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Original Research Paper

IN VITRO MICROPROPAGATION STUDIES ON SHOOT TIP EXPLANTS OF SOLANUM TORVUM (SWARTZ) – AN IMPORTANT MEDICINAL PLANT

Botany

KEYWORDS: Shoot tip, Micro propagation, Solanum torvum (Swartz) Shoots tip explants. Murashige and Skoog

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ABSTRACT

In vitro micropropagation protocol was developed for the annual medicinal herb Solanum torvum (Solanaceae) by the in vitro culture of shoot tip explants of mature plant. Shoot tips of 30-35 days old field grown eggplants were used for shoot tip explants culture of in vitro micropropagation. Surface sterilization of shoot tips was found to be the best in 0.1% HgCl2 solution for Three minutes. For primary establishment of isolated shoot tip on MS medium fortified with (BAP/Kn/TDZ) (0.5-5.0 mg/L), IAA (0.5 mg/L)+ (BAP/KN/TDZ) (1.0-3.0 mg/L) for multiple shoot induction. Multiple shoots proliferation was best observed at IAA (0.5 mg/L) + (3.0 mg/L) TDZ from the shoot explants within four weeks of culture. Shoot number per explants ranged between 2 and 6 Individual shoots were aseptically excised and sub cultured in the same media for shoot elongation. The elongated shoots were transferred to IBA (1.0mg/L–3.0mg/L) for root induction. Rooting was observed within two weeks of culture. Rooted plantlets were successfully hardened under culture conditions and subsequently established in the field conditions. The recorded survival rate of the plants was 86%. Plants looked healthy with no visually detectable phenotypic variations. The plantlets (12–16- week-old) were successfully acclimatized in soil with 87% survival frequency

INTRODUCTION

Medicinal plants are an important source of compounds for the pharmaceutical industry and traditional medicine. About 80% of the population living in developing countries still use traditional medicines derived from plants for their primary health care needs (Cunningham (1993) and De Silva (1997)

Solanum torvum commonly known as turkey berry, devil's-fig, or prickly *Solanum* (Pier 2003) is a plant of great economic importance. Its extract, rich in Solanine and Solasodine (steroidal alkaloids) have beneficial effect on bronchial asthma. It is cultivated in tropics for its immature edible fruits (Langeland and Burks 1998). Alkaloids are a class of nitrogen compounds structurally diverse, found in all plant groups, curring most of them in angiosperms (Henriques *et al.,* 2002; Hughes and Shanks, 2002). Many species of the Solanaceae have been regenerated by shoot organogenesis using young leaf explants. *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 base for the improvement of crop to induce somaclonal variations, *in vitro* mutations, herbicide tolerance, di-haploid induction, genetic transformation of economically important genes and development of somatic hybrids, efficient plant regeneration protocol is required. Such advance tech-niques in combination with conventional breeding give a momentum to the improvement of a crop. Thus, realizing the prospects for future research, relevant literature to attempt has been made to formulate a suitable Protocol for efficient micropropagation from seedling shoot tips explants of *S. torvum*.

Methodology:-

Young plants of *S. torvum* collected from outside and grown under partly shade conditions in the experimental garden of the Aromatic and medicinal plants. Department of Botany S.R.R. Govt. Degree & P.G. College Karimnagar. The shoot tips were collected from one year old Healthy plants shoot tips comprising the apical dome and 2 or 3 leaf primordial were excised. The shoot tip explants were washed in a mild non phytotoxic liquid detergent (2% Labolene) stirred for about 3 min and then washed in tap water. It was followed by a dip in a 0.1% (w/v) Mercuric Chloride ($HgCl_2$) solution for 2 minutes. Finally the axillary bud explants were washed thoroughly with sterile water before the inoculation on to sterilized nutrient agar media prepared in culture tubes. All the above operations were performed under aseptic conditions in a laminar air flow cabinet.

MS medium containing 3.0% sucrose and supplemented with different concentrations and combinations of cytokinins BAP/Kn/TDZ (0.5-5.0mg/L) individually and in combination with IAA (0.5mg/L) were used initial pH the culture media was adjusted to 5.8 before addition of 0.8% (w/v) agar-agar. The medium was dispensed into culture tubes (25 x 150mm) each containing 15ml of the culture medium, capable with non-absorbent cotton and was autoclaved at 121°C for 15 minutes. In each culture tube one shoot-tip explant was implanted. The cultures were maintained under than 16 h light provided with white fluorescent tubes (40 μ mol $m^{-2}s^{-1}$) at 25 ± 2°C.

