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RESEARCH ARTICLE

EFFECT OF GENOTYPE AND MEDIA ON DIRECT EMBRYOGENESIS OF CHILLI PEPPER (*CAPSICUM ANNUUM* L.)

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ABSTRACT

An *in vitro* anther culture method was standardized for producing double haploid plants in 10 F1 genotypes of Chilli pepper (*Capsicum annuum L.)*. This procedure was studied to optimize and study the effect of culture media and each genotype for androgenic response. Androgenesis varied depending upon the genotype and media. Ten genotypes were evaluated in 3 variations of media concentrations. All the genotypes showed differential expression in different media compositions. Direct embryogenesis for different genotypes varied from 4.1% to 38%, PH 53 showed the highest and lowest rate of embryogenesis was observed in PH 17 genotype. It was observed that MS medium with 0.1mg/L Kinetin and 0.5mg/L 2, 4 Dichlorophenoxyacetic acid (2,4-D) gave higher embryo frequency in PH 53 and lowest in PH 51 genotype. MS medium with 3mg/L 1-Napthalene acetic acid (NAA) and 1mg/L 6-Benzyl amino purine (BAP) gave higher embryo frequency in PH 59 lowest in PH 17 genotype where as MS medium with 0.5mg/L NAA, 1mg/L Kinetin and 0.5mg/L BAP gave higher embryo frequency in PH 57 followed by PH 43 genotype.

INTRODUCTION

Double haploidy is a phenomenon much sought after by the crop breeding community to lessen the process of generating new germplasm and reducing the process from 6 generations or more by the classical method down to single generation. The production of such doubled haploid plants via androgenesis is a powerful technique for evaluation of genetic diversity and an excellent material for plant breeding.

This technology has been effective in Chilli pepper and proved to be beneficial. Chilli pepper (*Capsicum annum L.*) which belongs to the genus Capsicum and family Solanaceae is a domesticated species originated from American tropics and was introduced in India by the Portuguese which has now become the most importantly used cuisine spice.

However, it is considered as a low output crop because of its low yield and diseases. New technologies are therefore needed to speed up the breeding programs to improve the quality of chilli pepper. Hence the technique of direct microspore embryogenesis has been introduced in Chilli pepper.

Economically Chilli pepper is an important vegetable crop and pollen embryogenesis through anther culture *in vitro* was first published by Wang *et al* in 1973, George and Narayanaswamy from India.

Several protocols in microspore induced embryogenesis have been reported in different genotypes by (Dumas de vaulx *et al.* 1981, Mityko *et al.*1995, Dolcet-sanjuan *et al.* 1997, Barany *et al.* 2001,Rodeva *et al.* 2004, 2006, Teodora Irikova *et al.* 2011, Yan Cheng *et al.* 2013, Başay and Ellialtıoğlu, 2013) by culture medium by (Sibi *et al.* 1979, Dumas de vaulx *et al.* 1981, Elcialitioglu *et al.* 2001, Supena *et al.* 2006, Teodora Irikova *et al.* 2011, Yan Cheng *et al.* 2013).

But embryogenesis in Chilli pepper is limited as efficient protocols for plant regeneration and development of normal plantlets from shoots (Nowaczyk and Kisisala 2006; Kothari *et al*, 2010) is lacking, which has been stated in many papers. The main aim of our study was to develop an efficient protocol for production of double haploids from different genotypes of Indian Chilli pepper which provides an opportunity to shorten the breeding cycle and fix agronomic traits.

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MATERIALS AND METHODS

10 F1 hybrid genotypes of NSL Chilli pepper (*Capsicum annuum L.*) (PH 8, PH 11, PH 17, PH 25, PH 43, PH 50, PH 53 and PH 57) were used to generate double haploids. Anther donor plants were grown under green house conditions at Nuziveedu Seeds Limited Hyderabad. Initiation of flowering was observed 8 weeks after sowing and young flower buds of all genotypes were harvested for about 6-8 weeks at their early flower production period for anther culture. Flower buds of each genotype were refrigerated at 4^0C for 2days to induce cold stress to the anthers for better embryogenic response.

