“Response of different growth harmones of leaf culture of solanum torvum”.

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Abstract:-
Plants are the major source of food, fuel, fibre and fodder. Besides these, they are also the sources of medicines, gene pool and aesthetic beauty. Increasing population, expanding urbanization, rapid industrialization and accelerated pace of developmental activities have resulted loss of valuable plant resources. A major concern of today is the rapid depletion of valuable plant resources especially in the tropics (D’souza, 1988; Tandon, 1994; Masen, 1997; Barooh and Das, 2009). There is an urgent need for systematic cultivation and conservation of these economically important plants including crop plants. Tissue culture methods could help in the plant conservation. Conservation of plant diversity and germplasms of wild species of crop plants is, therefore, of utmost importance to ensure protection of a healthy environment and meeting basic human needs of food, health care, clothing and fuel (Fay, 1992). The proper screening of wild relatives of crop plants including medicinal plants is the need of hour and their proper exploitation would need domestication and cultivation of these plants on large scale for scientific researches. Conservation and mass propagation of wild crops plants through tissue culture of wild crop plants including medicinal plants are being made to evaluate the morphogenic potentials for developing strategies for germplasm conservation as well as successful isolation and screening of secondary metabolites of pharmaceutical importance (Ahuja, 1994; Naseem and Jha, 1994, 1997; Chaturvedi and Sinha, 1979; Chaturvedi et al; 2004; Thind et al, 2008; Arya et al; 2009; Jadav, 2009; Kumar et al; 2010).

Keeping in view the above facts, of S. torvum were carried out to analyze regeneration potentiality of different explants sources with an aim to develop protocol for micro-propagation as well as a humble effort was also made to develop strategies for germplasm conservation through in vitro method within the ambit of facilities available in our lab.
Keywords :-

Germplasm, explants, tissue culture, shoot tip, callus, leaf primordia, Growth harmones, Auxin, Cytokinin.

**Introduction :-**

Tissue culture is a method of invitro culture of cell, tissue and organ in a sterile culture medium. This technique can be reffered to as “botanical laser” whose numerous uses are yet to be explored and fully understood. The tools of plant tissue culture are being applied to a wide range of biotechnology ventures and in particular to the clonal propagation and genetic up gradation of crop and medicinal plants (Jagannathan, 1988; Rao, 2008; Dhawan, 2009, Jha, 2010, Prasad 2010).

In recent years, tissue culture techniques have become useful tools in the hands of plant scientists of all disciplines because these techniques are more handy, less time consuming and less labour involving over the conventional methods of breeding and propagation (Chandra et al., 1985; Chaturvedi et al., 1994; Naseem and Jha, 1997; Bhojwani and Razdan, 2004, Sharma et al., 2008; Behera et al., 2009).

Plant tissue culture is used as a blanket term to include *in vitro* protoplast, cell, tissue and organ culture and this novel technology has proved to be of immense value for isolation and increased production of active constituents of medicinal plants besides plant propagation and improvement. It is a method of *in vitro* culture of cell, tissue and organ in an artificial nutrient medium under aseptic condition. By this techniques, living cells can be maintained *in vitro* for a considerable period of time.

Propagation of valuable economic plants through tissue culture is based on the principle of totipotency. During this principle, new plants may be raised in an artificial medium from very small parts of the plant called explant. The explant develops into a plant or grows into unorganized cells depending on the genetic potential of the tissue and the chemical as well as physical environments of the culture.

The protocols of plant tissue culture are being applied to the clonal propagation and genetic up gradation of economically important plants. Rapid advances have been made in tissue
culture techniques in the last three decades and pioneering work was carried out in India on the production of haploids and micropropagation of medicinal plants, forest trees, plantation crops on virus elimination (Jagannathan, 1988; Mathew and Philip, 2000; Bhojwani and Razdan, 2004, Karami et al., 2007; Josi et al., 2009). The culture technique have now achieved wide commercial importance (Johri, 1994; Agrawal 1996; Ahmad, 2008) and are used as valuable research tool for genetic upgradation of horticultural as well as agricultural plants besides micropropagation (Dhawan et al., 2008; Naseem et al., 2009; Singh et al., 2009; Ansari, 2010).

Commercial propagation of forest trees and garden plants using shoot-tip culture or nodal culture is now in frequent practice (Arya et al., 1994; Augustine and D’Souza, 1997; Thirunavoukkarasu and Debata, 1998; Kumar, 2002; Kumari and Shivanna, 2005).

