



DIRECT HIGH FREQUENCY PLANTLET REGENERATION FROM LEAF EXPLANTS OF *SOLANUM TORVUM* (SWARTZ). A MEDICINALLY IMPORTANT PLANT

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Tissue culture techniques of medicinal plants are important for the production of plant-based high quality medicine. *Solanum torvum* (Swartz) possesses immense medicinal importance of decoction of fruits is given for cough ailments and is considered useful in cases of liver and spleen enlargement. The plant is sedative and diuretic and the leaves are used as a haemostatic. The ripened fruits are used in the preparation of tonic and haemopoietic agents and also for the treatment for pain. It has antioxidant properties. It is intensively used worldwide in the traditional medicine as poison anti-dote and for the treatment of fever, wounds, tooth decay, reproductive problems and *in vitro* culture for commercial production was achieved by culturing the plant using different concentration and combination of plant growth regulators. Mainly, the concentration and combination of auxins and cytokinins in the nutrient MS medium is the key factor which determines successful plant regeneration. Direct high frequency plantlet regeneration has been obtained from 15-20days old seedling leaf explants of *Solanum torvum* (Swartz) using various phytohormones individually and in combination on Murashige and Skoog (MS) semi solid medium supplemented with BAP (1.0-5.0 mg/L), Kn (1.0-5.0 mg/L), IAA (0.5 mg/L)+ BAP (1.0-5.0 mg/L) and IAA (0.5 mg/L)+Kn (1.0-5.0 mg/L) for shoot proliferation. IAA (0.5 mg/L)+BAP (3.0 mg/L) proved to be best for induction of shoots for leaf explants. Individual shoots were aseptically excised and sub cultured in the same media for shoot elongation. The elongated shoots were transferred to Indole Butyric Acid (IBA) (1.0mg/L–5.0mg/L) for root induction. Rooting was observed within two weeks of culture. The rooted plantlets were successfully hardened under culture conditions and subsequently established in field conditions. The recorded survival rate of the plants was 86% and the plants were healthy with no visually detectable phenotypic variations.

Keywords: *Solanum torvum*, direct plant let regeneration, Leaf explants, High frequency shoot formation and *in vitro* Rooting

Abbreviations: BAP, 6-Benzyl Amino Purine;; Kin, Kinetin IAA, Indole Acetic Acid MS, Murashige and Skoog

The family *Solanaceae* is composed of approximately 90 genera and between 2,000 and 3,000 species. The family is widely distributed throughout tropical and temperate regions of the world, with center of diversity occurring in Central and South America and Australia. Within this family, *Solanum* is the biggest genus in the family, with the widest range of geographical distribution. A total of 1700 species of *Solanum* have been recorded all over the globe. 41 species represented in India, 19 are native, 8 naturalized, 10 cultivated and 4 cultivated experimentally, (Deb, 1979). *Solanum torvum*, commonly known as turkey berry, devil's-fig, or prickly *Solanum* (Pier 2003) is a plant of great economic importance. Its extract, which is rich in Solanine and Solasodine (steroidal alkaloids) has beneficial effect on bronchial asthma. It is cultivated in the tropics for its immature edible fruits (Langland and Burks 1998). Many species of

the *Solanaceae* have been regenerated by shoot organogenesis using young leaf explants eg. *Solanum surattense* (Gupta and Handra, 1982), *Solanum candidum*, *S.quitoense*, *Solanum sessiliflorum* (Hendrix *et al.* 1987), *Solanum melongena* (Mukherjee *et al.* 1991) and *Solanum commersonii* (Cardi *et al.* 1993). Arulmozhi and Ramanujam (1997) conducted *in vitro* culture studies on *Solanum trilobatum* L. with foliar and stem explants on MS medium containing IAA, BAP and KIN combinations. Madhavan *et al.* (1998) induced high frequency of shoot regeneration from mature seeds of *Solanum trilobatum* L. Callus was induced from root and shoot apical region and hypocotyls on MS medium supplemented with 2,4-D.

