ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 3, pp. 042-045, April, 2010

RESEARCH ARTICLE

IN VITRO INDUCTION OF CALLUSOGENESIS IN CHILI PEPPERS (*CAPSICUM ANNUUM* L.)

Aniel Kumar, O., Subba Tata, S* and Rupavati, T

Andhra University, Department of Botany, Visakhapatnam, India

ARTICLE INFO

Article History:
Received 13th February, 2010
Received in revised from
17th March, 2010
Accepted 27th March, 2010
Published online 1st April, 2010

Key words:
Capsicum annuum L.,
Callus,
Callusogenesis,
Fresh weight,
MS medium

ABSTRACT

In vitro callusogenesis and differential callus growth rate was studied from hypocotyl, cotyledon and leaf explants of three cultivars of *Capsicum annuum* L. viz., var. X-235, var. PC-1 and var. Pusa Jwala. Callus initiation was genotype dependent and var. X-235 had best callusogenesis response than var. PC-1 and var. Pusa Jwala. MS medium supplemented with 2,4-D(1.0mg/l) and BAP(2.0mg/l) was found to be the best medium for maximum frequency of callus induction recorded from hypocotyl explants of the 3 genotypes i.e., var. X-235(79%), var. PC-1(68%) and var. Pusa Jwala (64%). The hypocotyl explants manifested maximum callus fresh weight (1.16g) than cotyledon and leaf explants. The study revealed that the callus growth rate (fresh weight) of all the explants showed maximum increase in the first subculture (1st week) than the lateral subcultures (6th and 9th week). This rapid *in vitro* callusogenesis procedure can be used as a source for production of secondary metabolites from chili peppers.

INTRODUCTION

Plant tissue culture can potentially revolutionize the knowledge and application in several fields of the plant kingdom (Cooking, 1986). Considerable advance has been reached, especially in certain plant families as Solanaceae which is one of the best known (Flick *et al.,* 1983) even though some of the family members, such as *Capsicum* remain without an adequate *in vitro* exploration despite its commercial value as spice and vegetable throughout the world (Morrison *et al.,* 1986). *In vitro* studies of *Capsicum* species have been emphasized in the effects of growth regulators, some of which proved to be ineffective for the induction of morphogenetic responses but efficient for callus production (George and Narayanaswamy, 1973; Gunay and Rao, 1978). Standardized methods for callus production are an important goal in view of the industrial interest in some of its secondary metabolites (Williams *et al.,* 1988) particularly capsaicin (Ochoa-Alejo and Gomez-Peralta, 1990; Sukrasno and Yeoman, 1990). On the other hand, a friable and abundant callus is desirable in order to trigger somatic embryogenesis (Evans *et al*., 1981). In recent years several *Capsicum* explants and their *in vitro* callusogenesis potentiality have been explored with variable degrees of success (Agrawal *et al.,* 1989; Ashrafuzzaman *et al.,* 2009; Christopher and Rajam, 1994,1996; Gunay and Rao, 1978; Kintzios *et al.,* 1996; Marziah *et al.,* 1995; Rodeva and Grozeva, 2003; Rodeva *et al.,* 2006; Singh and Shukla, 2001; Shao and Caponetti, 1993 and Subhash and Prolaram, 1987). However, little information on induction and quality properties of callus from different explants of chili pepper has been provided in earlier *in vitro* culture studies (Kaparakis and Alderson, 2003). Despite of many reports, callusogenesis and callus growth rate in chili peppers is inadequate. Therefore the present investigation is taken up to study the callusogenesis and callus growth rate of hypocotyl, cotyledon and leaf explants of three genotypes of *C. annuum* L.

