Asian J. of Bio Sci. (April, 2008) Vol. 3 No. 1 : (61-665)

Somatic embryogenesis and plantlet regeneration in chilli (*Capsicum annuum* L)

P.N. JAYAPADMA, SUMANGALA BHAT* AND M.S.KURUVINASHETTI
Department of Biotechnology, University of Agricultural Sciences, DHARWAD, KARNATAKA (INDIA)

(Accepted : December, 2007)

Plant regeneration protocol through somatic embryogenesis, was developed from whole cotyledon explants of two local cultivars of chilli, Byadagi Dabbi and Sankeshwar Local. Explants cultured on Murashige and Skoog (1962) medium supplemented with different levels of thidiazuron (TDZ) (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0mg/l) induced somatic embryos and the best response was at 3mg/l TDZ. On an average, 51.3 percent explants responded in both the genotypes with over nine embryos per explant. Complete plantlet formation occurred on MS with 3mg/ABA or 2mg/l IBA. Among the different levels of ABA, IBA and higher levels of agar tried for maturation of somatic embryos and germination, ABA 3mg/l was found to be better.

Key words: *Capscicum annuum*, Chilli, Cotyledon, Somatic embryogenesis,

INTRODUCTION

Chilli (*Capsicum annuum* L.) also known as hot pepper is one of the most widely cultivated vegetable and spice crops in the world. It has become an essential component of diet of the rich and poor. Chilli is grown over an area of 1.7 million hectares in the world, with a production of 19.4 million tones. India is the largest producer of chillies with an area of 0.91 m.ha and production of about 0.97 metric tones. In India it is one of the important spices, earning foreign exchange. The susceptibility to many pathogens and insect pests limit the productivity of chilli crop. Selection of varieties tolerant to diseases and pests is a priority, but the progress is limited due to lack of resistance to many pests and diseases in the available germplasm. Transformation of crop plants with the desired genes from a different source to control pests and diseases is the focus of many plant genetic engineering research programmes, which require efficient and reliable regeneration protocol. Although chilli belongs to the Solanaceae family, whose members are easily amenable to tissue culture and transformation, it is recalcitrant to regeneration especially at the shoot elongation stage (Steinitz,*et al*., 1999; Ochoa-Alejo and Ramirez-Malagon, 2001; Pozueta,2001) and the responses are genotype specific (Christopher, T. and Rajam, 1996; Ramirez-Malagon and Ochoa-Alejo,1996). Though many crops are known to produce somatic embryos *in vitro*, only few reports are available on pepper somatic embryogenesis from zygotic embryos (Harini and Lakshmi Sita, 1993; Binzel, 1996; Bodhipadma, and Leungd, 2003), leaf (Kintzios *et al.*, 2001) and seed (Kaparakis, and Alderson, 2002) explants.

MATERIALS AND METHODS

Explant preparation:

Two popular and local cultivars of this region –Byadagi dabbi and Sankeshwar local were used in this study. Seeds were surface sterilized with 5%(v/v) sodium hypo chlorite for 20 minutes, rinsed 4-5 times with sterile distilled water and germinated on half strength MS medium (Murashige and Skoog,1962) under dark incubation. Whole cotyledon explants from 15-day-old in vitro grown seedlings were used as explants.

Induction, maturation and germination of somatic embryos:

Explants were cultured on MS medium with different concentrations of (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0mg/l) thidiazuron and incubated in 16/8hrs light and dark cycles for the induction of somatic embryos. Response of individual cultures to somatic embryogenesis and the number of somatic embryos produced in each explants was noted 30 days after inoculation. Clusters of somatic embryos obtained were transferred to MS supplemented with various levels (1,2,3,4mg/l) of ABA or Agar (8,10,12,14,16mg/l) or IBA (0.1,0.5,1.0,1.5,2mg/l) for maturation and development. Response in each cluster was noted 30-40days after culture for germination and plant formation.

Data collected on somatic embryo induction and their

* Author for Correspondence

maturation and germination were analyzed as a factorial experiment in a completely randomized block design with two factors and three replications per treatment. The observations in percentages were transferred to arc-sine values (sin-1vP) for statistical analysis.