The plant tissue culture methods also provide a base for the improvement of crop e.g. it is to induce somaclonal variations, *in vitro* mutations, herbicide tolerance, di-haploid

induction, genetic transformation of economically important genes and development of somatic hybrids for which efficient plant regeneration protocol is required. Such advanced techniques in combination with conventional breeding give a momentum to the improvement of a crop. Thus, realizing the prospects for future research “High frequency Plant regeneration in (*Solanum torvum* L.)” has been studied.

METHODOLOGY:

Plant material: Seeds of *S.torvum* were collected from Botanical Garden, Department, of Botany, Govt Degree College, Mahabubabad, Telangana State India. Dried mature seeds were soaked in Dilute Sulphuric acid (H_2SO_4) for 24 hrs and sterilized with 0.1% (w/v) aqueous Mercuric chloride ($HgCl_2$) for 3-5 minutes followed by washing 3 times with sterile distilled water. Later these were dried on sterile tissue paper under container air flow. 20 seeds per culture bottle were germinated aseptically on MS basal medium containing 3% (w/v) sucrose and 0.8% (w/v) agar. These culture bottles were incubated at $25 \pm 1^\circ C$ under 16 h photoperiod. Light was provided by cool white fluorescent tubes with an intensity of $50-60 \mu mol m^{-2} s^{-1}$.

Data analysis: 20 replicates were maintained for each treatment. Each treatment was repeated at least once with similar results. Data were recorded after 8 weeks of culture.

OBSERVATIONS AND RESULTS:

The role of cytokinin and Auxin- cytokinin combinations on direct plant regeneration and adventitious bud induction from leaf explants was studied in order to find out the efficient protocol and its potential on MS medium fortified with different concentrations of cytokinins alone and auxin's (0.5mg/L) in combination with various concentrations of cytokinins such as BAP/Kn/TDZ (1.0-5.0 mg/L). These explants enlarged 3-4-fold within one week of culture initiation. Morphogenic

changes were apparent after 6 weeks of culture. The leaf explants developed shoot primordia in large numbers directly from all cut surfaces in contact with the medium in all the concentrations and combinations. The results are presented in Table (1-2).

Effect of phytohormones on leaf explants:

Effect of BAP: Leaf explants were cultured on MS medium fortified with various concentrations of BAP (1.0-5.0 mg/L) as role of growth regulators and direct organogenesis was observed (Table -1) Maximum number of shoot bud proliferation (26.5 ± 0.32 shoots /explant) was found at (3.0 mg/L) BAP concentration. At 1.0, and 2.0 mg/L BAP concentration, 55 and 65% of cultures responded and 12.6 ± 0.42 and 18.3 ± 0.47 shoots/explant observed respectively. When the concentration was increased up to (3.0 mg/L) BAP, reduction of multiple shoots was observed. When 4.0 and 5.0 mg/L BAP was supplemented, 75 and 58 percentage of cultures responded and induced 20.3 ± 0.13 and 10.0 ± 0.12 shoots/explants The number of shoot bud induction was found to decrease as the concentration of BAP increased. At high concentration of BAP lesser number of shoots per explants were recorded.

Effect of Kn: Leaf explants were cultured on MS medium containing different concentrations of cytokinin Kn (1.0- 5.0 mg/L) as sole growth regulators and showed direct organogenesis/ shoot formation (Table-1). Experiments were also set to find out the difference between BAP and Kn in inducing the direct plant regeneration from cotyledonary explants in *S. torvum*. Maximum number of shoot buds (20.0 ± 0.12) were induced at 3.0mg/L Kn in comparison to BAP as a sole growth regulators. At above (3.0 mg/L) concentration, less number of shoots were recorded. At 4.0 and 5.0 mg/L concentration, Kn induced (18.0 ± 0.14 and 9.2 ± 0.13) shoots/explants with 70 and 53 percentage of cultures responding on MS + Kn. However, high induction ability was found in all the

Table 1: Effect of BAP, Kn and TDZ on Direct high frequency shoot bud Proliferation of *Solanum torvum* (Swartz) from Leaf explants.

