High efficiency direct *in vitro* regeneration from different explants of medicinal plant turkey berry (*solanum torvum sw*)

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Abstract

In the present investigation the role of different plant growth regulators (PGRs) on direct regeneration from various explants, cotyledon, hypocotyl and leaf was studied to find out the regeneration potentiality of the explant and also role of PGRs in Turkey berry/pea egg plant, *S. torvum*. The explants such as hypocotyl (0.4-0.8 cm long), cotyledon (0.6-0.8 cm^2) from 3 week old and leaves (1.0 cm^2) from 6 week old *in vitro* grown seedlings were excised and cultured on MS medium with various concentrations of BAP/KIN alone and also in combination with 0.5-1.0 mg/L IAA showed the direct shoots regeneration**.** All the explants have shown the maximum percentage of response (>90%) and highest number of direct induction of multiple shoots per explant (55±0.15 cotyledon, 52±0.13 leaf and 31±0.17 hypocotyl explants) was found at 0.5 mg/L IAA+2.5 mg/L BAP in comparison to different concentrations of cytokinins alone used and as well as in combination with IAA. Thus, it is clear that cotyledon explants were found to be more potential in producing high frequency number of shoots per explant among all other explants tested in the present investigations. Cytokinnes BAP/KIN alone or in combination with IAA was found more effective in inducing shoot regeneration in all the explants of *S. torvum*. However, 2.5 mg/L BAP/KIN with 0.5 mg/L IAA combination induced highest number of shoots. At 3.5 and 4.0 mg/L BAP in combination with 1.0 mg/L IAA, shoots were formed along with the callus. For *in vitro* rooting the elongated microshoots were transferred on to root induction medium (RIM) fortified with 0.25-2.0 mg/L NAA/IAA. Maximum percentage of response (92%), average number of roots (20.3±0.03) per micro shoot with highest length of roots (9.6±0.09) was observed at 1.0 mg/L IAA. *In vitro* rooted plantlets were transferred in to sterile vermiculate and soil (1:1) mix and subsequently these were acclimatized in the green house. The *in vitro* rooted plantlets were transferred into field. The survival percentage was found to be 90% and the plantlets were normal in morphology, flowering and fruiting. Thus, the regeneration protocol developed in the present investigation can be used for conservation and genetic transformation experiments in *S. torvum*, not only a medicinal plant but also a model plant.

Keywords: Plant growth regulators; cotyledon; leaf; hypocotyl explants; *in vitro* rooting; plantlet establishment.

INTRODUCTION

Solanum torvum Sw (Turkey Berry/Pea egg plant) is an ethnobotanical species growing wild in India. it is closely related to eggplant (*S. melongena* L.). Different parts of this plant are used in the treatment of various diseases. Fume of the burning seeds is inhaled for toothache (Bhakuni et al., 1969). In addition, the plant is reported to be a common ingredient in Thai cuisine (Arthan et al., 2002). The species contains steroidal alkaloids, viz., solasonine, torvogenin, torvoside and torvanol (Iida et al., 2005; Smith et al., 2008). It also possesses an antioxidants, antibacterial and antidiabetic activities (Agrawal et al., 2010). Fruits of *S. torvum* are generally consumed by local tribal community as vegetable and infrequently available in the local markets

(Choudhury et al., 2008, 2010; Deb et al., 2013). Fruits are effective in treatment of cough, cold, liver and spleen enlargement (Priyanaka et al., 2014).

Though *in vitro* regeneration systems have been successfully established in many varieties of *Solanum* species including eggplant, potato and tomato, no report is available on *S. torvum.* The purpose of this study was therefore to develop an efficient and reproducible *in vitro* regeneration protocol from different explants of *S. torvum* as a necessary first step for subsequent biotechnological studies and applications.

