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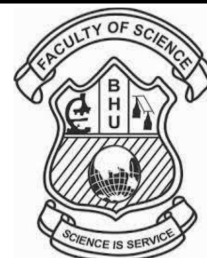

High Frequency Callus Mediated Plantlet Regeneration from Different Explants of Ethno-medicinal Plant Turkey Berry (*Solanum torvum* Sw)

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Abstract: We report on the influence of different plant growth regulators (PGRs) as well as the type of explants on callus mediated regeneration in Turkey berry/pea egg medicinal plant, *S.torvum*. The explants viz., 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 cultured on MS medium with various concentrations (0.5-4.0 mg/L) of BAP/KIN alone and also in combination with 0.5 mg/L NAA/IAA. The calli developed from all the explants have shown the maximum percentage of response (94% leaf and cotyledon, 88% hypocotyl) and highest number of multiple shoots induction (36.2±1.50 leaf, 33.2±0.25 cotyledon and 23.4±0.11 hypocotyl explants) per explant with maximum length of shoots (3.9±0.18 leaf, 4.0±0.11 cotyledon and 3.7±0.19 hypocotyl) at 0.5 mg/L NAA+2.5 mg/L BAP (leaf), 0.5 mg/L IAA+2.5 mg/L BAP (cotyledon) and 0.5 mg/L IAA+3.0 mg/L BAP (hypocotyl) in comparison to different concentrations of cytokinins used alone and as well as in combination with IAA/NAA. The calli induced from cotyledon explants were found to be more potent in inducing 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/NAA was found to be more effective in inducing callus mediated shoot regeneration in all the explants of *S. torvum*. 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 (90%), average number of roots (18.2±0.03) per micro-shoot with highest length of roots (7.8±0.09 cms) was observed at 1.0 mg/L IAA. *In vitro* rooted plantlets were acclimatized in the green house and transferred into field. The survival percentage was found to be 90% and the plantlets were found to be normal in morphology, flowering and fruiting. Thus, the regeneration protocol developed in the present investigation can be used for the conservation and genetic transformation experiments in *S. torvum*, not only as a medicinal plant but also as a model plant, based on its regeneration potentiality.

Index Terms: Plant Growth Regulators, Cotyledon, Leaf, Hypocotyl, *In Vitro* Rooting, Plantlet Establishment.

I. INTRODUCTION

Solanum torvum SW (Solanaceae), a wild relative of eggplant (*Solanum melongena*) is commonly known as **Turkey Berry/Pea egg plant.** Different parts of this plant are used in the treatment of various diseases. Fumes of the burning seeds are 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 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 the treatment of cough, cold, liver and spleen enlargement (Priyanaka et al, 2014).

In vitro plant regeneration plays an important role in conservation and multiplication of endangered, medicinal plants and also in genetic transformation and transgenic plants production. *In vitro* plant regeneration can be achieved either by direct or/and callus mediated regeneration. These pathways depend upon the type of the explant and also the concentration and combinations of auxin-cytokinin/

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cytokinin present alone in the nutrient medium. Regenerative efficiency is also dependent upon the genotype; age and physiological activity of the donor plant and explant type.

In vitro regeneration of *Solanum* sps has been achieved by many researchers using explants like leaf, stem, root, anthers, nodal and shoot tip (Rama Swamy et al, 2004). Recently regeneration protocols from different explants including somatic embryogenesis was reported in *S. torvum* (Ghansingh et al, 2021). However, there is no report on the induction of callus mediated regeneration in the species. Callus mediated regeneration is an alternative and efficient method for plant propagation over regeneration via organogenesis to produce large numbers of plants within a short period.

Nutrient medium supplemented with plant growth regulators (PGRs) viz., auxins or auxin-cytokinin combination and concentrations and also the culture conditions during the incubation period play a vital role in plant regeneration (Rama Swamy, 2006). The influence of these factors was also reported on *in vitro* regeneration in *Solanum lycopersicum* (Tan et al, 1987), *S.melongena* (Sharma & Rajam, 1995), *S.surattense* (Ugandhar et al, 2006), *S.nigrum* (Sharadaet al, 2019) and *S. torvum* (Ghansingh et al, 2021).

