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Short Communication

Assessment of double haploid culture conditions in bell pepper (*Capsicum annuum* L.)

Anjanabha Bhattacharya*, Bhavesh Palan, Kishor Mali and Bharat Char

Mahyco Research Centre, Maharashtra Hybrid Seeds Company Private Limited, Dawalwadi, Jalna, Aurangabad Road, Jalna 431 203, Maharashtra

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Abstract

The present study was performed to generate double haploid lines in bell pepper with high regeneration efficiency via anther culture. In total, 825 anthers were plated on five different modified MS media *viz*., MS I, MS II, MS III, MS IV and MS V. The number of embryoids formed was found to be highest on MS IV media (26 embryoids) as compared to others. Forty five embryoids were developed on five media combinations, out of which 19 were regenerated into plantlets. Therefore, the optimized media *i.e.* MS IV in this study may be suitable for the development of double haploid lines in other economically important bell pepper as well as hot pepper genotypes through anther culture.

Key words: Bell Pepper, double haploid, embryoids, Murashige and Skoog media, plantlet regeneration

Bell pepper (*Capsicum annuum*) is having high nutritive value as it contains vitamin C and many carotenoids (Irikova et al. 2011). To fulfill the growing demand of bell pepper in market, it is necessary to establish new cultivars and increase its production. This could be possible by means of double haploid production. Double haploid production has importance in vegetable breeding program as it reduces the breeding cycle and produces homozygous lines in less duration as compared to conventional breeding approaches. Anther culture is the most widely used technique for haploid plant production in pepper. Further, several factors were observed influencing androgenesis and low plant regeneration frequencies such as donor plant genotype, developmental stage of microspores, pretreatments to the anthers, media combinations and *in vitro* culture conditions (Ciner and Tipirdamaz 2002; Novaczyk and Kisiala 2006). Activated charcoal and $AgNO_3$ in the nutrient medium showed considerable improvement in androgenesis through anther culture (Novaczyk and Kisiala 2006). According to some researchers, the heat shock to the cultured anthers at 35±2^oC for 7-8 days promoted the anther culture towards direct embryogenesis (Ercan and Sensoy 2011; Luitel and Kang 2013). Spontaneous chromosome doubling in pepper haploids was observed and confirmed with flow cytometry of leaf nuclei (Dolcet-Sanjuan et al. 1997). Further, *in vivo* chromosome doubling was carried out in haploid plants of minipaprika with colchicine (0.5% w/v) treatment (Luitel and Kang 2012). A new sweet pepper cultivar, Haihua 3, was developed by anther culture technique and the cultivar showed wide adaptability, high tolerance to diseases and some characters better than that of its parents (Li and Jiang 1990). Therefore, the present study was aimed to determine the suitable culture media combinations for direct embryogenesis through anther culture to develop double haploid lines in bell pepper.

A single genotype of bell pepper, Gulshan Wonder, from Mahyco Research Centre, Dawalwadi, Jalna, India was used as anther donor plant in this study. The flower buds were collected with visual

*Corresponding author's e-mail: anjanabha.bhattacharya@gmail.com
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observation of same length of corolla and calyx showing anthers with uninucleate microspore. Excised flower buds were cold pretreated at 4^{o}C for 72 hrs in the dark. The buds were surface sterilized with 4% sodium hypochlorite for 10 min. The anthers were cultured without filament on five combinations of MS media with modifications in concentrations of growth hormones, activated charcoal and $AgNO_3$. The anther cultured plates were initially given heat stress by incubating at 35±2^{o}C for 5 days and then transferred to 25±2^{o}C in the dark until embryo development.

In total, 825 anthers were isolated and cultured (Figure 1-a) on five different (Murushige and Skoog) media combinations *viz*., MS I, MS II, MS III, MS IV and MS V (Table1). Maximum number of uninucleate pollens was found in flower buds with 5 mm length. Embryoids induction started at six weeks of culture and it was observed on MS I, MS III and MS IV media with varied frequencies in the range of 4% to 8.67% which was found to be higher as compared to that in bell pepper genotypes, California wonder (5.66 ± 0.57%) and lower than Feherozon (55.36 ± 1 %) on CP media (Koleva-Gudeva et al. 2007). In total, 45 embryoids were found to be produced from the 825 anthers cultured (Table 1). Media wise number of embryoids development was obtained in the range of 03-26. These results were found to be consistent with 6-30 embryoids on MS media (Luitel and Kang, 2013). Further, they concluded that bell pepper and/or sweet pepper genotypes were highly responsive to anther culture than hot pepper cultivars. The embryoids (embryo like structures) formed during embryogenesis were protruded from inside the anthers and finally developed into haploid/double haploid plantlets (Mishra, 2009).