In vitro plant regeneration plays an important role in conservation and multiplication of an endangered, medicinal plant and also in genetic transformation and transgenic plants production. *In vitro* direct regeneration was reported from different types of explants in various medicinal plants (Swartz et al., 1990; Desai & Mehta, 1990; Rajani & Urs, 1996; Jeong et al., 2001; Rama Swamy et al., 2005; Sharada et al., 2019).

Hence, the present study was conducted to find out the suitable plant growth regulators (PGRs) for efficient *in vitro* morphogenesis and direct regeneration from hypocotyl, cotyledon and leaf explants of *S. torvum.* This is also aimed at establishing an efficient reproducible regeneration protocol for future conservation, sustainable production of elite clones and transformation of *S. torvum.*

MATERIALS AND METHODS

Plant Material

Seeds of *S. torvum* were soaked for 24 hrs in sterile distilled water. The seeds were surface sterilized with 70% (v/v) alcohol for 2 minutes followed by two rinses in sterile distilled water. Later these were sterilized with 0.1% (w/v) $HgCl_2$ for 2-3 minutes and washed 3 times with sterile distilled water. These sterilized seeds were inoculated on MS (Murashige & Skoog's, 1962) medium without plant growth regulators (PGRs).

Culture Media and Culture Conditions

The explants viz., hypocotyl (0.4-0.8 cm long), cotyledon (0.6-0.8 cm^2) from 3 week old seedlings and leaves (1.0 cm^2) from 6 week old *in vitro* grown seedlings were excised and cultured on MS medium containing 3% (w/v) sucrose fortified with various concentrations of BAP/KIN alone and also in combination with 0.5-1.0 mg/L IAA (Tables 1-3). The pH of medium was adjusted to 5.8 with either 0.1 N HCl or 0.1 N NaOH, solidified with 0.8% (w/v) Difco-bacto agar and autoclaved at 121^0C under 15 psi for 15-20 minutes. Single explant was inoculated in each culture tube and incubated at 25^0C under white-fluorescent light of 40-60 µ mol $m^{-2}s^{-1}$ intensity for 16 hr. The cultures were transferred to fresh medium after an interval of 4 weeks.

In vitro Rooting

For *In vitro* rooting, the micro shoots developed through different explants consisting of 3-4 cm in height were excised and cultured on ½ strength MSO and MSO media without PGRs and MS medium augmented with various concentrations of auxins NAA/IAA (0.25-2.0 mg/L) Table 4.

Plantlet Establishment

The plantlets 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) mix, covered with polythene bags, maintained 80-85% relative humidity (RH) and kept in green house for 4 weeks. Later, they were transferred to earthenware pots containing garden soil and maintained in the research field.

Table 1: Effect of BAP / KIN on direct regeneration from hypocotyl, cotyledon and leaf explants of *S. torvum.*

Concn. of PGRs (mg/L)	% of cultures responding			Average no. of shoots / Explant± (SE)*			Average length of shoots (cms) ± (SE)*		
	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf
					BAP				
0.5	50	53	55	08.0±0.08	16.5±0.06	20±0.16	1.4±0.08	1.8±0.08	2.0±0.06
1.0	54	62	62	11.4±0.09	22.0±0.21	24±0.61	1.6±0.14	2.2±0.17	2.4±0.10
1.5	66	71	65	13.2±0.43	24.5±0.42	26±0.40	1.8±0.36	2.9±0.33	2.7±0.31
2.0	71	82	70	16.4±0.13	31.6±0.16	32±0.12	2.2±0.12	3.2±0.14	2.9±0.19
2.5	88	92	79	22.0±0.11	45.2±0.18	36±0.17	2.6±0.17	3.7±0.16	3.0±0.15
3.0	72	79	91	19.0±0.17	30.4±0.15	38±0.16	2.3±0.14	3.1±0.14	3.2±0.17
3.5	63	64	74	15.5±0.16	26.1±0.13**	34±0.17	2.0±0.19	2.5±0.19	2.5 ±0.16
4.0	53	56	51	11.6±0.05	16.5±0.11**	26±0.29	1.6±0.05	1.9±0.02	2.3±0.08
					KIN				
0.5	40	44	50	06.0±0.15	13.8±0.12	16±0.18	1.1±0.03	1.4±0.06	1.7±0.01
1.0	42	52	55	09.3±0.14	15.6±0.19	22±0.14	1.3±0.36	1.6±0.32	1.9±0.33
1.5	43	59	60	10.7±0.03	17.2±0.06	26±0.09	1.5±0.01	2.0±0.07	2.0±0.04
2.0	56	71	72	13.6±0.18	25.6±0.17	28±0.77	1.8±0.18	2.4±0.14	2.2±0.16
2.5	62	80	81	14.5±0.15	30.0±0.12	32±0.60	2.0±0.14	2.8±0.19	2.6±0.18
3.0	73	75	75	16.5±0.16	24.0±1.40	24±1.80	2.4±0.18	2.4±0.14	2.4±0.13
3.5	64	62	66	13.1±0.09	16.6±0.20**	20±0.25	2.1±0.65	2.2±0.63	2.2 ±0.65
4.0	50	51	52	08.7±0.15	08.8±1.40**	17±0.25	1.5±0.26	1.9±0.20	1.9±0.25

