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Somatic embryogenesis and plantlet regeneration from cotyledon and leaf explants of *Solanum surattense*

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Somatic embryogenesis and plant regeneration from the cotyledon and leaf explants of Indian Solanum (*Solanum surattense* Burm. f.), a medicinally important plant, is reported. Embryogenic callus was induced from cotyledon and leaf explants on Murashige and Skoog's medium fortified with 0.5-8.0 mg/L α-naphthalene acetic acid (NAA) + 0.5 mg/L N^6-benzylaminopurine (BAP). High frequency of somatic embryo formation was found at 6.0 mg/L NAA + 0.5 mg/L BAP and 4.0 mg/L NAA+ 0.5 mg/L BAP in cotyledon and leaf explants, respectively. Secondary somatic embryogenesis was also observed when primary somatic embryos were subcultured on the same somatic embryo induction medium. Well-developed cotyledonary stage embryos were germinated on MS medium supplemented with 0.5 mg/L indole-3-acetic acid (IAA) +1.0-8.0 mg/L BAP. Maximum percentage (71.2%) of somatic embryo germination and plantlet formation was found at 0.5 mg/L IAA+2.0 mg/L BAP, but they didn't germinate on ½ MSO and MSO media. The post transplantation survival rate of plants was 65-70%. Plants and flowers formed were morphologically similar to mother plants. The present protocol can be used for genetic transformation experiments in *S. surattense*.

Keywords: *S. surattense*, Indian solanum, somatic embryos, regeneration
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Introduction

Solanum surattense Burm.f. (Eng: Indian Solanum, Hindi: *Baigan Kateli* (Sanskrit: *Kantakari*, Malayalam: *Kantakari valutina*), a common vegetable, is alsoused in herbal and ayurvedic formulations[1,2]. It is a prostrate and diffuse herb very prickly on stem found in plains and low hills throughout India[2,3]. The plant extracts have been used against agricultural pests as repellant and contact poison and as molluscicide in public health and also to control vectors of malaria and dengue/DHF[4].

In vitro regeneration of *S. surattense* has been achieved by many researchers using explants like leaf, stem and root[5], anthers[6], nodal and shoot tip[7,8]. However, there is no report on the induction of somatic embryogenesis. Somatic embryogenesis is an alternative and efficient method for plant propagation over regeneration via organogenesis. The plants regenerated via somatic embryogenesis are of single cell origin with true-to-type and are produced in large numbers within a short period[9]. Many workers have emphasized that the somatic embryogenesis is a preferred method for rapid *in vitro* multiplication of plants[10,11] and also for production of artificial seeds or synthetic seeds[12,13], and *Agrobacterium*-mediated transformation and regeneration of transgenic plants[14]. In this communication, the authors report induction of somatic embryogenesis and plantlet regeneration from cotyledon and leaf explants of *S. surattense*.

Materials and Methods

Plant Material

The seeds of *S. surattense* were collected from the plants grown in the research field of Department of Botany, Kakatiya University. They were soaked in sterile distilled water for 24 h, later cleaned with 5% teepol (w/v) and thoroughly washed in running tap water 3-4 times. Subsequently they were surface sterilized with 0.1% (w/v) $HgCl_2$ for 3-4 min followed by rinsing with sterile distilled water for 2 to 3 times and germinated aseptically on Murashige & Skoog's[15] basal medium.

Culture Media

Cotyledon (3-week-old) and leaf (4-week-old) explants (0.8-1.0 cm^2) from axenic seedlings were inoculated on MS medium supplemented with different concentrations of α-naphthalene acetic acid

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(NAA) (0.5-10 mg/L) in combination with 0.5 mg/L N^6- benzylaminopurine (BAP). For maturation in the second culture phase, the somatic embryos developed from leaf and cotyledonary explants were transferred to MS medium fortified with 4.0/6.0 mg/L NAA + 0.5 mg/L BAP, respectively.

Embryo Germination and Plantlet Formation

For germination and plantlet formation cotyledonary stage somatic embryos were transferred to ½ MSO (half-strength MS salts without plant growth regulators), MSO (MS salts without plant growth regulators) and MS medium supplemented with 0.5 mg/L indole-3-acetic acid (IAA) + 1.0-8.0 mg/L BAP.

