Int.J.Curr.Microbiol.App.Sci (2017) *6*(5): 925-933

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 5 (2017) pp. 925-933**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.605.102

In vitro Androgenesis in Capsicum (*Capsicum annuum* L.)

Vivek Hegde[1]*, P.S. Partap[2], R.C. Yadav[3] and K.S. Baswana[2]

[1]Division of Crop Improvement, ICAR- Central Tuber Crops Institute, Thiruvananthapuram, Kerala, India
[2]Department of Vegetable Science, [3]Department of Molecular Biology and Biotechnology CCS Haryana Agricultural University, Hisar-125 004, Haryana, India
**Corresponding author*

Keywords

Capsicum
Anther culture,
Androgenesis,
Doubled Haploid,
Anther culture.

Article Info

Accepted:
12 April 2017
Available Online:
10 May 2017

A B S T R A C T

Experiment on anther culture in sweet pepper (*Capsicum annuum* L.) hybrids Bharat and Indra was carried out to investigate the responses of three anther developmental stages on the production of androgenic/microspore derived calli, using MS medium supplemented with various growth regulators in different ratios and with or without pre-culture heat treatment of anthers at 35°C for seven days. In general, heat treated anthers resulted in increased callus induction percentage in all growth regulator combinations and flower bud developmental stages. Most promising results were obtained on MS medium supplemented with 1.0 mg/l zeatin along with 0.2 mg/l 2, 4-D and 15.0 mg/l $AgNO_3$ with heat treatment reported maximum callus induction in both the hybrids, 54.02% and 60.92%, respectively when the anthers were at developmental stage-II (i.e. microspores at late uni-nucleate stage). Irrespective of anther developmental stage, zeatin was found superior in callus induction from cultured anthers followed by TDZ and Kinetin. Response of BAP was noted poor as compared to other cytokinins tested. The anther containing microspore at late uni-nucleate stage i.e. stage-II recorded maximum callus induction followed by anthers at stage-I having microspore at early uni-nucleate stage. The anthers at developmental stage-III, which contained mature pollen grains, were poor in callus production. Supplementation of MS medium with $AgNO_3$ 15.0 mg/l along with other growth regulators gave better results in all the stages of anther development.

Introduction

Capsicum (*Capsicum annuum* L.), commonly known as pepper, sweet pepper or bell pepper, belongs to the family Solanaceae and is one of the most important vegetable crops in the world. This indispensable item of kitchen occupying an important place in Indian diet is consumed daily in one or the other form as a condiment and spice, vegetable, adding colour, flavour, pungency and piquancy to various foodstuffs. It is an excellent source of vitamins A, B-complex, C, E and is rich in minerals like molybdenum, manganese, folate and potassium (Simonne *et al*., 1997).

In spite of number of different capsicum varieties found grown all over the world, the vulnerability of these genotypes consequent to the abiotic and biotic stresses including the extremes of temperature, moisture, light, nutrients, pH and the insect pests, fungi, bacteria and viruses has drastically restricted

their yield potential and quality (Ochoa and Ramirez, 2001; Egea *et al*., 2002 and Venkataiah *et al*., 2003). Although, the conventional plant breeding combined with improved agricultural practices has upgraded capsicum crop production and its quality, but the restricted gene pool has placed a limit to this technology. Hence, alternative biotechnological approaches, the more efficient and sustainable than conventional methods, are being used to produce uniform doubled haploid (DH) lines through *in vitro* regeneration of plants from microspore or pollen derived embryos or calli. Efficient methods to establish uniform DH lines are essential for researchers to map the genetic background of agronomically important traits as well as for breeders to shorten the breeding process required to produce homogenous plants that can be used directly as superior pure line varieties or in the production of hybrids, which would of greater benefit to the farmers. Also, haploids can be utilized to facilitate the detection of mutations and recovery of the unique recombinants.

According to various researchers, the androgenesis in pepper is often limited due to the difficulties encountered in plant regeneration and developing of shoots into normal plantlets (Nowaczyk and Kisiala, 2006; Kothari *et al*., 2010). The several reasons for this inefficiency in production of callus or embryo-like structures from anthers and subsequent plant regeneration include the genotypic dependence, collection of donor buds in the optimal stage, stress treatments as well as composition of medium and the culture conditions (Kothari *et al*., 2010 and Irikova *et al*., 2011).

Keeping these points in view, the present investigation was carried out to investigate the responses of three anther developmental stages from two hybrids on the production of androgenic/microspore derived calli cultured on MS medium supplemented with various growth regulators in different ratios and with or without pre-culture heat treatment of anthers at 35^{o}C for seven days.