Hormone concentration (mg/L)	% of cultures responding	Average No. of shoots / Explants \pm (SE)*	Average length of shoots (cms) \pm (SE)*
BAP			
1.0	55	12.6 \pm 0.42	1.4 \pm 0.52
2.0	65	18.3 \pm 0.47	2.0 \pm 0.16
3.0	82	26.5 \pm 0.32	3.4 \pm 0.14
4.0	75	20.3 \pm 0.13	3.0 \pm 0.12
5.0	58	10.0 \pm 0.125	1.2 \pm 0.05
Kn			
1.0	48	10.2 \pm 0.12	1.3 \pm 0.05
2.0	56	13.8 \pm 0.19	2.3 \pm 0.32
3.0	72	20.0 \pm 0.12	2.8 \pm 0.43
4.0	70	18.0 \pm 0.14	2.3 \pm 0.14
5.0	53	09.2 \pm 0.13	1.8 \pm 0.23
TDZ			
1.0	56	13.2 \pm 0.15	1.2 \pm 0.32
2.0	66	19.8 \pm 0.12	2.2 \pm 0.23
3.0	84	28.0 \pm 0.35	2.4 \pm 0.32
4.0	78	25.8 \pm 0.34	2.2 \pm 0.13
5.0	56	16.5 \pm 0.35	1.4 \pm 0.24

* Mean \pm Standard Error

concentrations of Kn as compared to BAP.

Effect of TDZ: TDZ was more responsive compared to BAP and Kn in inducing shoot buds from the explants (Table-1). TDZ (1.0 mg/L) supplementation, produced (13.2 \pm 0.15 shoots/ explants and 56% cultures responded). Highest percentage of response was observed at 3.0mg/L TDZ. The percentage of response was increased up to 3.0 mg/L TDZ and later gradually decreased at high concentration of TDZ (Table 1)

Effect of IAA + BAP: Auxin- Cytokinin combination such as IAA(0.5mg/L) + BAP (1.0-5.0mg/L) showed variable response (Table-2) Highest percentage of response was observed at (0.5 mg/L) IAA + (3.0 mg/L) BAP. The percentage of response and number of shoots proliferation was increased up to (1.0 mg/L) BAP and later gradually decreased at above (3.0 mg/L) BAP. At (1.0, 2.0 and 3.0 mg/L) BAP with IAA (0.5mg/L) induced (18.4 \pm 0.12, 21.3 \pm 0.23 and 28.2 \pm 0.17) shoots/explants which 55, 62 and 86 percentage of cultures. As the concentrations of BAP was gradually increased above (3.0 mg/L) there was decrease in the number of

shoots. (4.0 and 5.0 mg/L) BAP+ IAA (0.5 mg/L) induced (26.3 \pm 0.12 and 16.0 \pm 0.23) shoots/explants. This response is with 76 and 58 percentage. (Table -2), (Fig-I C). However more number of shoot buds/explants was induced at (0.5mg/L IAA with 3.0mg/L BAP. But comparatively lesser than of 0.5mg/L IAA + 3.0mg/L BAP combination.

Effect of IAA +Kn: Leaf explants cultured on MS medium supplemented with (0.5 mg/L) IAA and different concentrations of Kn (1.0 - 5.0 mg/L) exhibited variable response (Table - 2). Maximum shoot bud induction was recorded at (3.0 mg/L) (25.0 \pm 0.32 shoots/ explants) (Fig-1 B) with 80 % response.

Effect of IAA +TDZ: Leaf explants were cultured on MS medium amended with (0.5mg/L) IAA and various concentration of TDZ (1.0- 5mg/L). They showed variable results. Highest percentage of response was observed at (0.5mg/L) IAA+(3.0mg/L) TDZ. The percentage of response was increased up to 3.0mg/LTDZ and later gradually decreased at high concentration.

In vitro rooting: Fully elongated healthy shoots were transferred on to full strength MS

Table 2: Effect of IAA in combination with BAP, Kn and TDZ on Direct high frequency shoot bud proliferation of *Solanum torvum* (Swartz) from Leaf explants.

