

AFLP and PCR-based markers linked to *Rf3*, a fertility restorer gene for S cytoplasmic male sterility in maize

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Abstract The *Rf3* gene restores the pollen fertility disturbed by S male sterile cytoplasm. In order to develop molecular markers tightly linked to *Rf3*, we used amplified fragment length polymorphism (AFLP) technique with near isogenic lines (NILs) and bulk segregant analysis (BSA). A BC₁F₁ population from a pair of NILs with different *Rf3* locus was constructed and 528 primer combinations was screened. A linkage map was constructed around the *Rf3* locus, which was mapped on the distal region of chromosome 2 long arm with the help of SSR marker UMC2184. The closest marker E7P6 was 0.9 cM away from *Rf3*. Marker E3P1, 2.4 cM from *Rf3*, and E12M7, 1.8 cM from *Rf3*, were converted into a codominant CAPS and a dominant SCAR marker, and designated as CAPSE3P1 and SCARE12M7, respectively. These markers are useful for marker-assisted selection and map-based cloning of the *Rf3* gene.

Keywords Maize · Fertility restorer · Near isogenic lines · Bulk segregant analysis · AFLP · PCR-based markers · Marker-assisted selection · Map-based cloning

Abbreviations

AFLP Amplified fragment length polymorphism
BF Fertile DNA bulks

BS Sterile DNA bulks
BSA Bulk segregant analysis
CAPS Cleaved amplified polymorphic sequence
CMS Cytoplasmic male sterility
E/M *EcoRI/MseI*
P/M *PstI/MseI*
P/E *EcoRI/PstI*
MAS Marker-assisted selection
NILs Near isogenic lines
ORF Open reading frames
PC Primer combinations
PPR Pentatricopeptide repeat
RAPD Random amplified polymorphic DNA
RFLP Restriction fragment length polymorphism
SCAR Sequence characterized amplified regions
SNP Single nucleotide polymorphism
SSR Simple sequence repeat, microsatellite

Introduction

Plant cytoplasmic male sterility (CMS) is a maternally inherited trait, characterized by its inability to produce viable pollen without affecting the female fertility. It is often associated with novel mitochondrial open reading frames (ORFs) that are chimerical in structure and frequently co-transcribed with conventional mitochondrial genes (Schnable and Wise 1998). Nuclear genes designated fertility restorer (*Rf*) restore fertility to plant carrying CMS by suppressing the expression of deleterious mitochondrial ORFs or reducing CMS-associated proteins (Schnable and Wise 1998; Wise and Pring 2002). CMS/*Rf* system greatly facilitates hybrid seed production because it eliminates the need for tedious hand emasculation. Besides this practical

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exploitation, the isolation and characterization of ORFs associated with CMS and corresponding *Rf* genes also offer opportunity to examine the regulation of mitochondrial genes by nuclear genes in multicellular organisms (Maureen and Bentolila 2004).

In all, four restorer genes have been cloned and functionally characterized. The *Rf2* gene in T-CMS maize encodes a mitochondrial aldehyde dehydrogenase, which compensates the metabolic defect caused by mitochondrial URF13 protein associated with CMS (Cui et al. 1996). Other three *Rf* genes including *Rf* in petunia (Bentolila et al. 2002), *Rfk1/Rfo* in radish (Koizuka et al. 2003; Brown et al. 2003), and *Rf1* in rice (Kazama and Toriyama 2003; komori et al. 2004; Akagi et al. 2004) encode pentatricopeptide repeat (PPR) proteins, which are targeted to mitochondria and disrupt the accumulation of CMS-associated proteins. Except *Rf1* in rice which functions gametophytically, other cloned *Rf* genes work sporophytically. In CMS-S maize, male sterility and fertility are also determined by mitochondrial–nuclear interactions in the haploid male gametophyte (Buchert 1961). It was discovered that S cytoplasmic male sterility in maize was due to the expression of a chimeric mitochondrial gene region designated *orf355–orf77* (Zabala et al. 1997). The nuclear allele capable of restoring fertility to CMS-S plants is designated as *Rf3* (Duvick 1965), and the nonrestoring allele (*rf3*) does not transmit through the pollen. Several attempts have been made to tag *Rf3* with molecular markers. *Rf3* has been located on the long arm of maize chromosome 2 (2L) by translocation and inversion heterozygotes (Laughnan and Gabay 1978). Two RFLP markers, *whp* and *bnl7.14* in 2.09bin, were shown to be at a distance of 4.3 cM proximal and 6.4 cM distal to *Rf3* locus, respectively (Kamps and Chase 1997). Shi et al. (1997) and Tie (2000) found a RAPD and a SSR marker linked with *Rf3* locus, the distance between them and *Rf3* is 2.7 and 2.29 cM, respectively.

