Effect of Plant Growth Regulators on Somatic Embryogenesis and Plantlet Development of Turkey Berry (*Solanum torvum* SW)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

In the present study it was reported on direct somatic embryogenesis and plant regeneration from cotyledon and leaf explants of Turkey berry/pea egg plant (*Solanum torvum* SW), a medicinally important plant. Somatic embryogenesis has several advantages over other routes of *in vitro* plant regeneration. Somatic embryogenesis was induced directly from cotyledon and leaf explants on MS medium fortified with BAP (0.5 mg/L)+NAA (0.5-6.0 mg/L). High percentage of somatic embryogenesis (90%), maximum number of somatic embryos formation (62±0.18) along with high percentage (76%) conversion of somatic embryos into bipolar embryos was observed on cotyledon explants in 0.5 mg/L BAP+2.5 mg/L NAA. At the same concentration of BAP (0.5 mg/L)+NAA (2.5 mg/L) also resulted on the maximum percentage of somatic embryogenesis (92%), the highest number of somatic embryos formation (88±0.15) and the highest percentage (76%) of somatic embryos conversion into bipolar embryos in leaf explants. A mixture of globular, heart and torpedo-shaped embryos were germinated on MS medium supplemented with 0.5 mg/L IAA+1.0-4.0 mg/L BAP. Maximum germination frequency (75±0.14) of somatic embryos and plantlet formation was

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found in 0.5 mg/L IAA+2.0 mg/L BAP, but they didn't germinate on ½ MSO and MSO media. The survival rate of regenerated plants after field transfer was recorded to be 75%. These regenerated plants were found morphologically similar to donor plants. The present protocol can be used for conservation of the species and also for genetic transformation experiments in *S. torvum*.

1. INTRODUCTION

The species *S. torvum* (Turkey berry/Pea egg plant) is closely related to eggplant (*S. melongena L.*), native to India. Although invasive in some areas of introduction, this species is of particular interest as it has been identified as a potential source of resistance to bacterial wilt disease for cultivated susceptible *Solanaceous* species.

This plant is medicinally important as it was evidenced by many reports. Mythirayee et al. [1] have reported the presence of polyphenols from the fruits of *S. torvum*. Maiti et al. [2] have also determined the dominant steroidal glycoalkaloids, and solasonine and solamargine (two glycosilated compounds of solasodine) in *S. torvum*. Research on the therapeutic potential of *S. torvum* has been established mainly on its leaves [3] and the roots [4]. The fruits of *S. torvum,* locally known as terung pipit, are used commonly in traditional medicine as antihypertensive [5] and as antinematodal agents [6]. Apart from their therapeutic importance, the fruits of *S. torvum* are also served as dietary due to their high nutritional values [7]. The main constituents in the fruits of *S. torvum* are steroidal alkaloids, tannins and saponins [8]. The methanolic extract from the fruits of *S. torvum* showed the inhibiting activity against bacterial species commonly associated with pyogenic infections [8]. Arthan et al. [9] have also demonstrated the presence of isoflavonoid sulphate and steroidal glycosides in the fruits of *S. torvum* which displayed antiviral activity.

Regeneration systems have been successfully established in many varieties of *Solanums* including eggplant, potato, and tomato. But, inspite of its medicinal importance, the regeneration studies have not been reported in *S, torvum*. Hence, we have attempted to develop the regeneration protocol through somatic embryogenesis.

Somatic embryogenesis has several advantages over other routes of *in vitro* plant regeneration. 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 [10]. Somatic embryogenesis provides an efficient method for plant micropropagation, producing large numbers of elite and *true-to-type* plants [11]. Somatic embryogenesis is an alternative method for plant propagation over regeneration via organogenesis. Somatic embryogenesis is a preferred method for rapid *in vitro* multiplication of plants by producing artificial/synthetic seeds and also for *Agrobacterium tumefaciens* mediated genetic transformation and regeneration of transgenic plants [12].

Since there is no report on somatic embryogenesis in *S. torvum* a medicinal plant, we report in the present investigation to develop a protocol for plantlet regeneration through somatic embryogenesis. The purpose of this study was therefore to standardize an efficient and reproducible *in vitro* regeneration protocol from cotyledon and leaf explants of *S. torvum* as a necessary first step for subsequent biotechnological studies and applications.

2. MATERIAL AND METHODS

2.1 Plant Material

For somatic embryo induction and plantlet formation the seeds of Turkey berry/Pea egg plant (*S. torvum)* were soaked in sterile distilled water for 24 hrs. These were sterilized with 70% (v/v) alcohol for 2-3 minutes followed by 1% (w/v) aqueous solution of sodium hypochlorite for 3-5 minutes. Later, the sterilized seeds were washed thoroughly with sterile distilled water and germinated aseptically on MS (Murashige and Skoog's) [13] basal medium.

