Breeding Strategies and Genomic Insights into Male Sterility and Fertility Restorer Systems in Onion

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Abstract

Onion hybrid breeding is constrained by significant inbreeding depression, laborious emasculation, and a dearth of elite inbreds. The only option to increase output in onions is to take advantage of heterosis through male sterility mechanisms. In contemporary onion breeding, the problem of male sterility is very important. Marker-assisted selection (MAS) for identifying fertility restorer genes in addition to S, N and T cytoplasm with significant heterosis are more effective than the traditional hybrid development method. Several molecular markers linked to male-sterile cytoplasms, male-fertile cytoplasms, and nuclear-male fertility restorers have been used for crop improvement programs in onion. The molecular mechanisms of various intricate plant life processes may be elucidated with the use of recently developed omics technologies, which are very effective and reliable. In areas where genome sequence information is scarce, omics technologies like metabolomics, genomics, proteomics, transcriptomics etc. possess the capacity to offer up new study and opportunities in *allium* crops.

Key words: Cytoplasms, fertility restorer genes, heterosis, omics and onion

Introduction

Allium cepa L., or onion, is an important horticulture crop cultivated worldwide. Onion resistance to biotic and abiotic stressors along with yield and quality have all increased since domestication, due to a number of traditional breeding techniques (Mainkar et al. 2023). It is biennial in nature and in an open-pollination, it takes around 4 to 8 years for a progeny test to create a maintainer line that corresponds to its male-sterile line (Havey, 2000). Heterosis breeding works effectively in onions, but there are many complexities involved in improving them, such as the existence of multiple tiny hermaphrodite flowers, challenges with manual emasculation, bienniality, high inbreeding depression, and little hybrid seed production (Bal, 2023). Using sources of CMS, hybrid onion (*Allium cepa*) seed becomes commercially viable. A male-sterile line (A-line) along with its maintainer line (B-line) and a male-fertility restorer line (C-, or R-line) are required for the CMS system to produce hybrids (Goldman et al. 2002). Nevertheless, relatively few research teams have tried to use male sterile systems to utilize heterosis in onions. The absence of maintainers for male sterile genotypes, male sterility instability, high labour and time demands for seed production and hybrid breeding are the primary causes (Bal, 2023). By substituting tedious and time-consuming progeny testing, molecular markers can significantly speed up breeding procedures (Abbasi, 2023). Therefore, the discovery of closely related markers to the nuclear gene at the Ms locus would make it simple to isolate maintainer lines by marker-assisted selection at the early stages (seedling stage) in a short amount of time (Manjunathagowda, 2021). Breeders can quickly and economically choose desired features by using molecular markers (Bang et al. 2013). The complicated molecular process can be analyzed more quickly and accurately using a variety of omics techniques, which might eventually result in the creation of novel breeding program tactics for *allium* enhancement (Khandagale et al. 2020).

Genetic basis for heterosis

Since male sterility is accessible, heterosis in onions has been economically exploited. Onions have two very likely male sterility systems: genetic male sterility (GMS), which is controlled by dominant nuclear genes (Liu et al. 2019). The alternative is based on cytoplasmic genetic male sterility systems (CGMS), which comprise three lines: A line (cytoplasmic male sterile line), B line (isogenic maintainer line) and R line (fertility restorer line). This technique is most frequently employed to produce hybrid onion seeds. (Sharma et el. 2023). Male sterility in onions is controlled by the association of recessive nuclear genes with several sterile cytoplasms (CMS-S, CMS-R and CMS-T) in CGMS system. It has been linked to the association between recessive nuclear genes and sterile cytoplasm (CMS-S) (Jones and Emsweller, 1936a; Jones and Clarke, 1943). The recessive homozygous condition (msms) of the nuclear gene with sterile cytoplasm results in the male sterile phenotype, whereas the dominant form (Ms) of the nuclear gene results in the fertile phenotype (Chikh-Rouhou et al. 2025).

Mechanisms underlying male fertility restoration

For the creation of F_1 hybrid cultivars of onions, three different CMS and fertility restoration techniques have been discovered thus far. The first of these systems, known as CMS-S, was discovered in the cultivar 'Italian-Red' in 1925. (Jones and Emsweller, 1936b), the 'Rijnsburger' onion was found to have CMS-R or T-like (Banga and Petiet, 1958) and CMS-T, identified in the 'Jaune paille des Vertus' variety (Berninger, 1965). Due to the durability of S cytoplasm in a range of environmental circumstances and straightforward monogenic inheritance, it is now the most often utilized CMS source (Havey, 2000). Following crossing with the male-fertile line (R/C line), which is controlled by a single dominant allele (Ms) at the nuclear locus, the fertility restoration of male-sterile (CMS-S) cytoplasmic plants was discovered. The recessive allele (ms) at the fertility restoration locus is crossed with normal fertile (N) cytoplasm to create the maintainer line (Manjunathagowda, 2021). Additionally, strongly associated nuclear markers at the CMS genes and Ms Locus would make it easier to process F_1 breeding and development and enable molecular-assisted segregation analysis (Abbasi, 2023).

Marker-based identification of maintainer lines

Since only N-cytoplasmic plants would be kept forward until flowering and utilized for test crossing with A line (male sterile), it has been demonstrated that MAS for cytotype can minimize the number of test-crosses needed to isolate maintainers (Dehghani et al. 2021). Bang et al. (2013) identified the RAPD marker OBC14.1000, and the ACms.1100 marker was created to change a dominant marker into a codominant marker based on the marker OBC14.1000. In marker-assisted onion breeding, these markers would be perfect for allelic discrimination to forecast the genotype of a restorer-of-fertility gene with higher efficiency. Maintainer line development was considerably facilitated by the discovery of molecular markers closely associated with the nuclear (Ms) locus. Fertility restorer gene (Ms/ms) was identified (Saini et al. 2015), using two indicators associated with photosystem I subunit (PsaO) and putative oligopeptide transporter (OPT) as mentioned by Bang et al. (2011).

Omics-based insights for fertility restoration

Due to developments in genomics and molecular techniques, the whole mitochondrial genome has been sequenced, providing insight into the genetic causes of onion male sterility. The creation and identification of genetic markers associated with male sterility, namely the mitochondrial genes orf219, orf725 and cox1, have been made easier by genomic research, allowing marker-assisted selection of male-sterile lines in onions (Chikh-Rouhou et al. 2025). Kim et al. (2015) identified potential genes for fertility restoration using BSA and RNA-seq studies. Along with 14 contigs that demonstrated their perfect relationship with the Ms locus, many SNPs that distinguish between male-sterile and male-fertile bulks were found. The best candidate gene and most trustworthy marker for choosing the restorer line among these contigs is AcPMS1, which is engaged in the DNA repair pathway. These kinds of investigations revealed that nuclear and mitochondrial genes, as well as their interactions, control CMS in *alliums* (Khandagale et al. 2020).

Conclusion

It is possible to create consistent, high-yielding onion hybrids by successfully using stable male sterile lines. Male sterility is a rapid and cost-effective procedure for the production of onion hybrids. Marker-assisted selection aids in the discovery of nuclear male fertility restorers (NMSMS), male fertile maintainer lines (Nmsms), and cytoplasmic male sterile lines (CMS-S and CMS-T). Other molecular markers like as SNP, SSR, InDel and CAPS must be validated for allelic variation and co-dominant marker establishment.

Declaration

The authors declare no conflict of interest.

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