Marker-assisted selection (MAS) can improve the breeding process by selecting molecular marker linked with genes controlling the target traits (Francia et al. 2005). AFLP markers (Vos et al. 1995) combine the strengths of RFLP and RAPD markers and overcome their problems. This approach can generate complex band patterns in each reaction and cover the whole genome. It requires no probe or sequence information as needed by RFLP, and is reliable due to its high stringent polymorphase chain reaction (PCR) in contrast to RAPD (Zhu et al. 1998). Combination of AFLP and BSA has been shown to be an efficient way to generate a number of markers closely linked with important agronomic traits (Negi et al. 2000; von Stackelberg et al. 2003; Hagihara et al. 2005; Feng et al. 2005).

However, AFLP has limitations with large-scale, locus-specific application because of its intensity of labor, high costs and their dominant type of inheritance. Therefore, conversion of AFLP markers into sequence-specific PCR-based markers is required to facilitate large-scale population selection.

The objective of this study was to identify AFLP markers more closely linked with the *Rf3* of S-CMS in maize, and to convert them into PCR-based markers for the benefit of MAS and map-based cloning of *Rf3*. Furthermore, SSR markers on chromosome 2L reported previously were conducted to provide anchor points.

Materials and methods

Plant materials

A backcross population was set up using a cross among S-Mo17^{*rf3rf3*}, a S type cytoplasmic male sterile line, and a S-Mo17^{*Rf3Rf3*}, the near isogenic line (NIL) related to S-Mo17^{*rf3rf3*} (Xia and Zheng 2002). They share similar genetic background with only different *Rf3* locus and its adjacent region. Simultaneously, N-Mo17^{*rf3rf3*}, a sterility maintainer performed as recurrent male parent. Materials mentioned above and their deriving BC₁F₁ population, 343 plants of (S-Mo17^{*rf3rf3*} × S-Mo17^{*Rf3Rf3*}) × N-Mo17^{*rf3rf3*}, were grown on the farm of Huangzhong Agricultural University, Wuhan, China.

Male fertility

The BC₁F₁ plants of genotype (*Rf3/rf3*) produce 50% functional and 50% aborted pollen due to the gametophytic nature of fertility restoration, whereas the *rf3/rf3* plants are completely sterile. According to their ability in shedding pollen and stainability of pollen grains by 1% I-KI (Xia and Zheng 2002), each plant was scored as male fertile or sterile.

Isolation of genomic DNA and construction of DNA bulks

Genome DNA was prepared from parents and BC₁F₁ individuals by a CTAB procedure described by Zhang et al (1994) with minor modifications. 5 g leaf tissues were ground into powder in liquid nitrogen, then extracted at 65°C, de-proteinized with chloroform/isomyl alcohol, and then precipitated with isopropanol prior to treatment with RNase. Briefly dried DNA was solubilized in 0.5 ml TE (10 mM Tris/HCl pH 8.0, 1 mM EDTA). Equivalent amounts of DNA from ten fertile plants and ten sterile plants were used to

construct fertile (BF) and sterile (BS) DNA bulks in order to find markers linked to *Rf3*.

SSR and AFLP analysis

Thirteen microsatellite primers on maize chromosome 2.09bin were screened by parents and DNA bulks, their sequences were available on the maize genetic and genomics database (<http://www.maizegdb.org>). Special primers were used for linkage analysis as anchor points to distinguish *Rf3/rf3* parents and BF/BS bulks. The temperature and time profile is 30 cycles of 94°C for 30 s, 54–60°C for 30 s (modified when needed), and 72°C for 1 min. Electrophoresis was performed with 6% denaturing polyacrylamide gels. The DNA was visualized by silver staining following the protocol of Bassam et al. (1991).

AFLP marker analysis was performed as described by Vos et al. (1995) with some minor modifications, such as using three restriction enzyme combinations, *EcoRI/MseI* (*E/M*), *PstI/MseI* (*P/M*) and *EcoRI/PstI* (*P/E*). A total of 250 ng genomic DNA was digested and ligated with corresponding adaptors. Pre-amplification was performed with primers containing a single selective nucleotide. And selective amplification was carried out with primers containing two (only in the *PstI* primers) or three additional selective nucleotides. The electrophoresis and silver staining were performed as method in SSR.

