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Induction of Embryogenesis in Anthers of *Capsicum annuum* var. Arka Meghana

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Abstract

Arka Meghana is a high yielding hybrid variety of chilli suitable for cultivation in South India and is tolerant to powdery mildew and chilli mosaic disease. The present study is the first report on successful embryogenic induction during anther culture of Arka Meghana. The suitable stage for culture establishment was buds collected at six and nine days after bud initiation. Sterilization with 70 per cent ethanol for 30 seconds followed by 4 per cent sodium hypochlorite for 15 minutes was optimum for establishing aseptic cultures. The best growth regulator combinations for indirect embryogenesis and direct embryogenesis were MS + 4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25 per cent activated charcoal + 10 mgL^{-1} $AgNO_3$ and MS + 4.00 mgL^{-1} NAA + 0.50 mgL^{-1} BA + 0.25 per cent activated charcoal + 15 mgL^{-1} $AgNO_3$ respectively at culture conditions of 35°C initial incubation temperature for two days in darkness followed by incubation at 25°C with 12 hour photoperiod.

Keyword : Anther culture, Arka meghana, Callogenesis, Embryogenesis, Incubation conditions

Chilli (*Capsicum annuum* L.) is an important horticultural crop which belongs to the family Solanaceae. Chilli is consumed all around the world and is a source of a wide number of phytochemicals, minerals, dietary fiber and vitamins like vitamin C and A *i.e.,* beta-carotene and other carotenoid pigments like lycopene and zeaxanthin, which have anticancer properties (Anilkumar & Rao, 2018 and Olatunji & Afolayan, 2018). The active compound, capsaicin, responsible for the pungency of chilli is also proved to have medicinal uses.

Chilli possesses a high susceptibility to fungal and viral pathogens which constricts the production in a huge way to meet the current demand. The development of pure homozygous plant material is a significant step in the development of high yielding hybrids. Conventional breeding methods are tedious and will take 5-6 generations of continuous selfing for homozygous production. Moreover, the pure lines produced by conventional breeding need not be 100 per cent homozygous (Germana, 2006).

Doubled haploid production can introduce homozygosity in a single generation. Doubled haploids can be produced in a single generation by chromosome doubling of haploid plants by colchicine treatment. Haploids can be induced in plants using a variety of techniques like wide species hybridization and chromosome elimination, androgenesis, gynogenesis *etc.* (Forster *et al.,* 2007). Haploid induction through *in vitro* techniques can accelerate the plant breeding studies and plays an important role in the development of new cultivars and early release of varieties (Mityko & Fari, 1997 and Kele *et al.,* 2015). But there are many factors influencing *in vitro* haploid induction and subsequent production of plantlets like genotype, developmental stage of

gametes, pre-treatment, physical environmental factors (temperature, humidity, dark and light period) and the composition of the culture medium (Touraev *et al.,* 1996 and Debina *et al.,* 2016). Hence, a standard protocol is required for large-scale haploid production in crop plants.

Arka Meghana is a high yielding hybrid variety of chilli suitable for cultivation in South India. It is tolerant to powdery mildew and chilli mosaic disease. A successful protocol for haploid production in Arka Meghana is not yet established. Hence the present study was undertaken with the objective to induce embryogenesis in *Capsicum annuum var.* Arka Meghana by anther culture.

Material and Methods

Standardization of Microspore Stage Suitable for Androgenesis

F1 hybrid seeds of Arka Meghana were procured from IIHR, Bangalore. The seeds were germinated in protrays filled with 1:1 mixture of soil and composted coir pith. Two week old seedlings were transplanted into pots of 30 cm diameter and 45 cm height filled with potting mixture consisting of vermicompost, cow dung manure and soil in 1:1:1 ratio. The pots were maintained in open field conditions.

Buds of different stages were collected at three day intervals from Arka Meghana plants grown in open condition and anthers isolated from these buds were used as explants. Determination of the stage of microspores was carried out by aceto-orcein staining (1%) (Fig.1). The number of microspores at the late uninucleate stage and early binucleate stage per hundred microspores were counted and the percentage was calculated for each stage of bud (Table 1).

Surface Sterilization of Flower Buds

The buds were treated with 70 per cent ethanol followed by 4 per cent sodium hypochlorite for different time intervals. The treated buds were washed with autoclaved double distilled water three times, five minutes each.