*Mean ± Standard Error ** Callus + Shoots

Table 2: Effect of IAA + BAP on direct regeneration from hypocotyl, cotyledon and leaf explants of *S. torvum.*

Concn. of PGRs (mg/L)	% of cultures responding			Average no. of shoots / Explant± (SE)*			Average length of shoots (cms) ± (SE)*		
	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf
IAA+BAP									
0.5+0.5	42	55	60	09.2±0.09	22.6±0.15	28.0±0.15	1.6±0.18	2.0±0.13	2.4±0.16
0.5+1.0	46	59	65	12.0±0.02	25.3±0.46	34.8±0.47	1.8±0.15	2.4±0.19	2.8±0.15
0.5+1.5	51	70	76	19.3±0.40	36.7±0.91	40.1±0.96	2.0±0.33	3.1±0.36	3.2±0.37
0.5+2.0	64	84	82	26.1±0.16	44.2±0.17	45.8±0.15	2.8±0.14	3.6±0.17	3.5±0.19
0.5+2.5	92	92	94	31.0±0.17	55.0±0.15	52.0±0.13	3.2±0.16	4.2±0.19	4.0±0.12
0.5+3.0	78	86	86	19.4±0.16	42.6±0.13	41.5±0.17	2.6±0.13	3.3 0.14	3.3±0.14
0.5+3.5	53	76	75	15.2±0.15**	33.5±0.17**	33.4±0.16**	2.0±0.17	2.9±0.16	2.8±0.18
0.5+4.0	50	58	60	10.5±0.07**	26.2±0.06**	26.3±0.08**	1.8±0.04	2.5±0.08	2.5±0.06
1.0+0.5	49	48	61	07.6±0.13	13.6±0.14	18.5±0.12	1.3±0.06	1.6±0.06	1.7±0.04
1.0+1.0	51	51	62	09.3±0.19	14.0±0.12	20.0±0.17	1.4±0.35	1.8±0.37	1.9±0.30
1.0+1.5	59	55	74	11.6±0.04	16.0±0.09	22.4±0.06	1.5±0.01	2.0±0.03	2.1±0.06
1.0+2.0	71	61	82	14.2±0.16	17.6±0.17	25.9±0.19	1.9±0.19	2.2±0.19	2.2±0.12
1.0+2.5	83	74	90	16.7±0.18	25.3±0.16	28.6±0.14	2.2±0.18	2.6±0.14	2.5±0.17
1.0+3.0	90	86	96	24.6±0.15	30.5±0.15	30.0±0.18	2.8±0.14	2.9±0.23	2.8±0.29
1.0+3.5	80	71	89	17.3±0.14**	21.3± 0.40**	27.5±0.6**	2.1±0.12	2.4±0.36	2.7±0.36
1.0+4.0	69	67	81	11.0±0.20**	16.5±0.11**	24.6±0.84**	1.9±0.23	1.9±0.25	2.2±0.75