Callus mediated regeneration was reported in medicinal plants such as *Momordica dioica* (Hoque et al, 2000), *Solanum* species (Gleddie et al, 1983; Baburaj & Gunasekaran, 1994; Shahzad et al, 1999, Ugandhar et al, 2006, Sharada et al, 2019). Though the plant Turkey Berry is ethnomedicinally important, but there is no report on callus mediated regeneration. During the present investigations the callus mediated regeneration from various explants has been undertaken to standardize the protocol and also to know the influence of different types of PGRs and their concentration and combinations in the medium for morphogenetic event in *S.torvum.*

II. MATERIAL AND METHODS

A. Plant Material

For present investigations, the calli obtained on MS medium supplemented with 3.0-4.0 mg/L NAA from cotyledon and hypocotyl explants and 2.0 mg/L NAA from leaf explants were used after 4 weeks of culture. This callus was regularly sub cultured for 3 passages on the same fresh medium and PGRs. The friable callus induced after 3rd passage was used for the present investigations.

B. Culture Media and Culture Conditions

The fresh friable calli pieces approximately 0.5-1.0 cm^2 were transferred onto regeneration medium containing MS medium supplemented with different concentrations (0.5-4.0 mg/L) of BAP/KIN alone and also in combination with0.5 mg/L IAA/NAA (Tables 1-3). The pH of the media was adjusted to 5.7 with either 0.1N HCl or 0.1N NaOH, solidified with 0.8% (w/v) Difco-bacto agar and autoclaved at 121°C under 15 psi for 15-20 minutes. All the cultures were incubated at 25°C with 16h photoperiod under white-fluorescent light of 40-60 µ mol $m^{-2}s^{-1}$ intensity.

C. In Vitro Rooting and Plantlet Establishment

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

The *in vitro* rooted plantlets were 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 under 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.

D. Data Analysis

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

III. RESULTS

In the present investigation, the freshly isolated calli pieces were used after 3rd subculture, derived from different explants of *S.torvum.* Shoot organogenesis was found only from friable calli pieces in all the explants studied.

A. Regeneration from Cotyledon Derived Callus

Cotyledon derived callus was cultured on MS medium augmented with different concentrations (0.5-4.0 mg/L) of BAP/KIN and also in combination with 0.5 mg/L IAA (Tables 1-2).

Adventitious shoots were induced in all the concentrations of BAP/KIN used as a sole PGR. Maximum percentage of response (90%) and highest number of shoots formation (27.0±0.19) per explants were found at 2.5 mg/L BAP in comparison to KIN (Fig. 1a-b). As the concentration of PGRs increased there was a gradual enhancement in the shoot buds induction upto 2.5 mg/L BAP and 3.0 mg/L KIN. Beyond 2.5/3.0 mg/L concentration of BAP/KIN respectively, the

percentage of response and as well as shoots formation per explant was found to be decreased (Table 1).

To know the effect of auxin on enhancement of multiple shoots formation, IAA (0.5 mg/L) was added to MS medium along with different concentrations of cytokinin (BAP/KIN) (Table-2). Shoots formation per explant was enhanced in all the concentrations of BAP/KIN along with IAA. Maximum number of shoots per explant (35.2±0.25) was observed at 0.5 mg/L IAA+2.5 mg/L BAP (Fig. 1b) followed by 2.0 mg/L BAP. Highest percentage of responding cultures was also found at the same combination of PGRs.

B. Regeneration from Hypocotyl Derived Callus

Hypocotyl derived callus of *S. torvum* was cultured on MS medium fortified with various concentrations (0.5-4.0 mg/L) of BAP/KIN and also in combination with 0.5 mg/L IAA (Tables 1-2). Adventitious shoots were induced in all the concentrations of BAP/KIN alone and also along with IAA. The percentage of response was found to be lesser at low and high concentrations of BAP/KIN. Whereas more number of multiple shoots per explant (18.2±1.25) including highest percentage (85%) of responding cultures was also found at 2.5 mg/L BAP (Fig.1c) compared to KIN (Table 1). The percentage of response and as well as the developments of multiple shoots/explant were found to be more on BAP compared to KIN.

