"Response of different growth harmones of leaf culture of *solanum torvum*".

Chandan Kumar Singh, Md. Nizamuddin Ansari, Nidhi Kumari and Md.Naseem

Tissue culture lab, Univ. Department of Botany, B.R.A. Bihar,

University, Muzaffarpur-842001.

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 vasuable 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 peool 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 poeple 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 2years old) under different harmones 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 121^0C (1.06 Kg/cm^2) 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 2years 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 segements of 5x5mm were trimmed out. Transfer of explants into culture tubes and manipulations of tissue developed in vitro were carried cut under strictly aseptic conditions. The cultures were incubated in culture room maintained at 25 ± 2^0c 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 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 (2^{nd} to 4^{th}, 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 2^{nd} to 4^{th} 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 (1-5mgl^{-1}) or 2,4-D (1-5mgl^{-1}) .

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 (5mgl^{-1} NAA, 3mgl^{-1}, 2,4-D,, Table 2) after 12 days of culture.

Browning in culture was recorded above 5mgl^{-1} of 2,4-D / NAA.

EFFECT OF CYTOKININ

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

COMBINED EFFECT OF AUXIN AND CYTOKININ

NAA+Kn

Different concentrations of NAA (1-5mgl^{-1}) and Kn (1-5mgl^{-1}) 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 5mgl^{-1} each of NAA and Kn (Fig. 6) Callus on this combination developed after 12 days of culture.

Culture growth above 5mgl^{-1} each of NAA and Kn was inhibitory.

2 ,4-D+Kn

2,4-D (1-5mgl^{-1}) and Kn (1-5mgl^{-1}) 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 (1mgl^{-1} 2,4-D + 1mgl^{-1} Kn, (Table 2, Fig. 7). Explant finally turned brown on 5mgl^{-1} each of 2,4-D and Kn after 21 days of culture (Fig. 8)

Table 1: Response of 1st to 5th leaf primordia of young shoot of *Solanum torvum* in culture*

Explant	% response	Nature of response
1st leaf	-	Died
2nd leaf	a] 93.6	Enlargement, callus on surface
	b] 95.8	Do, leathery
3rd leaf	a] 90.4	Enlargement, callus
	b] 92.6	Do, leathery
4th leaf	a] 84.4	Do
	b] 86.2	Do
5th leaf	a] 58.6	Enlargement (yellowing)
	b] 49.6	Do (yellowing)

*Culturperiod : 21 days

Culture replicate : 20

Culture medium : a] MS+3mgl^{-1} 2,4-D

b] MS+5mgl^{-1} NAA + 5mgl^{-1} 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**

Hormones (MS medium)	Hormonal concentration (mg l^{-1})	Callusing (in days)	Colour	Nature	Other response
BM	-	-	-	-	-
NAA	1-3	-	-	-	Greening, curling
	5	15	-	-	Poor callus, yellowing
2,4-D	1-2	-	-	-	Swelling, curling
	3	12	Yellow	-	Callus on entire surface
	5	12	Brown	-	Yellowing
Kn	1-3	-	-	-	Enlargement, greening, curling,

	5	-	-	-	enlargement, greening.
NAA+Kn	1+1	-	-	-	Enlargement, greening, curling
	2+2	-	-	-	Do
	5+5	12	White	-	Enlargement, leathery, green, curling
2,4-D+Kn	1+1	-	-	-	Curling, greening, enlargement
	2+2	-	-	-	Do
	5+5	12	White	-	Bulging, enlargement, (Browning)

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

PLATE-1

(Leaf culture)