Shoot apices (1.0-1.5 cm) were trimmed from four week-old seedlings (Fig-a-b). and inoculated on a shoot bud induction medium consisting of MS basal medium supplemented with different concentrations of cytokines (0.5–5.0 mg/L) 6-Benzyl amino purine (BAP) or Kinetin (Kn) or Thioduzuron (TDZ) alone or in combinations of (0.5-5.0 mg/L) BAP/Kn/TDZ with (0.5 mg/L) Indole-3-acetic acid (IAA). The number of shoot buds and the percentage of explants forming shoot buds were counted after six weeks. The elongated shoots (about 1 cm long) obtained from shoot-tip explants were excised and cultured in 250 ml flasks containing 70 ml of rooting media consisting of MS medium supplemented with different concentrations of auxins, (0.5-2.0 mg/L) of IAA or indole-3-butyric acid (IBA) for the rooting of shoot buds. (Table -1). The number of roots (including the main roots and their branches). Axillary shoots were induced on four week-old rooted plantlets. These plantlets having 5-9 leaves were decapitated for inducing axillary shoot development by cutting the tips with a sterile blade. Axillary shoots developing in the axils of leaves of the decapitated plantlets were used for further multiple shoot bud induction by culturing on a medium containing (0.5-5.0 mg/L) BAP/Kn/TDZ alone or with (0.5mg/L) IAA and the number of shoot buds were counted after six weeks.

The shoot buds proliferated from axillary shoot-tip explants; they were excised and cultured on a rooting medium consisting of MS medium supplemented with different (0.5-2.0 mg/L) IAA or IBA. The rooted plantlets were gently removed from the flasks and the roots were washed in tap water to remove traces of agar.

Acclimatization

Plants with roots were transferred during two weeks, after washing of the agar with distilled water and to pots with a mixture of soilrite

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(1:1). Potted plantlets were covered with transparent polythene membrane to ensure high humidity and watered every three days with half strength MS salts solution for two weeks in order to acclimatize plants to field conditions. After two weeks the acclimatized plants were transferred to pots containing normal garden soil and maintained in greenhouse under natural day length conditions.

All cultures were maintained in a growth chamber at a temperature of 25±2°C and 16-h photoperiod provided by white fluorescent tubes (30 mol m-2S-1). All the experiments were repeated thrice and each treatment for shoot bud induction from the shoot-tip explants and rooting of the shoot buds consisted of ten replicates.

Results:-

Data on multiple shoot induction from shoot tip explants cultured on MS medium fortified with different concentrations of BAP/Kn/TDZ alone is presented in (Table -1) and BAP/Kn/TDZ in combination with (0.5mg/L) IAA is presented in (Table -2) for root induction from regenerated shoot tip explants cultured on MS medium supplemented with different concentrations of IAA and IBA is presented in (Table-3).

Effect of BAP:-

Direct multiple shoot proliferation was observed in shoot tip explants cultures callus formation was not observed in the above treatment. After 6 weeks of shoot tip explant culture developed multiple shoots (Fig-a). The various treatments tested on MS medium BAP at (3.0 mg/L) resulted in maximum number of shoots (3.2 ± 0.43). But at high concentration of BAP (5.0 mg/L) considerably the number of shoot induction was found to be reduced. As the concentration of BAP was increased up to 0.5mg/L the multiple shoots number was increased but as the concentration of BAP (3.0mg/L) to (5.0 mg/L) resulted the number of shoots were reduced. (Table-1).

Effect of Kn:-

Shoot tip explants were capable of directly developing multiple shoots on MS basal medium containing different concentrations of Kn (0.5-5.0 mg/L). Multiple shoot initiation from shoot tip explants was observed within six weeks of inoculation. Highest number of shoots (3.0 ± 0.45) was observed in the medium concentration of Kn with 75% cultures were responded. When the Kn was increased up to (0.5-3.0mg/L) the multiple number of shoots and frequency of responding was also increased (Table-1). (Fig-b).

Table – 1 Effect of various concentrations of BAP, Kn and TDZ on Direct Shoot bud proliferation of Solanum torvum (Swartz) from Shoot tip explants.