RESULTS AND DISCUSSION

Immature zygotic embryos are the most commonly used explants for somatic embryo induction. In this investigation, immature zygotic embryos were initially cultured on MS supplemented with different levels of 2,4-D (1,2,3,4mg/l) and higher levels of sucrose (30,40,60,80,100g/l) with or without coconut water to induce somatic embryos. These explants expanded many times their initial size without somatic embryos. However, there are reports on use of immature zygotic embryos as explants for somatic embryogenesis (Harini and Lakshmi Sita, 1993; Binzel, 1996). Lack of somatic embryo formation with 2,4-D in both the genotypes was confirmed by repeat experiments. Such genotype specific response is a common feature in plant tissue culture. On the other hand, whole cotyledon explants cultured on MS medium supplemented with different levels of TDZ produced somatic embryos at higher levels of TDZ (3.0,3.5,4.0mg/l)(Table 1) and the best response was at 3mg/l in both the genotypes. The frequency of somatic embryos induction was also similar in both the genotypes. On an average, 51.3 percent (Table 1) of the explants produced somatic embryos in both, but they differed in terms of the requirement of auxin and cytokinin for somatic embryogenesis (Gill and Saxena,1992). In the present study somatic embryo could be induced with relatively high concentration of TDZ but not on medium containing 2, 4-D. However, in chilli, there are no reports on the induction of somatic embryogenesis with TDZ alone, but somatic embryos formed only when 2,4-D was used along with TDZ. However Venkataiah

Table 1. Somatic embryo induction in whole cotyledon explants at different levels of TZD

MS+TZS (mg/l)	Genotype		Mean
	Sankeshwar Local	Byadagi Dabbi	
0.0	0.00* (0.00)^{i}**	0.00 (0.00)^{i}	0.00 (0.00)^{f}
0.5	0.00 (0.00)	0.00 (0.00)^{i}	0.00 (0.00)^{f}
1.0	42.00 (40.37)^{h}	46.63 (42.87)^{gh}	44.16 (41.62)^{h}
1.5	47.00 (43.26)^{gh}	51.00 (45.55)^{fg}	49.00 (44.40)^{de}
2.0	52.00 (46.12)^{fg}	54.00 (47.27)^{ef}	53.00 (46.70)^{d}
2.5	58.30 (49.77)^{de}	63.00 (52.73)^{d}	60.80 (51.25)^{e}
3.0	87.60 (69.47)a	88.00 (69.74)^{a}	87.83 (69.60)^{a}
3.5	87.00 (68.84)^{ab}	84.60 (66.94)^{ab}	85.80 (67.89)^{ab}
4.0	83.00 (65.66)^{bc}	80.00 (63.47)^{c}	81.50 (64.57)^{b}
Mean	50.77 (42.61)	51.92 (43.17)	51.53 (42.89)
	SEm±	Probability at 1%	
Verity	0.2822	NS	
Treatment	0.5987	S	
Interaction	0.8467	NS	

*-Figures indicate mean over three replication
**Figures in parenthesis indicate arcsine value
Figures followed by same letters are not significantly different from each other recording to Duncun`s multiple Range test (DMRT) at 1%

Table 2.Frequency of somatic embryos produced from whole cotyledon on MS medium with different levels of TDZ

MS+TDZ (mg/l)	Genotype		Mean
	Sankeshwar Local	Byadagi Dabbi	
1.0	4.5*	5.33	4.94
1.5	3.92	6.45	5.19
2.0	5.33	12.33	8.83
2.5	8.55	12.44	10.5
3.0	12.87	17.66	15.27
3.5	10.22	12.44	11.33
4.0	7.44	10.00	8.72
Mean	7.55	10.95	
	SEm±	Probability at 1%	
Variety	0.1555	S	
Treatment	0.2908	S	
Interaction	0.4113	S	

*-Figures indicate mean over three replication
Figures followed by same letters are not significantly different from each other according to Duncan's multiple Range test (DMRT) at 1%

Table 3.Percent response of somatic embryos to plantlet formation on medium with different levels of ABA

MS+ABA (mg/l)	Genotype		Mean
	Sankeshwar Local	Byadagi Dabbi	
1.0	5.00 (12.91)d	7.75 (16.13)c	6.37 (14.52)c
2.0	5.50 (13.49)d	8.25 (16.66)c	6.87 (15.07)c
3.0	14.50 (22.35)b	18.50 (25.45)a	16.50 (23.90)a
4.0	9.75 (18.17)c	14.25 (22.16)b	12.00 (20.16)b
Mean	8.68 (16.73)	12.18 (20.10)	10.43 (18.41)
	SEm±	Probability at 1%	
Verity	0.2532	S	
Treatment	0.3581	S	
Interaction	0.5064	NS	