0.5 mg/L IAA was added to the MS medium fortified with BAP/KIN to know the influence on induction of multiple shoots in *S.torvum* (Table 2). Percentage of responding cultures and shoots number per explant were found to be enhanced in comparison to the calli cultured on BAP/KIN as a sole growth regulator. Maximum frequency of shoot buds induction (23.4±0.11) per explant was observed at 0.5 mg/L IAA+3.0 mg/L BAP (Fig. 1d) than that of all other concentrations of BAP/KIN used. Highest percentage (88%) and lengthy shoots were also noted at the same concentration of BAP. It is also interesting to note that adventitious shoots formation along with the rooting was observed at 3.0-4.0 mg/L BAP in combination with 0.5 mg/L IAA (Fig.1d).

C. Regeneration from Leaf Derived Callus

Leaf derived callus of *S.torvum* was cultured on MS medium augmented with 0.5-4.0 mg/L BAP/KIN and also in combination with 0.5 mg/L IAA/NAA (Tales 1-3). Adventitious shoot buds were induced from the leaf derived callus in all the concentrations and combinations of PGRs used. As the concentration increased up to 2.5/3.0 mg/L BAP/KIN respectively, the gradual enhancement in the percentage of response as well as average number of shoots per explant was recorded (Tables 1-3). At 2.5 mg/L BAP showed the maximum number of shoots (36.4±0.13) formation per explant than that of all the other concentrations of KIN and BAP present alone in the medium. At high concentration of BAP/KIN, the percentage of response as well as number of shoots per explant was found to be reduced.

More number of shoots per explant was recorded in all the concentrations of BAP/KIN when IAA (0.5 mg/L) was added to the medium in comparison to cytokinins used alone (Table 2). The highest frequency of shoots (38.0±0.55) per explant was observed at 0.5 mg/L IAA+2.5 mg/L BAP compared to all other concentrations of BAP/KIN used. Even shoot length was also increased at the same concentration of PGRs.

To find out the efficacy of auxin on multiple shoots induction, NAA (0.5 mg/L) was also added to the medium along with BAP/KIN (0.5-4.0 mg/L). The shoots number per explant was found to be enhanced from calli cultured in all the concentrations of BAP/KIN along with 0.5 mg/L NAA compared to IAA used in *S.torvum* (Table 3). Maximum frequency of shoots (43.0±1.50) per explant was found at 0.5 mg/L NAA+2.5 mg/L BAP followed by 2.0 mg/L BAP and 3.0 mg/L KIN. The highest percentage (94%) of responding cultures with enhanced shoot length was also recorded at the same combination of PGRs tested in *S.torvum* (Fig. 1e, f).

D. In Vitro Rooting and Plantlet Establishment

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 MSO medium.

Roots formation was initiated after 10 days of culture in NAA/IAA supplemented medium. *In vitro* rooting was found in all the concentrations of NAA/IAA (Table 4). 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. 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 (90%) of response and also highest frequency of roots (18.2±0.03) were developed per micro-shoot (Fig. 1g).The *in vitro* rooted plantlets were hardened and shifted to earthenware pots containing garden soil and maintained under shady conditions in the research field. After one month, these plantlets were transferred into field (Fig.1h-i). The *in vitro* developed plants were found healthy with normal

morphological features, flowering and fruiting as that of donor plant.

Fig. 1: Callus mediated regeneration and plantlet establishment in *S. torvum*
(a-b) Cotyledon explants: (a) Meristemoids formation after 2 weeks of culture on MS+2.5 mg/L BAP, (b) Maximum frequency of shoot buds proliferation on MS+0.5 mg/L IAA+2.5 mg/L BAP, (c-d) Hypocotyl explants: (c) Shoot buds initiation on MS+2.5 mg/L BAP (after two weeks of culture), (d) Multiple shoots induction on MS+0.5 mg/L IAA+3.0 mg/L BAP (Note the formation of roots), (e-f) Leaf explants: (e) Shoot buds induction on MS+2.5 mg/L BAP after 2 weeks of culture, (f) Multiple shoots induction on MS+0.5 mg/L NAA+2.5 mg/L BAP after 4 weeks of culture, (g-i) *In vitro* rooting and plantlet establishment: (g) Profuse rhizogenesis on MS+1.0 mg/L IAA after 3 weeks of incubation, (h) Acclimatized plants growing in a plastic pot, (i) Acclimatized plant in an earthenware pot growing in the research field.