Hormone concentration (mg/L)	% of cultures response	Mean number of shoots /explants ±(S.E.)*
BAP		
0.5	65	2.0 ± 0.15
1.0	72	2.4 ± 0.25
2.0	78	2.8 ± 0.34
3.0	84	3.2 ± 0.43
4.0	76	1.8 ± 0.35
5.0	62	1.6 ± 0.23
Kn		
0.5	53	1.8 ± 0.52
1.0	64	2.0 ± 0.35
2.0	68	2.6 ± 0.36
3.0	75	3.0 ± 0.45
4.0	72	1.8 ± 0.32
5.0	64	1.0 ± 0.34
TDZ		
0.5	58	2.6 ± 0.35
1.0	60	3.2 ± 0.25
2.0	64	3.8 ± 0.35
3.0	73	4.2 ± 0.42
4.0	56	2.8 ± 0.34
5.0	50	1.8 ± 0.42

***SE Standard Error**

Table – 2 Effect of IAA in combination with various concentrations of BAP, Kn and TDZ on Direct shoot bud proliferation of Solanum torvum **(Swartz) from Shoot tip explants.**

Hormone concentration (mg/L)	% of cultures response	Mean number of shoots /explants ±(S.E.)*
IAA+BAP		
0.5+0.5	68	3.0 ± 0.23
0.5+1.0	76	3.4 ± 0.23
0.5+2.0	78	3.2 ± 0.24
0.5+3.0	80	4.0 ± 0.23
0.5+4.0	74	2.8 ± 0.32
0.5+5.0	60	2.6 ± 0.25
IAA+Kn		
0.5+0.5	58	2.8 ± 0.53
0.5+1.0	60	3.0 ± 0.35
0.5+2.0	65	3.2 ± 0.46
0.5+3.0	78	3.8 ± 0.25
0.5+4.0	70	2.3 ± 0.24
0.5+5.0	62	2.0 ± 0.53
IAA+TDZ		
0.5+0.5	60	3.4 ± 0.32
0.5+1.0	64	3.6 ± 0.35
0.5+2.0	68	4.0 ± 0.24
0.5+3.0	80	4.6 ± 0.32
0.5+4.0	72	3.2 ± 0.23
0.5+5.0	58	2.4 ± 0.32

SE Standard Error

Effect of TDZ:-

The result on shoot tip/axillary bud of S. torvum on MS medium supplemented with TDZ (0.5–5.0 mg/L) was observed. High percentage (73) of responding cultures were found at (3.0 mg/L) TDZ compared to all other concentrations tested. Whereas more number of shoots were regenerated from shoot tip explants at (2.0 mg/L) TDZ (3.8± 0.35 shoots/explant) followed by 3.0 mg/L TDZ. At 0.5, 1.0 and 2.0 mg/L TDZ (2.6 ±

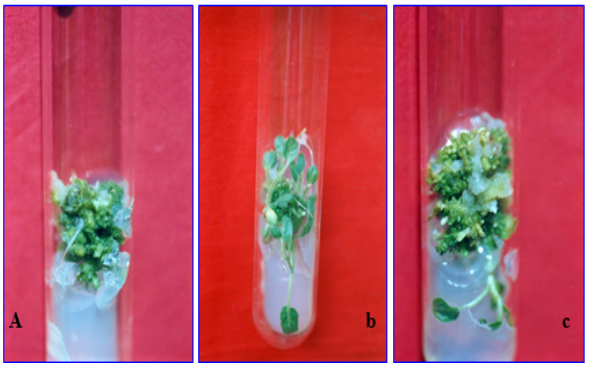



Figure1: In Vitro Micro propagation of Plantlet regeneration from shoot tip explants in S. torvum (Swartz). a) In Vitro Plantlets on MS+ (3.0/L) BAP b) Multiple shoots on MS+ (3.0 mg/L) Kn C) In Vitro shoots formation on MS+TDZ (3.0mg/L) after six weeks d) In Vitro rooting from Micro shoots on MS+ 3.0 mg/L IBA after after six week e) Hardening of plantlets

0.35), (3.2 ± 0.25) and 3.8 ± 0.35 shoots/explant. With 58, 60 and 64 percentage of cultures response was recorded. (Table- 2).

Effect of IAA + BAP:-

The results on shoot tip explants cultured on MS medium supplemented with (0.5 mg/L) IAA and different concentration (0.5 mg/L) of BAP are presented in (Table-2) and showed in. Direct multiple shoot proliferation was observed in shoot tip culture after six weeks of inoculation. Number of shoots reduced with increased level of BAP. The various treatments were tested on MS medium supplemented with (0.5 mg/L) IAA+ (0.5-5.0 mg/L) BAP. At IAA (0.5 mg/L) and BAP (3.0 mg/L) resulted in maximum number of shoots (4.0 ± 0.23) with 80 % cultures responded at (0.5, 1.0 and 2.0 mg/L) BAP within combination of (0.5 mg/L) IAA showed (3.0 ± 0.23), (3.4 ± 0.23) and (3.2 ± 0.24) shoots/ explants were induced. As the concentration of BAP was increased up to (3.0mg/L) the multiple shoots number was decreased but as the concentration of BAP (3.0mg/L) to (5.0 mg/L) resulted the number of shoots and percentage of responding were reduced. (Table- 2).