Hormone concentration (mg/L)	% of frequency of plantlet production	Average No. of shoots / Explants \pm (SE)*	Average length of shoots (cms) \pm (SE)*
IAA + BAP			
0.5 + 1.0	55	18.4 \pm 0.12	2.4 \pm 0.12
0.5 + 2.0	62	21.3 \pm 0.23	2.6 \pm 0.12
0.5 + 3.0	86	28.2 \pm 0.17	3.4 \pm 0.17
0.5 + 4.0	76	26.3 \pm 0.12	3.2 \pm 0.12
0.5 + 5.0	58	16.0 \pm 0.23	2.3 \pm 0.25
IAA + Kn			
0.5 + 1.0	52	14.2 \pm 0.12	1.6 \pm 0.35
0.5 + 2.0	60	20.4 \pm 0.34	2.4 \pm 0.13
0.5 + 3.0	80	25.0 \pm 0.32	3.2 \pm 0.34
0.5 + 4.0	72	23.8 \pm 0.32	3.0 \pm 0.24
0.5 + 5.0	56	14.2 \pm 0.45	2.0 \pm 0.34
IAA + TDZ			
0.5 + 1.0	56	16.5 \pm 0.35	1.2 \pm 0.32
0.5 + 2.0	64	23.0 \pm 0.54	2.0 \pm 0.32
0.5 + 3.0	87	29.5 \pm 0.35	2.6 \pm 0.42
0.5 + 4.0	78	24.0 \pm 0.62	2.8 \pm 0.42
0.5 + 5.0	59	16.5 \pm 0.34	2.0 \pm 0.46

* Mean \pm Standard Error

root induction medium (RIM) (Murashige and Skoog 1962) fortified with different concentration of IAA (0.5 – 2.0 mg/L) and IBA (0.5 – 2.0 mg/L).

Profuse rhizogenesis was observed on 1.5 mg/L IAA, compared to 0.5 -2.0 mg/L) IAA/IBA.

Acclimatization: Rooted plantlets were removed from the culture medium and the roots were washed under running tap water to remove agar. Then the plantlets were transferred to polypots containing pre-soaked vermiculite and maintained inside a growth chamber set at 28 °C and 70 – 80 % relative humidity. After three weeks they were transplanted to poly bags containing mixture of soil + sand + manure in 1: 1: 1 ratio and kept under shade house for a period of three weeks. The potted plantlets were irrigated with Hogland's solution every 3 days for a period of 3 weeks.

DISCUSSION:

We were successful in directly regenerating plants from leaf explants of *S. torvum* cultures on MS medium fortified with different concentrations of cytokinins i.e. BAP/Kn/ TDZ

(1.0 -5.0 mg/L) individually and also in combination with (0.5 mg/L) IAA. Maximum number of shoot buds was induced at (3.0 mg/L) TDZ in comparison to Kn/BAP as sole role growth regulators. When low level of auxin (0.5 mg/L) IAA were added to the medium containing BAP/Kn/TDZ, it was interesting to find out that the induction was enhanced in all the concentrations of cytokinins tested. However the shoot bud proliferation was found to be more on 0.5mg/LIAA in combination with Kn/BAP/TDZ compared to 0.5mg/L IAA. Probably IAA might have triggered the action of BAP. Kn/TDZ in a proper way for inducing more number of shoot buds per explants. But the combination of IAA+ TDZ induced highest number of plantlet regeneration among all hormonal combinations and concentrations used.