From the results, it was noticed that highest number of embryoids were developed on MS IV media while MS II and MS V media did not show embryoids formation. The embryoids developed were then transferred to MS media (without growth regulators) for further regeneration and kept at 25±2^{o}C with 16 hrs photoperiod (Fig. 1-b). After one week of subculture on MS media, the plantlet regeneration was observed. Further, these plantlets with shoots and roots (Fig. 1c) were transplanted into plastic cups for acclimatization (Fig. 1d and 3). Out of forty five embryos developed and sub-cultured on MS media, nineteen were found to be regenerated into plantlets. In another independent study, authors have reported that out of two bell pepper cultivars, Kandil showed no androgenic response while Odesa produced 3.01% and

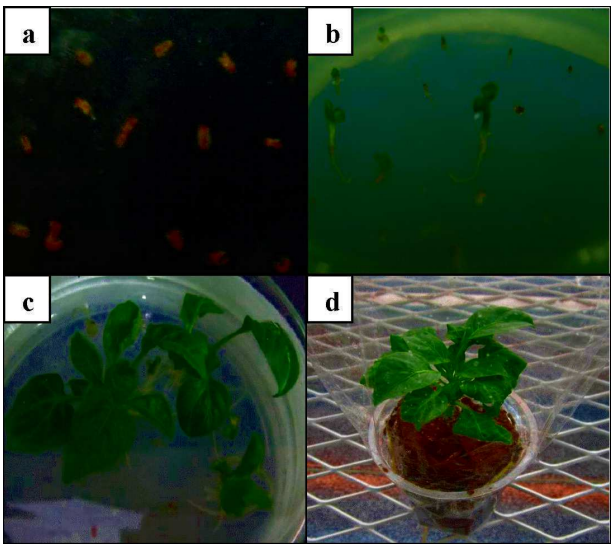

Fig. 1. Bell pepper anther culture

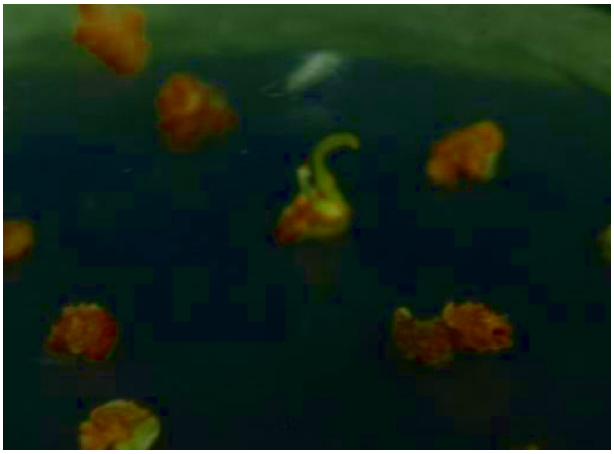

Fig. 2. Micro-plant developed from bell pepper anther culture

Fig. 3. Gulshan wonder DH plant

Table 1. Details of bell pepper anther culture on five different media combinations

S.No.	Media	No. of anthers cultured	No. of embryoids developed	Percent embryoids
1	MS I (MS+0.25% Activated Charcoal)	75	3	4.00
2	MS II (MS+1.0% Activated Charcoal)	100	0	0.00
3	MS III (MS+7.5mg/L $AgNO_3$+0.5% Activated Charcoal)	275	16	5.82
4	MS IV (MS+15mg/L $AgNO_3$+0.5% Activated Charcoal)	300	26	8.67
5	MS V (MS+15mg/L $AgNO_3$+1.0% Activated Charcoal)	75	0	0.00
	Total	825	45	-

Note: MS - MS0 + 1mg/L BAP + 5mg/L NAA + Cefotaxime 150mg/L + Carbenicillin 500mg/L

22.2% embryos and plantlets respectively (Ercan and Sensoy 2011). As compared to these results, we got higher percentage of plantlets regenerated from the embryoids (42.22%). It was noticed that some anthers of bell pepper genotype showed micro-plant development in the present study (Fig. 2) similar to that was observed in three genotypes out of 16 genotypes studied (Rodeva et al. 2004) by other researchers.

The anther culture technique was fruitfully used to regenerate the plantlets for development of double haploid lines in bell pepper genotypes. The modified MS media *i.e.* MS IV showed satisfactory number of embryoids with 8.67% frequency in the present study. The frequency of embryo formation and plantlet regeneration could further be increased with optimization of developmental stage of microspores, pretreatments to the anthers, media combinations and *in vitro* culture conditions. The spontaneously diploid plants can be employed to develop new lines in bell pepper through breeding. In summary, MS IV media could be used in case of other economically important pepper genotypes for the development of double haploid lines.

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