Materials and Methods

The popular F_1 sweet pepper hybrids, Bharat and Indra were planted in a screen house at Research Farm of Department of Vegetable Science, CCS HAU, Hisar. Throughout the growing period, the normal cultivation practices were implemented to raise healthy plants. Plants were maintained free from insect-pests and diseases. To stimulate development of fresh flower buds, the young set fruits were removed periodically. During flowering period of plants, flower buds which were in the proper stage of anther development were collected and their anthers were cultured in different media.

Flower buds were collected at three different stages of microspore development (Plate 1). The size and morphology of flower buds can be used as an indirect indication for determining the stage of microspore development. Anthers from flower buds of different stages were subjected to cytological examination by staining with 2 per cent aceto-carmine dye after squashing and observed under microscope at 100x magnification (Plate 1).

These buds were washed with tap water and surface sterilized for one minute in 70 per cent alcohol followed by 15 minutes in a flask containing 2 per cent NaOCl solution and then rinsed 3 to 4 times with sterile distilled water to remove the traces of NaOCl. The anthers were aseptically removed from the buds carefully and were placed on Petri-plates containing MS medium with different concentrations and combinations of hormones, with or without silver nitrate at 15 mg/l and 100 mg/l sucrose. The cultured

anthers were given heat shock treatment by keeping the inoculated Petri-plates in incubator at 35°C under dark condition for 0 and 7 days and then shifting the cultures to culture room at 25±2°C under photoperiod of 16 h light and 8 h dark. After three to four weeks, anthers were sub-cultured on same treatment for callus maintenance. Calli from anthers with size >7.0 mm were transferred to MS medium supplemented with BAP, kinetin, TDZ and Zeatin each of at 5 and 7.5 mg/l along with or without 0.2 mg/l NAA (shoot induction medium). Cultures were then incubated in culture room at 25±1°C temperature, under photoperiod of 16 h light and 8 h dark. The number of days taken to show callogenesis from the date of inoculation and Per cent response of anthers to callus initiation in various treatments on MS medium was recorded. The mean and standard errors were worked out from triplicate data obtained from various experiments. The per cent data transformed using angular transformation and analyzed following Completely Randomized Design (CRD).

Results and Discussion

The anther culture of capsicum hybrids Bharat and Indra was carried out on MS medium modified with addition of growth regulators and growth additives. Flower buds collected at three different stages of development (Plate 1) were used for anther culture. Effect of heat treatment of these anthers at 35°C for 7 days under dark condition on the response of cultured anthers along with different growth regulators was also studied. Results obtained have been summarized sequentially in table 1. By studying these factors, an effort was made to bring about maximum callus induction and regeneration. Importance of these factors found to affect anther culture in capsicum as encountered during the course of study is discussed below.

The genotype is the most important and often limiting factor in the pepper androgenic response (Wang and Zhang, 2001; Rodeva *et al*., 2004; Liu *et al*., 2007; Lantos *et al*., 2012). The results of the present *in vitro* anther culture study on capsicum hybrids Bharat and Indra amply demonstrated genotypic differences in response of anther culture. The androgenic potential was highest in hybrid Indra followed by Bharat. Maximum callus induction was reported in hybrid Indra followed by Bharat when the anthers were having microspores at late uni-nucleate stage (stage II). The androgenic potential of anthers at stage I and stage II were also high in hybrid Indra (Table 1). These results, i.e. genotype dependence on androgenic response are in consistent with the reports of several earlier workers (Dumas de Vaulx *et al*., 1981; Rodeva *et al.*, 2004; Lantos *et al*., 2009; Zhao *et al.*, 2010; Niklas *et al*., 2012; Liljana *et al*., 2013; Luitel and Kang, 2013 and Olszewska *et al.*, 2014).

The donor plant for androgenic embryos inductions was usually of hybrid forms, since higher the degree of heterozygosity, greater are the chances of producing androgenic embryos (Niklas *et al*., 2012). Liljana *et al*., (2013) reported that, some pepper genotypes as bell-shape and sweet ones had higher androgenic potential than the hot genotypes. Olszewska *et al.*, (2014) observed androgenic embryo development only in three out of the 17 Capsicum genotypes on medium with kinetin at 0.1 mg/l while, 12 genotypes induced androgenic embryos on medium with kinetin at 0.3 mg/l. Contrary to above results by various researchers, the present study in capsicum indicated the importance of heat pre-treatment of anthers, microspore developmental stages, genotype and growth regulators combinations for callus induction and its growth (Table 1). The hybrids under study showed recalcitrant behaviour to regeneration. Greening of calli (Plate 2d) was observed when transferred to the regeneration

media, while shoot differentiation failed to occur in both the hybrids. The inability of callus to regenerate despite greening might be due to the very low regeneration ability of the genotypes under investigation.

