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A REVIEW

Genetics and molecular mapping of fertility restoration genes for CMS-WA system in rice

■ N.V. KAYANDE AND N.S. KUTE

SUMMARY

For feeding the increasing population and enhancing productivity of rice, commercial exploitation of heterosis is essential. The combination of cytoplasmic male sterility (CMS) in one parent and restorer gene *(Rf)* to restore fertility in another are indispensable for the development of hybrid varieties. Searching for restorer genes is a good approach when phenotyping is very time-consuming and requires the determination of spikelet sterility in testcross progeny. The wild abortive (WA) cytoplasm is most widely used for hybrid seed production in rice. There are three major CMS types *i.e.* HL, BT and WA. *Rf3* for CMS-WA is located on chromosome 1, while *Rf1, Rf4, Rf5 and Rf6* correspond to CMS-BT, CMS-WA and CMS-HL, located on chromosome 10. Molecular mapping lead to the development of PCR-based markers linked to *Rf* genes and the application of MAS for restorer genes, which would increase the efficiency of selecting putative restorer lines. In addition to use of markers in MAS procedures, these markers can also be used to transfer *Rf* genes into adapted cultivars through a backcrossing programme in an active hybrid rice breeding programme.

Key Words : Hybrid rice, Molecular markers, Fertility restoration, Cytoplasmic male sterility

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Hybrid rice technology offers a potentially viable option for increasing rice yield potential beyond the level of inbred high-yielding varieties by exploiting heterosis, or hybrid vigor, on a commercial scale. Cytoplasmic male sterility (CMS) combined with a fertility restoration system has been found to be the most efficient genetic tool in commercializing this technology in rice (Lin and Yuan, 1980; Virmani and Wan, 1988). The CMS systems have been extensively studied for their cytological, physiological and genetic characteristics. They were usually categorized into Wild Abortive (WA), Bao Tai (BT) and Honglian (HL) based on the evidence from genetic and cytological studies (Rao, 1988; Li and Yuan, 2000). Among these three, the WA cytoplasm is the most widely used since it is a more stable system with complete pollen sterility (Shinjyo and Omura, 1966). In most of cases it was discovered that fertility restoration is controlled by two independent dominant nuclear genes with one stronger in action than the other (Young and Virmani, 1984; Virmani *et al.,* 1986). Identification of restorers and maintainers from large number of genotypes in the source nursery is the first and foremost step in the hybrid rice breeding. This is done by crossing the available genotypes with CMS lines and evaluating the progenies the test cross nursery for pollen and spikelet fertility. This conventional method of test crossing and evaluating the progenies based on morphological traits is laborious, time consuming and less accurate. Almost one year (two seasons) is required to identify the restorers. Moreover, the method is inefficient as only 15-20 per cent genotypes turn out to be restorers and 80 per cent of efforts in making crosses are wasted. Development of a MAS procedure involving the two main restorer QTLs on chromosomes 1 and 10 would significantly reduce the time

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
N.V. KAYANDE, Department of Agricultural Botany, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, AHMEDNAGAR (M.S.) INDIA
Email: navinchandra_kayande@rediffmail.com

Address of the Co-authors:
N.S. KUTE, Department of Agricultural Botany, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, AHMEDNAGAR (M.S.) INDIA

and labour in making and evaluating testcrosses in an active hybrid rice-breeding programme. This paper, besides investigating the inheritance of the fertility restoration of CMS-WA system and focused major achievements in identification of molecular markers linked to the *Rf* genes for marker-assisted selection (MAS) and to narrow down the region of the *Rf* locus to provide a more saturated map that is necessary for starting chromosome walking and cloning of the *Rf* genes.

