Utilization of Molecular Mechanisms and Male Sterility for Hybrid Breeding in Capsicum (*Capsicum annuum* L.)

Jagmeet Singh[1]*, Sandeep Kumar[1], Chander Parkash[1] and Dharminder Kumar[2]

[1]ICAR-Indian Agricultural Research Institute Regional Station, Katrain, Kullu, Himachal Pradesh, India 175129

[2]Krishi Vigyan Kendra (KVK), Saru, Chamba, Himachal Pradesh, India 176314

Corresponding Author: singhjagmeet877@gmail.com

Abstract

Both the cytoplasmic and nuclear male sterility mechanisms have been used to create hybrids in capsicum. In order to improve heterosis breeding and facilitate parental breeding, marker-assisted selection is essential. Markers associated with cytoplasmic male sterility would help shift the dominant NMS system of bell pepper to the CMS system. The markers, especially the co-dominant markers, can be used to discriminate between heterozygote and homozygote individuals and the efficacy of the hybrid breeding program would rise as a result. The precise mapping of new Rf genes and the identification of markers that are more closely associated to or co-segregate with the Rf genes in the CMS system for chili peppers would facilitate marker-assisted selection. It is simpler to quickly screen the germplasm for allele mining due to the related markers. Development or identification of sweet pepper's restorer lines is expected to make CMS hybrid technology widely available. Utilizing such stable CMS and restorer lines to create CMS-based sweet pepper hybrids would be advantageous and reduce the cost of hybrid development.

Key words: CMS, co-dominant markers, hybrids, marker-assisted selection and restorer lines

Introduction

The Solanaceae family, which contains capsicum, is made up of five major species namely, *C. annuum*, *C. baccatum*, *C. frutescens*, *C. chinense* and *C. pubescens* of cultivated peppers. According to Tang et al. (2024), *C. annuum* is the most extensively cultivated cultivar of them all. Cytoplasmic male sterility (CMS) breeding technologies expedite the production of hybrid seed for a number of crops. In order to reverse CMS and create male fertility in the F_1 hybrid offspring, the male parent's nuclear restorer-of-fertility (Rf) gene is usually utilized during hybrid development (Ortega et al. 2020). The development of commercial F_1 hybrid seeds frequently entails emasculation and physical pollination. To overcome this time-consuming limitation, earlier researchers have worked on employing mutants having male sterility (Swamy et al. 2017; Colombo and Galmarini 2017).

A male-sterile line, its maintainer line and a fertility-restorer line are necessary for a cytoplasmic male sterility system (Zhang et al. 2020). Reports indicate that the recovery of male fertility in chili pepper CMS is influenced by one dominant or double Rf gene (Peterson, 1958), one major-effect QTL, two complementary genes (Novak et al. 1971) and four additional minor-effect QTLs (Wang et al. 2004). Cytoplasmic male sterility (CMS) has not yet been effective in producing sweet pepper hybrids, while being utilized commercially to produce hot (syn. chili) pepper hybrid seed. The main reason for this is because the majority of sweet pepper genotypes either have unstable restorer lines with a limited capacity to restore fertility or lack the fertility restorer (Rf) allele (Mulyantoro et al. 2014 and Kumar et al. 2007). In order to produce male fertile CMS hybrids that are incapable of producing any commercial fruits, they cannot be utilized as the male parent (Lin et al. 2015).

Phenotypic and genotypic selection

The male-fertile phenotype was defined as plants that generated a lot of pollen grains and seeded fruit, whereas the male-sterile phenotype was defined as plants that produced neither pollen grains nor seedless fruit (Zhang et al. 2020). According to Gulyas et al. (2006), the hybrid and parent lines' fertility was evaluated using several techniques. In the first approach, plants were visually inspected for the generation of pollen and the presence or lack of anthers during the blooming stage. In the second procedure, the quantity of stained pollen grains was determined using microscopic analysis.

For the Rf allele for marker assisted breeding to be deployed effectively, markers that are tightly linked to the fertility restoration locus (Rf allele) are required (Lin et al. 2015). The structure and genetic diversity of the chili pepper genome have been completely revealed over the last five years by a number of excellent genome and resequencing projects. These initiatives have laid the groundwork for the creation of a high-throughput genotyping system, which is essential for gene identification and molecular breeding (Tang et al. 2024).

Molecular mapping for fertility restoration gene

Fine-grained gene mapping may help identify markers that are towards the locus or overlap. In order to breed maintenance lines and restorer lines that might potentially fully restore CMS fertility, it would be simpler to filter capsicum germplasm and transfer the "rf" gene more quickly (Jindal et al. 2020). Lee et al. (2008) identified a CMS-associated "pr" (partial restoration) locus that affects fertility restoration. Reproduction is only partially restored by the main "Rf" gene when paired with the recessive "pr" gene (Jindal et al. 2020).

Using the CRF-S870 marker and MAB, the Rf allele from the hot pepper line AVPP9905 was successfully transferred to many sweet pepper genotypes (Lin et al. 2015). It was discovered that the chili pepper (*Capsicum annuum* L.) strong restorer inbred line IVF2014032 has a single dominant locus (CaRf032) for the fertility restoration of CMS. CA00g82510 is one gene candidate for CaRf032 that shows promise. Five KASP markers that co-segregated with CaRf032 were effectively used to genotype 63 restorer and 38 maintainer lines. These markers revealed SNPs (single-nucleotide polymorphisms) in CA00g82510 of 77013 and IVF2014032 (Zhang et al. 2020).