MATERIALS AND METHODS

Seeds of three genotypes of *C. annuum* L. viz., var. X-235, var. PC-1 and var. Pusa Jwala were obtained from Sutton & Seeds, Calcutta, India. The seeds were surface sterilized with 0.1% $HgCl_2$ and repeatedly washed in sterile distilled water. The seeds were then germinated on MS basal medium (Murashige and Skoog, 1962) for germination. The hypocotyl, cotyledon and leaf explants were derived from 14-15 days old *in vitro* germinated seedlings cultured on MS medium supplemented with various concentrations (0.5-1.5mg/l) of auxins like 2,4-D(2,4.dichlorophenoxyacetic acid), NAA(1-Naphthaleneacetic acid)) and cytokinin BAP(6- benzyl aminopurine) at the concentrations of (1.0-3.0mg/l) either alone or in combinations and subculture at every three weeks to the same medium. All media were supplemented with 3% sucrose and 0.8% agar, the P^H of the media was adjusted to 5.8 with 1N NaOH or 1N HCl prior to autoclaving. The percentage of callusogenesis was

*Corresponding author: *s_tata_s@yahoo.co.in*

recorded after three weeks of culture. The callus induction frequency was determined as follows:

$$\text{Callus induction (\%)} = \frac{\text{No. of explants showing callus}}{\text{No. of explants inoculated}} \times 100$$

For callus growth rate the explants viz., hypocotyl, cotyledon and leaf were placed on MS medium supplemented with 2,4-D (1.0mg/l) and BAP(2.0mg/l) and sub culture at every three weeks interval on the same medium. For determining the fresh weight, the callus was blotted on a sterile blotting paper immediately after removing it from the medium then placed in a pre-weighed sterile vial and weighed. The increase in fresh weight was calculated by substracting the initial weight from the final weight taken at the end of 3, 6 and 9 weeks of culture (1st, 2nd and 3rd subculture). The differences in growth rate were expressed in terms of percentage increase or gain in fresh weight at 3 week interval. All the cultures were maintained in a tissue culture chamber at 25 ± 2^0C under 16hr photoperiod. All the experiments were repeated thrice, each treatment for callus induction from all the explants consisted of twenty replicates respectively.

RESULTS AND DISCUSSION

The frequency of callus induction and callus growth rate was studied in different explants of three genotypes of chili peppers were cultured on MS medium supplemented with varying concentrations of growth regulators (Table 1&2). Callus induction (callusogenesis) was obtained from hypocotyl, cotyledon and leaf explants of 3 genotypes of *Capsicum annuum* L. viz., var. X-235, var. PC-1 and var. Pusa Jwala were cultured on MS medium supplemented with 2,4-D(0.5-1.5mg/l), NAA (0.5-1.5mg/l) and BAP(1.0-3.0mg/l) either alone or in combinations. Among the auxins tested NAA and 2,4-D were found to be effective in inducing callus in all the explants. BAP also induce callusogenesis but rate of proliferation was low. However, when higher concentrations of 2,4-D and NAA above (1.0mg/l) were used, decreased callus induction and callus browning was observed. Among the various concentrations and combinations of auxins and cytokinins tested 2,4-D(1.0mg/l) + BAP(2.0mg/l) was found to be most effective in inducing callus in hypocotyl, cotyledon and leaf explants of 3 genotypes of *C. annuum* L. Higher frequency of callus induction was recorded from hypocotyl (79%) cotyledon (72%) and leaf (70%) explants of genotype var. X-235 than that of cultivars var. PC-1 and var. Pusa Jwala (Table 1). This may be due to growth regulators to promote the wound reaction, where the cells at the cut ends undergo mitosis which leads to callus formation. This may also be due to internal stimuli of the plant hormones and genotype influence. Nevertheless, our results agree with those from Agarwal *et al.,* (1989), Ashrafuzzaman *et al.,* (2009), Christopher and Rajam (1994,1996), Fari and Czako (1981), Gunay and Rao (1978), Kintzios *et al.,* (1996), Marziah *et al.,* (1995), Ochoa–Alejo and Ireta-Moreno (1990), Rodeva and Grozeva (2003), Rodeva *et al.,* (2006), Singh and Shukla (2001), Shao and Caponetti (1993), Subhash and Prolaram (1987) and Szasz *et al.,*(1995). On the other hand these results differ from what has been reported by Phillips and Hubstenberger (1995) who found that MS medium fortified with 2,4-D ranks as the best callus inductor in *Capsicum*. The calli derived from all the explants on MS medium supplemented with 2,4-D(1.0mg/l) and BAP(2.0mg/l) from all the explants were subsequently subcultured at 3, 6 and 9 weeks on the same medium for callus growth rate . The hypocotyl calli produce maximum fresh weight (1.16g) than cotyledon and leaf explants. Among the 3 cultivars the var. X-235 hypocotyl calli showed maximum growth rate i.e., 0.63(75.0%),0.92(46.0%) and 1.16(26.0%) at 3, 6 and 9 weeks of culture respectively (Table 2). The calli growth rate from all the explants showed maximum increase in first (3rd week) subculture than the second (6th week) and