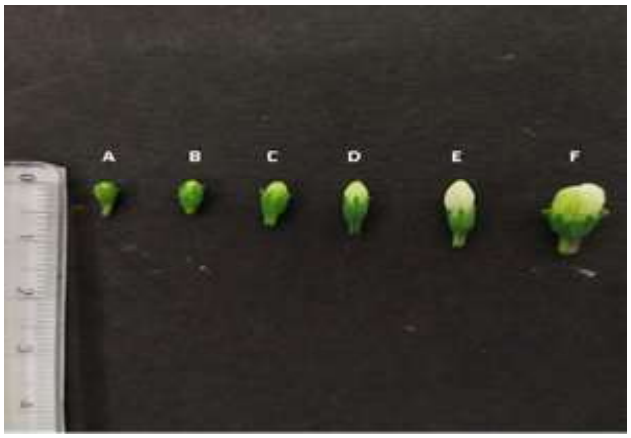

Fig. 1 : Flower buds of Arka Meghana collected at three day intervals after bud initiation: (A) Three days after bud initiation (B) Six days after bud initiation (C) Nine days after bud initiation (D) Twelve days after bud initiation (E) Fifteen days after bud initiation (F) Eighteen days after bud initiation

Table 1

Percentage of uninucleate and binucleate microspores in different stages of bud

Stage of bud	Late uninucleate microspore (%)	Early binucleate microspore (%)
3 days after bud initiation	5	0
6 days after bud initiation	57	34
9 days after bud initiation	21	49
12 days after bud initiation	7	12
15 days after bud initiation	0	6
18 days after bud initiation	0	0

Inoculation

The flower buds after surface sterilization were transferred to autoclaved petri plates and blotted with sterile blotting paper. Sterile forceps were used to remove the sepals from the bud and open the petals. Care was taken to remove the filament from the anthers.

The anthers were placed onto the media with their concave face touching the medium. The anthers (maximum of four) from the same buds were cultured in a petri plate. Anthers were inoculated onto nine media combinations *viz.,* full strength MS [Murashige and Skoog (1962)] medium and CP [De Vaulx- (1981)] media with different concentrations of plant growth

TABLE 2
Composition of media used for the study

Treatment	Basal Medium	Supplements	Reference
T_1	MS	0.10 mgL^{-1} kinetin + 0.004 mgL^{-1} 2,4-D	Irikova and Rodeva (2004)
T_2	MS	2.00 mgL^{-1} IAA + 0.30 mgL^{-1} BA	Gonzalez -Garcia (2002)
T_3	MS	4.00 mgL^{-1} NAA + 1.00 mgL^{-1} BA + 0.25% Activated charcoal	Ciner and Tipirdamaz (2002)
T_4	MS	4.00 mgL^{-1} NAA + 1.00 mgL^{-1} BA	Comlekcioglu and Ellialtioglu (2018)
T_5	MS	4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% Activated charcoal + 10mgL^{-1} $AgNO_3$	Comlekcioglu and Ellialtioglu (2018)
T_6	MS	4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% Activated charcoal + 15mgL^{-1} $AgNO_3$	Buyukalaca *et al*. (2004)
T_7	MS	4.00 mgL^{-1} NAA + 0.50 mgL^{-1} BA+ 0.25% Activated charcoal + 15 mgL^{-1} $AgNO_3$	Keles *et al*. (2015)
T_8	CP	2.00 mgL^{-1} Kinetin + 0.10 mgL^{-1} 2,4-D	Irikova *et al*. (2011)
T_9	CP	0.01 mgL^{-1} Kinetin + 0.01 mgL^{-1} 2,4-D	De Vaulx *et al*. (1981)

regulators such as kinetin, BA, NAA, IAA and 2, 4-D along with silver nitrate and activated charcoal as additives (T_1 - T_9) under varying culture conditions (Table 2).

Incubation

The anthers inoculated in MS media (T_1-T_7) were retained in the same media for regeneration. Anthers inoculated in CP medium (T_8 and T_9) were transferred after 12 days to CP medium supplemented with 0.01 mgL^{-1} kinetin for regeneration. Culture conditions are detailed in Table 3. The cultures were initially incubated at 25°C for 2/8 days in nine media combinations. The six media combinations that showed good response were tried in initial incubation condition of 35°C for 2 days also. After initial incubation, all the cultures were transferred to the culture room at 25 ± 2 °C with 12 hours light photoperiod.