Fig.1

Fig.2

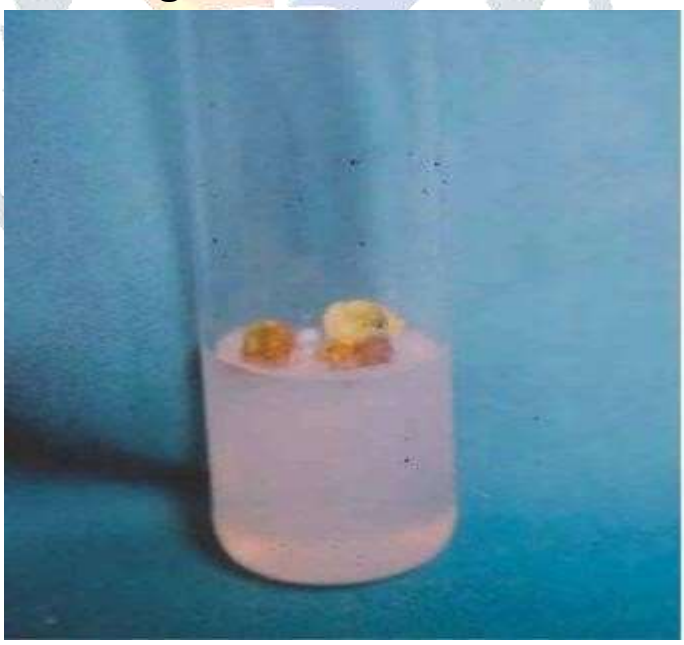

Fig.3

Fig.4a

Fig.4b

Fig.5

Fig.6

Fig.7

Fig.8

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

Fig. 2 : Explant showing greening and curling on MS + 2mgl^{-1} 2,4-D, 15 days old culture (x1.8)

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

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

Fig. 4b : 21 days old culture on the same combination of hormone as in Fig. 32a

Fig. 5 : Explant showing enlargement, curling and greening on MS + 2mgl^{-1} NAA + 2mgl^{-1} Kn; 18 days old culture

(x1.8)

Fig. 6 : 20 days old culture showing leathery leaf and curling on MS + 5mgl^{-1}NAA + 5mgl^{-1} Kn; mark superficial callus

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

Fig. 8 : 20 days old culture showing enlargement, curling & browning of explant on MS + 5mgl^{-1} 2,4-D+5mgl^{-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.

Reference:-

1. **Agrawal N 1996** Tissue culture studies on some plants of medicinal importance; Ph.D. Thesis, BRA Bihar University, Muzaffarpur

2. **Ahmad MS 2008** Tissue culture studies on *Gmelina arborea* Rox. under salt stress; Ph.D. Thesis, BRA Bihar University, Muzaffarpur

3.Ahuja PS 1994 Role of plant tissue culture in the improvement of medicinal and aromatic plants; Proceedings of **XVII** Plant Tissue Culture Conference; p**1** BHU, Varanasi

4.Ansari MN, Prakash A, Singh CK & Naseem M 2010 Exploration and pharmacopoeial use of some antidiabetic drug plants of Muzaffarpur; J. curr. Sci **15(2)** pp 413-418

5.Arya S, Rathi N & Arya ID 2009 Micropropagation protocol for *Glycyrrhiza glabra* L.; Phytomorphology **59 (1&2)** 71-76

6.Ausgustine AC & D' Souza L 1997 Micropropagation of an endangered forest tree - *Zanthoxylum rhetsa* Roxb; Phytomorphology **47(3)** 319-323

7.Badola KC, Mohinder P, Bhanderi HCS & Pal M 1993 Vegetative propagation of banbaigan (*Solanum torvum* Sw.) by rooting branch cuttings; Indian Forester 199 **(12)** 1027-1028

8.Barooh M & Das R 2009 Biodiversity, biopiracy and inntellectual property rights: An Indian context; Proceedings of Souvenir, National

9.Behera KK, Sahoo S and Prusti A 2009 Ef fi ci ent *in vitr o* mi c ro propagation of greater Yam (*Dioscorea alata* L. ev. Hinjicatu) through nodal vine explants; Indian J. Plant Physiol. **14(3)** 250-256

10.Bhojwani SS & RazdanMK 2004 Haploid production In : Plant Tissue Culture- Theory and Practice, pp. 167-213, (The Amsterdam, Netherlands: Elsevier Science Publisher B.V)

Conference on Frontiers in Plant Physiology towards Sustainable Agriculture **(ISPP)** pp. 100-113, AAU Jorhat, India

11. Chandola A, Shankhdhar SC & Shankhdhar D 2009 *In vitro* callus induction and regeneration in *withania somnifera;*Proceedings of National Conference on Frontiers in Plant Physiology towards Sustainable Agriculture **(ISPP)**, pp 134, AAU Jorhat, India