*-Figures indicate mean over three replication
**Figures in parenthesis indicate arcsine values
Figures followed by same letters are not significantly different from each other according to Duncan's multiple Range test (DMRT) at 1%

Table 4.Percent response of somatic embryos to plantlet formation on medium with different levels of agar

MS+ Agar (g/l)	Genotype		Mean
	Sankeshwar Local	Byadagi Dabbi	
8.0	0.00* (0.0)e	0.00 (0.0)e	0.00 (0.0)c
10.0	0.00 (0.0)e	0.00 (0.0)e	0.00 (0.0)c
12.0	3.33 (10.49)c	4.33 (11.99)b	3.83 (11.24)b
14.0	7.33 (15.69)a	8.66 (17.01)a	8.00 (16.40)a
16.0	2.33 (8.74)d	3.33 (10.49)c	2.83 (9.61)b
Mean	2.60 (6.98)	3.26 (7.91)	2.93 (7.45)
	SEm±	Probability at 1%	
Variety	0.1662	S	
Treatment	0.2628	S	
Interaction	0.3716	NS	

*-Figures indicate mean over three replication
**Figures in parenthesis indicate arcsine values
Figures followed by same letters are not significantly different from each other according to Duncan's multiple Range test (DMRT) at 1%

et al. (2003) reported only multiple shoot bud induction from cotyledon explants on medium with TDZ alone. This difference in response may be because of difference in endogenous auxins and cytokinines in cotyledon explants of different genotypes. Similarly, direct differentiations of somatic embryos were observed from leaves and cotyledons in *Azardirachta* when cultured on MS with 1.0Mm TDZ.

Somatic embryos in clusters were in different developmental stages (globular, heart, torpedo and cotyledon shape) in every explant. These embryos were transferred to maturation medium for further development. But in chilli itself, Harini and Laxmisita (1993) and Binzel *et al.* (1996) reported that the entire process of somatic embryo initiation and development was a one step process when zygotic embryos were used as the initial explants. In both the genotypes here, every somatic embryo clusters had many malformed embryos and their frequency increased with time or when cultured on the same medium. It was necessary to change the medium to achieve maturation. Clusters of embryos when transferred to MS medium supplemented with various levels of ABA (1, 2, 3,4mg/l), IBA (0.5, 1.0, 1.5, and 2mg/l) and higher levels of agar (12, 14,16g/l), few embryos matured, germinated and developed into complete plants. Medium with 2 -3mg/l ABA, 1.5 -2mg/l IBA or 14g/l agar were desirable for this purpose, but the genotypes had significant differences. Cultivar Byadagi Dabbi responded better (12.1%) than Snakeshwar Local (8.68%). Embryo germination was significantly enhanced (16.5%) on 3mg/l IBA (Table 5). Application of stress in the form of higher levels of sucrose, agar or addition of ABA is known to cause maturation of somatic embryos (Pratibha Devi *et al.*, 2004).

Among the different levels of ABA, IBA and higher

Table 5.Per cent response of somatic embryos to plantlet formation on medium with different levels of IBA

MS+IBA (mg/l)	Genotype		Mean
	Sankeshwar Local	Byadagi Dabbi	
0.1	0.00* (0.00)d	0.00 (0.00)d	0.00 (0.00)d
0.5	5.00 (12.91)c	5.00 (12.91)c	5.00 (12.91)b
1.0	5.00 (12.91)c	6.25 (14.29)c	5.62 (13.60)b
2.0	12.50 (20.60)b	16.25 (23.72)a	14.37 (22.16)a
Mean	5.62 (11.60)	6.875 (12.73)	6.25 (12.17)
	SEm±	Probability at 1%	
Variety	0.3696	S	
Treatment	0.5224	S	
Interaction	0.7388	NS	

*-Figures indicate mean over three replication
**Figures in parenthesis indicate arcsine values
Figures followed by same letters are not significantly different from each other according to Duncan`s multiple Rang test (DMRT) at 1%

levels of agar tried for maturation and germination, ABA 3mg/l (16.5%) was the best followed by IBA (14.37%) Agar had no effect (Table 3, 4 and 5). Somatic embryos developed shoots and roots on these media usually and formed plantlets after 25-30 days of transfer. Plantlets developed in the presence of IBA (2mg/l) were found to be more robust and healthy with expanded leaves and well developed roots compared to plantlets on ABA medium. Though somatic embryos could be induced at a very high frequency in chillies, their conversion to plantlets is very low (2-3 plantlets/cluster of somatic embryos). In most cases numerous leafy structures that appear on regeneration medium do not have clear root/shoot axis, possibly due to precocious germination of somatic embryos. In plant transformation experiments, number of plants that can be realized per culture is more important than the number of somatic embryos produced. Further increase in the conversion rate of the embryos is desired in chilli.