Cytoplasmic male sterility in rice :

Cytoplasmic male sterility (CMS) is a maternally inherited trait characterized by the inability of a plant to produce functional pollen that is associated with abnormal open reading frames (ORFs) found in mitochondrial genomes and, in many cases, male fertility can be restored by fertility restorer (Rf) genes associated with nuclear genes encoding pentatricopeptide repeat (PPR) proteins (Chase and Babay-Laughnan, 2004; Hanson and Bentolila, 2004). Cytoplasmic male sterility (CMS) is the foundation of utilization of crop heterosis. Most types of CMS are caused by the expression of aberrant chimeric genes locating on mitochondrial genomes (Chase and Babay Laughnan, 2004). The combination of Cytoplasmic Male Sterility (CMS) in one parent and a restorer gene *(Rf)* to restore fertility in another are indispensable for the development of hybrid varieties. Plant cytoplasmic male sterility (CMS) caused by lesion or rearrangement of mitochondrial genome is unable to produce functional pollens (Jing *et al.,* 2001). Jones (1926) first reported the existence of male sterility in rice. Later Sampath and Mohanty (1954) reported the role of cytoplasm in causing male sterility in rice. The first cytoplasmic male sterile line in cultivated rice designated as BaoTai (BT) was developed by Shinjyo and Omura (1966) from the cytoplasmic source of Chinsurah Boro II variety. After that, a series of elite CMS lines have been developed by breeders in China. The first CMS line used to develop commercial F_1 rice hybrid was developed in China in 1973 from a CMS plant occurring naturally in a wild population of *Oryza sativa f. spontanea* in Hainan Island, designated as Wild abortive (WA-type).The cytoplasm induces the cytoplasmic male sterility (CMS) through interaction with the cell nucleus (Virmani *et al.,* 1981).

Hybrid breeding based on CMS/*Rf* systems has achieved great success all over the world. Reliable CMS systems can eliminate labour intensive steps of emasculation and hand pollination in F_1 seed production and breeding programmes (Newton, 1988). Since rice is strictly a self-pollinated crop, hybrid seed production must be based on male sterility systems. The CMS based hybrid breeding system involving male sterile (CMS/A), maintainer (CMS/B) and restorer (R) lines is popularly known as three line breeding (Virmani *et al.,* 1993). Till date, more than 40 different CMS sources have been reported in rice (Virmani and Shinjyo, 1988). Wild abortive (WA), BaoTai (BT), Honglian (HL), Gambiaca, Dian 1(D), Lead rice (Ld), Dissi (DIS) and Assam Rice Collection (ARC) sources are currently used in hybrid rice breeding world-wide. The WA-CMS system, discovered in China (Yuan, 1977; Lin and Yuan, 1980), is the most widely used CMS source, which accounted at one stage for 90 per cent of the rice hybrids produced in China and 100 per cent of the hybrids developed outside China (Sattari *et al*., 2008). However, extensive research work on identification of restorers and maintainers and the inheritance of fertility restoration has been done on the WA cytoplasmic source only.

Inheritance of *Rf* for CMS-WA :

Information on the genetics of fertility restoration in a CMS system facilitates breeding and /or selection of restorer lines used in hybrid breeding programmes.(Govinda Raj and Virmani, 1988).The discovery of cytoplasmic male sterility (CMS) not only facilitates hybrid seed production, but also provides an excellent system for the study of nucleus-cytoplasm interaction. High yield potential of CMS derived F_1 hybrids depends upon their high pollen fertility and spikelet fertility, which determined by the number and mode of action of restorer genes present in their restorer parent. Knowledge of the genetic control of male fertility restoration is also useful to transfer fertility restoring genes to promising breeding lines and undertake improved restorer breeding programme. It may also help in efficient transfer of restorer genes in other agronomically desirable genotypes (Sohu and Phul, 1995). In indica rice, hybrid varieties have been developed using Wild abortive (WA) CMS source. However, reports regarding the number, positions, and the effects of these *Rf* genes are inconsistent in various studies. The genetics of fertility restoration is monogenic in WA-CMS lines (Shen *et al.,* 1996; Wang, 1980; Mishra, 2001; Anandkumar and Subramaniam , 1992), digenic (Virmani *et al.*,1986 ; Bharaj *et al.*, 1991, 1995; Yao *et al.* 1997; Zhang *et al.,* 1997; Komori *et al.*, 2003; Ahmadikhah and Karlov, 2006), digenic with different types of interaction (Govinda Raj and Virmani, 1988; Sohu and Phul, 1995; Sharma *et al.,* 2001; Sattari *et al*., 2008; Sheeba *et al.,* 2009) trigenic (Sarkar *et al.,* 2002; Govinda Raj, 1983; Kumar and Chakrabarti, 1983) and trigenic interactions (Huang *et al.*, 1987). These findings suggest that indica rice exhibits genetic diversity of *Rf* genes.