*Mean ± Standard Error ** Callus + Shoots

Table 3: Effect of IAA+KIN on direct regeneration from hypocotyl, cotyledon and leaf explants of *S. torvum.*

Concn. of PGRs (mg/L)	% of cultures responding			Average no. of shoots / Explant± (SE)*			Average length of shoots (cms) ± (SE)*		
	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf
IAA+KIN									
0.5+0.5	44	48	55	10.0±0.03	13.7±0.12	20.4±0.72	1.1±0.11	1.6±0.11	1.7±0.15
0.5+1.0	49	56	62	12.6±0.04	18.5±0.49	26.6±0.45	1.4±0.15	1.8±0.16	1.9±0.16
0.5+1.5	56	64	74	15.4±0.51	24.9±0.91	30.2±0.99	1.7±0.36	2.1±0.35	2.1±0.34
0.5+2.0	64	72	86	16.3±0.12	27.6±0.16	35.6±0.16	2.0±0.18	2.5±0.12	2.4±0.13
0.5+2.5	85	88	91	19.0±0.19	35.0±0.18	39.0±0.17	2.4±0.17	3.0±0.16	3.2±0.18
0.5+3.0	72	76	81	17.4±0.16	26.4±0.15	32.4±0.14**	2.1±0.14	2.8±0.17	2.8±0.16
0.5+3.5	54	61	69	15.3±0.13	22.8±0.13	29.0±0.12	1.6±0.12	2.2±0.19	2.5±0.14
0.5+4.0	52	54	60	11.0±0.07	20.0±0.06	22.3±0.06	1.3±0.04	1.9±0.08	1.9±0.06
1.0+0.5	42	48	50	08.3±0.14	11.0±0.14	19.4±0.17	1.2±0.09	1.8±0.06	1.4±0.09
1.0+1.5	54	64	66	12.5±0.03	17.3±0.07	24.4±0.09	1.3±0.33	1.9±0.34	1.6±0.37
1.0+1.0	50	53	52	10.0±0.19	13.6±0.19	21.0±0.15	1.6±0.04	2.3±0.03	1.7±0.03
1.0+2.0	73	71	72	15.5±0.18	21.6±0.16	30.7±0.17	1.9±0.16	2.7±0.19	2.0±0.12
1.0+2.5	86	79	84	18.4±0.14	24.5±0.18	36.3±0.18	2.1±0.15	3.2±0.17	2.2±0.15
1.0+3.0	90	86	93	22.3±0.16	22.2±0.16	40.2±0.16	2.5±0.24	2.9±0.24	2.8±0.27
1.0+3.5	82	72	79	19.4±0.11**	17.5±0.8**	33.0±0.84**	2.1±0.34	2.4±0.38	2.3±0.36
1.0+4.0	71	66	64	14.5±0.34**	12.6±0.17**	21.3±0.35**	1.8±0.22	2.0±0.26	1.9±0.24

*Mean ± Standard Error ** Callus + Shoots

Table 4: Effect of different types of auxins on *in vitro* rooting in *S. torvum.*

Concn. of PGRs (mg/L)	% of cultures with rooting	Average No. of shoots/Explant±(SE)*	Average length of shoots (cms)±(SE)*
½MSO	-	-	-
MSO	16	(4 -6) feeble	3.6±0.16
		NAA	
0.25	67	09.0±0.04	4.5±0.06
0.50	90	18.8±0.05	4.1±0.09
0.75	63	14.2±0.08	6.6±0.16
1.0	56	12.4±0.09**	5.8±0.18
1.5	45	12.0±0.19**	4.4±0.23
2.0	42	10.0±0.10**	2.6±0.14
		IAA	
0.25	67	08.2±0.22	7.0 ± 0.09
0.50	75	14.5±0.14	6.8 ± 0.08
0.75	87	16.8±0.12	8.3 ± 0.13
1.0	92	20.3±0.03	9.6 ± 0.09
1.5	56	11.2±0.15	6.2 ± 0.14
2.0	45	10.0±0.07	5.7 ± 0.02

*Mean ± Standard Error ** Callusing with roots

Data Analysis

A minimum of 20 replicates were maintained for each treatment. Each experiment was repeated at least twice and data were recorded after 4 weeks of culture.