It is of paramount importance to select flower buds at optimum stage of pollen development (Dumas de Vaulx *et al*., 1981; Lantos *et al*., 2009). Stages are however difficult to be identified. For large scale production of microspore derived plants, the indirect method of correlating microspore development with anther and/or flower bud size and morphology is often used to provide a way to select anthers at proper stage of development. In order to identify and select the starting material at the onset of the culture, an initial analysis of the flower bud morphology, the size of the anthers and the developmental stage of the microspores were performed by 2 per cent aceto-carmine staining on squashes of tissue. Anthers from buds with petals slightly longer than the sepals (Plate 1, 1b) were the most responsive for embryogenesis induction. These preferentially contained microspores in the late-uninucleate phase (Plate 1, 3b), as determined by aceto-carmine staining. In the present investigation, maximum callus induction in both the hybrids Indra (60.92%) and Bharat (54.02%) was observed from the anthers at developmental stage II (i.e. microspores being at late uninucleate stage), which was found to coincidence with flower bud and anther morphology. At developmental stage II, corolla (petals) and calyx (sepals) were of equal length or with petals slightly longer than sepals and anthers showing light anthocyanin tinge in the distal end. This was in accordance with the result obtained by Dumas de Vaulx *et al.*, (1981) who used anthers from flower buds where the microspores were at the late uni-nucleate stage, i.e. equivalent to a bud size having sepals and petals of equal length. Similar bud size was reported to be best for callus induction and regeneration by Lantos *et al*., (2009).

Thermal shock given to plants can alter the mode of division of microspores (Sax, 1935). In most solanaceous crops placing excised buds at high or low temperature was found to be effective for higher response of cultured anthers (Kristiansen and Anderson, 1993 and Nowaczyk *et al*., 2006). In the present study, heat treatment of cultured anthers at 35^{o}C for seven days was found to influence callus induction frequency. After 7 days of cultured anthers with heat treatment, responsive anthers were swelled and increased in size. Later, 20 to 30 days of culture, callus emerged from sides of the responsive anther's sacs (Plate 2 b&c).

Maximum callus induction was reported in both the hybrids *i.e.* Indra (60.9%) and Bharat (54.0%), when the anthers were pre-treated at 35^{o}C for seven days. Without heat treatment the callus per cent decreased to 43.7% and 36.8% in hybrid Indra and Bharat, respectively. Heat treatment increased the androgenic callus formation in all the treatments in both the hybrids (Table 1). The results are in conformity with the results of earlier other investigators. Nowaczyk *et al*., (2006) incubated in darkness in the temperature of 35°C for 8 days and transferred to culture room with the temperature of 25°C. Taskin *et al.*, (2011) obtained embryos from anthers after heat pretreatment at 35°C. Similarly, Luitel and Kang (2013) cultured pepper anthers and kept them in dark at 35°C for seven days. Although the mode of action is not clear, a thermal treatment seems to activate the anthers for androgenesis.

Table.1 Effect of MS media supplemented with different growth regulators, anther developmental stage and heat treatment on anther culture response of Capsicum hybrids