TABLE 3
Incubation conditions

Incubation Temperature	Days in Darkness
25°C	2
25°C	8
35°C	2

RESULTS AND DISCUSSION

Standardisation of Microspore Stage Suitable for Androgenesis

The flower buds collected at six days after bud initiation had 57 per cent late uninucleate and 34 per cent early binucleate microspores whereas, the flower buds collected at nine days after bud initiation had 21 per cent late uninucleate and 49 per cent early binucleate microspores. The buds collected at six and nine days after bud initiation were observed to be most suitable for anther culture in our study. This was in accordance with the results obtained by Supena *et al*. (2006) in Indonesian hot chilli genotypes and Lantos *et al*. (2009) in Hungarian and Spanish chilli genotypes.

It is essential to correlate the microspore stage with the size and morphology of the flower buds for ease of explant collection. Morphological observation of the flower buds collected at six days after bud initiation, had most of the microspores in the late uninucleate stage with an almost uniform length of calyx and corolla. Similar results are reported by Buyukalaca *et al*. (2004) in U-247 and U-238

genotypes of *Capsicum annuum* L. and Nowaczyk *et al.* (2014) in hybrids of cross between *Capsicum frutescens* L. and *Capsicum annuum* L. The flower buds collected at nine days after bud initiation had the corolla slightly longer than the calyx and the majority of the microspores in the early binucleate stage. Ciner and Tipirdamaz (2002) in Malatya genotype of chilli observed that, buds with petals slightly longer than sepals had most of the microspores in the uninucleate stage while Testillano *et al.* (1995) by immunolocalization and cytological studies reported that in American variety of *Capsicum annuum* L., the buds with petals slightly longer than sepals had a majority of microspores in the early binucleate stage. Microspore stage and corresponding morphology of flower buds in *Capsicum annuum* appears to be genotype dependent and need to be verified for each genotype in anther culture.

Surface Sterilization of Flower Bud

Standardisation of optimum surface sterilization of flower buds of Arka Meghana grown in pots under open condition was carried out by treating the buds with 70 per cent ethanol followed by 4 per cent sodium hypochlorite for different time intervals as given in Table 4. Treatment of the buds with 70 per cent ethanol for 30 seconds followed by 4 per cent sodium hypochlorite for 15 minutes gave the highest percentage of uncontaminated culture without browning. Ciner and Tipirdamaz (2002), Rodeva *et al.* (2004), Supena *et al.* (2006), Lantos *et al.* (2011) and Barroso *et al.* (2015) have also reported the use of sodium hypochlorite for effective surface sterilization of buds for anther culture.

TABLE 4

Response of anthers to different surface sterilization treatments

Treatment	70% ethanol (seconds)	4% sodium hypochlorite (minutes)	Uncontaminated culture (%)
T_1	30	10	77.28
T_2	30	12	88.00
T_3	30	15	96.25
T_4	45	10	82.20
T_5	45	12	90.00
T_6	45	15	98.53 *

*Browning of anthers observed

Anther Culture

On incubation at 25 °C with initial darkness for two days, callogenesis (3.17%) was observed only in one treatment with MS medium *viz.,* T_5 (MS + 4.00 mg L^{-1} NAA + 0.10 mgL^{-1} BA + 0.25 per cent activated charcoal + 10 mgL^{-1} $AgNO_3$)at sixth week of inoculation (Table 5). All the treatments incubated