As per the literature reviewed it is observed that, most of the investigations tend to indicate that fertility restoration of the WA cytosterility system is controlled by two pairs of independent dominant fertility restoring genes with one pair being stronger in action than other (Yang and Lu, 1984; Li and Yuan, 1986; Govinda Raj and Virmani, 1988; Pradhan and Jachuk, 1999; Teng and Shen, 1994). Bharaj *et al.* (1995) reported that the stronger gene was located on chromosome 7 and the weaker one on chromosome 10 in IR36. The nature of genetic control and mode of action of *Rf* genes are still not

clear. Further, the fertility restorers selected in these studies were not always suitable for commercial exploitation due to either weak restoration ability or lack of good combining ability for developing commercial hybrids. So there is need of more research in this area to have better knowledge regarding the genetics of *Rf* genes. The information on the inheritance of fertility restoration will help in designing strategies for breeding elite hybrid parents. The use of restorers with more than one independent gen for fertility restoration is likely to produce the hybrids with higher fertility restoration and consequently increased yield. The identified new restorers would be useful in future hybrid breeding programmes so as to develop hybrids with diverse genetic background.

Molecular markers for fertility restoration genes in CMS-WA systems of rice :

In the 1990s, many advances occurred in the application of molecular markers in rice (Mackill and Ni, 2001; Temnykh *et al.,*2001).Molecular markers are particularly useful for accelerating the process of introducing a gene or quantitative trait loci (QTL) into an elite cultivar or breeding line via backcrossing . Markers linked to the gene can be used to select plants possessing the desired trait, and markers throughout the genome can be used to select plants that are genetically similar to the recurrent parent (Young and Tanksley, 1989; Hospital *et al.*,1992; Semgan *et al*., 2006).Mapping studies conducted on *Rf* genes for the WA-CMS system have not produced consistent results to confirm their chromosomal locations and the number of significant QTLs on account of researchers using different sources of restorer genes and divergent genetic backgrounds of the experimental populations. Hence, the chromosomal location of the *Rf* loci for WA-CMS remains to be resolved.

Molecular marker technology is the powerful tool for determining genetic variation in rice varieties (Xu *et al.,* 1974).The use of DNA markers for indirect selection offer the greatest benefits for quantitative traits with low heritability as these are the most difficult characters to assess in the field experiments. Many fingerprinting techniques based on polymerase chain reaction (PCR) such as simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD), expressed sequence tags (ETS), sequence tagged sites (STS), and amplified fragment length polymorphism (AFLP) markers are capable of detecting polymorphism between A lines and R lines have been developed over the past several years. Characterization of WA-CMS is essential for designing markers for its precise identification and also for understanding the molecular basis of male sterility expression. Despite the importance of WA-CMS, its fertility restoration is poorly understood at molecular level. The utility of CMS lines is determined by availability of well characterized and effective fertility restoration systems. Recent advances in molecular marker technology have enabled several research groups to determine the chromosomal locations of the two *Rf* genes (*Rf3* and *Rf4*) for the WA-CMS system. Two major fertility-restorer genes, *Rf3* and *Rf4*, are required for the production of viable pollen in WA-type CMS and the genes have been mapped to chromosomes 1 and 10, respectively (Jing *et al.,* 2001; Yao *et al.,* 1997; Zhang *et al.,* 2002; Ahmadikhah *et al.,* 2006; Sattari *et al.,* 2008, Sheeba *et al.,* 2009; Alavi, 2009). Using RFLP markers, Zhang *et al.* (1997) mapped one of the two *Rf* loci (*Rf3*) on chromosome 1 between RG140 and RG532 at a distance of 1.9 cM from each.Yao *et al.* (1997) confirmed the location of *Rf3* on chromosome 1 and mapped the second *Rf* locus (*Rf4*) on chromosome 10 at 3.3 cm from G4003. Tan *et al.* (1998) identified one QTL that, in a region between S10019 and C1361 on chromosome 10, explained 71.5 per cent of the phenotypic variance. Jing *et al.* (2001) mapped an *Rf* locus (*Rf4*) governing fertility restoration on the long arm of chromosome 10 using SSLP markers. Zhang *et al.* (2002) also mapped the *Rf4* gene on chromosome 10 at 0.9 cm from the marker Y3-8 and anchored to the RFLP marker S10019.