RESULTS

The present investigations have been undertaken to find out the efficiency of the explant and also the PGRs concentration and combinations for direct regeneration in *S. torvum.* The explants such as cotyledon, hypocotyl and leaf were cultured on MS medium augmented with different concentrations of cytokinins BAP/KIN single and also in combination with IAA (Tables 1-4). These explants were enlarged 3-4 fold after 10 days of culture. The shoot primordia were directly initiated in many numbers after 2 weeks of culture from the cut ends of the explants viz., hypocotyl, cotyledon and leaf in contact with the medium in all the concentrations and combinations of PGRs used (Figure 1). Whereas the organogenesis was totally inhibited on MSO medium.

Regeneration from Hypocotyl Explants

Hypocotyl explants of *S.torvum* were cultured on MSO and MS medium supplemented with different concentrations of BAP/KIN alone and also in combination with 0.5/1.0 mg/L IAA (Tables 1-3). High percentage (92%) of responding cultures was found at 0.5 mg/L IAA+2.5 mg/L BAP in comparison to all other concentrations of BAP/KIN alone and IAA+KIN. More number of adventitious shoots formation was also observed in all the concentrations of BAP alone (Table 1) and combined with IAA. When 0.5 mg/L

Figure 1: Direct regeneration and plantlet establishment of *S. torvum.*

a-c) hypocotyl explants: a) Adventitious shoot buds formation on MS+ 2.5 mg/L BAP+0.5 mg/L IAA (After 10 days of culture); **b)** Multiple shoots induction on MS+ 2.0 mg/L BAP+0.5 mg/L IAA after 3 weeks (Note the proliferation of shoot buds); **c)** Multiple shoots development and elongation on MS + 0.5mg/L IAA + 2.5mg/L BAP after 4 weeks of culture; **d-f) Cotyledon explants: d)** Adventitious shoot buds formation on MS+ 2.5 mg/L BAP (After 10 days of culture); **e)** Multiple shoots induction on the same medium after 3 weeks (Note the proliferation of shoot buds); **f)** Multiple shoots development on MS + 0.5mg/L IAA + 2.5mg/L BAP after 4 weeks of culture; **g-i) Leaf explants: g)** Proliferation of shoot buds from the cut ends on MS+ 3.0 mg/L BAP (after 10 days); **h)** Shoot buds formation on MS+0.5 mg/L IAA + 2.5 mg/L BAP after 2 weeks of culture; **i)** Multiple shoots proliferation and elongation on MS + 0.5 mg/L IAA + 3.0 mg/L BAP after 4 weeks of culture; **j-m) *In vitro* rooting and plantlet establishment: j)** Profuse rhizogenesis on MS + 1.0 mg/L IAA after 3 weeks of culture; **k)** Showing profuse rhizogenesis with well developed roots on MS + 1.0 mg/L IAA after 4 weeks (Note the healthy roots); **l)** Acclimatized plants growing in a pot; **m)** Acclimatized plants transferred to field (Note the plants similar to donor plants).