12. Chandra R Upadhya MD & Jha KK 1985 Callus single cell suspension culture and plantlet regeneration in potato (*Solanum tuberosum* L.) Egypt, J. Bot., **28 (1-3)** : 131-139

13. Chaturvedi HC & Sinha M 1979 Mass clonal propagation of *Solanum Khasianum* through tissue culture; Indian J. Exp. Biol. **17** 153-157

14. Chaturuvedi HC, Sharma AK, Prasad RN, Mishra P, Bhattacharya A & Sharma M 1994 Role of tissue culture in improved production of some horticultural crops; Proceedings of xvii Annual Plant Tissue Culture Conference, p**8** BHU, Varanasi, India

15. Chaturvedi HC, Sharma M, Sharma AK, Jain M, Agha BQ & Gupta P 2004 *In vitro* germplasm preservation through regenerative excised root culture for conservation of phytodiversity, Indian J. of Biotechnol. vol **3** pp 305-315

16. Chopra R N, Nayar SL & Chopra IC 1956 "Glossary of Indian Medicinal Plants" CSIR Publication, New Delhi

17. CPR Environmental Education Centre 2001 Medicinal plants; http://cpreec.org/edu/medi-pln.htm.4p

18. Dhawan AK, Dewan M & Dhingra HR 2008 Salt adaptation of cell lines in *Jatropha*; A biodiesel plant; Proceedings of Golden Jubilee Conference on "Challanging and Emerging Strategies for Improving Plant Productivity" **(ISPP)** pp **36**, IARI, New Delhi, India

19. Dhawan AK 2009 Micropropagation in sugarcane : Applications of nonpurine cytokinins and polyamines; Proceedings of National Conference on Frontiers in Plant Physiology towards Sustainable Agriculture **(ISPP),** pp. **21** AAU Jorhat, India

20. D'Souza L 1988 Tissue culture of forest trees and woody perennials: Potentials, Problems and Present status; Proceedings of the National Seminar on Plant Tissue Culture, pp 170-177 (New Delhi : ICAR Publication)

21. Fay MF 1992 *In vitro* Cell : Conservation of rare and endangered plants using *in vitro* methods; Dev. Biol. **28** 1-4

22. Horozog F & Gautier-Beguin D 2001 Uncultivated plants for human nutrition in Coted' Invvorie; http://www.fao.org/docrep/W3755e/w 3735e10 .htm.12p

23. Jadhav BB 2009 Recent trends in conservation, utilization and applications of medicinal plants; Proceedings of Souvenier; National conference on Frontiers in plant physiology towards Sustainable Agriculture, **(ISPP)** pp 28-30, IARI, AAU Jorhat, India

24. Jagannathan V 1988 Present status of plant tissue culture in India; Proceedings of the National Seminar on Plant Tissue Culture, pp 1-10 (New Delhi: ICAR Publication)

25. Jha S 2010 Biomedicinals from plant tissue culture;Proceedings of National Seminar on Application of Biotechnology & Development of Bihar pp 35, Central University of Bihar & BIT Mesra, India

26. Johri MM 1994 Basic features underlying plant embryogenesis and their relevance in micro propagation; Proceedings of **XVII** Annual Plant

27. Joshi R, Shukla A & Kumar P 2009 *In vitro* flowering in hill maize: A novel technique for future; Indian J. Pl. Physiol. **14**(3) 299-302 Proceedings of the National Seminar on Plant Tissue Culture, pp Tissue Culture Conference, pp **15,** BHU, Varanasi *Desmodium oojeinense* Roxb; Phytomorphology **55** (166-171)

28. Karami O, Kordestani GK & Mohamadi M 2007 Direct somatic embryogenesis and plant regeneration in strawberry (*Fragaria ananssa*); Indian J. Pl. Physiol. **12** (4) 322-326

29. Kumar A 2002 Tissue culture studies on some species of Verbenaceae;

30. Kumar A, Ahmad MS & Naseem M 2010 *In vitro* plant regeneration from organ cultures of *Gmelina arborea* Roxb; J. Indian Bot. Soc. **89** (1&2) 197-203