Growth regulators (mg/l)			Per cent callus formation in Bharat						Per cent callus formation in Indra					
			Wthout heat treatment			With heat treatment			Wthout heat treatment			With heat treatment		
BAP	2,4-D	$AgNO_3$	Anther developmental stage			Anther developmental stage			Anther developmental stage			Anther developmental stage		
			I	II	III	I	II	III	I	II	III	I	II	III
0.1	0.2	-	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.2 (6.2)	0.0 (0.0)
0.1	0.2	15	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.5 (7.0)	4.6 (12.4)	0.0 (0.0)
0.5	0.2	-	1.2 (6.2)	6.9 (15.2)	0.0 (0.0)	12.3 (20.5)	24.1 (29.4)	0.0 (0.0)	1.2 (6.2)	13.8 (21.8)	0.0 (0.0)	14.6 (22.4)	31.0 (33.8)	1.5 (7.0)
0.5	0.2	15	4.2 (11.8)	15.3 (23.0)	1.2 (6.2)	21.5 (27.6)	32.6 (34.8)	3.1(10.0)	6.5 (14.8)	21.8 (27.9)	1.9 (7.9)	23.8 (29.2)	39.5 (38.9)	5.4 (13.4)
1.0	0.2	-	1.2 (6.2)	6.5 (14.8)	0.0 (0.0)	13.0 (21.1)	23.8 (29.2)	1.2 (6.2)	1.9 (7.9)	14.2 (22.1)	0.0 (0.0)	15.3 (23.0)	30.7 (33.6)	1.9 (7.9)
1.0	0.2	15	4.6 (12.4)	14.9 (22.7)	1.2 (6.2)	21.8 (27.9)	32.2 (34.6)	3.8 (11.3)	6.9 (15.2)	21.8 (27.9)	2.3 (8.7)	24.1 (29.4)	39.1 (38.7)	6.1 (14.3)
Kin	2,4-D	$AgNO_3$												
0.1	0.2	-	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	2.7 (9.4)	0.0 (0.0)
0.1	0.2	15	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.2 (6.2)	1.5 (7.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	3.1 (10.1)	6.9 (15.2)	0.0 (0.0)
0.5	0.2	-	1.2 (6.2)	11.5 (19.8)	0.0 (0.0)	13.8 (21.8)	28.7 (32.4)	1.2 (6.2)	1.9 (7.9)	18.4 (25.4)	0.0 (0.0)	16.1 (23.6)	35.6 (36.6)	1.9 (7.9)
0.5	0.2	15	6.5 (14.8)	21.5 (27.6)	1.5 (7.0)	23.8 (29.2)	38.7 (38.5)	5.4 (13.4)	8.8 (17.3)	28.4 (32.2)	2.3 (8.7)	26.1 (30.7)	45.6 (42.5)	7.7 (16.1)
1.0	0.2	-	1.5 (7.0)	11.9 (20.1)	0.0 (0.0)	14.6 (22.4)	29.1 (32.7)	1.2 (6.15)	2.3 (8.7)	18.8 (25.7)	0.0 (0.0)	16.9 (24.2)	36.0 (36.9)	1.9 (7.9)
1.0	0.2	15	6.9 (15.2)	21.8 (27.9)	1.9 (7.9)	24.1 (29.4)	39.1 (38.7)	5.8 (13.9)	9.2 (17.7)	28.7 (32.4)	2.3 (8.7)	26.4 (30.9)	46.0 (42.7)	8.1 (16.5)
TDZ	2,4-D	$AgNO_3$												
0.1	0.2	-	0.0 (0.0)	0.8 (4.1)	0.0 (0.0)	1.2 (6.2)	9.2 (17.6)	1.2 (6.2)	0.0 (0.0)	1.2 (6.2)	0.0 (0.0)	3.5 (10.7)	16.1 (23.6)	2.3 (8.7)
0.1	0.2	15	0.0 (0.0)	2.7 (9.4)	0.0 (0.0)	4.2 (11.8)	19.9 (26.5)	3.5 (10.7)	0.0 (0.0)	9.6 (18.0)	1.2 (6.2)	6.5 (14.8)	26.8 (31.2)	5.8 (13.9)
0.5	0.2	-	2.3 (8.7)	18.0 (25.1)	0.0 (0.0)	18.0 (25.1)	35.3 (36.4)	1.2 (6.2)	3.5 (6.2)	24.9 (29.9)	0.0 (0.0)	20.3 (26.8)	42.2 (40.5)	3.1 (10.0)
0.5	0.2	15	9.2 (17.7)	26.8 (31.2)	1.5 (7.0)	26.4 (30.9)	44.1 (41.6)	5.4 (13.4)	10.7 (14.8)	33.7 (35.5)	2.3 (8.7)	28.7 (32.4)	51.0 (45.5)	7.7 (16.1)
1.0	0.2	-	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.2 (6.2)	1.9 (7.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.9 (7.9)	7.7 (16.1)	1.2 (6.2)
1.0	0.2	15	0.0 (0.0)	1.2 (6.2)	0.0 (0.0)	3.5 (10.7)	11.9 (20.1)	1.2 (6.2)	0.0 (0.0)	1.5 (7.0)	0.0 (0.0)	4.6 (12.4)	18.8 (25.7)	1.5 (7.0)
ZTn	2,4-D	$AgNO_3$												
0.1	0.2	-	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.9 (7.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.8 (4.1)	7.7 (16.1)	0.0 (0.0)
0.1	0.2	15	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	3.1 (10.0)	5.4 (13.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.0 (12.9)	12.3 (20.5)	1.2 (6.4)
0.5	0.2	-	3.8 (11.3)	25.3 (30.2)	0.0 (0.0)	21.1 (27.3)	42.5 (40.7)	1.2 (6.2)	6.1 (7.9)	32.2 (34.6)	0.0 (0.0)	23.4 (28.7)	49.4 (44.7)	2.7 (9.4)
0.5	0.2	15	12.6 (20.8)	36.4 (37.1)	1.9 (7.9)	29.9 (33.1)	54.0 (47.3)	6.9 (15.2)	14.6 (17.3)	43.3 (41.1)	2.7 (9.4)	32.2 (34.5)	60.5 (51.1)	9.2 (17.7)
1.0	0.2	-	4.6 (12.4)	25.7 (30.4)	0.0 (0.0)	21.8 (27.9)	42.9 (40.9)	1.5 (7.0)	6.9 (8.7)	32.6 (34.8)	0.0 (0.0)	24.1 (29.4)	49.8 (44.9)	3.1 (10.0)
1.0	0.2	15	13.0 (21.1)	36.8 (37.3)	2.3 (8.7)	30.3 (33.4)	54.0 (47.3)	7.3 (15.6)	15.3 (17.7)	43.7 (41.4)	3.5 (10.7)	32.6 (34.8)	60.9 (51.3)	9.6 (18.0)
CD at 5%			0.8	1.4	1.0	0.9	1.2	0.9	1.1	0.8	0.6	1.7	0.9	1.4