REFERENCES

Steinitz, B., Wolf, D., Matzevitch-Josef, T. And Zelcer, A., (1999). Regeneration *in vitro* and genetic transformation of pepper (*Capsicum spp*.): the current state of the art. *Capsicum and Eggplant Newsletter*, **18**: 9-15.

Ochoa-Alejo, N. And Ramirez-Malagon, R. (2001) *In Vitro* chilli pepper Biotechnology. *In vitro Cellular and Development Biology-Plant*, **37**: 701-729.

Pozueta, J.R., Houline, G., Canas, L., Schantz, R. And Chamero, J. (2001). Enhanced regeneration of tomato and pepper seedling explants for *Agrobacterium* mediated transformation. *Plant cell, Tissue and Organ Culture*, **67**: 173-180.

Christopher, T. And Rajam, M.V. (1996). Effect of genotype explant and medium on *in vitro* regeneration of red pepper. *Plant Cell, Tissue and Organ Culture*, **46**: 245-250

Ramirez-Malagon, R. And Ochoa-Alejo, N. (1996). An improved and reliable chilli pepper (*Capsicum annuum* L.) Plant regeneration method. *Plant Cell Reports*, **167**: 226-231

Harini, I. And Lakshmi Sita, G. (1993). Direct somatic embryogenesis and plant regeneration from immature embryos of chilli (*Capsicum annuum* L.). *Plant Science Limerick*, **89**: 107-112.

Binzel, M.L., Sankhla, N., Joshi, S. And Sankhla, D. (1996). Induction of direct somatic embryogensis and plant regeneration in pepper (*Capsicum annuum* L.). *Plant Cell Reports*, **15**: 536-540.

Bodhipadma, K. And Leungd, W.M., (2003). *In vitro* fruiting and seed set of *Capsicum annuum* L. cv. sweet banana. *In vitro Cellular and Development Biology Plant*, **39**: 536-539.

Kintzios, S., Drossopoulos, J.B. And Lymperopoulos, C. (2001). Effect of vitamins and inorganic micronutrients on callus growth and somatic embryogenesis from leaves of chilli pepper. *Plant Cell, Tissue and Organ Culture*, **67**: 55-62.

Kaparakis, G. And Alderson, P.G. (2002). Influence of high concentrations of cytokinins on the production of somatic embryos by germinating seeds of tomato, aubergine and pepper. *The Journal of Horticultural Science and Biotechnology*, **77**: 186-190.

Murashige, T. And Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, **15**: 475-497.

Huetteman, C.A. And Preece, J.E. (1993). Thidiazuron: A potent cytokinin for woody plant tissue culture. *Plant Cell, Tissue and Organ Culture*, **33**:105-119.

Malik, K.A. And Saxena, P.K. (1992). Somatic embryogenesis and shoot regeneration from intact seedling of *Phaseolus acutifolius*, *P. aureus*, *P. coccineus* and *P. wrightii*. *Plant Cell Reports*, **11**: 163-168.

Gill, R. And Saxena, P.K. (1992). Direct somatic embryogenesis and regeneration of plants from seedling explants of peanut (*Arachis hypogea*): promotive role of thidiazuron. *Canadian Journal of Botany*, **70**: 1186-1192.

Venkataiah, P., Christopher, T. And Subhash, K. (2003). Thidiazuron induced high frequency adventitious shoot formation and plant regeneration in *Capsicum annuum* L. *Journal of Plant Biotechnology*, **5**: 245-250.

Pratibhadevi, Radha, P., Sitamahalaxmi, L., Syamala, D. And Manojkumar, S.(2004). Plant regeneration via somatic embryogenesis in Mungbean (*Vigna radiata* L.). *Scientia Horticulturae*, **99**:1-8.