Table 1: Effect of BAP/KIN on adventitious shoots induction from cotyledon, hypocotyl and leaf derived callus cultures of *S. torvum.*

Concn. of PGRs (mg/L)	% of cultures responding			Average no. of shoots/Explant ±(SE)*			Average length of shoots (cms) ±(SE)*		
	Cotyledon	Hypocotyl	Leaf	Cotyledon	Hypocotyl	Leaf	Cotyledon	Hypocotyl	Leaf
BAP									
0.5	32	41	41	07.0±0.04	07.6±0.06	09.3±0.05	1.2±0.06	1.5±0.04	1.6±0.07

1.0	49	52	57	10.8±0.05	08.8±0.09	11.0±0.06	1.6±0.09	1.7±0.07	1.9±0.17
1.5	60	60	64	12.4±0.08	10.4±0.11	15.4±0.80	2.1±0.16	1.9±0.10	2.5±0.35
2.0	81	74	72	18.5±0.09	13.5±0.12	26.7±0.12	2.8±0.18	2.3±0.14	3.0±0.18
2.5	90	85	81	27.0±0.19	18.2±1.25	36.4±0.13	3.4±0.23	3.0±0.32	3.2±0.12
3.0	79	79	76	16.3±0.10	16.4±0.84	30.7±0.11	2.6±0.14	2.1±0.13	3.1±0.13
3.5	54	56	51	09.5±0.08	10.5±0.14	18.5±0.12	1.4±0.08	1.8±0.11	2.4±0.10
4.0	35	43	44	06.0±0.05	08.5±0.09	12.3±0.06	1.0±0.06	1.2±0.07	1.9±0.01
KIN									
0.5	34	32	30	07.4±0.13	06.3±0.14	05.2±0.13	1.6±0.12	1.0±0.07	1.0±0.09
1.0	38	40	35	08.0±0.19	07.8±0.17	08.4±0.16	1.5±0.09	1.1±0.08	1.0±0.33
1.5	42	45	41	08.9±0.11	08.2±0.10	10.7±0.06	2.0±0.07	1.5±0.06	1.5±0.07
2.0	53	61	54	11.8±0.16	09.5±0.12	14.3±0.12	2.4±0.14	1.6±0.08	1.7±0.18
2.5	64	64	62	16.5±0.22	10.8±0.26	17.8±0.14	2.8±0.09	2.1±0.10	2.5±0.11
3.0	82	74	70	20.3±0.14	13.4±0.12	21.2±0.18	3.0±0.04	2.7±0.14	2.7±0.18
3.5	40	60	60	07.2±0.12	08.8±0.19	13.4±0.09	2.0±0.18	1.8±1.03	1.9±0.61
4.0	30	51	33	07.0±0.09	04.8±0.16	06.3±0.10	1.2±0.03	1.0±0.06	1.7±0.24

*Mean ± Standard Error

Table 2: Effect of IAA + BAP on adventitious shoots induction from cotyledon, hypocotyl and leaf derived callus cultures of *S. torvum.*