Tissue culture techniques have become a big way to rescue endangered rare plants known for their medicinal, timber and ornamental value (Purohit and Dev, 1996; Augustine D’Souza, 1997; Naomita and Rai, 2000). Keeping these facts into consideration the present investigation on tissue culture of *solanum torvum* was undertaken to explore the possibilities of regeneration and morphogenesis in explants of diverse origin. *S. torvum* is a multipurpose wild crop species used as fuel, pulp, medicine and gene pool for improvement of brinjal varieties. *Solanum torvum*, commonly known as titbaigun and devil's fig is a bushy perennial wild plant measuring 150-300cm in height and usually growing in sub tropical areas throughout the world as a weed of disturbed areas. In Muzaffarpur, it is found growing in pastures, road sides and wastelands but not significantly in cultivated land. It prefers moist and fertile soil and also tolerates drought and saline soils.

Fruits are eaten as vegetable and used as ingredient of pickles, it is said to be good for enlargement of the spleen (Chopra et al., 1986).

Fruits contain a number of potentially pharmacologically active chemicals including sapogenin, steroid, sterolin, chlorogenin and solasonine (Chopra et al., 1956; Badola & others, 1993; Herzog and Gautier-Beguin, 2001) Tapia and others (1996) reported that aqueous extracts of turkey berry (*S. torvum*) were lethal to mice or depressed the erythrocytes, leukocytes and
platelets in their blood. Extracts of the plant are reported to be useful in the treatment of hyperactivity, colds and cough (Null, 2001; CPR Environmental Education Centre, 2001), pimples, skin diseases and leprosy. This plant is also used medicinally for the treatment of epilepsy (Wagner et al., 1999).

Conservation of germplasm of this wild crop is highly needed for developing perennial brinjal variety, a common vegetable for millions of people of the world and its medicinal uses are also required to be investigated in right perspectives. In this background, it is necessary to multiply this plant through *in vitro* methods. Calli and regenerants obtained through *in vitro* methods can be used for germplasm conservation as well as for biochemical analysis. For rapid multiplication of these wild plants, micropropagation is being increasingly applied to supplement conventional methods of propagation (Mascarenhas and Murlidharan, 1989; Sarthi and Annexavier, 2006; Mathew and Prasad, 2007; Bahera et al., 2008 and Chandola et al., 2009). Hence the present studies were aimed at invitro regeneration of *S. torvum* through direct and callus mediated shoot regeneration using explants taken from in vivo grown plant (about 2 years old) under different hormones regimes.

**Materials & Method-**

The experimental plant, *Solanum torvum* Swartz belonging to family Solanaceae is a bushy perennial wild plant. Tissue culture studies on vegetative parts (node internode, leaf & shoot-tip) of this plant were carried out under normal in vitro conditions. The methodology of tissue culture experiment include the following steps:

A. Preparation of culture media.

B. Preparation of Explants (leaf explants).

C. Inoculation and Transfer. D. Maintenance of cultures.

E. Rooting and transfer of plantlets.

Nutritional requirements for optimal growth of a tissue in vitro is supplied by culture media. Murashige and skoog’s medium was used as culture media as this medium was
suitable for regeneration and callus induction. Various growth regulators and adjuvants used as supplement 2,4-D & kn. The sequence of steps involved in preparing the medium was as follows-

I. Required quantities of agar (0.8% w/v) and sucrose (3% w/v) were weighed out.

II. Sucrose was dissolved in some amount of distilled water to give a concentrated solution and was filtered through the Whatman filter paper No.1 (9.0 cm) to remove the particulate impurities, if any.

III. Appropriate quantities of various stock solutions and growth regulators were added.

IV. Agar was dissolved in distilled water (in about ¼ of the final volume of the medium) by heating in a water bath. The dissolved agar solution & sucrose solution were mixed with stock solution.

V. The final volume of the medium was made upto 1 litre / required volume with distilled water.

VI. After proper mixing, the pH of the medium was adjusted to 5.8 using 0.1N NaOH or 0.1N HCl wit the help of "Systronic" digital pH meter model no. 335.

VII. About 20 ml of the medium was poured into the culture tube (25 x 100mm )

VIII. The culture tubes were plugged with non-absorbent cotton wrapped in cheese cloth. The cotton plugs were wrapped with aluminium foils to prevent wetting during autoclaving.