Effect of IAA + Kn:-

Morphogenetic response of shoot tip culture on various concentration of cytokinin such as Kn in combination with (0.5 mg/L) IAA is presented in (Table -17) on MS + (0.5 mg/L) IAA + (3.0 mg/L) Kn resulted in maximum number of shoots (3.8 ± 0.25) shoots/ explants as the concentration of Kn was increased, considerably the number of shoot induction was found to be reduced. At (0.5 mg/L) Kn supplemented with (0.5mg/L) IAA resulted in lowest number of shoots (2.8 ± 0.53 shoots/ explant) were induced.

Effect of IAA+TDZ:-

Whereas with the addition of IAA + TDZ induced maximum number of shoots/ explants was developed on MS medium (0.5 mg/L) IAA + TDZ (3.0mg/L) resulted (4.6 ± 0.32 Shoots/ explant). Highest percentage of response was observe at (0.5 mg. /L) IAA+ (3.0 mg/L) TDZ. The percentage of response was increased up to 3.0 mg/L TDZ and later gradually decreased at high concentrations. High frequency of shoots (4.6 ± 0.32) were induced per explant at (3.0 mg./L) TDZ and the induction of ability was decreased as the concentration of TDZ increased. At 0.5,1.0 and 2.0 mg/L TDZ induced (3.4± 0.32, 3.6± 0.35 and 4.0± 0.24 shoots / explants) with 60,64 and 68 % cultures were responded. Percentage of response was gradually increased up to (3.0mg/L) TDZ and after wards decreased the percentage of responding cultures. Low number of shoots per explants at (5.0mg/L) TDZ + (0.5 mg/L) IAA was recorded.

In vitro **rooting:-**

Fully elongated healthy shoots were transferred on to half strength MS 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 on MS medium containing 1.5 mg/L IBA whereas 96% of plants produced roots with 5.6 ± 0.38 roots/ explant. (Table -3).

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 + S and + 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.

Table -20: Rooting ability of regenerated shoots from Shoot tip and Nodal explants culture of Solanum torvum **(Swartz) cultured on MS medium supplemented with IAA and IBA.**

Growth Hormones (mg/L)		Percentage of response	Average no of roots (S.E)*
IAA	IBA		
00	00	23	1.0 ± 0.12
0.5	-	60	5.3 ± 0.37
1.0	-	70	7.2 ± 0.38
2.0	-	73	5.6 ± 0.38
-	0.5	54	4.3 ± 0.36
-	1.0	73	8.3 ± 0.87
-	2.0	70	6.3 ± 0.36

*** Mean ± Standard Error**

Acclimatization:-

Discussion:-

The result of present investigation show that the shoot tip explants from mature plants of *S. torvum* could be induced to produce multiple shoots *in vitro*. Maximum number of shoots was induced on MS medium fortified with various concentrations of BAP, Kn and TDZ. The BAP is the most efficient cytokinin in promoting adventitious shoot formation in many plants (Pirek, 1987). BAP was superior to Kn in inducing high frequency shoot regeneration in many numbers of plants (Devendra *et al*., 2010; Johnson and Manickam, 2003; Johnson *et al*., 2004; Johsnon *et al*., 2007). Combination of auxin and cytokinin favored shoot bud differentiation in many plants (Sudha *et al*., 2005; Sanjaya Rathore *et al*., 2005).

These results are also in agreement with those on *Tectona grandis* (Gupta *et. al*., 1980) *Abizzia lebbeck* (Gharyl and Maheshwari 1982) multiple shoot induction was also observed in *Ziziphus manritiana* (Sudharshan *et. al*., 2000) and *Vanilla plantifolia* (Geetha *et. al*., 2000) shoot tips cultured on MS + cytokinin alone as it was observed in the present studies.

Zamen *et.al*., (1996) have studied the effect of different cytokinins viz, BAP, Kn, 2-ip and Zeatin on multiple shoot induction from shoot tip culture in mulberry. According to their observation, BAP and Kn were superior to 2-ip and Zeatin. The superiority of BAP over other cytokinins for multiple shoot formation has been reported as it was observed in the present investigations in Mulberry by Hossian *et. al*., (1991).

Das and Mitra (1990) and Roy *et. al*., (1993) have also reported the requirement of both auxin and cytokinin for induction of multiple shoos in *Eucalyptus tereticorvis* and Jack fruit respectively. Thus, a combination of both auxin and cytokinin improved the efficiency of multiple shoots development although it depended on the

combination and concentrations employed.