Figures in parenthesis are angular transformed values

Table.2 Effect of MS media supplemented with different growth regulators used for shoot induction from androgenic callus

Different growth regulators (mg/l)		Greening (%)			
BAP	**NAA**	**Bharat**		**Indra**	
5.0	-	0.00	(0.0±0.0)	0.00	(0.0±0.0)
5.0	0.2	31.11	(33.9±1.4)	33.33	(35.3±0.0)
7.5	-	0.00	(0.0±0.0)	0.00	(0.0±0.0)
7.5	0.2	33.33	(35.3±0.0)	35.56	(36.6±1.3)
Kinetin	**NAA**				
5.0	-	6.67	(15.0±0.0)	8.89	(17.1±2.2)
5.0	0.2	48.89	(44.4±1.3)	51.11	(45.6±1.3)
7.5	-	8.89	(17.1±2.2)	8.89	(17.1±2.2)
7.5	0.2	51.11	(45.6±1.3)	53.33	(46.9±0.0)
TDZ	**NAA**				
5.0	-	0.00	(0.0±0.0)	0.00	(0.0±0.0)
5.0	0.2	0.00	(0.0±0.0)	0.00	(0.0±0.0)
7.5	-	0.00	(0.0±0.0)	0.00	(0.0±0.0)
7.5	0.2	0.00	(0.0±0.0)	0.00	(0.0±0.0)
Zeatin	**NAA**				
5.0	-	0.00	(0.0±0.0)	0.00	(0.0±0.0)
5.0	0.2	15.56	(23.1±1.7)	17.78	(24.8±1.7)
7.5	-	0.00	(0.0±0.0)	0.00	(0.0±0.0)
7.5	0.2	15.56	(23.1±1.7)	17.78	(24.8±1.7)
CD at 5%		2.86		3.11	

Figures in parenthesis are angular transformed values

Plate.1 Flower buds, anther morphology and developmental stages of pepper microspores: Based on anther morphology and microspore stage of development the flower buds collected were divided into three different groups (1a, 2a & 3a). Colour of the anthers is viewed as good indicators of stage of microspore development (1b, 2b & 3b). Stages of microspore development in anthers were determined using a stereo microscope, (1c, 2c & 3c). Group-1 flower buds consisted of anthers with uni-nucleate microspores (1a–c); Group-2 flower buds consisted of anthers with 80% uni-nucleate and 20% bi-nucleate microspores (2a–c); and Group-3 flower buds consisted of anthers with pollen grains (3a–c).

