RESEARCH PAPER | Botany | Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

In vitro plantlet regeneration from tendril explants of spine gourd (*Momordica dioica* Roxb. ex. wild)

KEYWORDS: Maturation, embryogenic callus, in vitro propagation, L-glutamic acid.

S.Raju	Ravi Chithakari	Md. Mustafa
Research Scholar, Department of Botany, Kakatiya University, Warangal-500 602	Research Scholar, Department of Botany, Kakatiya University, Warangal-500 602	Assistant Professor, Department of Botany, Kakatiya University, Warangal-500 602

ABSTRACT *The effect of amino acids in combination with cytokinin was observed for the maturation and regeneration of embryogenic callus in spine gourd. Highest percentage (80%) embryogenic callus was obtained on MS medium fortified with 2.0 mg/L each of 2, 4-D and BAP in tendril explants. Maximum numbers of shoots (8 shoots/ embryogenic calli) were observed on MS medium supplemented with 4.0 mg/L BAP in combination with 2.0 mg/L L-glutamic acid from embryogenic calli derived from tendril explants. Addition of the amino acid L-glutamic acid provides plant cells with an immediately available source of nitrogen, which generally can be taken up by the cultured cells more rapidly than inorganic nitrogen. All shoots were rooted on MS medium augmented with IBA (3.0 mg/L). The regenerated plantlets were acclimatized in plastic pots and transferred to field condition with 70% survivability.*

Introduction:
Momordica dioica Roxb. is commonly known as 'spine gourd'. It is a perennial, rhizomatus, dioecious, climbing creeper belongs to cucurbitaceae family. It was originated in Indo Malayan region (De Wilde and Duyfjes, 2002; Joseph John and Antony, 2007) and it was spread over to India, China, Nepal, Bangladesh, Myanmar, Pakistan and Srilanka (Trivedi and Roy, 1972). Spine gourd had different vernacular names family Viz; Kartloi in bengali, akakara in telugu, kakor and parora in hindi and aegarvalli in tamil (Bhavana *et al.*, 2010). High nutritional value and good taste the young fruits are used as vegetable in India, Bangladesh and Srilanka. Apart from that every part of spine gourd had medicinal properties to cure various diseases and disorder of human being. Viz; the leaves are used as an aphrodisiac, to remove intestinal parasites, treatment of fever, asthma and piles. The roots (paste) of female plant were used to heal bleeding piles and also used for the treatment of kidney stones, jaundice, ulcers. The fruit is considered pungent, bitter, hot, alexiteric, stomachie and luxative and plays a role in cures for biliousness, asthma, leprosy, bronchitis, fever, tumors, urinary discharges, excessive salivation, and heart disease, Juice of the fruit is a domestic remedy for inflammation (Kumar and Prajapati, 2003). The dried fruits are powedered and used to induce sneezing, leading to nasal clearing. Ethanol extracts of fruits are used to protect and heal the kidneys (Jain and Singhai, 2009). Spine gourd is effective against acute renal failure (Jain and Singhai, 2009). Ethanol and aqueous extracts of spine gourd had antioxidant and hepato-protective activity (Jain *et al.*, 2008). Because of its dioecious nature and vegetative mode of propagation and long period of seed dormancy and low percentage of germination, this vegetable plant cultivation was commercially not developed (Ali *et al.*, 1991). Although the conventional methods for spine gourd cultivation had several limitations for large scale propagation. Here we first time reported plantlet regeneration from tendril explants of spine gourd in this study, which is really a boost for the in vitro propagation of plantlets from a simple explants without much somaclonal variations.

Material and methods:
Young tendril explants were collected from spine gourd plants during rainy season which were grown in research field in the Department of Botany, Kakatiya University. These tendril explants were washed thoroughly under running tap water for few minutes and surface sterilized with 0.1% mercuric chloride for 5 to 6 minutes, then rinsed 4 to 5 times with sterilized distilled water. These sterilized tendril explants were inoculated on MS medium fortified with 2, 4-D (0.5 to 3.0 mg/L) alone and in combination with BAP (0.5 to 3.0 mg/L). These cultures were maintained at 25 ± 2° C temperature and 2000 lux light intensity with a photo period of 16 hours. After 4 weeks the embryogenic callus was developed from the explant. These embryogenic calli were subcultured on MS medium supplemented with BAP (1.0 to 5.0 mg/L) alone and in combination with L-glutamic acid (2.0 mg/L). After 4 weeks of culture the *in vitro* regenerated shoots were transferred to half strength MS medium fortified with 3.0 mg/L IBA for rooting. After 4 weeks of culture the regenerated plantlets were acclimatized and transferred to field.

