAP or TDE. They were tested in combination for inducing more number of shoots from cotyledonary node explant of *S. surattense*. Cotyledonary node explants exhibited varying response for shoot induction with different concentrations of TDE+BAP combination (Table 2). The number of shoots induced at the lowest concentration combination of 0.5 mg L^{-1} BAP+10% TDE, was 4.8 shoots/explant with 60% of response. This shoot number is comparatively higher than the shoots obtained with individual treatment of either BAP or TDE. The shoot number slightly increased (5.6) in combination of 1.0 mg L^{-1} BAP and 20% TDE. With increasing concentrations of TDE and BAP, maximum number of shoots were formed at 30% TDE+1.5 mg L^{-1} BAP which gave 6.5 shoots/explant with 90% of response (Table 2, Fig. 1a). Beyond that concentration shoot number decreased at 40% TDE+2.5 mg L^{-1} BAP (5.8 shoots) and 50% TDE+2.5 mg L^{-1} BAP (2.5 shoots) were induced respectively. The highest shoot number (8.5) was recorded with combination of 30% of TDE and 1.5 mg L^{-1} BAP containing medium, with 100% of response (Fig. 1b,c). These

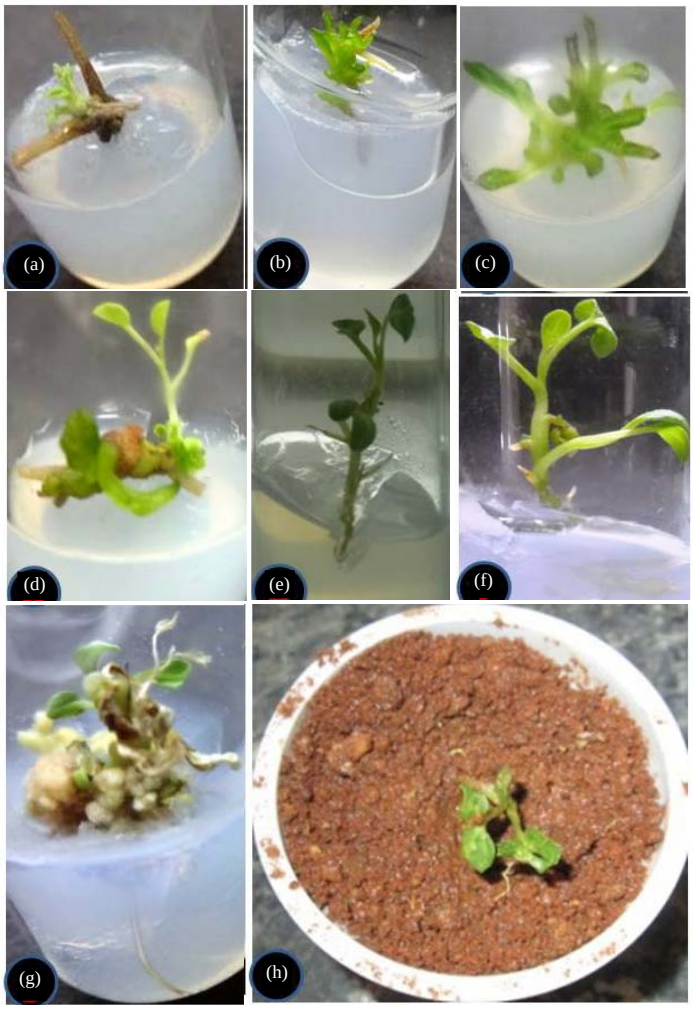

Fig. 1(a-h): Plant regeneration form cotyledonary node of *Solanum surattense* Burm.f (a) Explant culture, (b–d) Shoot proliferation and elongation after 6 week culture. (e) Micro cuttings (f-g) Root initiation and developed roots after 4 week culture and (h) Regenerated plantlets on plastic cup

results clearly suggest the shoot production can be increased with BAP and TDE combination at optimum concentration.

***In vitro* rooting and acclimatization:** Rooting of *in vitro* plants is the most important step in plant regeneration before taking them for acclimatization stage. In this study, the efficiency of rooting from micro shoots of *S. surattense* by using auxins and TDE was tested (Fig 1e, f). Successful rooting was achieved from *in vitro* derived shoots when different concentrations of auxins and TDE were tested either alone or in combination. In this study, MS medium supplemented with different concentrations of NAA, IBA (0.0, 0.2, 0.4, 0.6 and 0.8 mg L^{-1}) was used for root, regeneration (Table 3). Well-developed roots were observed when medium was enriched with 0.4 mg L^{-1} of IBA combined with 20% of TBE with maximum of 6.2 number of roots and 5.8 cm of root length after 4 weeks of culture (Fig 1g, Table 4).

DISCUSSION

Cytokinins play a key role in shoot organogenesis. Among the two cytokinins tested, BA (1.5 mg L^{-1}) was found to induce more shoots/explant (5.5) and greater shoot length (2.2 cm) than Kin (Table 1). The different concentrations of cytokinins influenced shoot bud proliferation and multiplication in cotyledonary node explant of *S. surattense.* Similar results were obtained by Jawahar *et al.*[22] in *S. trilobatum* and Govindarajan *et al.*[23] in *Psoralea corylifolia*. Pawar *et al.*[1] reported that high frequency of multiple shoots

Table 3: Effect of auxins on root formation from *in vitro* micro-cuttings of *Solanum surattense* Burm.f.