Sheeba *et al*. (2009) reported the usefulness of the marker RM6100 in marker-assisted selection for fertility restoration in segregating populations and identification of restorers from germplasm lines for their fertility restoration ability. RM6100 amplified the *Rf-4* linked allele in a majority of the restorers with a selection accuracy of 94.87 per cent. RM6100 was very close to the gene at a genetic distance of 1.9 cm. The accuracy of the marker RM6100 in predicting fertility restoration was validated in 21 restorer and 18 maintainers with 94.9 per cent efficiency. Ahmadikhah and Karlov (2006) identified that *Rf4* was flanked by two SSR markers RM171 and RM6737 at distances of 3.2 and 1.6 cm, respectively. Sattari *et al.,* (2008) reported that from F_2 populations, generated from the crosses between the parents of good-performing rice hybrids, *Rf3* was located at a distance of 2.8 cm from RM490 on chromosome 1 and *Rf4* was located at 1.6 cm from RM1108 on chromosome 10.Alavi *et al.* (2009) reported in a F_2 population developed from the cross Neda-A×IR36, three SSR markers (RM1, RM3233, RM3873) and one CAPS marker (RG140/EcoRI) on the short arm of chromosome 1 were linked to *Rf3*. *Rf3* flanked by two SSR markers RM1 and RM3873 at distances of 5.6 and 14 cm, respectively.

Jing *et al.* (2001) studied a F_2 population consisting of 210 excessive sterile individuals from a cross between Zhenshan 97A and a strong restorer line IR24 was used for mapping of *Rf4.* The genetic distance from *Rf4* locus to RM171 and RM228 on long arm of chromosome 10 was 3.7 cm and 3.4 cm, respectively, which were the two closest SSR markers flanking the *Rf4* locus. The two SSR markers gave promise of application in molecular marker-assisted selection (MAS) for fertility restorer lines of the CMS-WA system. Bazrkar *et al.* (2008) tagged four *Rf* genes for WA–CMS system using SSR markers on chromosomes 1(*Rf3*), 7(*Rf4*), 10(*Rf6*) and12 (*Rf7*) by recessive class analysis. A new *Rf* locus designated as *Rf7*

on chromosome 12 was found to be linked to RM7003 at a genetic distance of 13.3 cm. Molecular marker (RM 6344) linked to *Rf4* locus on chromosome 7; RM443 and RM315 were flanking the *Rf3* gene at a genetic distance of 4.4 and 20.7 cm on chromosome 1, respectively. The *Rf6* was flanked on both sides with SSR markers RM 258 and RM591 at a genetic distance of 4.4 and 23.3 cm located on chromosome 10.

Khatibani *et al.* (2009) reported that microsatellite markers RM443 and RM315 were flanking *Rf3* gen at a genetic distance of 4.4 and 20.7 cM on chromosome 1, respectively while RM6344 was closely linked at a distance of 6.6 cM to *Rf1* on chromosome 7. The third gene *Rf2* was flanked on either side with SSR markers RM258 and RM591 on chromosome 10 at a genetic distance of 4.4 and 22 cm, respectively.

Summary and outlook :

Molecular tagging of the fertility restorer genes will help in the characterization of restorer lines without involving a CMS line or extensive test crossing with cytoplasmic male sterile (CMS) lines. In addition, pyramiding of the restorers, selection for the restorer genes independent of environmental influence on restoration and identification of plants with restorer genes at every seedling stage would be possible by marker assisted selection (MAS). Ultimately, identification of closely linked markers would help in map based cloning of fertility restorer genes to understand the molecular and biochemical basis of fertility restoration and the possible role of Rf genes in other biological processes. Development of a MAS procedure involving the two main restorer QTLs on chromosomes 1 and 10 would significantly reduce the time and labour in making and evaluating testcrosses in an active hybrid rice-breeding programme. In particular, the development of PCR based markers would empower researchers in local agricultural research systems to apply the technology in local hybrid rice breeding programs and seed purity assessments.

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