Surface sterilization and anther extraction

The fresh and cold treated flower buds were initially washed with sterile distilled water. Then these were treated with 70% ethanol for 15 seconds and then with 2.5% sodium hypochlorite and a drop of Tween-20 for 8 minutes and rinsed in sterile distilled water 3 times or until traces of sodium hypochlorite were removed.

Then these buds were carefully dissected and individual anthers were separated and placed horizontally on the medium such that the anther was in contact with the medium. Care was taken to avoid anther damage.

Microspore stage

Anthers containing microspores mainly at the late uninucleate and early binucleate stage were selected. At this stage the corolla was little longer than the calyx. Sepals and anthers are light violet in color. The stage of the microspores was observed accurately in acetocarmine squashes.

Culture medium

Anthers were cultured on MS (Murashige and Skoog, 1962) basal medium with different concentrations of hormones: **C4** - 0.1mg/L kinetin+ 0.5 mg/L 2,4-D, **C5** - 3mg/L NAA + 1mg/L BAP and **C11** - 0.5mg/L NAA+1mg/L Kinetin +0.5mg/L BAP and with 30 g/L sucrose and 8 g/L agar-agar with pH of the medium adjusted to 5.8 for direct embryogenesis. Other mediums used were**C2** - MS basal medium with 0.1mg/L Kinetin is for embryo germination. **C12** - ½ MS medium with 0.02mg/L kinetin for shoot elongation and rooting.

Culture conditions

The cultures on **C4, C5 & C11 media** were incubated in dark for 8 days at 9^0C, later at 28^0C for 4 to 6 weeks in dark to induce direct embryogenesis. Then these embryos were transferred to C2 medium and were kept in light with 16 hours photo period and 60-65% humidity at 25^0C. After few days the embryo germinated plants were transferred to C12 medium for shoot and root elongation. Later plants which measured 4-5cms in length were transferred to cocopeat pots. Ploidy levels were checked by root tip squash method using acetocarmine stain, flow cytometry and chloroplast counting in stomatal guard cells. Haploid plants were treated with Colchicine for chromosome doubling (Arjunappa *et al*. 2016).

Observation of growth

Some parameters related to the growth of explants were observed regularly. Those parameters were the initiation of embryogenesis, embryo germination and plant development

RESULT AND DISCUSSIONS

Effect of genotype on direct embryogenesis

Genotype plays an important role in androgenic reaction in Chilli pepper as it is most often limiting factor stated by Comlekcioglu *et al*. 2001; Wang and Zhang 2001; Rodeva *et al*. 2001; 2004. Though different media compositions were maintained equally for all the genotypes there was a varied response in each genotype. The number of embryos produced varied from genotype to genotype.

Table I Effect of genotype and media on embryo and plant frequencies

Genotype	Media	No, of anthers inoculated	No, of Embryos produced	Embryos frequency	No, of plants produced	Plants frequency
PH 8	C4	550	180	32.7%	75	41.7%
	C5	776	69	8.9%	64	92.8%
	C11	626	53	8.5%	23	43.4%
PH 11	C4	1654	599	36.2%	36	6.0%
	C5	274	0	0.0%	0	0.0%
	C11	533	1	0.2%	1	100.0%
PH 17	C4	203	0	0.0%	0	0.0%
	C5	587	24	4.1%	2	8.3%
	C11	484	3	0.6%	1	33.3%
PH 43	C4	222	7	3.2%	7	100.0%
	C5	486	18	3.7%	2	11.1%
	C11	516	48	9.3%	12	25.0%
PH 50	C4	204	27	13.2%	4	14.8%
	C5	252	8	3.2%	4	50.0%
	C11	1322	107	8.1%	2	1.9%
PH 51	C4	1255	9	0.7%	0	0.0%
	C5	219	15	6.8%	0	0.0%
	C11	1327	53	4.0%	1	1.9%
PH 53	C4	363	138	38.0%	1	0.7%
	C5	804	159	19.8%	6	3.8%
	C11	538	196	36.4%	7	3.6%
PH 57	C4	520	72	13.8%	52	72.2%
	C5	324	11	3.4%	11	100.0%
	C11	448	95	21.2%	20	21.1%
PH 58	C4	126	3	2.4%	0	0.00%
	C5	457	25	5.5%	1	4.00%
	C11	594	25	4.2%	7	28.00%
PH 59	C4	127	4	3.1%	0	0.0%
	C5	352	25	7.1%	5	20.0%
	C11	386	10	2.6%	4	40.0%