Culture Conditions

All the media were supplemented with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar (Bacto-agar, Difco). After adding growth regulators, the *p*H of the medium was adjusted to 5.8±0.1 with 0.1 N HCl or 0.1 NaOH and autoclaved at 121°C and 1.06 kg/cm^2 for 15-20 min. All the cultures were incubated at 25±2°C under a 16 h photoperiod. Light intensity of 40-50 µmol m^{-2} S^{-1} was provided by using cool-white, fluorescent tubes. The cultures were transferred to fresh medium after an interval of 4 weeks. For each hormonal treatment 20 replicates were raised and the experiments repeated at least twice. Data on somatic embryogenesis and germination were statistically analyzed using Mean and Standard Error.

Acclimatization

The plants were taken out and washed with sterile distilled water under aseptic conditions to remove agar medium. They were shifted to micro pots (plastic pots) containing sterile vermiculite: soil (1:1), covered with polythene bags in order to maintain 80-85% relative humidity and kept in culture room for 3 weeks. Later, they were transferred to earthen pots containing garden soil and maintained in the research field.

Results and Discussion

Induction of Somatic Embryogenesis

Cotyledons and tender leaves cultured on various concentrations of NAA in combination with 0.5 mg/L BAP became swollen, and generally dedifferentiated and developed friable callus after 8-10 days of culture. Within 15-20 days of culture, the globular embryos were formed directly on the surface of callus (Fig.1 a). When the explants of primary somatic