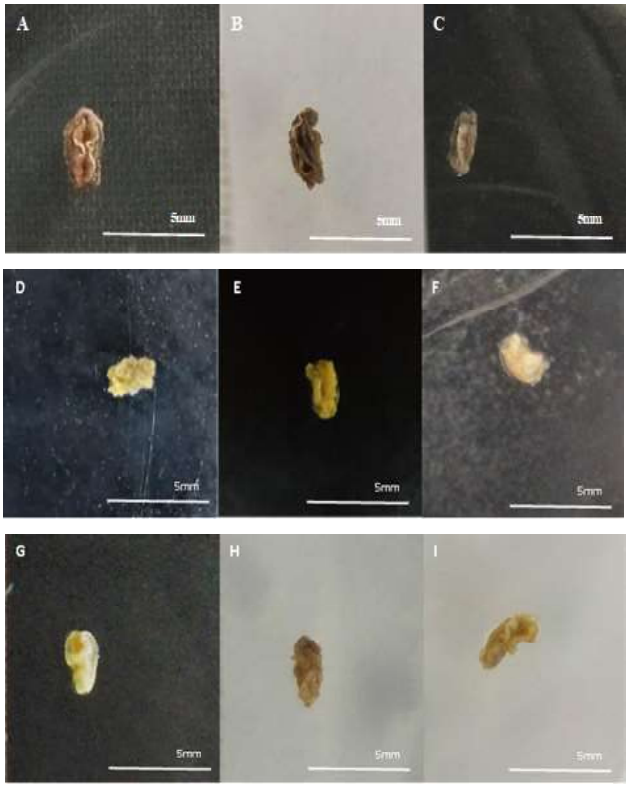

Fig. 2 : Callogenesis in anthers six weeks after inoculation at 25°C incubation temperature and eight days darkness: (A) T_1 (MS medium + 0.10 mgL^{-1} Kinetin + 0.004 mg L^{-1} 2,4-D) (B) T_2 (MS medium + 2.00 mgL^{-1} IAA + 0.30 mgL^{-1} BA) (C) T_3 (MS medium + 4.00 mgL^{-1} NAA + 1.00 mgL^{-1} BA + 0.25% activated charcoal) (D) T_4 (MS medium + 4.00 mgL^{-1} NAA + 1.00 mgL^{-1}BA) (E) T_5 (MS medium + 4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 10mg L^{-1} $AgNO_3$) (F) T_6 (MS media + 4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 15$mgL^{-1}AgNO_3$) (G) T_7 (MS media + 4.00 mgL^{-1} NAA + 0.50 mgL^{-1} BA + 0.25% activated charcoal + 15 $mgL^{-1}AgNO_3$) (H) T_8 (CP Media + 2.00 mgL^{-1} Kinetin + 0.10 mgL^{-1} 2,4-D) (I) T_9 (CP Media + 0.01 mgL^{-1} Kinetin + 0.01 mgL^{-1} 2,4-D)

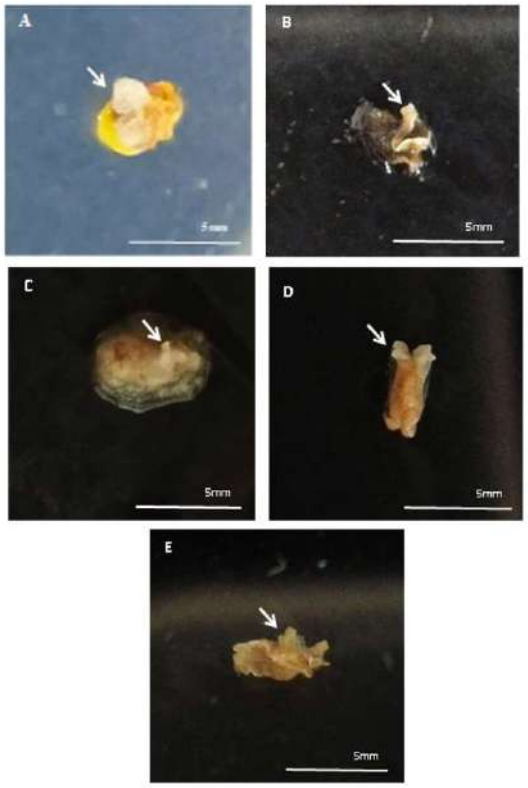

Fig. 3 : Direct embryogenesis from anthers of *Capsicum annuum* var. Arka Meghana (A) Globular embryo in T_1 (MS media + 0.10 mgL^{-1} Kinetin + 0.004 mgL^{-1} 2,4-D) incubated at 25°C and eight days darkness (B) Embryo in T_5(MS media + 4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 10mgL^{-1} $AgNO_3$) incubated at 35°C and two days darkness (C) Heart shaped embryo in T_6 (MS media + 4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 15$mgL^{-1}AgNO_3$) incubated at 35°C and two days darkness (D) Heart shaped embryos in T_7 (MS media + 4.00 mgL^{-1} NAA + 0.50 mgL^{-1} BA+ 0.25% activated charcoal + 15 $mgL^{-1}AgNO_3$) incubated at 35°C and two days darkness (E) Embryo in T_7 (MS media + 4.00 mgL^{-1} NAA + 0.50 mgL^{-1} BA+ 0.25% activated charcoal + 15 mgL^{-1} $AgNO_3$) incubated at 35°C and two days darkness (Bar = 5mm)

at 25 °C for eight days in darkness showed a response at sixth week of inoculation with callogenesis varying from 3.75 to 15.38 per cent. (Fig.2). Among the nine treatments, the highest callogenesis of 15.38 per cent was observed in the treatment T_5 at the sixth week of inoculation (Table 6). Embryogenesis (1.25%) was observed in the treatment T_4 (MS + 4.00 mgL^{-1} NAA + 1.00 mgL^{-1} BA) at sixth week of inoculation (Fig.3).

