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Short Communication

DEVELOPMENT OF EMBRYO RESCUE TECHNIQUE FOR WIDE HYBRIDIZATION OF CAPSICUM SPP

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INTRODUCTION

Capsicum peppers are native plants of the New World tropics. They belong to the family Solanaceae which also includes potato, tomato and tobacco (Agrawel *et al.*, 1983). Five different specices, *Capsicum annuum, C. frutescens, C. chinense, C. baccatum and C. pubescens,* are regarded as domesticated peppers (Campos *et al.*, 2006). Interspecific hybridization is essential for introduction of genes from wild and related species into commercial varieties for disease and pest resistance, nutritional quality, yield and adaptation to stresses (Husain *et al.*, 1999, Ramirez *et al.*, 1996). Several interspecific hybrids were obtained within *C. annuum* complex, such as introducing Tobacco Mosaic Virus (TMV) resistance from *C. frutescens* to *C. annuum*, resistance to PVY and TEV from *C. chinense* to *C. annuum* partial resistance to CMV from *C. baccatum* to *C. annuum*) or multiple-flower character from *C. chinense* into *C. annuum* (Agrawel *et al.* 1983,1989). Successful crosses between complexes are much more uncommon due to expression of unilateral or bilateral incompatibilities (Campos *et al.*, 2006).

However, diseases namely, (TMV), root rot, Tomato Spotted Wilt Virus (TSWV), etc. leads to a considerable declining in yield in *Capsicum annuum* varieties; including chilli, bell pepper, capsicum etc. To overcome this problem, wide hybridization could be used to transfer the responsible genes from one species to another, but there has been very little success due to incompatibilities. To bypass these post-fertilization barriers, embryo-rescue, ovule culture and manipulations with protoplasts have been successfully used. The embryo rescue technique have been used with MS (1962) modified medium (Murashige *et al.*, 1962). It is observe that the stage of development of the wide hybrid embryos at the time of culture is utmost importance; and the growth is strongly influenced by the age (Hogenboom, 1973).

Therefore the present study was carried out to develop a method to overcome post fertilization barriers using embryo rescue techniques in order to transfer the important traits from *C. frutescence* to *C. annuum*. Age of the embryo and compositions of the media are the major concerned area for this study.

MATERIALS AND METHODS

The experiment was carried out from September 2015 to February 2016 at the tissue culture laboratory of Horticultural Crops Research and Development Institute (HORDI), Sri Lanka. Wide hybrids were prepared using *Capsicum annuum* and *C. frutescence*. Two recommended varieties, Hungarian Yellow Wax (HYW), Lanka Yellow Wax (LYW) and one inbred line (1782) of *C. annuum* and three accessions of *C. frutescence*, C-8, C-12, C-17 were used for the study. Plants were established in plant house and inter specific hybrids were made. *C. annuum* varieties were used as the female parent and the *C. frutescence* as the male parent. To culture immature embryos, MS media containing different hormone concentrations were used (Table 1)

Table 1: Media composition.

	MS-A	MS-B	MS-C	MS-D
NAA (mg/l)	0.1	0.1	0.1	0.1
GA_3 (mg/l)	0.2	0.3	0.4	0.5
BAP (mg/l)	-	0.1	0.2	0.3
Casein hydrolysate (mg/l)	500	500	500	500

Collected mature and immature fruits were washed by using running tap water for 30 minutes. There after pods were exposed to laminar air flow and further sterilized using 10% sodium hypochlorite solution for 10 minutes and dipped in 70% ethanol for 3 to 5 minutes followed by rinsing three times with sterile distilled water. Cultures were placed in a culture room under a controlled temperature (25 ±2°C with 1800-2000 lux, light intensity, (33-37 $\mu E/m^2/s$) with 16 hours light/8 hr dark/ day).

Germination was recorded at 14 days after the culture establishment. Germinated plantlets (10-11cm height) were transferred to potting media for further growth. Healthy rooted plantlets were then transplanted to clay pots (10 cm diameter) containing a mixture of sterilized top soil 2: sand 1: coir dust 1 mixture.

RESULTS AND DISCUSSION

HYW and 1782 successfully produced well developed pods with all the crosses made between selected *C. frutescence.* However in cross combinations with LYW, successful pod formation was observed only from the LYW X C-8 cross. LYW x C-12 and LYW x C-17 crosses did not produce completely developed pods and immature pod drop was observed at 5 days after pollination. For these incompatible combinations where pod development was observed only for 5 days, embryos were cultured on embryo rescue medium at 5 days after pollination only.

Premature embryo germination percentage in different media combinations were analyzed using SAS (Statistical analysis software) statistical package (version 9.1") to determine the significance of differences between treatments at 0.05% probability. MS-A, MS-B and MS-C showed lower germination of immature embryo (less than 10 %) compare to the MS-D (18% -41%) for the embryos collected 10-15 days after pollination. Germination ability at different age of the embryo showed significant difference. MS-A, MS-B and MS-C media did not show any embryo germination ability for embryo collected 5-10 days after pollination. Significantly higher pre mature embryo germination percentage (18% -41%) for all tested ages and embryo rescue ability for 5 days old embryos (18%) were observed only with MS-D medium. Low concentrations of auxins have promoted normal growth, and gibberellic acid has caused embryo enlargement (Agrawal *et al.*, 1989). Selected rescue medium from this study also containing low concentrations of growth regulators NAA, BAP and GA_3 as 0.1 mg/l 0.3 mg/l and 0.5 mg/l, respectively.

CONCLUSION

Immature wide hybridized embryos collected 5-15 days after pollination germinated well in the embryo rescue MS medium, supplemented with Casein hydrolysate 500 mg/l, NAA 0.01 mg/l,GA_3 0.5 mg/l,BAP 0.1 mg/l . Immature embryos at the age of 5 days after pollination from incompatible crosses could be used to produce completely developed plants using selected embryo rescue medium.

This developed embryo rescue technique can be used to overcome incompatible barriers in wide hybridization of *C. annuum* and *C. frutescens.*

REFERENCES

Agrawal, S. and Chandra, N. 1983. Differentiation of multiple shoot buds and plantlets in cultured embryos of *Capsicum annuum* L. val'. mathania. *Curt. Sci. 52:645-646.*

Agrawal, S., Chandra, N. and Kothari, S.L.. (1989) Plant regeneration in tissue culture of pepper *(Capsicum annuum* L. cv. mathania). *Plant Cell Tiss. Org. Cult. 16:47-55.*

Campos, K.P., Pereira, T.N.S., Costa, F.R., Sudré, C.P., Monteiro, C.E.S. and Rodrigues, R. (2006) Interspecific hybridization among cultivated germplasm in Capsicum. In: The 17th. International Pepper Conference. Naples, Florida, p. 20-20.

Hogenboom, N.G. 1973. A model for incongruity in intimate partner relationships.Euphytica 22 (2): 219-233.

Husain, S., Jain, A. and Kothari, S.L. 1999. Phenylacetic acid improves bud elongation and *in vitro* plant regeneration efficiency in *Capsicum annuum* L. *Plant Cell Rep. 19:64-* 68.

Ramirez-Malagon, R. and Ochoa-Alejo, N. (1996) An improved and reliable chili pepper (*Capsicum annuum* L.) plant regeneration method. *Plant Cell Rep. 16:226-231 Rep. 15:536-540.*

Williams, E.G., I.M. Verry, and W.M. Williams. 1982. Use of embryo culture in interspecific hybridization. In: *Plant Improvement and Somatic Cell Genetics.* Ed. I.K. Vasil, W.R. Scowcroft, and K.J. Frey. Academic Press, New York. 119–128.