Conversion of AFLP markers into PCR-based markers

Gel with polymorphic DNA fragments was excised with a scalpel blade and transferred into a microcentrifuge tube. Distilled water (50 μ l) was added, each piece was crushed with a pipette tip and the mixture was boiled for 20 min. The solution was centrifuged and the supernatant was used as template for PCR amplification with corresponding selective AFLP primers for 30 cycles at 94°C for 30 s, 58°C for 1 min and 72°C for 1 min. The product was electrophoresed on 1.5% agarose gel, and the appropriate size of fragment was inserted into a pGEM-T easy vector (Promega) and sequenced (Shanghai Sangon). A search for sequences homologous with the AFLP fragments was conducted in Genbank (<http://www.ncbi.nlm.nih.gov/BLAST>) and Clustal W (<http://www.ebi.ac.uk/clustalW>) was used to compare marker sequences for homology.

Primers were designed with Primer Premier 5.0 and synthesized commercially. The primers based on the original sequences of AFLP fragments were used to amplify the corresponding region from S-Mo17^{*Rf3Rf3*} and S-Mo17^{*rf3rf3*} to detect polymorphism. Sequences

whose polymorphism was not obtained in this process were used to design external primers according to their flanking sequences. Restriction endonucleases required for digesting PCR products to get CAPS markers were purchased from Promega.

Linkage analysis

Codominant markers or dominant markers with fragments present in S-Mo17^{*Rf3Rf3*} and BF pool but not in S-Mo17^{*rf3rf3*} and BS pool were applied for linkage analysis in 343 BC₁F₁ plants. Mapmaker/Exp, version3.0 software (Lander et al. 1987) was employed to calculate genetic distance on a minimum LOD score of 3.0 between *Rf3* and associated markers, and to draw the linkage map.

Results

Segregation analysis of male fertility

The BC₁F₁ generation yielded 178 semi-fertile (having 50% or more stainable pollen) and 165 sterile plants. This segregation ratio fits a monogenic Mendelian inheritance model of 1 fertile (*Rf3rf3*):1 sterile (*rf3rf3*) ($\chi^2 = 0.419$, $P > 0.95$) in this population, it confirmed that fertility restoration was conditioned by one dominant restorer gene in these maize materials.

Identification of molecular markers linked to *rf3*

The fertile parent, bulk and sterile parent, bulk were compared to screen SSR and AFLP markers. One of 13 SSRs on chromosome 2.09bin, UMC2184, was detected to show codominant polymorphism between parents and bulks. No differences were revealed between our study materials with other 12 SSRs, thus UMC2184 should be employed to analyze linkage relationship between itself and *rf3* and perform anchor point to other markers.

The total 528 (144 of *E/M*, 192 of *P/M*, 192 of *P/E*) AFLP primer combinations (PC) were screened for their polymorphism between parents and bulks. Approximately 29,000 fragments were scored, with an average of 55 fragments per PC. Each *P/E* (both are hexa-nucleotide-required endonucleases) PC generated an average of 40 bands, while 62 bands were revealed by the other PC, *P/M* and *E/M* (*MseI* recognizes tetra-nucleotide site) on average. The number of bands was consistent with expected results with respect to the site length recognized by restriction enzyme. What's more, *P/E* PC provided clearer bands than

others. We totally identified 31 polymorphic fragments which were present in the fertile parent and bulk while absent from the sterile parent and bulk. About 21 were shown by the *P/E* PC. Polymorphic bands were named as the PC where they originated from, and were further confirmed whether they were linked to *Rf3* by amplifying them from the individuals composing the bulks. Only five polymorphic fragments from E3P1, E4P6, E7P6, E10P7 and E12M7 were present in ten fertile individuals and absent from ten sterile individuals. A representative gel from PC E10P7 is shown in Fig. 1. Its linkage relationship with *Rf3* was analyzed subsequently using the BC₁F₁ population.

Conversion of AFLP markers into PCR-based markers

In order to convert AFLP markers into simple single-locus PCR-based markers, the AFLP markers, confirmed to be linked to *rf3*, were gel-purified, re-amplified, cloned and sequenced. Eighty percent fragments except one from E10P7 were re-amplified successfully. Homologous analysis among other four marker sequences show that sequences from E7P6 and E4P6 were homologous with only one nucleotide difference, originated from *Eco*RI selective base. Therefore, only AFLP markers from PC E3P1, E7P6, E12M7 were selected to conduct

conversion. The locus-specific primers were designed based on their sequences for amplifying parents and bulks to detect polymorphism. A summary of every converted marker is shown in the Table 1.