All the treatments with initial incubation at 35 °C in two days darkness showed callogenesis at the second week of inoculation with responses varying from 4 to 15.54 per cent. Callogenesis varying from 10.66 to 34.48 per cent and embryonic calli induction varying from 3.17 to 17.24 per cent was observed in the fourth week of inoculation (Fig.4). At the sixth week of inoculation callogenesis varied from 11.11 to 37.93 per cent and embryonic callus induction varied from 3.17 to 19.54 per cent. Among the treatments, the maximum callogenesis of 37.93 per cent and embryonic calli induction of 19.54 per cent was observed in the treatment T_5 at sixth week of inoculation (Table 7).

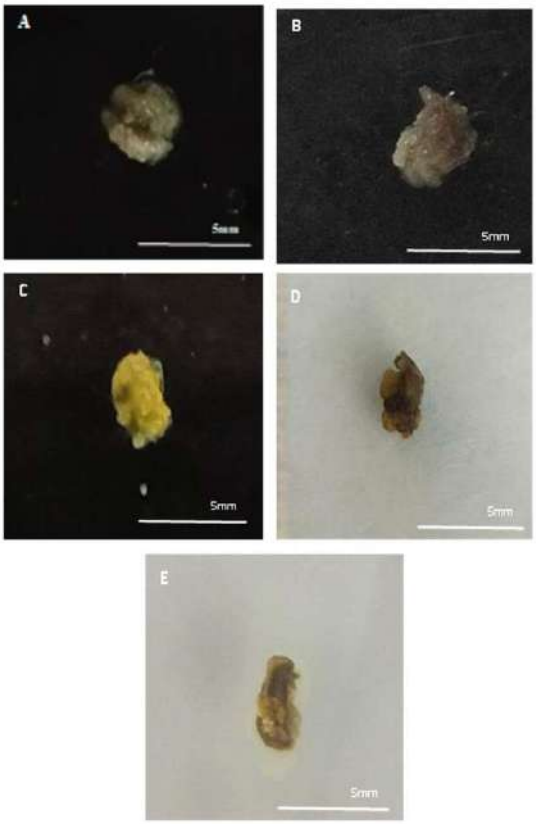

Fig. 4 : Embryonic calli induced from anther culture at 35°C incubation temperature and two days darkness (A) T_5 (MS media + 4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 10mgL^{-1} $AgNO_3$ (B) T_5 (MS media + 4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 10mgL^{-1} $AgNO_3$) (C) T_5 (MS media + 4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 10mgL^{-1} $AgNO_3$) (D) T_4 (MS media + 4.00 mgL^{-1} NAA + 1.00mgL^{-1} BA) (E) T_8 (CP medium +2.00 mg L^{-1} Kinetin + 0.10 mgL^{-1} 2,4-D) (Bar = 5mm)

TABLE 5

Response of anthers to different media combinations at 25 °C incubation temperature and two days darkness

Treatment	Basal medium	Supplements	Reference	Number of anthers inoculated	Callogenesis/ Embryogenesis (%)								
					2nd week			4th week			6th week		
					C	EC	E	C	EC	E	C	EC	E
T_1	MS	0.10 mgL^{-1} Kinetin + 0.004 mgL^{-1} 2,4-D	Irikova and Rodeva (2004)	50	0	0	0	0	0	0	0	0	0
T_2	MS	2.00 mgL^{-1} IAA + 0.30 mgL^{-1} BA	Gonzalez -Garcia (2002)	52	0	0	0	0	0	0	0	0	0
T_3	MS	4.00 mgL^{-1} NAA + 1.00 mgL^{-1} BA + 0.25% activated charcoal	Ciner and Tipirdamaz (2002)	51	0	0	0	0	0	0	0	0	0
T_4	MS	4.00 mgL^{-1} NAA + 1.00 mgL^{-1} BA	Comlekcioglu and Ellialtioglu (2018)	56	0	0	0	0	0	0	0	0	0
T_5	MS	4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 10$mgL^{-1}AgNO_3$	Comlekcioglu and Ellialtioglu (2018)	63	0	0	0	0	0	0	3.17	0	0
T_6	MS	4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 15$mgL^{-1}AgNO_3$	Buyukalaca *et al.* (2004)	55	0	0	0	0	0	0	0	0	0
T_7	MS	4.00 mgL^{-1} NAA + 0.50 mgL^{-1} BA+ 0.25% activated charcoal + 15 $mgL^{-1}AgNO_3$	Keles *et al.* (2015)	53	0	0	0	0	0	0	0	0	0
T_8	CP	2.00 mgL^{-1} Kinetin + 0.10 mgL^{-1} 2,4-D	Irikova *et al.* (2011)	57	0	0	0	0	0	0	0	0	0
T_9	CP	0.01 mgL^{-1} Kinetin + 0.01 mgL^{-1} 2,4-D	De Vaulx *et al.* (1981)	51	0	0	0	0	0	0	0	0	0