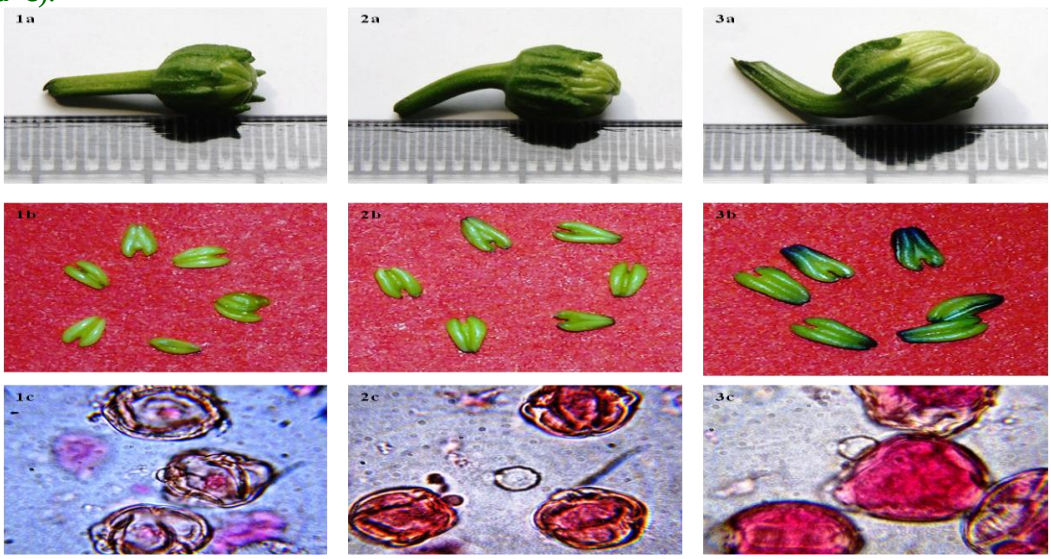
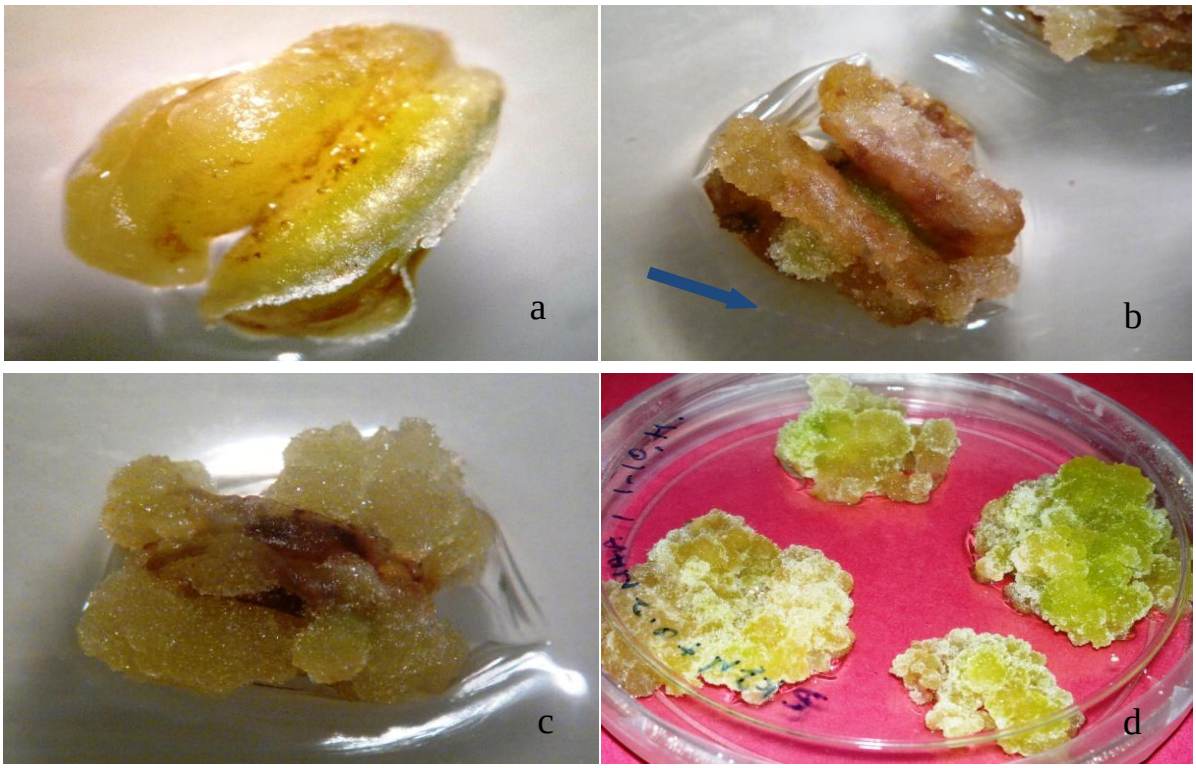


Plate.2 (a) Splitting of cultured anthers after heat treatment, (b) Emerging callus from the cultured anther, (c) Androgenic callus development and (d) Greening of androgenic callus



Results on effect of MS media supplemented with three concentrations of individual plant growth regulators like BAP, kinetin, TDZ and zeatin along with 2,4-D at 0.2mg/l and $AgNO_3$ at 15mg/l, and three flower bud developmental stages along with heat treatment at 35°C for seven days on androgenic response of two capsicum hybrids are presented in table 1. Anther response is enhanced with the suitable balance of auxins and cytokinins in the culture medium. In the present experiment, the results showed the need for growth regulators i.e. both auxins and cytokinins to achieve androgenesis from anthers. It was found that the addition of silver nitrate ($AgNO_3$) in combination with cytokinins and auxins had a profound influence on callus induction frequency in both the hybrids. Callus induction frequency also depends on type of cytokinins used (BAP, kinetin, TDZ and zeatin). The growing medium supplemented with zeatin was found superior for callus induction from cultured anthers followed by TDZ and kinetin (Table 1).