IX. The culture vessels were transferred to appropriate baskets and autoclaved at 121°C (1.06 Kg/cm²) for 20 minutes.

X. Slants were prepared by keeping the tubes titled during cooling.
Leaf segments from youngest shoots were collected from in vivo grown mature plant (about 2 years old) of *Solanum torvum* during March to November were used as explants and were surface sterilized. Following all protocols for sterilization of tissues organ explants required size of leaf segments of 5x5mm were trimmed out. Transfer of explants into culture tubes and manipulations of tissue developed in vitro were carried out under strictly aseptic conditions. The cultures were incubated in culture room maintained at 25 ± 2°C with a relative humidity of about 60% under continuous fluorescent light (2000 lux, cool & white). Calli obtained from different explants were taken out of the culture tubes aseptically and kept in a presterilized culture tubes. A callus is an amorphous mass of loosely arranged thin walled parenchymal cells developing from proliferating cells of the parent tissue. The unique feature of callus is that the abnormal growth has logical potential to develop normal root, shoots and embryoids ultimately forming plants. Microshoots obtained from regeneration callus in *S. torvum* were cultured on MS and rooting media for rhizogenesis. Special care was adopted in transferring the plantlet from culture tube to the pot.

**Result and discussion:**

Leaves of different ages (2nd to 4th, from shoot apex to base) collected during March to November from growing shoots of mature plant (about 2 years old) were used as explants and these leaf segments were surface sterilized as per the protocol. Leaf explants (5x5mm) were aseptically cut and cultured on MS medium either alone or in combination with various growth hormones. Observations were recorded at regular intervals and results have been presented in Table 1 and 2.

**a] Selection of suitable leaf primordia for culture**

Leaves of different ages (1st to 5th, from shoot apex to base) collected from young shoots were cultured on suitable media (Table 1) and differential results were noticed in culture. The optimal response was noticed in 2nd and 3rd leaf primordia and 2nd to 4th leaves were selected for culture experiments.

**b] Effect of growth hormones on leaf culture**

There was no response of leaf segment on MS basal medium. In general, leaf enlargement and curling were observed on almost all the combination of hormones tested (Table 2, Figs.
1,2,3,4a,4b). However in some combinations of hormones, callusing was also recorded. Out of all the explants tested, leaf segments were least responsive.

No significant morphogenic changes were encountered in leaf cultures on MS medium fortified with NAA (1-5mg/l) or 2,4-D (1-5mg/l).

Greening, curling and enlargement in leaf size were observed on these hormones (Table 2, Figs. 1,2,3). Poor callusing was noted on some combinations of hormones (5mg/l NAA, 3mg/l, 2,4-D,, Table 2) after 12 days of culture.

Browning in culture was recorded above 5mg/l of 2,4-D / NAA.

**EFFECT OF CYTOKININ**

Kn was used in MS medium within a range of 1-5mg/l. Curling and leaf enlargement (Fig. 4a,4b) were noted on 1-3mg/l Kn supported media. Kn above 5mg/l was not suitable for leaf culture, leaf segments turned brown.

**COMBINED EFFECT OF AUXIN AND CYTOKININ**

**NAA+Kn**

Different concentrations of NAA (1-5mg/l) and Kn (1-5mg/l) in various combinations were used and it was found that the explant enlarged and turned leathery (Table 2, Figs. 5,6). White superficial callus grew on margin of explant and explant turned leathery on 5mg/l each of NAA and Kn (Fig. 6) Callus on this combination developed after 12 days of culture.

Culture growth above 5mg/l each of NAA and Kn was inhibitory.

**2,4-D+Kn**

2,4-D (1-5mg/l) and Kn (1-5mg/l) were used in different combinations and it was found that leaf explant enlarged and turned leathery (Figs. 7,8).
Greening and curling of explant was prominent on lower concentration of 2,4-D and Kn (1mg/l 2,4-D + 1mg/l Kn, (Table 2, Fig. 7). Explant finally turned brown on 5mg/l each of 2,4-D and Kn after 21 days of culture (Fig. 8).