Using primers derived from internal sequences of E12M7 markers, we amplified a 170 bp fragment from fertile parent and bulk, but no product from sterile parent and bulk. Further confirmation of this marker linked to *rf3* was accomplished by examining two individual bulks. The same result as originated AFLP marker E12M7 was obtained (Fig. 2a). This marker was then designated as SCARE12M7, and employed for linkage analysis in BC₁F₁ population.

The AFLP marker E3P1 is 271 bp with AT-rich (60%), the internal primers were not designed successfully. To acquire its flanking sequences, the convenient homologous search was applied. A maize GSS sequence from Genbank (accession No.: CL993366) is high homologous with our query (*E* value = 3e-127) and long enough to cover the sequence studied. According to the flanking sequence, the appropriate external primers were designed and a 359 bp fragment was produced from both fertile and sterile parents. The fragments were cloned into pGEM-T easy vector and sequenced. Sequences comparing analysis indicated that a SNP resided in the site recognized by *Mse*I.

Fig. 1 A representative image of BSA–AFLP analysis from E10P7. Arrow indicates fertile parent, bulk, and individuals—specific band (*Rf3*, BF: fertile parent and bulk; *rf3*, BF: sterile parent and bulk)

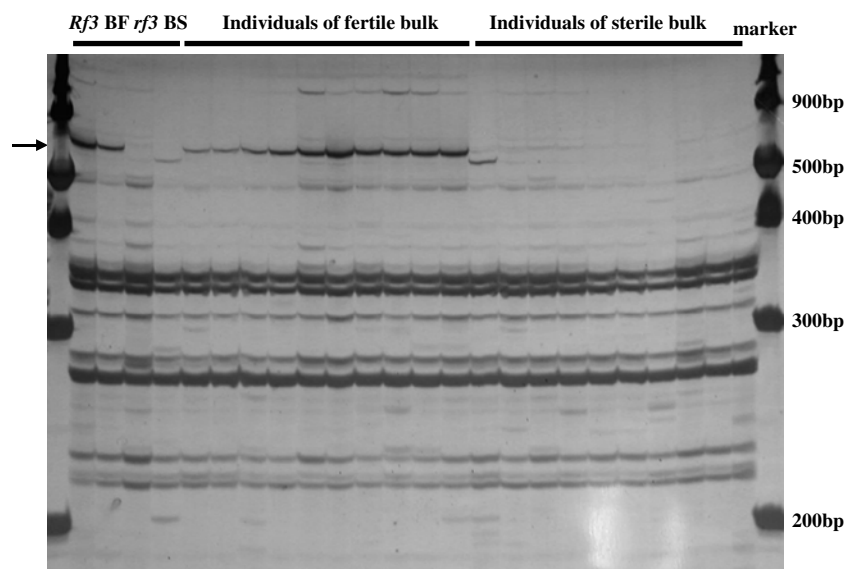
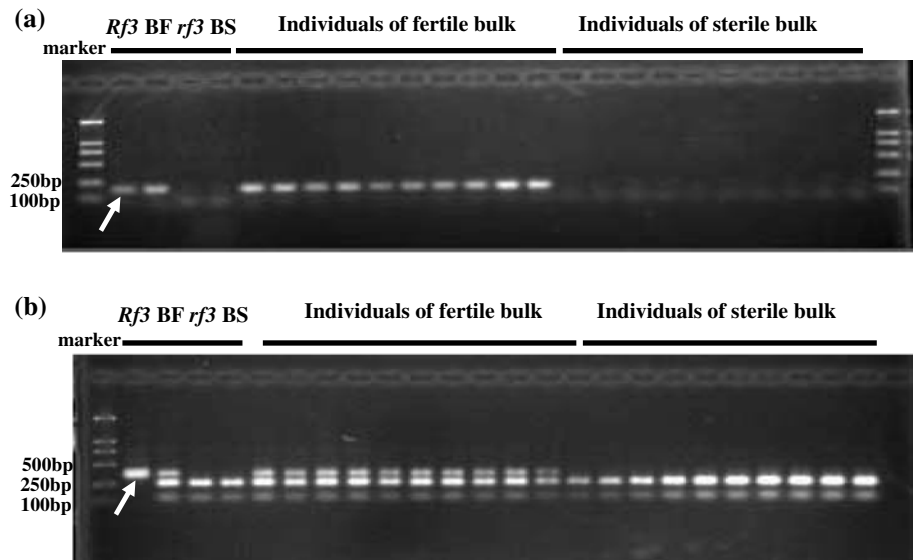