Table 1. Frequency of callus induction (%) from hypocotyl, cotyledon and leaf explants of three genotypes of *Capsicum* L.

MS + Growth Regulators (mg/l)			*C. annuum* L. varieties								
			var. X-235			var. PC-1			var. Pusa Jwala		
2,4. D	NAA	BAP	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf
-	-	-	-	-	-	-	-	-	-	-	-
0.5	-	-	49.0	41.0	32.0	36.0	35.0	29.0	30.0	24.0	26.0
1.0	-	-	57.0	45.0	42.0	48.0	40.0	36.0	41.0	37.0	32.0
1.5	-	-	52.0	39.0	40.0	45.0	31.0	25.0	37.0	32.0	23.0
-	0.5	-	36.0	29.0	28.0	29.0	24.0	20.0	24.0	21.0	15.0
-	1.0	-	55.0	40.0	43.0	50.0	40.0	35.0	44.0	31.0	29.0
-	1.5	-	50.0	38.0	36.0	46.0	35.0	30.0	42.0	27.0	20.0
-	-	1.0	37.0	29.0	29.0	33.0	26.0	25.0	29.0	19.0	18.0
-	-	2.0	48.0	34.0	31.0	45.0	32.0	27.0	38.0	23.0	29.0
-	-	3.0	44.0	30.0	27.0	39.0	25.0	24.0	34.0	22.0	20.0
-	0.5	1.0	63.0	53.0	52.0	55.0	48.0	53.0	52.0	42.0	39.0
-	1.0	2.0	73.0	70.0	68.0	69.0	62.0	60.0	63.0	60.0	54.0
-	1.5	3.0	65.0	62.0	56.0	59.0	54.0	50.0	55.0	52.0	45.0
0.5	-	1.0	56.0	49.0	45.0	60.0	47.0	39.0	54.0	44.0	39.0
1.0	-	2.0	79.0	72.0	70.0	68.0	65.0	63.0	64.0	62.0	58.0
1.5	-	3.0	61.0	52.0	49.0	59.0	45.0	43.0	51.0	49.0	38.0

Values of twenty replicates (20X3)

third (9th week) subcultures respectively. This may be due to less totipotent and reduces mitotic activity as the calli tissues are getting older. Similar results were recorded in *Capsicum annuum* by De Donato *et al.,* (1989) and *Capsicum annuum* var. blue star by Kaparakis and Alderson (2003). Finally, the study on callus induction and callus growth rate from different explants i.e., (hypocotyl, cotyledon and leaf) of the genotypes *Capsicum annuum* L. offers the possibility to further investigation on pharmaceutical aspects for production of secondary metabolites.

Table 2. Mean fresh weights of hypocotyl, cotyledon and leaf calli of three genotypes of *Capsicum* L. at 3, 6 and 9 weeks.