Results:
The tendril explants were inoculated on MS medium with various concentrations of 2, 4-D (0.5 to 3.0 mg/L) alone and in combination with BAP (0.5 to 3.0 mg/L). Maximum amount of embryogenic callus with a mean fresh weight (1.64 ± 0.03) was observed on MS medium fortified with 2.0 mg/L 2, 4- D and 2.0 mg/L BAP (Table:1**,** fig. a & b). These embryogenic calli were subcultured on MS medium with different concentrations of BAP (1.0 to 5.0 mg/L) in combination with L-glutamic acid (1.0 to 5.0 mg/L). Maximum mean numbers of shoots (8.62 ± 0.94) with a mean length of shoot (1.95 ± 0.15) was observed on MS medium supplemented with 4.0 mg/L BAP in combination with 2.0 mg/L L-glutamic acid. After 4 weeks of subculture the plantlets were transferred to rooting medium. Maximum numbers of roots (30) were developed on MS medium supplemented with 3.0 mg/L IBA. After 4 weeks the regenerated plantlets were acclimatized and transferred to field conditions.

Discussion:
The regenerated calli was obtained from tendril explants in spine gourd on MS medium fortified with equal amount of (2.0 mg/L) of auxin (2, 4-D) and cytokinin (BAP). Similar kind of results also observed in leaf explants of *M. dioica* (Devendra *et al.*, 2009; Thiruvengadam *et al.*, 2011 and Mustafa *et al.*, 2013), petiole explants of *M. dioica* (Thiruvengadam *et al.*, 2012), leaf and root explant of *Momordica charantia* (Munsur *et al.*, 2007), leaf and stem explants of *M. charantia* (Saima *et al.*, 2007).

Highest number of shoots (8) were observed on MS medium

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

fortified with BAP (4.0 mg/L) in combination with L-glutamic acid(2.0 mg/L). Similar results were observed in intermodal explants of *Momordica charantia* (Thiruvengadam *et al.*, 2012), in leaf explants of *Cucumis anguraia* L. (Thiruvengadam *et al.*, 2013), in cotyledon explants of *Cucumis sativus* L. (Selavaraj *et al.*, 2007) in leaf and petiole explants of *Melothria maderaspatana* (Baskeran *et al.*, 2009).

Conclusion:

The present study reports that embryogenic callus was obtained on MS medium fortified with auxin (2, 4-D) in combination with cytokinin (BAP) in equal volumes. Here cytokinin influencing the formation of green spots in the callus. Although cultured cells are normally capable of synthesizing all of the required aminoacids, the addition of certain amino acids (L-glutamic acid) provides cultured plant cells with an immediately available source of nitrogen, which generally can be taken up by the cells more rapidly than inorganic nitrogen.