2.2 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 NAA-α-naphthalene acetic acid (0.5-6.0 mg/L) in combination with 0.5 mg/L BAP-N^6-

benzylaminopurine. For further proliferation of somatic embryos in the second culture phase, the explants along with the somatic embryos were cultured on MS medium augmented with 0.5 mg/L BAP+2.5 mg/L NAA (Tables 1-2).

2.3 Embryo Germination and Plantlet Formation

For germination and plantlet formation the bipolar (torpedo-shaped) stage embryos were transferred onto ½ strength MSO (MS medium without growth regulators), MSO and MS medium fortified with different concentrations of BAP (1.0-4.0 mg/L)+0.5 mg/L IAA-Indole-3-acetic acid (Table 3).

2.4 Culture Conditions

All the media were supplemented with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar (Bactoagar, Difco). After adding plant growth regulators-PGRs, the pH of the medium was adjusted to 5.8±0.1 either 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 with light intensity of 40-50 µmol m^{-2} s^{-1} provided by using coolwhite, fluorescent tubes. The cultures were transferred to fresh medium after an interval of 4 weeks.

2.5 Histological Study

To ascertain the embryogenic nature of differentiating structures, cultured tissues were subjected to a histological study. Tissue bearing somatic embryos at different developmental stages was fixed in aceto-alcohol (1:3) and then dehydrated in ethanol-xylon series, embedded in paraffin wax, sectioned at 10 µ thickness and stained with haematoxylin and basic Fuschin. Total number of embryos and the percentage of globular, heart and torpedo-shaped embryos and cotyledonary embryos were scored.

2.6 Plantlet Establishment

The plants were taken out and washed with sterile distilled water under aseptic conditions to remove remains of agar medium. They were shifted to plastic pots containing sterile vermiculite: soil (1:1), covered with polythene bags, maintained 80-85% relative humidity and kept in culture room for 4 weeks. Later, they were transferred to earthenware pots containing garden soil and maintained in the research field.

2.7 Data Analysis

Data were recorded after 4 weeks of culture. Each experiment was repeated at least thrice and 20 replicates were maintained for each experiment.

3. RESULTS

3.1 Direct Somatic Embryogenesis from Cotyledon Explants

Results on somatic embryogenesis of *S. torvum* from cotyledon explants are presented in Table 1 and shown in Fig. 1. Cotyledon explants were swollen after 4 days of culture and globular somatic embryos were induced directly from the explant after 10 days of culture (Fig. 1a-b). Somatic embryogenesis was induced from the cotyledon explants cultured on all the concentrations of NAA + 0.5 mg/L BAP except at 6.0 mg/L NAA.

Callus was induced from all the explants at 6.0 mg/L NAA. The highest percentage of somatic embryogenesis was observed at 0.5 mg/L BAP+2.5 mg/L NAA. Maximum frequency of somatic embryos formation was also found at the same concentration of NAA. As the concentration of NAA in combination with BAP increased, the percentage of somatic embryo induction and as well as somatic embryo number per explant were enhanced upto 2.5 mg/L NAA (Table 1). But at high concentration of NAA + 0.5 mg/L BAP, the percentage of somatic embryogenesis was found to be reduced.

Average number of somatic embryos formation per explant was also decreased under concentrations higher than 2.5 mg/L NAA+0.5 mg/L BAP. Globular embryos were converted into bipolar embryos on all the concentrations of NAA. The embryo conversion was found to be dependent on the level of NAA. The highest percentage of bipolar/torpedo-shaped embryos formation was recorded at 2.5 mg/L NAA. The smallest percentage of conversion was observed at 4.0 g/L NAA.

3.2 Direct Somatic Embryogenesis from Leaf Explants

Leaf explants of *S. torvum* were cultured on MS medium augmented with different concentrations of NAA in combination with 0.5 mg/L BAP (Table 2). Somatic embryogenesis was initiated directly from the explant in all the concentrations of NAA

except at the highest concentration (6.0 mg/L NAA). Somatic embryoids were formed 10 days after culture. As in the cotyledon explant, the somatic embryogenesis was inhibited at 0.5 mg/L BAP+6.0 mg/L NAA and callus was induced. The highest percentage of somatic embryogenesis was observed at 0.5 mg/L BAP+2.5 mg/L NAA followed by 0.5 mg/L BAP +2.0 mg/L NAA.