Beneficial effect of auxin and cytokinins added to the culture media on haploid callus and embryo induction was reported by Nowaczyk *et al.*, (2006); Supena and Custers (2011); Lantos *et al.*, (2012); Luitel and Kang (2013) and Olszewska *et al.*, (2014). In both the hybrids, the percentage of callusing anthers was significantly higher on medium supplemented with zeatin 0.5 at mg/l along with 2, 4-D at 0.2 mg/l and $AgNO_3$ at 15.0 mg/l (Plate 2b&c). In hybrid Bharat, the MS medium supplemented with zeatin at 0.5, 1.0 mg/l along with 2,4-D at 0.2 mg/l and $AgNO_3$ at 15.0 mg/l reported maximum callus induction (54.0%) when the anthers were at developmental stage II (i.e. microspores were at stage of late uni-nucliate). Callus induction response under BAP was the poor as compared to other cytokinins tested in capsicum hybrid Bharat. Similarly in Indra, the MS medium supplemented with zeatin at 1.0 mg/l along with 2,4-D at 0.2 mg/l and $AgNO_3$ at 15.0 mg/l was reported maximum callus induction (60.9%). These results were at par

(60.5%) with the medium containing zeatin at 0.5 mg/l along with 2,4-D at 0.2 mg/l and $AgNO_3$ at 15.0 mg/l when the cultured anthers were at developmental stage II. Irrespective of anther developmental stages, the zeatin was found superior for callus induction from cultured anthers followed by TDZ and kinetin. BAP was the poor responder as compared to other cytokinins. The supplementation of $AgNO_3$ 15.0 mg/l along with growth regulators gave better results in all stages of anther development in both the hybrids studied. $AgNO_3$ acted as an inhibitor of ethylene production during *in vitro* plant tissue culture (Santana *et al*., 2006). Nowaczyk and Kisiala (2006) cultured anthers on medium with an addition silver nitrate at 5 mg/1, which resulted in a significant improvement of androgenesis effectiveness in anther culture of pepper.

The effect of different cytokinins, BAP, kinetin, TDZ and zeatin along with auxin NAA on regeneration of androgenic callus was studied in both the hybrids, but shoot induction was not achieved in the present study. Hence, greening of callus in particular medium was recorded and their results are presented in table 2. Maximum greening of callus in both the hybrids was observed on MS medium supplemented with kinetin at 7.5 mg/l along with NAA at 0.2 mg/l recorded maximum per cent greening of callus i.e. 51.11%, and 53.33% respectively in both the hybrids Bharat and Indra. This results was followed by MS media supplemented with kinetin at 5.0 mg/l along with NAA at 0.2 mg/l. However, no further organogenesis was noticed (Plate 2d). Therefore, further efforts are needed to critically investigate microspore genesis with the aim of breaking existing barriers, refinement of conditions facilitating embryo induction using the alternative responsive genotypes or responsive genotype could be crossed with recalcitrant genotype to produce recombinant lines with higher androgenic capabilities and developing better androgenesis to generate successful double haploid plants in capsicum for direct use as pure line varieties and in the production of hybrids.

References

Dumas de Vaulx, R., Chambonnet, D. and Pochard, E. 1981. *In vitro* anther culture in red pepper (*Capsicum annuum* L.): improvement of the rate of plant production in different genotypes by treatments at 35°C. *Agronomie*, 1: 859–864.

Egea, C., Dickinson, M.J., Candela, M. and Candela, M.E. 2002. β-1,3-glucanase isoenzyme and genes in resistant and susceptible pepper (*C. annuum*) cultivars infected with *Phytopthora capsici*. *Physiologia Plantarum,* 107: 312–318.

Irikova, T., Grozeva, S. and Rodeva, V. 2011. Anther culture in pepper (*Capsicum annuum* L.) *in vitro*. *Acta Physiologiae Plantarum*, 33: 1559-1570.

Kothari, S.L., Joshi, A., Kachhwaha, S. and Ochoa, A.N. 2010. Chilli peppers—a review on tissue culture and transgenesis. *Biotechnol. Adv.*, 28: 35-48.

Kristiansen, K. and Andersen, S.B. 1993. Effects of donor plant temperature photoperiod, and age on anther culture response of *Capsicum annuum* L. *Euphytica*, 67: 105-109.

Lantos, C., Juhasz, A., Somogyi, G., Otvos, K., Vagi, P., Mihaly, R., Kristof, Z., Somogyi, N. and Pauk, J. 2009. Improvement of isolated microspore culture of pepper (*Capsicum annuum* L.) *via* co-culture with ovary tissues of pepper or wheat. *Plant Cell, Tissue and Organ Culture*, 97: 285–293.