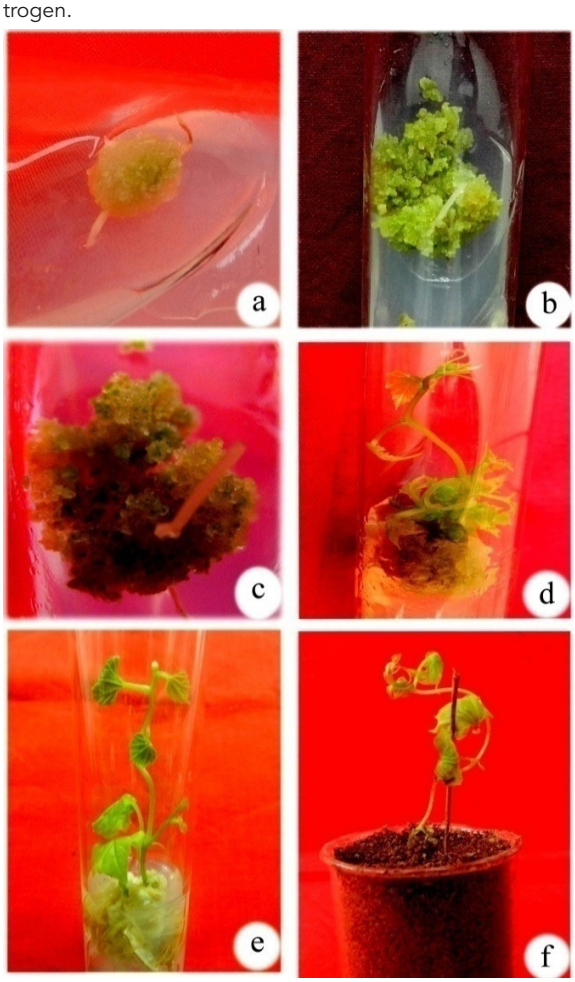

Fig: In vitro plantlet regeneration from tendril explants of spine gourd (Momordica dioica Roxb. ex. Wild

a & b) Induction of callus on MS +2.0 mg/L 2,4-D +2.0 mg/L BAP

c) Subculture of tendril derived callus on MS + 4.0 mg/L BAP + 2.0 mg/L L-glutamic acid

d) Regeneration of plantlets on MS + 4.0mg/l BAP + 2.0 mg/l L-glutamic acid

e) Rooting of plantlets on MS ½ strength + 3.0mg/l IBA.

f) Hardening of plantlets of Momordica dioica Roxb.

Table 1: In vitro callus induction in tendril explants on MS medium suppliented with different
concentrations of 2, 4 – D (0.5 – 2.5 mg/L) , NAA (0.5 – 2.5 mg/L) and IAA (0.5 -2.5 mg/L) either alone or in combination BAP (0.5 – 2.5 mg/L) in *M. dioica* Roxb. after 4 weeks of culture.

Ms medium with plant growth regulators (mg/L)		% explants responded	Weight of callus (g) (Mean±S.E)	Nature of callus
2, 4-D	BAP			
0.5	--	25	0.17 ± 0.08	Soft white
1	--	37	0.45 ± 0.05	Soft white
1.5	--	44	1.13 ± 0.06	Soft white
2	--	75	1.30 ± 0.03	Soft white
2.5	--	50	1.00 ± 0.04	Soft white
0.5	0.5	43	0.34 ± 0.06	Soft green
1	1	55	0.65 ± 0.04	Soft green
1.5	1.5	78	1.32 ± 0.05	Friable green
2	2	85	1.64 ± 0.03	Friable green
2.5	2.5	85	1.38 ± 0.02	Friable green

Data represents average of three replicates; each replicate consists of 15 cultures.

Table:2 *In vitro* **shoot regeneration via indirect organogenesis in tendril calli cultured on MS + BAP (1.0 – 5.0 mg/L), TDZ (0.5 - 2.5 mg/L) and Kn (1.0 – 5.0 mg/L) in combination with L- glutamic acid (2.0 mg/L) in M. dioica Roxb., after 4 weeks of culture.**

MS medium with Plant growth regulators (mg/L)		Tendril derived calli	
BAP	L-glutamic acid	No. of shoots/ explant (Mean±S.E)	Shoot length (cm) (Mean±S.E)
1.0	---	2.18±0.08	3.25±0.98
2.0	---	4.32±0.06	2.80±0.96
3.0	---	5.64±0.04	2.50±0.68
4.0	---	6.25±0.02	1.50±0.88
5.0	---	4.98±0.05	1.85±0.78
1.0	2.0	2.75±0.82	2.35±0.69
2.0	2.0	5.96±0.25	2.12±0.16
3.0	2.0	6.75±0.02	1.50±0.88
4.0	2.0	8.62±0.94	1.95±0.85
5.0	2.0	5.25±0.98	1.84±0.74

Data represents average of three replicates; each replicate consists of 15 cultures.