Maximum frequency of somatic embryos per explant was observed at 0.5 mg/L BAP+2.5 mg/L NAA. The smallest number of somatic embryos induction was recorded at 0.5 mg/L BAP+4.0 mg/L NAA. As the concentration of NAA increased, there was an increase in the average number of somatic embryos development per explant up to 2.5 mg/L NAA.
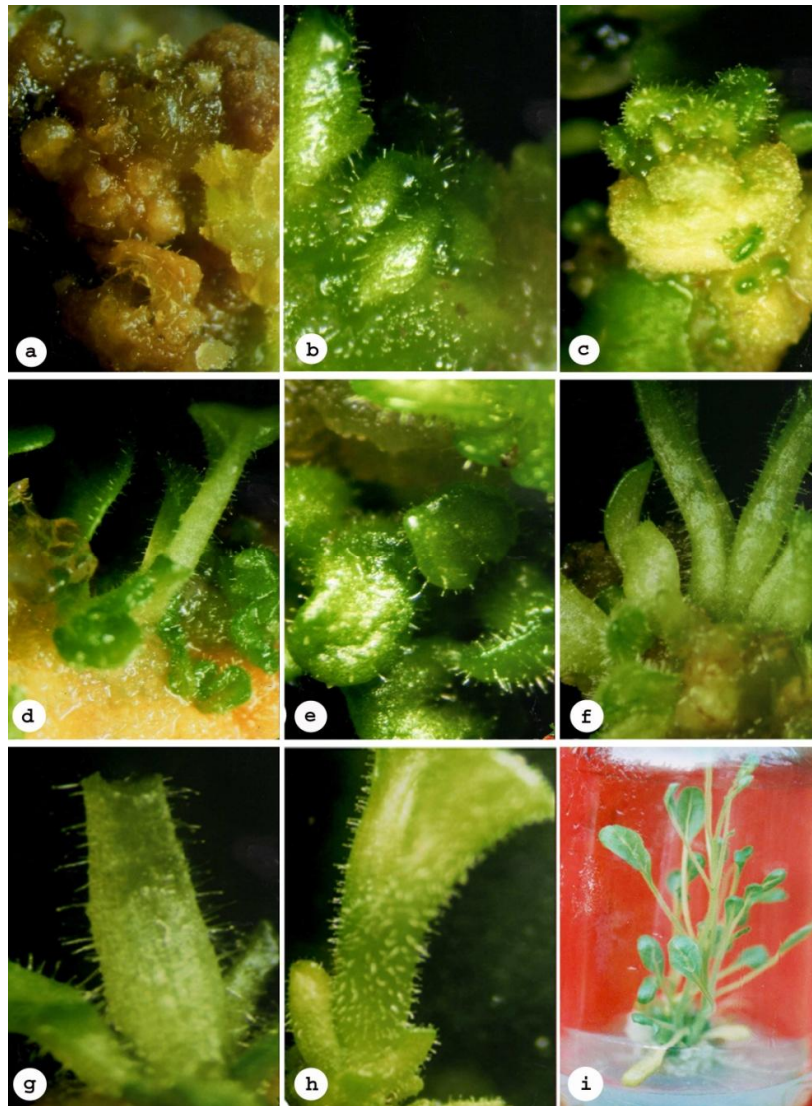

Fig. 1. Induction of direct somatic embryogenesis on MS+0.5 mg/L BAP+2.5 mg/L NAA from cotyledon and leaf explants of *S. torvum*: a-b) Cotyledon explants: Many globular embryoids and different stages of somatic embryoids on MS+0.5 mg/L BAP+2.5 mg/L NAA respectively; c-d) Leaf explants: c) Globular, heart and torpedo shaped embryos; d) Cluster of torpedo shaped embryoids after 4 weeks of 1st subculture; e-h) Somatic embryoids germination on 0.5 mg/L IAA+ BAP: e) Cotyledonary stage and torpedo-shaped embryos; f) A group of torpedo and cotyledonary stage embryos on MS+0.5 mg/L IAA+2.0 mg/L BAP (enlarged view) respectively; g) An enlarged view of single torpedo- shaped embryo; h) An enlarged and elongated cotyledonary stage embryo; i) Plantlets formation

The conversion of somatic embryos from globular to torpedo-shaped was found in all the concentrations of NAA tested except at 6.0 mg/L NAA. Globular embryos were not converted into bipolar on MS medium supplemented with 0.5 mg/L BAP+4.0 mg/L NAA. Maximum percentage of bipolar embryos was recorded at 0.5 mg/L BAP+2.5 mg/L NAA (Fig.1c-d). Even after 2nd subculture on the same fresh medium, bipolar somatic embryos did not mature further. But the somatic embryos number per explant was enhanced.