Role of male sterility in hybrid breeding

According to Zhang et al. (2020), three component lines are essential for a CMS breeding system: a fertility-restorer line, a male-sterile line, and its maintainer. The male parent frequently has a nuclear restorer-of-fertility (Rf) gene to counteract CMS and promote male fertility in the F_1 hybrid offspring (Ortega et al. 2020). Only once the right B-line (maintainer) and R-line (fertility restorer) have been found for a stable CMS line (A-line) can CMS be used in pepper hybridization. The R-line would be used as the male parent for the development of hybrid, while the B-line would be utilized to create a new cytoplasmic male sterile (CMS) line and to multiply A-line seeds (Swamy et al. 2019). In order to increase commercial agricultural yields (Jindal et al. 2020), markers associated with the modifier genes can be used to breed C-lines that can restore the hybrid population's full fertility (Lee et al. 2008).

Conclusion

Nuclear restoration enables the exploitation of the cytoplasmic male sterile system for the production of hetrotic seeds with high yield while removing the need for costly manual emasculation and intensive labour. In a more segregated population, it is possible to calculate the physical distance between the marker and the locus with greater accuracy. Additionally, after several generations, populations that were almost homozygous for CMS and the restorer could be identified thanks to the use of this marker, suggesting that it may be used in actual breeding. By employing these markers to conduct genotypic choices, breeders can reduce the time and cost associated with gene transfer.

Declaration

The authors declare no conflict of interest

References

Colombo N and Galmarini CR. 2017. The use of genetic, manual and chemical methods to control pollination in vegetable hybrid seed production: a review. *Plant Breeding* 136: 287-299.

Gulyas G, Pakozdi K, Lee JS and Hirata Y. 2006. Analysis of fertility restoration by using cytoplasmic male-sterile red pepper (*Capsicum annum* L.) lines. *Breeding Science* 56: 331-334.

Jindal SK, Dhaliwal MS and Meena OP. 2020. Molecular advancements in male sterility systems of capsicum: A review. *Plant Breeding* 139:42-64.

Kumar S, Singh V, Singh M, Rai S, Rai SK, Rai M. 2007. Genetics and distribution of fertility restoration associated RAPD markers in inbreds of pepper (*Capsicum annuum* L.). *Scientia Horticulturae* 111: 197-202.

Lee J, Yoon JB and Park HG. 2008. Linkage analysis between the partial restoration (pr) and the restorer-of-fertility (Rf) loci in pep per cytoplasmic male sterility. *Theoretical and Applied Genetics* 117: 383-389.

Lin SW, Shieh HC, Wang YW, Tan CW, Schafleitner R, Yang WJ and Kumar S. 2015. Restorer breeding in sweet pepper: Introgressing Rf allele from hot pepper through marker-assisted backcrossing. *Scientia Horticulturae* 197: 170-175.

Mulyantoro, Ou SL, Chen SY, Liu SC, Lo TC, Wahyono A, Chen SY and Ku HM. 2014. Conversion of the genic male sterility (GMS) system of bell pepper (*Capsicum annuum* L.) to cytoplasmic male sterility (CMS). *Plant Breeding* 133: 291-297.

Novak F, Betlach J and Dubovsky J. 1971. Cytoplasmic male sterility in sweet pepper (*Casicum annuum* L.). I. Phenotype and inheritance of male sterile character. *Zeit-schrift fur Pflamenzuchtung* 65:129-140.

Ortega FA, Barchenger DW, Wei B and Bosland PW. 2020. Development of a genotype-specific molecular marker associated with restoration-of-fertility (Rf) in chile pepper (*Capsicum annuum*). *Euphytica* 216: 43.

Peterson PA. 1958. Cytoplasmically inherited male sterility in capsicum. *The American Naturalist* 92: 111-119.

Swamy BN, Hedau NK, Chaudhari GV, Kant L and Pattanayak A. 2017. CMS system and its stimulation in hybrid seed production of *Capsicum annuum* L. *Scientia Horticulturae* 222: 175-179.

Swamy BN, Sharma VK, Arpita S, Kumar R, Behera TK, Manisha M, Chandresh C and Mukesh K. 2019. Maintenance and fertility restoration of CMS system in sweet pepper (*Capsicum annuum*). *Indian Journal of Agricultural Sciences* 89: 1855-1859.

Tang B, Yang H, Yin Q, Miao W et al. 2024. Fertility restorer gene CaRf and PepperSNP50K provide a promising breeding system for hybrid pepper. *Horticulture Research* 11: uhae223.

Wang LH, Zhang BX, Lefebvre V, Huang SW, Daubeze AM and Palloix A. 2004. QTL analysis of fertility restoration in cytoplasmic male sterile pepper. *Theoretical and Applied Genetics* 109:1058-1063.

Zhang Z, Zhu Y, Cao Y, Yu H, Bai R, Zhao H, Zhang B and Wang L. 2020. Fine mapping of the male fertility restoration gene CaRf032 in *Capsicum annuum* L. *Theoretical and Applied Genetics*

* * * * * * * * *