Concn. of PGRs (mg/L)	% of cultures responding			Average no. of shoots/Explant ±(SE)*			Average length of shoots (cms) ±(SE)*		
	Cotyledon	Hypocotyl	Leaf	Cotyledon	Hypocotyl	Leaf	Cotyledon	Hypocotyl	Leaf
IAA+BAP									
0.5+0.5	52	37	45	10.8±0.16	08.2±0.07	10.4±0.04	1.8±0.03	1.9±0.07	1.8±0.10
0.5+1.0	58	45	59	12.6±0.10	9.8±0.09	16.2±0.12	2.0±0.13	2.0±0.18	2.0±0.16
0.5+1.5	66	59	72	16.8±0.08	18.2±0.15	18.5±0.15	2.4±0.08	2.0±0.09	2.2±0.04
0.5+2.0	81	65	84	26.4±0.19	20.4±0.17	30.7±0.20	3.0±0.18	2.7±0.13	2.8±0.18
0.5+2.5	94	76	90	33.2±0.25	21.6±0.56	33.3±0.55	4.0±0.11	3.0±0.11	3.5±0.11
0.5+3.0	88	88	82	21.8±0.16	23.4±0.11**	22.5±1.02	2.6±0.13	3.7±0.19	3.9±0.16
0.5+3.5	75	75	72	15.6±0.12	14.4±0.14**	19.6±1.50	1.8±0.10	2.9±0.10	3.0±0.15
0.5+4.0	58	64	53	12.2±0.23	11.8±0.16**	15.3±0.15	1.6±0.07	2.0±0.16	2.0±0.12
IAA+KIN									
0.5+0.5	48	32	38	09.6±0.06	05.6±0.03	07.4±0.36	1.3±0.15	1.6±0.12	1.3±0.17
0.5+1.0	54	42	46	10.0±0.12	07.0±0.21	09.5±0.71	1.4±0.10	1.6±0.33	1.4±0.35
0.5+1.5	62	50	51	12.3±0.09	08.5±0.32	13.7±0.14	1.6±0.07	1.9±0.10	1.7±0.11
0.5+2.0	71	58	57	16.8±0.08	10.0±0.64	19.4±0.32	2.2±0.11	1.8±0.18	1.9±0.19
0.5+2.5	78	64	64	20.5±0.16	10.7±0.12	22.6±0.02	2.8±0.16	2.0±0.11	2.2±0.14
0.5+3.0	86	71	73	24.6±0.19	12.3± 0.14	28.5±0.53	3.1±0.26	2.3±0.29	2.6±0.28
0.5+3.5	70	80	62	16.4±0.11	16.8±2.03	16.2±0.32	2.6±0.16	2.4±0.22	2.5±0.26
0.5+4.0	56	67	53	11.2±0.13	13.2±0.88	13.5±0.08	1.4±0.09	1.8±0.13	2.0±0.15

*Mean ± Standard Error; ** With Roots

Table 3: Effect of NAA+BAP/KIN on adventitious shoots induction from leaf derived callus cultures of *S. torvum.*

Concn. of PGRs (mg/L)	% of cultures responding	Average no. of shoots/Explant±(SE)*	Average length of shoots (cms)±(SE)*
NAA+BAP			
0.5+0.5	49	15.0±0.06	2.0±0.02
0.5+1.0	61	16.5±0.08	2.5±0.16

0.5+1.5	76	25.4±0.13	3.0±0.03
0.5+2.0	83	30.5±0.18	3.8±0.14
0.5+2.5	94	36.2±1.50	3.9±0.18
0.5+3.0	81	23.4±0.13	3.6±0.11
0.5+3.5	75	18.0±0.14	2.9±0.16
0.5+4.0	63	16.5±0.19	2.4±0.15
NAA+KIN			
1.0+0.5	43	11.0±0.04	1.6±0.16
1.0+1.5	51	12.5±0.23	1.7±0.35
1.0+1.0	60	16.7±0.39	1.7±0.13
1.0+2.0	62	23.2±0.64	1.9±0.17
1.0+2.5	76	29.3±0.18	2.2±0.12
1.0+3.0	82	34.5±0.15	2.6±0.29
1.0+3.5	72	26.3±0.62	2.4±0.26
1.0+4.0	66	16.8±0.88	2.0±0.15

*Mean ± Standard Error

Table 4: Effect of NAA/IAA on *in vitro* rooting of micro-shoots developed from different explants of *S. torvum.*

Concn. of PGRs (mg/L)	% of cultures with rooting	Average No. of roots/Explant±(SE)*	Average length of roots(cms)±(SE)*
NAA			
0.25	63	08.0±0.12	3.5±0.06
0.50	80	09.8±0.15	3.8±0.09
0.75	61	11.3±0.08	5.2±0.16
1.0	59	12.9±0.11**	6.3±0.18
1.5	42	11.7±0.10**	4.9±0.23
2.0	40	09.0±0.10**	2.6±0.14
IAA			
0.25	63	10.2±0.13	6.0 ± 0.09
0.50	72	11.5±0.10	5.8 ± 0.18
0.75	81	15.7±0.16	6.5 ± 0.23
1.0	90	18.2±0.03	7.8 ± 0.09
1.5	65	12.2±0.19	5.9 ± 0.14
2.0	49	11.3±0.17	4.7 ± 0.02