C. annuum L. varieties	Callus fresh weight (grams)			
	Initial	3rd week	6th week	9th week
		Hypocotyl		
var. X-235	0.36±0.0012	0.63±0.0036(75.0%)	0.92±0.0029(46.0%)	1.16±0.0040(26.0%)
var. PC-1	0.23±0.0036	0.40±0.0018(70.0%)	0.56±0.0037(40.0%)	0.69±0.0045(23.0%)
var. Pusa Jwala	0.18±0.0030	0.26±0.0039(44.4%)	0.35±0.0027(34.6%)	0.42±0.0044(20.0%)
		Cotyledon		
var. X-235	0.31±0.0014	0.53±0.0029(70.9%)	0.75±0.0014(41.5%)	0.91±0.0020(21.0%)
var. PC-1	0.19±0.0022	0.32±0.0024(68.4%)	0.43±0.0024(34.3%)	0.50±0.0014(16.0%)
var. Pusa Jwala	0.15±0.0011	0.21±0.0036(40.0%)	0.25±0.0030(19.0%)	0.28±0.0028(12.0%)
		Leaf		
var. X-235	0.37±0.0020	0.62±0.0030(67.5%)	0.84±0.0031(35.0%)	0.98±0.0038(16.0%)
var. PC-1	0.24±0.0036	0.39±0.0041(62.5%)	0.50±0.0045(28.2%)	0.56±0.0072(12.0%)
var. Pusa Jwala	0.22±0.0032	0.30±0.0048(36.3%)	0.35±0.0050(16.0%)	0.38±0.0056(8.0%)

Mean of twenty replicates (20X3)
Figures in parenthesis indicate percentage increase/gain in fresh weight

CONCLUSIONS

Based on our data we can suggest that MS medium supplemented with 2,4-D(1.0mg/l) and BAP(2.0mg/l) is the best way to obtain an efficient callusogenesis in genotypes of *C. annuum* L. Among the genotypes var. X-235 responded better than var. PC-1 and var. Pusa Jwala. Moreover, it was also found that hypocotyl explants in chili was the best source for callus formation and callus growth rate. This protocol will pave way for future pharmaceutical experiments, to study the secondary metabolites production from callus cultures.

ACKNOWLEDGEMENTS

One of the authors (O. Aniel Kumar) is grateful to UGC-SAP, Department of Botany, AndhraUniversity, Visakhapatnam.

REFERENCES

Agrawal, S., Chandra, N. and Kothari, S.L. 1989. Plant regeneration in tissue cultures of pepper (*Capsicum annuum* L. cv. mathani). Plant Cell Tissue and Organ Culture. 6: 47-56.

Ashrafuzzaman, M., Hossain, M.M., Razi Ismail, M., Shahidul Haque, M., Shahidullah and Shahin-uz-zaman, S.M. 2009. Regeneration potential of seedling explants of chilli (*Capsicum annuum*). African Journal of Biotechnology. 8(4): 591-596.

Christopher, T. and Rajam, M.V. 1994. *In vitro* clonal propagation of *Capsicum* spp. Plant Cell Tissue and Organ Culture. 38: 25-29.

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

Cooking, E.C. 1986. Plant cell biology in the 21st Century: The needs of plant cell and tissue culture. In: C. E. Green (eds.). VI. International Congress of Plant Tissue and Cell Culture, University of Minnesota, Minneapolis (Abstracts). p.2.

De Donato, M., Perucco, E., Chiavazza, P. and Panei E. 1989. Activation of nuclear DNA replication and mitosis in excised cotyledons of *Capsicum annuum*. Plant Sci. 63: 217-225.

Evans, D.A., Sharp, W.R. and Flick, C.E. 1981. Growth behavior of cell cultures: Embryogenesis and organogenesis. In: T. A. Thorpe (eds.), Plant tissue culture methods and applications in Agriculture. Academic Press, New York. p. 45-113.

Fari, M. and Czako, M. 1981. Relationship between position and morphogenetic response of pepper hypocotyl explants cultured *in vitro*. Scientia Horticulture. 15: 207-213.

Flick, C.E., Evans, D.A. and Sharp, W.R. 1983. Organogenesis. In: D. A. Evans, W. R. Sharp., P. V. Amirato and Y. Yamada (eds). Hand book of Plant Cell Culture. Vol. 1. McMillan Publishing Co., New York. p. 13-81.