Lantos, C., Juhasz, A.G., Vagi, P., Mihaly, R., Kristof, Z, and Pauk, J. 2012. Androgenesis induction in microspore culture of sweet pepper (*Capsicum annuum* L). *Plant Biotechnol. Reports*, 6: 123-132.

Liljana, R. Gudeva, K., Gulaboski, R., Ivanovska, J.E., Trajkova, F. and Maksimova, V. 2013. Capsaicin-inhibitory factor for somatic embryogenesis in pepper anther culture. *Electronic J. Biol.*, 9(2): 29-36.

Liu, F., Zhao, H., Chen, B. and Zhang, Y.Y.

2007. Embryogenesis of microspore derived multicells in *Capsicum annuum* L. *Fen. Zi Xi Bao Cheng. Wu Xue. Bao*, 40: 371–379.

Luitel, B.P. and Kang, W.H. 2013. *In vitro* androgenic response of minipaprika (*Capsicum annuum* L.) genotypes in different culture media. *Hort. Environ. Biotechnol*., 54(2): 162-171.

Niklas, N.A., Olszewska, D. Kisiała, A. and Nowaczyk, P. 2012. Study of individual plant responsiveness in anther cultures of selected pepper (*Capsicum spp*.) genotypes. *Folia Horticulturae*, 24/2: 141-146.

Nowaczyk, P. and Kisiala, A. 2006. Effect of selected factors on the effectiveness of *Capsicum annuum* L. anther culture. *J. Applied Genetics*, 47(2): 113-117.

Nowaczyk, P., Kisiała, A. and Olszewska, D. 2006. Induced androgenesis of *Capsicum frutescens* L. *Acta Physiologiae Plantarum*, 28 (1): 35-39.

Ochoa, N.A. and, Ramirez, M.R. 2001. *In vitro* chilli pepper biotechnology. *In Vitro Cellular and Developmental Biol. Plant*, 37: 701-729.

Olszewska, D., Kisiala, A., Niklas, N.A. and Nowaczyk, P. 2014. Study of *in vitro* anther culture in selected genotypes of genus *Capsicum*. *Turkish J. Biol.*, 38: 118-124.

Rodeva, V., Irikova, T. and Todorova, V. 2004. Anther culture of pepper (*Capsicum annuum* L.): comparative study on effect of the genotype. *Biotechnology and Biotechnological Equipment*, 3: 34-38.

Santana, B.N., Canto, F.A., Iglesias, A.L.G., Montalvo, P.M.C., Lopez, P.G. and Barahona, P.F. 2006. Improvement of *in vitro* culturing of Habanero pepper by inhibition of ethylene effects. *Hort. Sci.*, 41: 405–409.

Sax, K. 1935. The effect of temperature on nuclear differentiation in microspore development. *J. Arnold Arboretum*, 16: 301-310.

Simonne, A.H., Simonne, E.H., Eitenmiller, R.R., Mills, H.A. and Green, N.R. 1997. Ascorbic acid and provitamin A contents in unusually colored bell peppers (*Capsicum annuum* L). *Food Components Analysis*, 10: 299-311.

Supena, E.D.J. and Custers, J.B.M. 2011. Refinement of shed-microspore culture protocol to increase normal embryos production in hot pepper (*Capsicum annuum* L). *Scientia Horticulturae*, 130:769-774.

Taskin, H., Buyukalaca, S., Keles, D. and Ercan E. 2011. Induction of microspore-derived embryos by anther culture in selected pepper genotypes. *African J. Biotechnol.*, 10(75): 17116-17121.

Venkataiah. P., Christopher, T. and Subhash, K. 2003. Thiadiazuron induced high frequency adventitious shoot formation and plant regeneration in *Capsicum annuum* L. *J. Plant Biotechnol.*, 5: 245–250.

Wang, L.H. and Zhang, B.X. 2001. Advancement in the anther culture of *Capsicum annuum* L. *China Vegetables*, 3: 52-53.

Zhao, J.Z., Xue, X., Zhang, Z.Q., Yang, B.Z. and Zhou, S.D. 2010. Influences of carbon sources and plant growth regulators on anther culture efficiency of pepper. *Agri. Sci. Technol. Hunan*, 11(4): 102-105.

How to cite this article:

Vivek Hegde, P.S. Partap, R.C. Yadav and Baswana, K.S. 2017. *In vitro* Androgenesis in Capsicum (*Capsicum annuum* L.). *Int.J.Curr.Microbiol.App.Sci.* 6(5): 925-933.
doi: https://doi.org/10.20546/ijcmas.2017.605.102