The anthers from all the 10 F1 genotypes were cultured on 3 induction media (C4, C5 and C11). The anthers from genotype PH 53 showed the highest rate of embryo frequency of 38% when cultured on C4 medium compared to other genotypes (Table.1). The anthers of genotype PH 11 responded well and gave embryo frequency of 36.2%, where as genotype PH 8 demonstrated 32.7% of embryogenesis. Genotype PH 57 responded well with 21.2% embryogenesis. The anthers of genotype PH 17 gave the lowest embryogenesis of 4.1%. Thus these results show considerable variation in the embryogenesis response by different genotypes. Genetic pre determination of the genotype is the reason for different androgenic responsiveness and ratio between exogenous and endogenous plant growth regulators was studied by (Irikova *et al*. 2011).

Different stages of Androgenesis

Figure I Embryo developmental stage after 4 weeks of culture

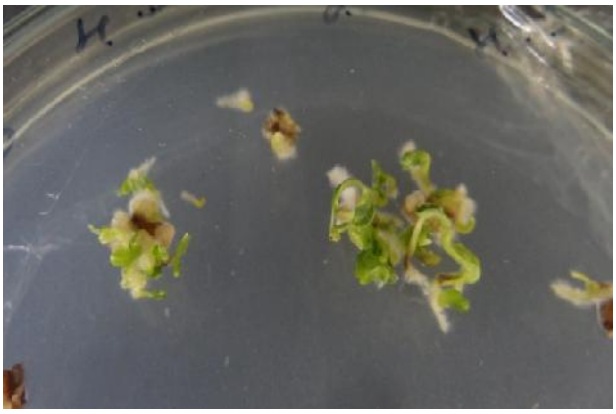

Figure II Embryo germination on C2 medium

Figure III Rooted plant on C12 medium

Figure IV DH plant in fruiting stage

The effectiveness of androgenesis in different hybrids and among individual plants of different genotypes was tested by Nowaczyk *et al*.2009.

Effect of media composition on direct embryogenesis

The type of media and concentration of growth regulators are crucial factors for determining embryogenesis in anther culture of capsicum (ozkum cinu and tipirdamaz 2002).

It was observed that though all the genotypes were simultaneously inoculated on MS media with 3 different concentrations of growth hormones **(C4, C5 and C11)**, the number of embryos varied from 0 to 38% from genotype to genotype. The anthers of genotypes PH 8, PH 11, PH 53 and PH 50 responded well on C4 (32.7%, 36.2%, 38% and 13.2% respectively) where as genotypes PH 43, and PH 57 showed better response on C11 medium with 9.3%, and 21.2% respectively. Embryo frequency of PH 17, PH 58, PH 51, and PH 59 were notably good on C5media with 4.1%, 5.5% 6.8% and 7.1% respectively.

However, genotypes PH 8, PH 57 and PH 50 showed higher rate of plant frequency with 92.8%, 100% and 50% on C5 medium. Whereas genotypes PH11, PH 57 and PH 59 showed a great plant frequency with 100%, 75% and 40% on C11 medium.

The plant frequency of PH 43 on C4 was found to be 100%. Higher kinetin concentration of 0.2 and 0.3mgL in RI medium showed increased induction of androgenesis in Capsicum was stated by (Gemesene *et al* 1998)

Thus this concludes that embryogenesis differs in different media compositions for different genotypes, showing media concentrations also has a major impact on direct embryogenesis. This is due to genotypic genetic predetermination, exogenous and endogenous ratio of plant growth regulators which induces changes in the start of cell division and callus formation.

CONCLUSION

From our study we have found that the factors such as genotype and media play an important role in direct embryogenesis in Chilli pepper. Each genotype showed different embryogenesis response in different media. Different types of growth regulators and their varied concentrations in the MS media played an important role such as highest embryo frequency was observed in PH53 genotype in MS medium with 0.1mg/L Kinetin and 0.5mg/L2,4-D (C4) and lowest embryo frequency in PH 53, PH 51 genotype. Thus demonstrating that different genotypes showed varied response in different media demonstrating that genotype and media play an important role in embryogenesis.

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