Khanan *et. al*., (1998) have recorded the maximum number of shoots / culture (22.5) on MS medium containing 5.0 mg/L BAP in strawberry shoot tip culture. Gupta *et. al*., (1997) reported the multiple shoot bud induction from shoot apex cultured on MS medium containing BAP in cotton. Nasir *et. al*., (1997) have studied the shoot meristem culture in 16 cultures of cotton using several media formation. They observed the best shoot developmentation MS media containing Kn alone compared to other media with NAA / IAA in combination with Kn. These results are to the present observation in *S.torvum* which contain with cytokinins showed the increased number of shoots/ explant. Sharma and Dhiman (1998) have also observed the similar results when they have cultured the shoot tips of F1 hybrids of *Paulownia*.

The capacity of shoot bud differentiation and shoot proliferation from shoot tip explants of *S. torvum* depended on hormonal variation. There was good shoot bud induction and proliferation response only in the presence of cytokinin and no response in the basal medium. Similar results are well documented in several medicinal plants (Pattnaik and Chand, 1996), *Bixa ovellana* L. (Sharon and Marie, 2000), *Emblica officinale* (Verma and Kant, 1996) and *Withania somnifera* (Deka *et.al*., 1999). From our study it was clear that 3.0 mg/L BAP, KN and TDZ were significantly more effective for inducing shoot organogenesis. Well-developed shoot lets when transferred to rooting medium containing (1.5mg/L) IBA/IAA induced higher frequency of roots than. Similar effect of IBA was reported in *Ocimum americanum*, *O.canum* and *O.sanctum* (Pattnaik and Chand, 1996) and also in *Heracleum candicans* (Wakhlu and Sharma, 1999). However, IBA (1.5mg/L) was found to be the best rooting hormone than other auxins. Auxin support *in vitro* multiplication similar results were observed in Sunflower (Patil *et. al.,* 1993), in Mulberry (Naik and Lata, 1996) and in Coriander (Stephan and Jayabalan, 1998). From our experimental data, it is evident that BAP and Kn are the best suited for inducing multiple shoots and IBA for rooting and *in vitro* flowering. In conclusion, this communication describes an efficient rapid propagation system of *S. torvum*. When the explants cultured on MS medium supplemented with BAP and NAA/2, 4-D individually showed only callus formation without multiple shoot induction. This may be due to the fact that requirement of cytokinin and auxin depends on the endogenous levels these substances in the tissues used for the culture (Gupta, 1998).

Shoot tip explants were found to be an excellent explants source to induce direct organogenesis than nodal explants in *S.torvum*. The shoot tips are better than nodal segment for multiple shoot production because of the higher cytokinin to auxin ratio present in the shoot tip. Similarly the shoot tip was found to be the superior explants for micropropagation in many number of plants, for example *Cannabis sativa* (Ren Wang *et al*., 2009); *Boehmeria nivea* (L) Gaud (Sut *et al*., 2004), *Ocimum sanctum* (Girija *et al*., 2006), *Alternanthera sessils* (Wesely *et al*., 2011), and *Stevia rebaudiana* Bert., (Arpita *et al*., 2011). The percentage of shoots forming roots and the number of roots per shoot significantly varied depending on concentrations of NAA.

The maximum number of roots was obtained in medium containing 1.0 mg/ L NAA/IBA. Root development was; however, slow at lower concentrations of NAA/IBA. Jabeen *et al*., (2005) reported that NAA was a more effective rooting agent for *S. torvum*. On the other hand Sundari *et al*. (2010) observed only 3-4 roots on MS medium supplemented with combination of IAA (5.58 M) IBA (4.92 M). NAA was also found to promote rooting in many numbers of plants (Gyana Ranjan Rout, 2004; Mohammad Anis *et al*., 2003; Kambaska Kumar *et.,al* 2009).

Conclusion

Research work has mainly been focused on the development of regeneration protocol, Somaclonal variations and their physiological as well as morphological aspects in *S.torvum* plant. An efficient plant regeneration protocol is a pre-requisite for the exploitation of various biotechnological techniques. However, practical utility of the basic protocol is still far away. It can serve as a platform for the transfer of economically important traits through genetic engineering, inducing somaclonal variations, *in vitro* mutations, double-haploids induction, development and utilization of somatic hybrids, determining herbicide or pesticide tolerance limits in *S. torvum* plant. Therefore, a remark-able progress can be made in *S. torvum* improvement through the combination of conventional and biotechnological approaches.

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