*Mean ± Standard Error; **with callus

IV. DISCUSSION

Callus mediated regeneration was successfully established in medicinally important species *S.torvum*. Callus from different explants viz., cotyledon, hypocotyl and leaf was cultured on MS medium fortified with different concentrations of cytokinins BAP/KIN individually and also in combination with 0.5 mg/L IAA/NAA (leaf derived callus). Adventitious multiple shoots were induced in all the concentrations and combinations of PGRs used. Maximum number of shoots per explant was induced at 2.5 mg/L BAP in comparison KIN used as a sole growth regulator. Similarly, Oceania et al (2015) and Papry et al (2016) have obtained the maximum shoot multiplication through callus cultures on MS medium augmented with 3.0 mg/L and 2.0 mg/L BAP respectively in *Solanum lycopersicum.* While the addition of low level of auxin (0.5 mg/L IAA) to cytokinin (BAP/KIN) showed the enhancement in the development of shoots per explant from all the concentrations of cytokinins tested. However the shoot buds proliferation was found to be maximum at 0.5 mg/L IAA+2.5 mg/L BAP among all other PGRs combinations and concentrations used except in hypocotyl derived callus cultures. Whereas 0.5 mg/L NAA+2.5 mg/L BAP combination

showed superiority in inducing maximum frequency of shoots per explant in leaf cultures.

Hoque et al (2000) have also reported the high frequency of plant regeneration on MS medium containing 2.5 mg/L BAP in combination with 0.5 mg/L NAA from cotyledon derived callus in *Momordica dioica.* They have also found the maximum number of shoots per explant on BAP compared to KIN and induction was higher on NAA+BAP than KIN as observed in *S. torvum*. Similar observations were also made in medicinal plants like *Theobrama cacao* (King & Rao, 1981), *Piper longum* (Bhatt et al, 1992), *Adhatda vasica* (Azad & Amin, 1998) and *Passiflora caerulea* (Jas Rai et al, 1999) as it was observed in the present investigations. Whereas De Langhe& De Bruijne (1976) and Rama Swamy et al (2001) have noted the efficacy of IAA+BAP combination than NAA+BAP for inducing maximum number of shoots from leaf derived callus cultures of *Solanum lycopersicum* and *S.surattense* respectively. Shahzadet al (1999) have reported the efficacy of NAA+BAP in plant regeneration from leaf derived callus cultures of *S.nigrum* in conformity with the present results.

Kumlay & Ercisli (2015) have also recorded the maximum number of shoots per explant on MS medium containing BAP+GA_3 in *S. tuberosum*. Sreenu et al (2019) have also observed the highest mean number of shoots differentiated *de novo* on MS medium augmented with TDZ+NAA in *Solanum trilobatum*. *In vitro* micropropagation through callus cultures is an important technique to multiply and propagate a species in large numbers. But, the plantlets regenerated through callus cultures may show genetic variability and deviate from the normal diploids, which is reflected in various kinds of morphological abnormalities. But, in the present investigations, all the plants regenerated through callus cultures of *S.torvum* have shown normal flowering and fruiting without any variable characters.

CONCLUSION

Based on our observations, the leaf derived callus was found to be more potential in producing high frequency of shoots per explant among all other explants tested in the present investigations. Cytokinins BAP in combination with IAA/ NAA was effective in inducing enhancement of shoot regeneration in all the explants of *S.torvum* studied. However, 2.5/3.0 mg/L BAP with 0.5 mg/L IAA/NAA combination induced highest number of shoots per explant. Hence, the present regeneration protocols developed through hypocotyl, cotyledon and leaf derived callus cultures can be used for mass-scale propagation of the species Turky berry and also for genetic transformation studies to introduce agronomically important traits to enhance the secondary metabolites production as a model system.

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