Auxins (mg L^{-1})				
NAA	IBA	Response (%)	Mean number of roots	Root length (cm)
0	0	0.0	0.0	0.0
0.2	0	75.0±2.7^{d}	1.6±0.2^{c}	1.1±0.4^{e}
0.4	0	85.2±1.6^{c}	2.5±0.5^{e}	1.6±0.5^{b}
0.6	0	65.5±3.2^{c}	1.3±0.1^{d}	3.8±1.2^{c}
0.8	0	45.6±1.3^{d}	1.3±0.3^{d}	2.5±0.6^{a}
0	0.2	80.3±3.1^{e}	3.6±0.6^{c}	3.5±0.2^{a}
0*	0.4*	89.6±1.9^{c*}	5.5±1.1^{b*}	5.6±1.5^{b*}
0	0.6	71.5±3.8^{b}	4.2±1.3^{b}	5.1±1.0^{e}
0	0.8	62.0±2.6^{c}	2.9±0.2^{a}	3.4±0.9^{a}

For each auxin tested, means followed by the same letter within a column are not significantly different according to Tukey's test ($p<0.05$). *Best results are indicated. Measurements were recorded after 4 week of culture on MSB_5 medium supplemented with auxins. Values represent the mean±SE, NAA α: Naphthalene acetic acid, IBA: Indole-3-butyric acid

Table 4: Combined effects of IBA and TDE on root formation from *in vitro* micro cuttings of *Solanum surattense* Burm.f.

Plant growth regulators (mg L^{-1})				
IBA	TDE (%)	Response (%)	Mean number of roots	Root length (cm)
0	0	0.0	0.0	0.0
0.4	10	55.0±3.3^{b}	2.5±0.5^{a}	3.2±0.9^{e}
0.4*	20*	95.6±3.8^{d*}	6.2±1.5^{a*}	8.6±3.6^{a*}
0.4	30	82.5±2.9^{e}	5.1±1.7^{c}	5.8±3.2^{c}
0.4	40	78.5±2.1^{a}	4.5±1.1^{d}	3.2±0.5^{b}
0.4	50	50.2±1.7^{c}	2.8±0.6^{c}	2.5±0.2^{d}

Means followed by the same letter within a column are not significantly different, according to Tukey's test ($p<0.05$). *Best results are indicated. Measurements were recorded after 4 week of culture on MSB_5 medium supplemented with IBA+ *Turbinaria*. Values represent the mean±SE

was produced from shoot tip and leaf explants of *S. xanthocarpum* only in the presence of BA and Kin. These results were in agreement to the present study in which the cytokinin was proved to be more effective for multiple shoot initiation. BA and Kin are the most widely used cytokinins for shoot regeneration in *Psoralea* and *Enicostemma* plant species[24,25]. Recent reports indicate that, cotyledonary node explants possess high capacity, for efficient *in vitro* regeneration[26,27]. Cotyledonary node is an ideal explant that possesses potent axillary meristematic cells that are competent for rapid regeneration. Similar to the present results, greater shoot induction (11.5 shoots) was achieved from cotyledonary node explants of *Ricinus communis* with 1.5 mg L^{-1} BA and 10 shoots were obtained/nodal explant of *Exacum bicolor* with 10 μM BA[27,28]. Greater shoot induction of 4.8 shoots was obtained/per cotyledenory node explant of tomato with using 30% of *Sargassum wightii* seaweed extract[13]. Esserti *et al.*[20] reported that seaweed extract increased adventitious shoot regeneration from *Nicotiana benthamiana* leaf discs explants when compared to the conventional regeneration medium. *In vitro* rooting was obtained with a combination of 0.4 mg L^{-1} of IBA and 20% of TBE with maximum of 6.2 numbers of roots from elongated shoots. Similar to this result, well-developed roots were obtained (4.0 roots/shoot) in cotton using IBA combined with seaweed extract[29]. However, it was noted that IBA and NAA above 0.4 mg L^{-1} concentration produced stumpy roots with average root lengths. Whereas, lowest roots number (1.3) and root length (2.9 cm) were obtained when micro shoots were cultured on MS media containing NAA at 0.8 mg L^{-1} of concentrations. *In vitro* rooting of *C. alba* was significantly improved with addition of suitable concentration of IBA (0.3 mg L^{-1})[30]. In contrast to this, maximum root number was achieved with NAA in cotton[26] and Finger millet[31]. Overall, it is noticed that the addition of TDE significantly improved *in vitro* regeneration in *Solanum surattense*. Rooted plantlets were successfully acclimatized in a potting medium containing soil and perlite (3:1) and grew naturally in a greenhouse (Fig 1h). Survival rate of the regenerated plant of *Solanum* was 90%.

CONCLUSION

In this study, an efficient and rapid protocol was developed for multiple shoot regeneration from cotyledonary node explants of *S. surattense*. The BAP at 1.5 mg L^{-1} in combination with 30% *Turbinaria* extract (TDE) produced a maximum of 8.5 shoots/cotyledonary node. Moreover, the presence of TDE also improved root formation from elongated shoots along with IBA. From this study, it can

be concluded that the high frequency *in vitro* regeneration of *Solanum surattense* is possible by supplementing seaweed extract (TDE) in combination with phytohormones.

SIGNIFICANCE STATEMENTS

This study discovers the possible synergistic effect of seaweed extract and plant growth regulators on *in vitro* regeneration of *Solanum surattense*. This study will help the researcher to uncover the significant application of seaweed extract in tissue culture of valuable medicinal plants that many researchers never explored. Thus, a new concept on the use of seaweed extract combination and possibly other combinations, may be arrived at.

ACKNOWLEDGMENTS

The authors are thankful for support assisted by University Grants Commission (UGC), New Delhi, India under the SAP-DRS-I program. The authors also thank Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India for providing necessity facilities to carried out the research in Department of Botany.

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