IAA added to the medium the percentage of responding cultures was increased along with the enhanced number of shoots per explant (Table 2). Maximum frequency number of shoots (31±0.17) was induced at 0.5 mg/L IAA + 2.5 mg/L BAP followed by 1.0 mg/L IAA + 3.0 mg/L BAP (24±0.15) (Figure 1a-c). At 3.5/4.0 mg/L BAP in combination with 1.0 mg/L IAA, shoots were formed along with the callus. Hypocotyl explants were also cultured on MS medium supplemented with different concentrations of KIN (0.5-4.0 mg/L) in combination with 0.5-1.0 mg/L IAA (Table 3). As the concentration of KIN increased in combination with 0.5-1.0 mg/L IAA, the enhancement (less than IAA+BAP combination) in the percentage of cultures response and as well as shoots formation per explant was recorded. Maximum frequency number of shoots formation was found at 1.0 mg/L IAA+3.0 mg/L KIN. The induction of multiple shoot buds from the explant with callus found at 1.0 mg/L IAA + 3.5-4.0 mg/L KIN.

Regeneration from Cotyledon Explants

Cotyledon explants were cultured on MSO and MS medium supplemented with different concentrations of BAP/KIN and also in combination with 0.5-1.0 mg/L IAA (Tables 1-3, Figure 1d-f). Adventitious shoots were induced from cut ends of the explants in all the concentrations used. Highest (96%) percentage of response was noted at 0.5 mg/L IAA+2.5 mg/L BAP followed by 2.0 mg/L BAP (92%) (Tables 1-2). The percentage of response and also average number of shoots per explant was found to be more in all the concentrations of BAP than KIN (Figure 1d-f). Cotyledon explants were also cultured on MS medium fortified with 0.5/1.0 mg/L IAA+0.5-4.0 mg/L BAP/KIN (Tables 1-3). The morphogenetic response in shoots formation was enhanced in all the concentrations of BAP/KIN in combination with 0.5-1.0 mg/L IAA. But, the auxin-cytokinin combination IAA+BAP, induced more number of shoots than that of IAA+KIN (Table 3). Maximum number of shoots formation (55.0±0.15) was recorded at 0.5 mg/L IAA+2.5 mg/L BAP by showing its superiority (Figure 1d-f). Whereas at high concentrations (3.5/4.0 mg/L) of BAP/KIN induced the formation of both callus and shoots.

Regeneration from Leaf Explants

The results on multiple shoots formation from leaf explants of *S. torvum* on MS medium supplemented with different concentration of BAP/KIN singly and also in combination with 0.5-1.0 mg/L IAA are presented in Tables 1-3. Organogenesis was totally inhibited on MSO medium without PGRs. Maximum percentage of response was found at 0.5 mg/L IAA+2.5 mg/L BAP, and also showed maximum frequency number of shoots formation in comparison to KIN alone (Table 1, Figure 1g-i). At higher concentrations of BAP/KIN, less number of shoots was formed.

Leaf explants were also cultured on MS medium supplemented with different concentrations of BAP/KIN in combination with 0.5-1.0 mg/L IAA (Tables 1-3). Many adventitious shoots proliferation was observed at 0.5 mg/L IAA + 2.5 mg/L BAP. More number of shoots formations was observed when 0.5-1.0 mg/L IAA added to MS medium along with BAP/KIN. Percentage of response and average number of shoots formation per explant were found to be more in all the concentrations of BAP than KIN in combination with IAA .

Profuse rhizogenesis was also found from shoots developed on MS+2.5/3.0 mg/L BAP in combination with 0.5/1.0 mg/L IAA after 6 weeks of 1st subculture. Feeble rooting was also recorded from the shoots developed directly from leaf explants on MS+0.5 mg/L IAA+2.5 mg/L KIN.

In vitro Rooting

The shoots developed through hypocotyl, cotyledon and leaf cultures were transferred onto ½ strength MSO, MSO and MS medium augmented with different concentrations (0.25-2.0 mg/L) of auxins NAA/IAA (Table 4).

In vitro rooting was absent on half-strength MS medium without PGRs in *S. torvum.* Poor and moderate rooting was observed on MSO medium. The percentage of response was also very less (10%) with 4-6 feeble roots on MS basal medium.