Individual embryos developed into distinct bipolar structures and passed through each of the typical developmental stages (globular, heart, torpedo/bipolar) 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 originated from the explant (Fig. 1b-c). It is also interesting to note that a cluster of torpedo-shaped embryoids development was observed (Fig. 2a, c).

3.3 Somatic Embryo Germination and Plantlet Formation

Somatic embryos did not germinate on ½ strength MSO and also on MSO medium. The highest frequency (75%) of embryo germination was noticed on medium containing 0.5 mg/L IAA+2.0 mg/L BAP (Fig. 1e-h). Embryo germination frequency was reduced at high concentration of BAP (Table- 3). It was also recorded the conversion of cotyledonary stage embryos in a group (Fig. 1e-f).

Histological sections of embryo forming explants clearly revealed a globular-shaped embryo (Fig. 2), a heart-shaped embryo with a notch and two cotyledons (Fig. 2b) and torpedo-shaped embryo with shoot and root poles (Fig. 2c). Upon transfer to a medium containing 0.5 mg/L IAA+2.0 mg/L BAP, the embryos turned green with folded cotyledons, which subsequently developed into whole plantlets(Fig. 1h).

Table 1. Induction of direct somatic embryogenesis from cotyledon explants of *S. torvum*.

Concentration of PGRs (mg/L)	Cultures with somatic embryogenesis (%)	Somatic embryos/ Explant (Average n^0)	Somatic embryos converted into bipolar embryoids (%)
BAP+NAA			
0.5 + 0.5	51	31 ± 0.06	34
0.5 + 1.0	59	41 ± 0.48	52
0.5 + 1.5	72	48 ± 0.96	66
0.5 + 2.0	81	56 ± 0.14	69
0.5 + 2.5	90	62 ± 0.18	76
0.5 + 3.0	79	52 ± 0.15	51
0.5 + 4.0	60	36 ± 0.13	24
0.5 + 6.0	Callus	---	---

*Mean ± Standard Error

Table 2. Induction of direct somatic embryogenesis from leaf explants of *S. torvum*

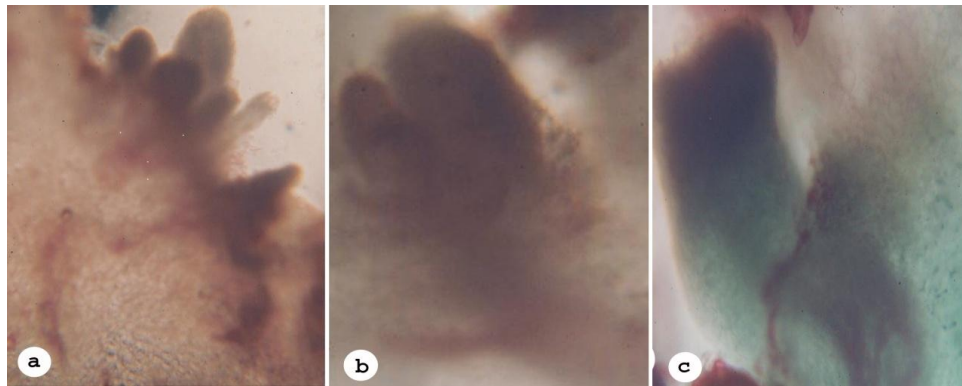| Concentration of PGRs (mg/L)	Cultures with somatic embryogenesis (%)	Somatic embryos/ Explant (Average n^0)	Somatic embryos converted into bipolar embryoids (%)
BAP+NAA			
0.5 + 0.5	42	34 ± 0.17	32
0.5 + 1.0	56	52 ± 0.46	49
0.5 + 1.5	76	67 ± 0.94	46
0.5 + 2.0	84	72 ± 0.16	58
0.5 + 2.5	92	88 ± 0.15	76
0.5 + 3.0	78	61 ± 0.89	56
0.5 + 4.0	30	43 ± 0.14	20
0.5 + 6.0	Callus	---	---

*Mean ± Standard Error

Fig. 2. Histological sections of somatic embryogenesis: a) Different stages of somatic embryos; b) Heart-shaped embryo with clear notch; c) Torpedo-shaped embryo

Table 3. Effect of IAA + BAP on germination of somatic embryos in *S. torvum*

Concentration of PGRs (mg/L)	Germination Frequency (Mean ± SE)
½ MSO	---
MSO	---
IAA + BAP	
0.5 + 1.0	32 ± 0.44
0.5 + 1.5	54 ± 0.93
0.5 + 2.0	75 ± 0.14
0.5 + 2.5	65 ± 0.16
0.5 + 3.0	35 ± 0.88
0.5 + 4.0	22 ± 0.85

Data scored after five weeks of culture
*Mean ± Standard Error

Plantlets regenerated via somatic embryogenesis transferred to polycups containing mixture of sterile vermiculite: soil 1:1 resulted on 75% survival rate. A total of 30 regenerated plants transferred to earthenware pots from the polycups and maintained in the research field under shady conditions. These plants are remained normal and similar to donor plants.