Similarly, Hoque *et al.* (2000) have reported the high frequency of plant regeneration on MS medium containing 2.0mg/L BAP in combination with 0.5 mg/L IAA from cotyledon-derived callus in *Momordica dioica*. Of the cytokinins used Kn proved as most effective than BAP in inducing shoots,

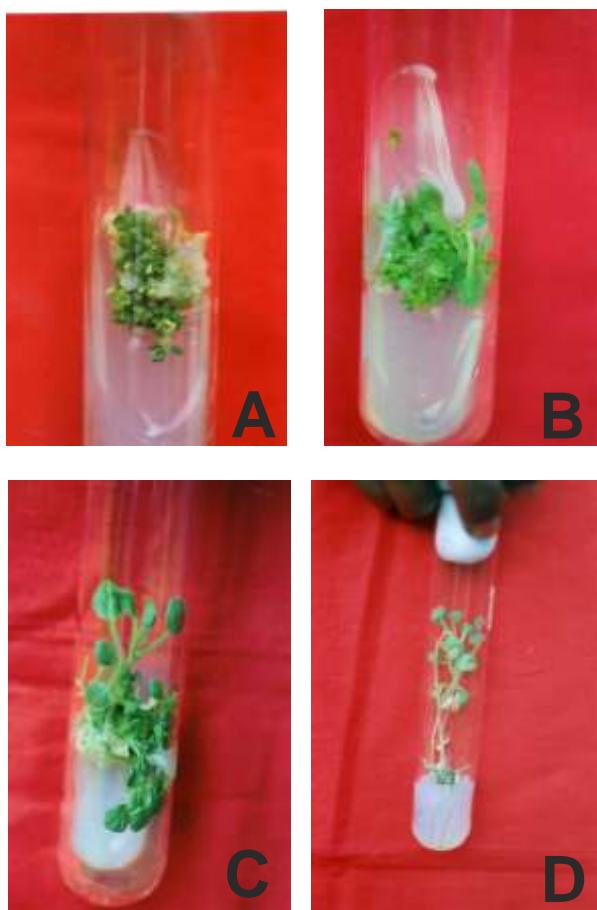


Figure 1 (A-D): Direct High Frequency *In Vitro* Propagation of *Solanum torvum* from Leaf Explants Culture A) on MS+0.1mg/L IAA+3.0 mg/L TDZ B) Induction of Multiple shoots on MS+0.5mg/L IAA+3.0mg/L Kn C) Formation of shoots on MS+0.5 mg/L IAA +3.0mg/L BAP D) *In Vitro* rooting from Micro shoots on MS+ 3.0 mg/L IBA after six week

the same findings were recorded in *Capsicum* Spp.(Venkataiah and Subhash 2002).

The leaf explants cultured on MS medium supplemented with IAA+BAP combination was found to be more effective in inducing maximum number of shoots in *Capsicum* Spp (Gunay and Rao,1978). Similar results have been reported in *Capsicum annuum* (Christopher and Rajam 1996) and *Solanum melongena* cv Pusa round (Sharma and Rajam1995). The combination of IAA+BAP showed superiority over IAA+Kn in all the explants tested. Similar finding were also reported in *Petunia* (Rao *et al.*1973), *Lycopersicon esculentum* (Kantha *et al.*1976) and *Solanum incanum* (Sadanandam and

Farooqui,1997) and in two species of Niger (Jadimath *et al.* 1998).

Shakazad *et al.* (1999) have also observed the highest frequency of direct shoot regeneration on lower levels of Auxin and high level of Cytokinin (4.0 mg/L) BAP+(0.5mg/L) IAA) in leaf explants of *S. nigrum*. Similar results were obtained in *Dalbergia canceolats* (Dwari and Chand 1996). However, at lower levels of BAP, the frequency of shoot regeneration was decreased similar to our present observations. Highest numbers of shoots per explant were developed on MS Medium containing (0.5mg/L) IAA+ (2.5mg/L) BAP in leaf explants of *Solanum sisymbriifolium* (Rao *et al.* 1998,) compared to all other concentrations of BAP alone and also in combination with (0.5mg/L) IAA.