Effect of NAA on Rooting

Roots formation was initiated after 10 days of culture. As the concentration of NAA increased, the percentage of response was also enhanced upto 0.50 mg/L NAA. Profuse rhizogenesis along with the formation of shoots was also observed at 0.50 mg/L NAA in comparison to rest of the concentrations used. Lengthy roots were also found at the same concentration of NAA. As the concentration of NAA increased beyond 0.75 mg/L, gradual enhancement in the callus induction was observed along with the induction of roots.

Effect of IAA on Rooting

Roots were induced after 08 days of culture and without any callus induction in all the concentrations of IAA used. Less percentage of response with low number of roots formation was observed at 0.25 mg/L IAA. At 1.0 mg/L IAA, maximum percentage of response and also highest frequency number of roots were developed (Figure 1j-k).

Plantlet Establishment

The *in vitro* rooted plantlets from different explants were hardened to plastic pots containing sterile vermiculate: soil (1:1) mix. The maximum percentage of plant survival rate was observed from hypocotyl culture followed by cotyledon and leaf culture (Figure 1).

After one month, polythene bags were removed and plantlets were shifted from walk-in-chamber to earthenware pots containing garden soil. These plantlets were maintained under shady conditions in the research

field. After one month, these plants were shifted into field (Figure 1m). These, acclimatized plants were healthy with normal morphological features, flowering and fruiting as that of donor plants .

DISCUSSION

In the present investigation the role of different PGRs on direct regeneration of various explants, cotyledon, hypocotyl and leaf was studied to findout the regeneration potentiality of the explant and also importance of cytokinins and auxin-cytokinin combinations in *S. torvum*. The explants such as cotyledon, hypocotyl and leaf cultured on MS medium fortified with different concentrations of BAP/ KIN alone and also in combination with 0.5/1.0 mg/L IAA showed the direct shoots regeneration. Whereas at 0.5/1.0 mg/L IAA+2.5 mg/L BAP/3.0 mg/L KIN induced the shoots along with rhizogenesis only in leaf explants.

Maximum number of shoots per explant was directly developed from the cut ends of the explants at 3.0 mg/L BAP/KIN as a sole growth regulator in all the explants studied (Figure 1). When 0.5/1.0 mg/L IAA added to the MS medium containing BAP/KIN showed the enhanced shoot buds proliferation in all the explants used (Tables 1-3). But, the induction of shoot buds proliferation was decreased when the concentration of IAA was increased to 1.0 mg/L in all the explants and concentrations of BAP/KIN tested. High frequency of shoots was induced on MS medium supplemented with 0.5 mg/L IAA+2.5-3.0 mg/L BAP. Maximum number of shoots was developed per explant in leaf explants followed by cotyledon and hypocotyl explants. Less number of shoot buds proliferation was observed in hypocotyl explants. Thus, from the foregoing, it is evident that BAP showed superiority over KIN in inducing direct organogenesis from all the explants used in *S. torvum* (see Figure 1).

Similarly, the combination of IAA+BAP was found to be more efficient in inducing maximum number of shoots from different types of explants in *Solanum melongena* (Sadanandam & Farooqui, 1991; Sharma & Rajam, 1995), *Physalis* spp (Bapat & Rao, 1977), *S. lycopersicum* (Praveen & Rama Swamy, 2011), *S. surattense* (Rama Swamy et al., 2004, 2005) *S. nigrum* (Sharada et al., 2019) and *S. torvum* (Ghan singh et al., 2021).

Highest number of shoots per explant was developed on MS medium containing 0.5 mg/L IAA+2.5 mg/L BAP in leaf explants of *Solanum sisymbrifolium* (Rao et al., 1997) compared to all other concentrations of BAP alone and also in combination with 1.0 mg/L IAA. When IAA concentration was increased to 1.0 mg/L, the shoot bud induction efficiency was reduced in *S. sisymbrifolium* similar to the present findings in *S. torvum*. Tejavathi & Bhuvana (1998) have observed the maximum shoot regeneration on IAA+BAP/2-ip and superiority of BAP over KIN in *S. viarum* similar to the present observations.