*C = Callus; EC = Embryogenic callus; E = Embryo

TABLE 6

Response of anthers to different media combinations at 25°C incubation temperature and eight days darkness

Treatment	Basal medium	Supplements	Reference	Number of anthers inoculated	Callogenesis/ Embryogenesis (%)								
					2nd week			4th week			6th week		
					C	EC	E	C	EC	E	C	EC	E
T_1	MS	0.10 mgL^{-1} Kinetin + 0.004 mgL^{-1} 2,4-D	Irikova and Rodeva (2004)	74	0	0	0	0	0	0	5.40	0	0
T_2	MS	2.00 mgL^{-1} IAA + 0.30 mgL^{-1} BA	Gonzalez -Garcia (2002)	70	0	0	0	0	0	0	4.28	0	0
T_3	MS	4.00 mgL^{-1} NAA + 1.00 mgL^{-1} BA + 0.25% activated charcoal	Ciner and Tipirdamaz (2002)	66	0	0	0	1.51	0	0	4.54	0	0
T_4	MS	4.00 mgL^{-1} NAA + 1.00 mgL^{-1} BA	Comlekcioglu and Ellialtioglu (2018)	80	0	0	0	0	0	0	3.75	0	1.25
T_5	MS	4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 10$mgL^{-1}AgNO_3$	Comlekcioglu and Ellialtioglu (2018)	65	0	0	0	3.07	0	0	15.38	0	0
T_6	MS	4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 15$mgL^{-1}AgNO_3$	Buyukalaca *et al.* (2004)	58	0	0	0	0	0	0	8.62	0	0
T_7	MS	4.00 mgL^{-1} NAA + 0.50 mgL^{-1} BA+ 0.25% activated charcoal + 15 $mgL^{-1}AgNO_3$	Keles *et al.*(2015)	81	0	0	0	2.46	0	0	8.64	0	0
T_8	CP	2.00 mgL^{-1} Kinetin + 0.10 mgL^{-1} 2,4-D	Irikova *et al.* (2011)	59	0	0	0	1.69	0	0	6.78	0	0
T_9	CP	0.01 mgL^{-1} Kinetin + 0.01 mgL^{-1} 2,4-D	De Vaulx *et al.* (1981)	54	0	0	0	1.85	0	0	7.40	0	0

*C = Callus; EC = Embryogenic callus; E = Embryo

TABLE 7

Response of anthers to different media combinations at 35°C incubation temperature and two days darkness

Treatment	Basal medium	Supplements	Reference	Number of anthers inoculated	Callogenesis/ Embryogenesis (%)								
					2nd week			4th week			6th week		
					C	EC	E	C	EC	E	C	EC	E
T_4	MS	4.00 mgL^{-1} NAA + 1.00 mgL^{-1}BA	Comlekcioglu and Ellialtioglu (2018)	63	4.76	0	0	11.11	3.17	0	11.11	3.17	0
T_5	MS	4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 10$mgL^{-1}AgNO_3$	Comlekcioglu and Ellialtioglu (2018)	87	13.79	0	0	34.48	17.24	0	37.93	19.54	1.15
T_6	MS	4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 15$mgL^{-1}AgNO_3$	Buyukalaca *et al.* (2004)	75	4.00	0	0	10.66	0	1.33	13.33	0	1.33
T_7	MS	4.00 mgL^{-1} NAA + 0.50 mgL^{-1} BA+ 0.25% activated charcoal + 15 $mgL^{-1}AgNO_3$	Keles *et al.* (2015)	110	15.54	0	0	31.81	0	1.82	33.63	0	2.72
T_8	CP	2.00 mgL^{-1} Kinetin + 0.10 mgL^{-1} 2,4-D	Irikova *et al.* (2011)	72	12.50	0	0	26.38	6.94	0	26.38	9.72	0
T_9	CP	0.01 mgL^{-1} Kinetin + 0.01 mgL^{-1} 2,4-D	De Vaulx *et al.* (1981)	103	6.79	0	0	12.62	0	0	13.59	0	0