Direct shoot regeneration occurred on MS medium containing BAP/Kn and in combination with IAA in hypocotyl, stem and leaf cultures of *Solanum viarum*. We have also observed the maximum shoot regeneration on IAA+BAP and superiority of BAP over Kn. Similar to the present observation. Jas Rai *et al.* (1999) reported the induction of large number of shoot buds from leaf disc explants of *Passiflora caerulea* when cultured on MS medium fortified with BAP+IAA. A Desai and Mehta (1990) have also reported direct shoot regeneration from seedling explants of *Passiflora edulis* on MS medium supplemented with BAP+ Coconut water. Similarly, maximum numbers of shoots were obtained when leaf explants of moth bean and pigeon pea were cultured on MS medium containing BAP+IAA (Kunjumon *et al.* 1996).

Neeta *et al.* (2001) have reported the direct plantlet regeneration from different explants i.e. hypocotyl, epicotyls, cotyledon and leaves cultured on IAA+BAP combination, but they found the highest average number of shoot buds per leaf explant in *Azadirachta indica*. Similarly, it was also reported in *Capsicum annuum* (Christopher and Rajam 1996) and 7 Genotypes of *Capsicum annuum* (Venkataiah and Subhash 2001). As with our results on

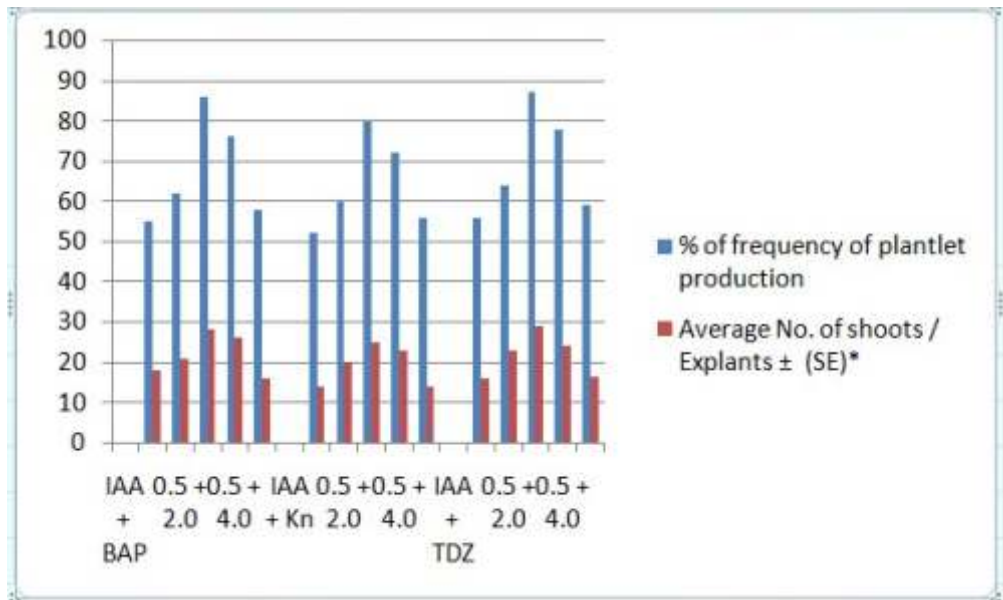


Figure 2: Effect of IAA in combination with BAP, Kn and TDZ on Direct high frequency shoot bud proliferation from leaf explants of *Solanum torvum* (Swartz).