George, L. and Narayanaswamy, S. 1973. Haploid *Capsicum* through experimental androgenesis. Protoplasma. 78: 467-480.

Gunay, A.L. and Rao, P.S. 1978. *In vitro* plant regeneration from hypocotyls and cotyledon explants of red pepper (*Capsicum*). Plant Science Letters. 11: 365-372.

Kaparakis, G. and Alderson, P.G. 2003. Differential callus type formation by auxins and cytokinin in *in vitro* cultures of pepper (*Capsicum annuum* L.). Plant Biosystems. 137(3): 275-280.

Kintzios, S., Drossopoulos, J., Manousaridou, M. and Holevas, C.D. 1996. Competence for callus induction on mature pepper leaves depends upon specific developmental stages of the donor plant. Sci. Hortic. 65: 341-347.

Marziah, M., Zaliha A. and Kow, C.W. 1995. Studies of tissue culture of different chilli cultivars (*Capsicum annuum* L.). Asia Pacific Journal of Molecular Biology and biotechnology. 3(2): 96-105.

Morrison, R.A., Koning, R.E. and Evans, D.A. 1986. Handbook of Plant Cell Culture. Pepper, In: D.A. Evans, W. R. Sharp and P. V. Ammirato (eds.). Macmillan, New York. Vol. 4. p. 552-573.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Ochoa-Alejo, N. and Gomez-Peralta, J.E. 1990. Activity of enzymes involved in capsaicinoid biosynthesis in fruit and callus of chili pepper (*Capsicum annuum* L.). Abstracts VIIth International Congress on Plant Tissue and Cell Culture. IAPTC. Amsterdam. p. 363.

Ochoa-Alejo, N. and Ireta-Moreno, L. 1990. Cultivar differences in shoot-forming capacity of hypocotyl tissues of chili peppers (*Capsicum annuum* L.) cultured *in vitro*. Scientia Hort. 42: 21-28.

Phillips, G.C. and Hubstenberger, J.F. 1985. Organogenesis in pepper tissue culture. Plant Cell and Organ Culture. 4: 261-269.

Rodeva, V. and Grozeva, S. 2003. Induction of callusogenesis and regeneration *in vitro* in pepper (*Capsicum annuum* L.). Plant Science (Bulgaria). 40: 302-307.

Rodeva, V., Grozeva, S. and Todorova, V. 2006. *In vitro* answer of Bulgarian pepper (*Capsicum annuum* L.) varieties. Genetika. 38(2): 129-136.

Shao, W. and Caponetti, J. 1993. Callus production, organogenesis and plantlet formation in three cultivars of sweet pepper. J. of the Tennessee Academy of Science. 68(4): 113-118.

Singh, R.R. and Shukla, R. 2001. *In vitro* shoot differentiation in *Capsicum annuum* (L.) var. Accuminatum. J. Indian. Bot. Soc. 80: 301-303.

Subhash, K. and Prolaram, B. 1987. Induction of multiple shoots by gamma rays from cotyledon cultures of *Capsicum*. Current Science. 56(24): 1289-1291.

Sukrasno, N. and Yeoman, M.H. 1990. Phenolics in callus cultures of *Capsicum frutescens*. Abstracts VII International Congress on Plant Tissue and Cell culture. IAPTC. Amsterdam. p. 323.

Szasz, A., Nervo, G. and Fari, M. 1995. Screening for *in vitro* shoot forming capacity of seedling explants in bell pepper (*Capsicum annuum* L.) genotypes and efficient plant regeneration using thidiazuron. Plant Cell Reports. 14: 666-669.

Williams, P.D., A.K. Wilkinon, Lewis, J.A. Black, G.M. and Marituna, F. 1988. A method for the rapid production of fine plant cell suspension cultures. Plant Cell Reports. 7(6): 459-462.

****