Leaf explants showed the efficiency in inducing high frequency of direct regeneration compared to cotyledon and hypocotyl explants in *S. torvum.* Whereas Ugandhar et al. (2006) had observed that the cotyledon explants were superior to hypocotyl and leaf explants in *S. surattense*.

From the foregoing discussion, it is evident that the different explants cultured on the same concentration of cytokinins and auxin-cytokinin combinations show varying results in the same species/either through callus mediated organogenesis/direct regeneration. Morphogenic response of explants depends on many factors including balancing of exo-and endogenous auxin-cytokinin levels (Rama Swamy et al., 2004). Effect of these growth substances on morphogenesis *in vitro* was well documented (Rao et al., 1989). Endogenous hormones are triggered in explants by exogenous supply which interacts with each other and leads to the establishment of some sort of balance to achieve regeneration (Vasil & Nitsch, 1975). Thus, the morphogenetic expression in a particular direction is manifested as a result of the cumulative effect of many factors viz., growth substances, nutrient medium, temperature, humidity, photoperiod etc. These are the important morphogenetic tools which can control the internal milieu of the cell (Rama Swamy, 2006).

In vitro rooting was successfully achieved in *S.torvum*. The shoots regenerated from all the explants were rooted on NAA/IAA. Profuse rhizogenesis was observed on MS medium augmented with 1.0 mg/L IAA in comparison to all other concentrations of auxins used in *S.torvum*. Maximum percentage of responding cultures was also observed at the same concentration of IAA. IAA showed superiority in inducing *in vitro* rooting followed by NAA. Whereas Komalavalli & Rao (2000) have observed the efficacy of IBA compared to IAA/NAA in medicinal plant *Gymnema sylvestre*. Recently, Rama Swamy et al. (2004) have reported the best *in vitro* rooting on ½ strength MS medium containing 1.0 mg/L IBA followed by 1.0 mg/L IAA in *S. surattense* a medicinal herb.

Hardening/acclimatization technique mainly depends on the physiological changes take place in a plant body to withstand the natural environmental conditions. Little is known about the physiological changes which occur during acclimatization. Studies related to hardening/ acclimatization were most often limited to observations on visual plant quality, rooting, histochemical changes and plant survival (Van Huylenbroeck & Debergh, 1996). During acclimatization an increase in enzymatic activity of both catalase and superoxide dismutase was observed in *Spathiphyllum* and *Calathea* (Van Huylenbroeck & Debergh, 1996). They suggested that during acclimatization the changes in humidity combined with light intensity can generate activated oxygen species (AOS). The physiological function of the observed changes can be considered as a protective mechanism against AOS.

During acclimatization process, three important factors are involved. They are: photosynthesis, carbon metabolism and activity of enzymes related to oxidative stress. Light intensity plays an important role in acclimatization. A sudden increase in light intensity during acclimatization leads to photo inhibition and severe stress (Van Huylenbroeck & De Riek, 1995).

CONCLUSION

Thus, it is clear that leaf explants were found to be more potential in producing high frequency number of shoots per explant among all other explants tested in the present investigations. Cytokinins BAP/KIN alone or in combination with IAA was found to be effective in inducing direct shoot regeneration in all the explants of *S. torvum*. However, 2.5 mg/L BAP/KIN with 0.5 mg/L IAA/NAA combination induced highest number of shoots per explant. Thus, the plants regenerated *in vitro* by direct organogenesis may exhibit greater genetic stability than those produced from callus. Based on our observations on *in vitro* direct regeneration reproducible protocol, the plant *S. torvum* can be used as model system (organism) as like as tobacco and cress plants. In the present investigation the regeneration protocol developed from hypocotyl, cotyledon and leaf explants can be used for mass-scale propagation of the species and also for genetic transformation studies to introduce agronomically important traits to enhance the secondary metabolites production and also to develop resistance against pests/insects.

Conflict of Interest

The authors declare that there is no conflict of interest.

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