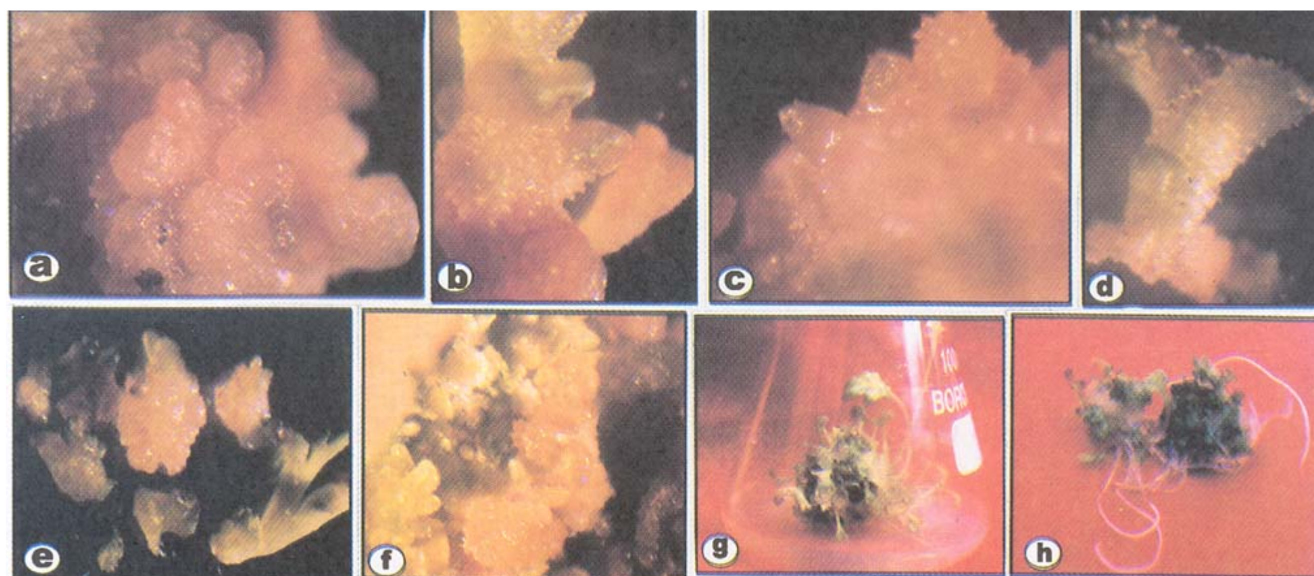

Fig 1—Regeneration via somatic embryogenesis in leaf explant and cotyledonary cultures of *S. surattense*: *a,* Formation of globular embryos on MS+4.0 mg/l NAA + 0.5 mg/L BAP; b, Heart shaped somatic embryo which differentiated on MS+4.0 mg/L NAA + 0.5 mg/L BAP; c, Torpedo-shaped embryo on MS+4.0 mg/L NAA +0.5 mg/L BAP; d, Cotyledonary stage somatic embryo which differentiated on MS+6.0 mg/L NAA + 0.5 mg/L BAP; e, Isolated somatic embryos in different stages; f, Development of somatic embryos in different stages on MS+6.0 mg/L NAA + 0.5 mg/L BAP; & g-h, Conversion of cotyledonary stage embryos into plantlets on MS+0.5 mg/L IAA + 2.0 mg/L BAP.

embryos were cut into fragments and cultured on the same induction medium, secondary somatic embryos were induced within two weeks. The production of secondary somatic embryos from primary embryos frequently occurred when somatic embryos were maintained for an extended period of 3-4 months on the induction medium in both the explants studied.

Thus, proliferation of somatic embryos occurred in two ways: 1 Formation of primary somatic embryos from the explants somatic embryogenesis; and 2 Proliferation of secondary somatic embryos from primary embryos through repetitive embryogenesis.

Among the various concentrations of NAA tested in combination with 0.5 mg/L BAP, the percentage of explants responded for somatic embryo formation was found to be higher at 6.0 mg/L NAA + 0.5 mg/L BAP in cotyledon explants (Table 1) with a maximum of 24.0±0.27 somatic embryo production. There was generally an increased tendency of somatic embryo formation with the increasing concentration of NAA in combination with BAP. However, at the concentration of 10 mg/L NAA + 0.5 mg/L BAP somatic embryogenesis was inhibited.

Maximum number of somatic embryos/explant and higher percentage of response for somatic embryos formation have been found at 4.0 mg/L NAA+ 0.5 mg/L BAP in leaf explants of *S. surattense* (Table 2). With the increase of NAA concentration up to 4.0 mg/L + 0.5 mg/L BAP, there was a gradual enhanced somatic embryos induction. It was found that at higher concentration of NAA (10 mg/L) in combination with 0.5 mg/L BAP, the induction of somatic embryogenesis was inhibited as observed in cotyledon explants.

In the present investigation, leaf explants showed maximum percentage of somatic embryogenesis and high frequency of somatic embryo induction/explant (38.0±0.27) compared to cotyledons (24.0±0.25).

The calli developed from leaf and cotyledon explants containing globular embryos were transferred to maturation medium containing MS medium supplemented with 4.0/ 6.0 mg/L NAA + 0.5 mg/L BAP, respectively. Individual embryos enlarged into distinct bipolar structures and passed through each of the typical developmental stages (globular, heart, torpedo and cotyledonary) (Fig.1 b-f) after 4-6 weeks of culture. The development of somatic embryos was asynchronous. As a result, various stages of embryo development could be observed in the same cluster of embryos originally from the explant. When these embryos with different developmental stages were transferred to the same medium, further germination in them was not observed.

Embryo Germination and Plantlet Formation

Germination of cotyledonary stage somatic embryos started after two weeks of culture on germination medium containing various concentrations of BAP (1.0-8.0 mg/L) in combination with 0.5 mg/L IAA (Table 3). Somatic embryos did not germinate on ½ MSO and MSO media. The highest frequency (71.2%) of somatic embryo germination was observed on MS medium supplemented with 0.5 mg/L IAA + 2.0 mg/L BAP (Fig.1.g-h).