31. Kumari M V &Shivanna MB 2005 Cellus mediated regeneration of Ph.D. Thesis, BRA Bihar University, Muzaffarpur, India

32. Mascarenhas AF & Muralidharan EM 1989 *Curr. Sci.* **58:** 606-613

33. Masen F 1997 The potential for genetic improvement in silviculture; Agronomia-costarricense **21** 49-53.

34. Mathew D and Prasad MC 2007 Multiple shoot and plant regeneration from immature leaflets of *in vitro* origin in curryleaf (Murraya Koenigii Sprng); Indian J. Plant Physiol. **12(1)** 18-22

35. Mathew MM & Philip VJ 2000 *In vitro* adventitious shoot formation from embryos of *Areca catechu* Linn; Phytomorphology **50** 221-228

36. Naseem M & Jha K K 1994a Differentiation and regeneration in *Cleome* leaves cultured *in vitro;* Egypt. j. Bot. **34** 37-49

37. Naseem M & Jha K K 1994b Rapid clonal multiplication of *Gynandropsis gynandra* (L.) Briq. through tissue culture; Proceedings of XVII Annual Plant Tissue Culture Conference; pp 25, BHU, Varansadi, India

38. Naseem M & Jha KK 1997 Rapid clonal multiplication of *Cleome gynandra* DC. through tissue culture; Phytomorphology **47** 405-411

39. Naseem M, Ansari MN, Prakash A, Ahmad MS & Singh CK 2009 Direct and callus mediated plant regeneration from shoot tip and nodal explants of *Vernonia divrgens;* Proceedings of National Conference on Frontiers in Plant Physiology towards Sustainable Agriculture **(ISPP)** pp. 133, AAU, Jorhat, India

40. Naomita VD & Rai VR 2000 *In vitro* regeneration of *Crotolaria lutescens* (Dalz.) an endemic and rare species of W estern Ghat; Phytomorphology **50** 291-296.

41. Null G 2001 The biochemical activity of plants; http://www.garynull.com/ Documents/phytochemicals/phytochemcials5.htm.15p

42. Prasad AB 2010 Biotechnology in perspective of Bihar; Proceedings of National Seminar on Application of Biotechnology & Development of Bihar, pp 17-18, Central University of Bihar & BIT (Mesra), Patna, India

43. Purohit SD & Dev A 1996 Micropropagation of *Sterculia urens* Roxb - an endangered tree species; Plant Cell Rep. **15** 704-706

44. Rao IU 2008 Understanding *in vitro* flowering of bamboos by using plant growth regulators; Proceedings of Golden Jubilee Conference on "Challenging and Emerging Strategies for Improving Plant Productivity" **(ISPP)**, pp 52-53, IARI, New Delhi, India

45. Shanthi P & Annexavier SR 2006 Micropropagation of *Acmella calva* (DC) R.K. Jansen from nodal explants; Indian J. Pl. Physiol. **11**(1) 89-99 138 , AAU, Jorhat, India

46. Sharma A, Patna V, Arora DK & Kant U 2008 Micropropagation of *Adenium obesum L.* through apical bud explants - A caudiciform succulent ornamental plant; J. Indian Bot. Soc. **87** 232-236

47. Singh SJ, Sajeevn RS, Reddy PC & Nataraja KN 2009 Development and standardization of multiple shoot inductions protocol in *Morus indica* var.,Proceedings of National Conference on Frontiers in Plant Physiology towards Sustainable Agriculture **(ISPP)**, pp.

Tandon P 1994 Role of tissue culture in plant conservation; Proceedings of XVII Annual Plant Tissue Culture Conference, pp 41 eds. V.S. Jaiswal & U Jaiswal BHU, Varanasi, India

48. Thind SK, Jain N & Gosal SS 2008 Micropropagation of *Aloe vera* L. and estimation of potentially active secondary constituents; Phytomorphology **58 (1&2)** 65-71

49. Tapia ARA, Astudillo VR & Uribe H 1996 Resutados preliminaries del efetco de *Solanum torvum* y *Plantago major* sobre la proliferation de celulas hematopoyeticas *in vivo* e *in vitro*; pp 102-104 (Resumen de Ponencias de Primer Congreso Nacional de Plantas Medicinales de Mexico, Tlaxcala, Tlax, Mexico)

50. Thirunavoukkarasu M & Debata BK 1998 Micropropagation of *Gmelina arborea Roxb.* through axillary bud culture; Indian J. Plant Physiol **3(2)** 82-85