<table>
<thead>
<tr>
<th>Table 1: Response of 1st to 5th leaf primordia of young shoot of <em>Solanum torvum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Explant</strong></td>
</tr>
<tr>
<td>1 leaf</td>
</tr>
<tr>
<td>2 leaf</td>
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<tr>
<td></td>
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<tr>
<td>3 leaf</td>
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<td></td>
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<tr>
<td>4 leaf</td>
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<tr>
<td>5 leaf</td>
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*Culturperiod*: 21 days
*Culture replicate*: 20
*Culture medium*: a) MS+3mg/l 2,4-D
b) MS+5mg/l NAA + 5mg/l Kn

Thus, results on 2,4-D and Kn supplemented media was almost similar to that of NAA and Kn supported media.
Table 2: Response of different growth hormones on leaf cultures of *Solanum torvum*

<table>
<thead>
<tr>
<th>Hormones (MS medium)</th>
<th>Hormonal concentration (mg l⁻¹)</th>
<th>Callusing (in days)</th>
<th>Colour</th>
<th>Nature</th>
<th>Other response</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
<td>-</td>
<td>15</td>
<td>Greening, curling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA</td>
<td>1-3</td>
<td></td>
<td>Poor callus, yellowing</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>15</td>
<td>Brown</td>
<td>Yellowing</td>
<td></td>
</tr>
<tr>
<td>2,4-D</td>
<td>1-2</td>
<td></td>
<td>Swelling, curling</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>Yellow</td>
<td>Callus on entire surface</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12</td>
<td>Brown</td>
<td>Yellowing</td>
<td></td>
</tr>
<tr>
<td>Kn</td>
<td>1-3</td>
<td></td>
<td>Enlargement, greening, curling</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>enlargement, greening.</td>
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<tr>
<td><strong>NAA+Kn</strong></td>
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<td></td>
<td>1+1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Enlargement, greening, curling</td>
</tr>
<tr>
<td></td>
<td>2+2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Do</td>
</tr>
<tr>
<td></td>
<td>5+5</td>
<td>12</td>
<td>White</td>
<td>-</td>
<td>Enlargement, leathery, green, curling</td>
</tr>
<tr>
<td><strong>2,4-D+Kn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1+1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Curling, greening, enlargement</td>
</tr>
<tr>
<td></td>
<td>2+2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Do</td>
</tr>
<tr>
<td></td>
<td>5+5</td>
<td>12</td>
<td>White</td>
<td>-</td>
<td>Bulging, enlargement, (Browning)</td>
</tr>
</tbody>
</table>

*Culture period*: 21 days  
*Culture replicate*: 20  
*Culture medium*: MS+3% sucrose & 0.8% agar
PLATE-1

( Leaf culture )

Fig.1

Fig.2

Fig.3
Fig. 1: Explant showing enlargement and curling on MS + 3mg\textsuperscript{l-1} NAA; 15 days old culture (x1.8)

Fig. 2: Explant showing greening and curling on MS + 2mg\textsuperscript{l-1} 2,4-D; 15 days old culture (x1.8)

Fig. 3: Explant showing browning in culture on MS + 5mg\textsuperscript{l-1} 2,4-D; 18 days old culture

Fig. 4a: Explant showing greening, enlargement and curling on MS + 3mg\textsuperscript{l-1} Kn; 15 days old culture

...21 days old culture on the same combination of hormone as in Fig. 32a

Fig. 5: Explant showing enlargement, curling and greening on MS + 2mg\textsuperscript{l-1} NAA + 2mg\textsuperscript{l-1} Kn; 18 days old culture
Fig. 6 : 20 days old culture showing leathery leaf and curling on MS + 5mg l⁻¹ NAA + 5mg l⁻¹ Kn; mark superficial callus

Fig. 7 : Explant showing curling and greening on MS+1mg l⁻¹ 2,4-D + 1mg l⁻¹ Kn; 15 days old culture (x1.8)

Fig. 8 : 20 days old culture showing enlargement, curling & browning of explant on MS + 5mg l⁻¹ 2,4-D+5mg l⁻¹ Kn

Conclusion:-
The present investigation on tissue culture of *Solanum torvum* was undertaken to explore the possibilities of regeneration and morphogenesis in explant of diverse origin. Conservation of germplasm of this wild crop is highly needed for developing perennial brinjal variety, a common vegetable for millions of people of the world and its medicine uses are also required to be investigated in right perspectives. In this background, it is necessary to multiply this plant through invitro methods. Calli and regenerats obtained through in vitro methods can be used for germplasm conservation as well as biochemical analysis. Further, on the basis of present findings it can be inferred that calli obtained from different explant sources can be employed as an ideal system for preservation of germplasm which will be used as gene pool for the improvement of brinjal cultivation by biotechnology tools.

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