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INTERSPECIFIC HYBRIDIZATION AND EMBRYORESCUE TECHNIQUES FOR CHILLI (*CAPSICUM ANNUUM* L.) CROP IMPROVEMENT *

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INTRODUCTION

Chilli is an important spice grown as a cash crop throughout the world. Diversity exits within and among *Capsicum* spp. and their landraces. Cultivated species, *C. frutescens*, *C. pubescence*, *C. chinense* and *C. baccatum* show resistance to some diseases such as *Tobacco Mosaic Virus*, anthracnose and tolerance to adverse climatic conditions and are rich in flavours and aromas. *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV) and leaf curl diseases, root rot and anthracnose are the major diseases reported in Sri Lanka, which leads to considerable yield losses.

The crosses between such species, aiming at transferring genes, may be inconsistent or incompatible due to the existence of a set of pre and post-zygotic incompatibilities (Hogenboom, 1973). Embryo rescue helps to overcome post embryonic incompatibility and to continue the breeding programs. Field tolerant character of PGRC Accession No.11642 *C. frutescens*) can be used to develop chilli varieties with tolerance to chilli leaf curl disease (Senanayake *et al.*, 2014). The present study was carried out to develop a methodology to overcome post fertilization barriers using embryo rescue techniques in order to transfer the important traits from *C. chinense*, and *C. frutescence* to *C. annuum*.

* *See "Tropical Agriculturist" Volume 164 for details.*

MATERIALS AND METHODS

In the first experiment wide hybridization was done using three species *viz*. *C. annuum*, *C. chinense* and *C. frutescens*. Several single crosses and selected reciprocal crosses were made between *C.annuum* varieties, MI Hot, KA2, MICH3, Galkiriyagama, MI-green and *C. frutescens* accessions, PGRC Acc.No.11642 and *C. chinense*, "Kaum miris" and "Purple Nai Miris" during *Yala* 2013, *Maha* 2013/2014 and *Yala* 2014 seasons. About 28-31 days old pods were harvested and sterilization was done. Embryos were detached from the endosperm and placed on MS media containing different hormone concentrations and organic supplements as follows, MS1- 0.5 mg/l GA3+0.05 mg/l NAA+500 mg/l Yeast extract + 500 mg/l Casin hydrolysate + Coconut water (150 ml), MS2- with 0.5 mg/l GA3+0.05 mg/l NAA+500 mg/l Yeast extract, MS3- with 0.5 mg/l GA3+0.05 mg/l NAA+500 mg/l Yeast extract+ 500 mg/l Casein hydrolysate, MS4- with 0.5 mg / l GA3+0.1 mg/l NAA.

The media was solidified with 7.5 % Nutrient Agar and pH was adjusted to 5.7-5.8 prior to autoclaving. All the cultures were maintained at 25±2°C temperature and 1,800-2,000 lux (33-37 $\mu E/m^2/S$) light intensity (12 light/ 8 dark hours/ day). Germination was recorded at 8-10 days after the culture establishment. Germinated plantlets were transferred on to fresh culture media (30 ml) for further growth. Healthy rooted plantlets were then transplanted in clay pots (10 cm diameter) containing a mixture of sterilized top soil 2: sand 1: coir dust 1 and hardening of plants was done for two weeks in a net house. Finally, plants were transferred to 30 cm (diameter) x 30 cm (height)black colour plastic pots containing sand: partially burn paddy husk: top soil: cow dung (1:1:2:2) mixture.

RESULTS AND DISCUSSION

Germinations on different media was analyzed using pair wise comparisons in MINI TAB (14 version). MS2 and MS4 media did not show any embryo germination. MS1and MS3 showed the embryo germination (5% and 23%, respectively) and regenerating plantlets. There was a significant higher embryo germination percentage (23%) in MS3 culture medium. However, this value was less than 50%. The plant recovery percentage was also low. It has been reported that embryo rescue in plants of some species needs lower temperatures. In the case

of embryo culture of peach (*Prunus percisa* L.), cherry (*Prunus* spp.) plum (*Prunus domestica* L.) and pear (*Pyrus* spp.) chilling treatment of embryos is an essential factor for embryos germination (Sharmal *et al.*, 1996).

Therefore, the second experiment was conducted to understand whether cold stress has any influence on embryo germination and plant recovery percentage.MS3 was selected as culture medium. Harvested immature F_1 hybrid chilli pods (28-31 days old) were covered with an aluminum foil and kept in cold temperature (10-12 °C) for 07 days. All the sterilization, culture establishment procedure and data recording were done as in the experiment 1. Germinated embryo counts were analyzed using T test (MINITAB 14 version).

There was a significant difference between cold and non-cold treatments. Under the cold condition (10 -12 °C / 7 days) embryo germination percentage was better (38%) than that of none cold condition (20%)in MS medium with 0.5mg/l GA3+0.05mg/l NAA+500mg/l Yeast extract + Casein hydrolysate. Embryo germination percentage was comparatively higher in *C. annuum*/*C. Chinense* cross combination (24-50%) than that of *C. frutiescence* / *C. annum* cross combination (26-31%).Seed formation in the pods was found to be less.

A few number of seeds were found in *C. frutiescence*/ *C. annum* cross combinations (data are not shown). Shifriss *et al.* (1997) mentioned that *C. frutiescence* carries mitochondrial genes for male sterility. There is a possibility that the hybrids developed in this study show varying degrees of male sterility in all cross combinations when *C. frutiescence* used as female parent. Further studies with more number of cross combinations (*C. frutiescence*/*C. annum*) are needed to confirm the success of *C. frutiescence*/*C. annuum* cross combinations and their embryo germination.

CONCLUSION

The embryo rescue technique developed in this study can be used for the chilli crop improvement through inter specific hybridization. Hybridity testing with molecular tools should also be carried out for the confirmation of crosses.

REFERENCES

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

Sharmal, D.R., R. Kaur and K. Kumar. 1996. Embryo rescue in plants a review. Euphytica 89: 325-337.

Senanayake, D.M.J.B., W.A.R. Dhammika, E.S.C. Edirisinghe, W.W.K..S. Gunawardena, K.S.I. Senevirathne, A.M.A. Navoditha and P.J.K. Dassanayake. 2014. Preliminary Identification of Chilli Leaf Curl Virus disease resistant chilli accessions. Annals of Sri Lanka Department of Agriculture, 16: 373.