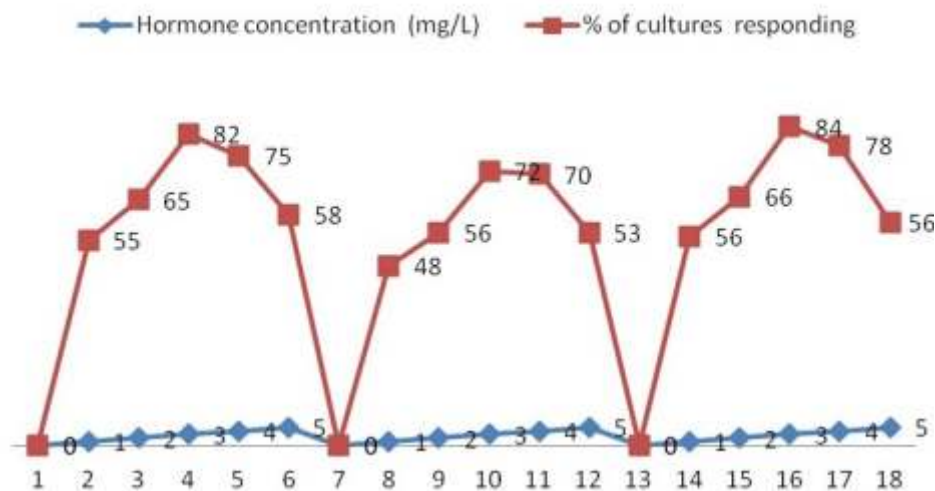


Figure 3:Effect of IAA (0.5mg/L) in combination with BAP, Kn and TDZ (1.0-5.0 mg/L) on % of Culture responding

S.torvum, large numbers of adventitious shoot buds were induced by TDZ, but not by other cytokinins (Blakesey 1990). Variation in the activity of different cytokinins can be explained by their differential uptake rate reported in different genomes.

TDZ, when used in combination with auxins, drastically reduced the percentage of response. Moreover, there was no significant increase in the number of shoots regeneration per explants. In contrast, Yildirim and Turker (Blakesey

1990) observed a significant increase in the percentage of explants forming shoot and the mean shoot number per explants when TDZ was used in combination with IAA.

A similar pattern of shoot bud development from leaf explants was reported by (Banerjee *et al.* 2004). Although it was difficult to count the number of shoot buds, it was clearly visible that the number of shoot buds increases with every increase in TDZ concentration. In lower concentrations of TDZ the shoots elongated

with some expanded leaves, whereas in the higher concentration the shoot buds were highly vitrified and fail to elongate. The efficacy of TDZ for induction of direct shoot organogenesis is well documented in several woody plants (Graham and Millam 1997, Leblay *et al.* 1991, Preece and Imel 1991)) Moreover some researchers (Hsia and Korban 1997) have reported TDZ to be an essential growth regulator for shoot induction from leaf explant of Ericaceae family. It was emphasized that the efficiency of TDZ may be due to its ability to induce cytokinin accumulation (Victor *et al.* 1999) or enhance the accumulation and translocation of auxin within the tissue (Murthy *et al.* 1998). TDZ is also useful in suspected promoting regulated morphogenesis in plants through the modulation of endogenous cytokinin and auxin (Gill and Saxena 1992). Heutteman and Preece (1993) emphasized the potential use of TDZ in the regulation of adventitious shoot production and hypothesize on the synergism existing between TDZ and both endogenous and exogenous auxin.

From the foregoing discussion, it is evident that cotyledon and Leaf explants were found to be more potential in producing high frequency number of shoots among all other explants tested in the present investigation Cytokinins BAP / Kn/TDZ alone or in combination with IAA was effective in inducing shoot regeneration in all the explants of *S.torvum*. However, (3.0 mg/L) Kn / BAP with (0.5 mg/L) IAA combination induced highest number of shoots in Cotyledon and leaf explants. Thus, the plants regenerated *in vitro* by direct organogenesis may exhibit greater genetic stability than those produced from callus (Lee and Phillips, 1988). The regeneration protocols developed from Cotyledon and leaf explants in the present investigation can be used for mass propagation of the species and also for genetic manipulation studies to introduce agronomically important traits.

CONCLUSIONS:

The present research work has mainly been

focused on the development of regeneration protocol and exploitation of somaclonal variations and their physiological as well as morphological aspects in *S.torvum* plant. An efficient plant regeneration protocol is a prerequisite for the exploitation of various biotechnological techniques and it has been developed for *S. torvum*. Though the practical utility of the basic protocol developed may be a long-drawn process, in future the regeneration protocol developed can definitely serve as a biotechnological platform for the transfer of economically important traits through genetic engineering, inducing somaclonal variations, *in vitro* mutations, double-haploid induction, development and utilization of somatic hybrids, determining herbicide or pesticide tolerance limits in *S. torvum*.

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