After 30 days of somatic embryo germination, the plantlets were transferred to micropots containing sterile vermiculite: soil (1:1) mixture. Later, they were shifted to earthen pots after hardening in the culture room and maintained in the research field under shady conditions. The survival percentage of plants was

Table 1—Effect of various concentrations of NAA and 0.5 mg/L BAP on somatic embryogenesis in cotyledon explants of *S. surattense*

Growth regulators (mg/L) NAA+BAP	Percentage of response for somatic embryo formation	Average number of somatic embryos/explant (S.E)*
0.5+0.5	31.0	5.7 ± 0.32
1.0+0.5	50.7	6.9 ± 0.23
2.0+0.5	60.2	15.8 ± 0.27
3.0+0.5	76.2	20.0 ± 0.23
4.0+0.5	80.4	22.0 ± 0.35
6.0+0.5	85.0	24.0 ± 0.27
8.0+0.5	76.2	15.0 ± 0.37
10.0+0.5	—	—

Table 2—Effect of various concentrations of NAA and 0.5 mg/L BAP on somatic embryogenesis in leaf explants of *S. surattense*

Growth regulators (mg/L) NAA+BAP	Percentage of response for somatic embryo formation	Average number of somatic embryos/explant (S.E)*
0.5+0.5	25.4	8.7 ± 0.23
1.0+0.5	56.0	9.9 ± 0.35
2.0+0.5	72.0	15.8 ± 0.73
3.0+0.5	80.0	25.3 ± 0.32
4.0+0.5	87.0	38.0 ± 0.27
6.0+0.5	75.0	25.0 ± 0.35
8.0+0.5	60.8	20.0 ± 0.32
10.0+0.5	—	

*Mean ± Standard Error

Table 3–Effect of IAA + BAP on germination of somatic embryo in *S. surattense*

Medium + growth Regul ators (mg/L)	Germination frequency (mean ± S.E)*
½ MSO	—
MSO	—
MS+ IAA (0.5) + BAP (1.0)	31.6 ± 0.56
MS+ IAA (0.5) + BAP (2.0)	71.2 ± 1.21
MS+ IAA (0.5) + BAP (3.0)	63.0 ± 0.72
MS+ IAA (0.5) + BAP (4.0)	35.5 ± 0.31
MS+ IAA (0.5) + BAP (6.0)	23.0 ± 0.25
MS+ IAA (0.5) + BAP (8.0)	11.3 ± 1.30

- Data represents mean ± standard error of three experiments each consisting of 20
- Somatic embryos

found to be 65-70%. The plants were normal, and morphological and floral characters were found to be similar to the donor plants.

In the present investigation, the results on somatic embryogenesis have shown that auxin, such as NAA along with cytokinin BAP are essential for inducing the somatic embryogenesis from cotyledon and leaf explants of *S. surattense*. The auxin or auxin in combination with cytokinin used in the medium can greatly influence the frequency of induction and also on maturation of somatic embryos. The requirement of cytokinin in addition to auxin was observed in *Terminalia arjuna*[16] and *Psoralea corylifolia*[17], as it was observed in the present studies; whereas, somatic embryogenesis was reported on medium containing NAA alone in *S. melongena*[18]. Direct somatic embryogenesis was also reported by adding BAP to the medium and also the number of embryos further increased by enriching the medium with NAA in *Hippeastrum hybridum*[19] and *Brimeura amethystine*[20].

New gene products are needed for the progression from the globular to the heart-staped stage and these new products are synthesized only when an exogenous auxin is removed[21]. But, according to our observations in *S. surattense* for morphogenesis of somatic embryos, auxins and cytokinin combination is required. At higher concentration of auxin, probably the population of embryogenic cells drops due to their disruption and elongation and the embryogenic potential of the culture is lost[22]. Maturation process is a critical step in somatic embryogenesis, which leads to the complete plantlet formation. In the present investigation, both auxin and cytokinin combination favoured the maturation and germination of somatic embryos. Similarly, somatic embryos maturation on MS medium containing the combination of NAA and BAP was observed in *Cajanus cajan*[23], *Prunus avium*[24] and *Hardwickia binata*[25].

In conclusion, for induction of *in vitro* somatic embryogenesis the type of primary explant, choice of genotypes and hormonal concentration plays an important role[26]. During the present investigations, it was found that the high concentration of auxin in combination with less concentration of cytokinin induced the somatic embryogenesis and maturation of somatic embryos in *S. surattense*. However, for germination of somatic embryos, low level of auxins and high concentrations of cytokinin combination is required. Secondary embryogenesis observed in *S. surattense* has great potential for its mass propagation and repetitive embryogenesis can also be used for synthetic seed production and genetic transformation[27].

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