*C = Callus; EC = Embryogenic callus; E = Embryo

Embryogenesis of 1.33 and 1.82 per cent were observed in the treatments T_6 (MS + 4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25% activated charcoal + 15mg L^{-1} $AgNO_3$) and T_7 (MS + 4.00 mgL^{-1} NAA + 0.50 mgL^{-1} BA+ 0.25% activated charcoal + 15 mgL^{-1} $AgNO_3$) respectively at fourth week of inoculation. At sixth week of inoculation, treatment T_5 exhibited 1.15 per cent embryogenesis whereas T_7 exhibited 2.72 per cent embryogenesis.

Among the different hormones tried, embryogenesis was observed only in media supplemented with NAA and BA. The results are in accordance with the reports by Ciner and Tipirdamaz (2002), where a combination of NAA and BA in the MS medium induced embryogenesis in Malatya genotype of chilli. Cheng *et al.* (2013) and Phuong (2021) also reported the significant influence of NAA and BA on embryogenesis in Chinese chilli genotypes and Vietnam local chilli varieties respectively.

The treatments T_6 and T_7 varied only in the concentration of BA. T_6 recorded 1.33 per cent of embryogenesis whereas T_7 recorded 2.72 per cent embryogenesis. Thus, increased concentration of BA exhibited comparatively better embryogenesis in Arka Meghana variety of chilli. This result is concordant to the report by Kele *et al.* (2015) in Charleston, Bell, Capia and Green genotypes of chilli. However, Buyukalaca *et al.* (2004) in U-247 and U- 238 genotypes of chilli and Ozsan and Onus (2017) in Benino, Kanyon, Belissa and Filinta cultivars of chilli have reported better embryogenesis in the medium with a lower concentration of BA.

The influence of silver nitrate on embryo induction in Arka Meghana was dependent on culture condition. Percentage of embryo induction in the presence of silver nitrate was better in T_6 compared to T_5. Increased concentration of silver nitrate exhibited a better response. Similar results are reported by Nervo *et al.* (1995), Buyukalaca *et al.* (2004) and Keles *et al*. (2015) in *Capsicum annuum* L.

Initial incubation at 35 °C in darkness for two days exhibited better response to embryonic calli induction and embryogenesis compared to incubation at 25 °C for two and eight days darkness. Incubation temperature of 25 °C exhibited only 1.25 per cent direct embryogenesis and no indirect embryogenesis was observed. According to Morrison *et al.* (1986) and Rajakaruna *et al.* (2018), initial incubation at 35 °C was better for embryo initiation than incubation temperature of 25 °C. Buyukalaca *et al.* (2004), Keles *et al.* (2015) and Atasoy *et al.* (2021) have also reported embryogenesis in anthers of *Capsicum annuum* L. inoculated at 35 °C and one or two days in darkness.

To conclude, among the treatments tried in *Capsicum annuum var.* Arka Meghana the best treatment for indirect embryogenesis and direct embryogenesis was MS + 4.00 mgL^{-1} NAA + 0.10 mgL^{-1} BA + 0.25 per cent activated charcoal + 10 mgL^{-1} $AgNO_3$ (T_5) and MS + 4.00 mgL^{-1} NAA + 0.50 mgL^{-1} BA + 0.25 per cent activated charcoal + 15 mgL^{-1} $AgNO_3$ (T_7) respectively at culture conditions of 35 °C initial incubation temperature for two days darkness followed by incubation at 25 °C with 12 hour photoperiod. The most suitable initial incubation condition was determined to be 35 °C initial incubation temperature in two days darkness. It was observed that the hormonal combination of NAA and BA was better suited for embryogenesis and the increased concentration of silver nitrate in the medium supplemented the embryogenic potential. The embryos induced could be further used for regeneration of haploid plantlets of Arka Meghana for doubled haploid production and crop improvement.

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