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

REFERENCE

[1] De Wilde, WJJO. & Duyfjes, BEE., (2002). Synopsis of Momordica (Cucurbitaceae) in SE- | Asia and Malesia. Bot Zh. 87, 132-148. | | [2] Joseph John, K., Antony, V.T., (2007). Momordica sahyadrica sp. Nov. (Cucurbitaceae), an | endemic species of Western Ghats of Inida. Nord J Bot 24 (5), 539-542. | | [3] Bhavan, B., Mukesh, D., Chauhan, N.S., Dixitn V.K. & Saraf, D.K., (2010). Phyto- | Pharmacology of Momordica dioica Roxb,. Ex. Wild: A Review. Int Journal of Phy. Med. | 2(2010),01-09. | | [4] Jain, A., Soni, M., Deb, L., Jain, A., Rout, S.P., Gupta, V.B. & Krishna, K.L., (2008). | Antioxidant and hepato-protective activity of ethanolic and aqueous extracts of Momordica | dioica Roxb, leaves. J. Ethnopharmacol. 115, 61-66. | | [5] Kumar, U. & Prajapati, N.D., (2003). Agro's dictionary of medicinal plants. Agrobios, | Agrohouse, Jodhpur, India. 216 pp. | | [6] Jain, A. & Singhai, A.K., (2009). Effect of Momordica dioica Roxb, on gentamicin model of | acute renal failure. Nat. Prod. Res. 2, 1-10. | | [7] Saima, M., Muhamad, Z., Riaz-ur-Rehman., & Chaudhary, M.F., (2007). In vitro | Regeneration from Direct and Indirect Oranogenesis of Momordica charantia,. Pakistan J. | Bio. Sci., 1(22), 4118-4122. | | [8] Devendra, N. K., Subhash, B., & Seetaram, Y.N., (2009). Callus growth and regeneration in | Momordica dioica Roxb. Wild Cucurbitaceae. American-Eurasian jJournal of Agriculture | 3(4), 743-748. | | [9]Ali M., Okubo H., Fujii T. & Fujieda. K., (1991). Techniques of propagation and breeding of | kakrol (Momordica dioical\Roxb.). Scientia Hort 47,335-343. | | [10]Baskaran. P., Velayutham. P. & Jayabalan. N., (2009). In vitro regeneration of Melothria | maderaspatana via indirect organogenesis. In Vitro cell . Dev. Biol.-Plant. 45, 407-413. | | [11]Thiruvengadam. M., Jeyakumar. J. J., Kamaraj. M., Lee. Y. J. & Chung. I.M., (2013). Plant | regeneration through somatic embryogenesis from suspension cultures of gherkin (Cucumis | angurai L.). Australian Journal of Crop Science.7(7), 969-977. | | [12]Trivedi. R.N. & Roy. R.P., (1972). Cytological studies in some species of Momordica. | Gentica 43,282-291. | | [13]Thiruvengadam. M., Praveen N., & Chung.III.M., (2012). In vitro regeneration from | internodal explants of Bitter melon (Momordica charantia L.) via indirect organogenesis. | African Journal of Biotechnology Vol. 11(33), 8218-8224. | | [14]Mustafa. Md., Swamy. T.N., Raju. S., Peer Mohammad. S.K. & Suresh V., (2012). | Regeneration of plantlets from nodal cultures of Momordica dioica Roxb. Int. J. Pharm | Biosci. 3(4)(B), 92-96. | | [15]Selvaraj. N., Vasudevan. A., Manickavasagam. M., Kasthurirengan S. & Ganapathi. A., | (2007). High frequency shoot regeneration from cotyledon explants of cucumber via | organogenesis. Sci. Hortic. 112, 2-8. | | [16]Munsur, MAZ Al., Haque, M.S., Nasiruddin K.M., & Hasan M. J., (2007). Invitro | regeneration of bitter gourd (Momordica charantia L.) from leaf segments and root tips. | Progress Agric. 18(2), 1-9. | | [17]Thiruvengadam M., Praveen N. Lee Y. J. & Chung. III. M., (2012). An efficient regeneration | from petiole derived callus of male and female spine gourd (Momordica dioica Roxb. Ex. | Wild.). J. Med. Plants Res.. 6(17), 3330-3337. | | [18]Thiruvengadam M. & Chung III-Min., (2011). Establishment of an efficient Agrobacterium | tumbifaciens –mediated leaf disc transformation of spine gourd (Momordica dioica Roxb. | Ex Wild). Afr. J. Biotechnol. 10(83), 19337-19345. |