4. DISCUSSION

Somatic embryogenesis was induced directly from the explants viz., cotyledon and leaf in *S. torvum* on MS medium fortified with different concentrations of NAA (1.0-6.0 mg/L) in combination with 0.5 mg/L BAP except at 6.0 mg/L NAA. The present investigations showed that auxins such as NAA along with cytokinin BAP are required for inducing the somatic embryogenesis of *S. torvum*. For Somatic embryogenesis the nature of growth regulators and their combinations used in the culture medium play a vital role. The type of auxin or auxin in combination with cytokinin used in the induction medium can greatly influence somatic embryo frequency in *S. torvum*. The requirement of cytokinin in addition to auxin was observed in medicinal plants like *Terminalia arjuna* [14] and *Psoralea corylifolia* [15], as it was observed in the present investigation. Whereas somatic embryogenesis was induced on medium containing NAA alone in *Solanum melongena* [16,17]. Recently, Rama Swamy et al. [12] have also reported the essentiality of both auxin-cytokinin combination for inducing somatic embryogenesis in *S.surattense* a medicinal plant.

BAP induced the direct somatic embryogenesis and also the number of embryos further increased by enriching the medium with NAA in *Hippeastrum hybridum* [18] and *Brimeura amethystine* [16]. Sahrawat and Chand [15] have also observed the high frequency somatic embryogenesis in hypocotyl explants on MS medium supplemented with NAA (1.4 µM)+BAP (2.2 µM), as observed here in *S. torvum*.

In the present investigation, leaf explants showed the maximum frequency number of somatic embryos production and also conversion into

bipolar embryos at 5.0 mg/L BAP+2.5 mg/L NAA compared to cotyledon explants. The same response was also observed in *S. surattense* [12].

Somatic embryo maturation 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. For somatic embryos germination, heart/ torpedo shaped/ bipolar embryos in the present investigations required the combination of 0.5 mg/L IAA+1.0-4.0 mg/L BAP.

This is probably because of conversion of some of the heart-shaped embryos to torpedo or cotyledonary stage embryos and their subsequent germination in the presence of IAA+BAP. Thus, a combination of auxin-cytokinin seems to be necessary for maturation and germination of bipolar somatic embryos in *S. torvum*. Prakash et al. [19] have reported that TDZ (1.0 mg/L) in combination with GA_3 (1.0 mg/L) was found to be comparatively more effective than BAP for somatic embryo maturation in *Pimpinella tirupatiensis,* an endangered medicinal plant. The requirement of auxin-cytokinin combination was also reported in *S. surattense* for germination of torpedo stage embryos [12], as it was noted in the present investigations.

According to Zimmerman [20], new gene products are needed for the progression from the globular to the heart-stage and these new products are synthesized only when an exogenous auxin is removed. But, according to our observations in *S. torvum* for induction 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 are lost [21]. Similarly, in the present investigation embryogenesis was inhibited at 6.0 mg/L NAA + 0.5 mg/L BAP.

5. CONCLUSION

Thus, somatic embryogenesis always appeared to be dependent on the type of auxin/cytokinin/auxin + cytokinin and their concentrations in the medium. The type of PGR and its concentration also varies from genotype to genotype. High concentration of auxin in combination with less concentration of cytokinin induced the somatic embryogenesis and maturation of somatic embryos in *S. torvum*. However, for germination of somatic embryos, low level of auxins and high concentration of cytokinin combination is required.

Regeneration via embryogenesis is better for obtaining genetically uniform plants than through organogenesis. It is evident from the present studies that the somatic embryogenesis in this species will be useful in the improvement of medicinally important species. Somatic embryogenesis is also preferred, because it allows production of plants without somaclonal variation and also used for genetic transformation studies. These somatic embryoids induced in *S. torvum* can also be used for development of synseeds for germplasm storage, conservation and also for an exchange.

Thus, for induction of *in vitro* somatic embryogenesis and plantlet formation, the type of primary explant, genotype and growth regulators concentration and combinations play an important role. The protocol developed in the present investigation can be used for mass-scale propagation of *true-to-type* of the medicinally important plant *S.torvum*.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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