Table 1 Description of simple single-locus markers converted from *Rf3*-linked AFLP markers

Marker name	Primer sequences	PCR conditions			Genetic character
SCARE12M7	5' ATGGAGATTGAAGGGACG3'	94°C	–56°C	–72°C	Dominant
	5' ACACGGAAGACCATGACC3'	30 s	–30 s	–60 s	
CAPSE3P1 (<i>Mse</i> I)	5' CGGCTTGGATGCTACAACACA3'	94°C	–58°C	–72°C	Codominant
	5' CCAGAAGGCTTTAAGATGA 3'	30 s	–45 s	–60 s	

Fig. 2 **a** BSA analysis of SCAR marker converted from E12M7, *arrow* indicates a 170 bp band specific for fertile parent, bulk, and individuals. **b** BSA analysis of CAPs marker converted from E3P1, *arrow* indicates a 347 bp band specific for fertile parent, bulk, and individuals, this band is cut into 238 and 109 bp bands in sterile parent and individuals, all the three bands are present in fertile bulk and individuals due to their heterozygosity in *Rf3* locus. Marker is DNA ladder 2000 from Takara (*Rf3*, BF: fertile parent and bulk; *rf3*, BF: sterile parent and bulk)



Polymorphism distinguishing *Rf3* and *rf3* alleles were displayed by cutting this 359 bp fragments from fertile parent once into a 347 bp fragment and a 12 bp invisible piece while from sterile parent twice into 238 and 109 bp fragments and a 12 bp invisible piece. This difference can be displayed distinctly through electrophoresis in 2% agarose gel, as indicated in Fig. 2b. Further confirmation in the individuals of bulks indicated the undoubted linked character to the *rf3* locus (Fig. 2b). This marker was thus converted into a codominant CAPS marker and named as CAPSE3P1 for subsequent linkage analysis in BC₁F₁ population.

Linkage analysis

In our study, one SSR marker, two AFLP marker and two PCR-based markers, converted from AFLP markers, were chosen to analyze all members in the segregated population. Linkage analysis revealed a single linkage group with a total size of 11.1 cM and demonstrated that this group was located on the maize chromosome 2L anchored by marker UMC2184 lying on 2.09bin (<http://www.maizegdb.org>). Markers E7P6 and SCARE12M7, flanking *Rf3* most closely, delimited a 2.7 cM window with 0.9 and 1.8 cM from *Rf3*, respectively, and the codominant marker CAPSE3P1 was 2.4 cM from *Rf3* on the same side with E7P6 (Fig. 3).

Discussion

Near isogenic lines were successfully applied in screening molecular markers tightly linked to interested trait (Komori et al. 2003). Generally, fertile NIL plants are

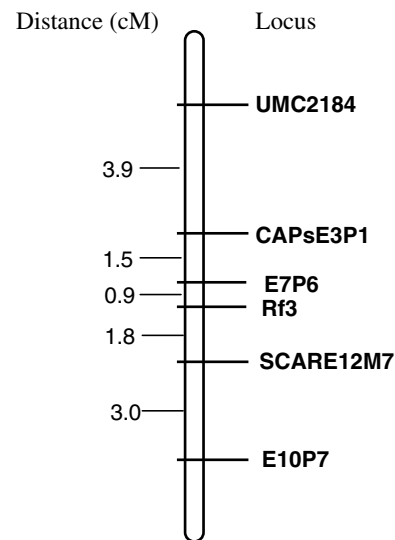


Fig. 3 Genetic linkage map of maize S-CMS restorer gene *Rf3* and associated molecular markers on the long arm of chromosome 2L. This map was based on 343 BC₁F₁ plants (*top*, towards the centromere; *bottom*, towards the telomere)

generated by transferring corresponding *Rf* gene from exotic germplasm into an elite CMS line through multi-generation backcross. Theoretically, selected fertile NILs plants will share probably uniform genetic background with original sterile plants other than the restorer gene and its vicinity. When molecular markers are screened in the NILs materials by BSA method, only those differing in the *Rf* locus and its adjacent region will exhibit polymorphism. In our study, we totally identified 31 polymorphic fragments in the fertile parent and bulk while absent from the sterile parent and bulk. According to present and absent

fragments oppositely, approximately 62 polymorphic fragments from total 29,000 were revealed by AFLP analysis. The low polymorphism was expected because we employed NILs and BSA methods. The markers, RAPD Eo8-1.2, and SSR UMC1525, bnlg1520, previously showed to link to *rf3* (Shi et al. 1997; Tie 2000), displayed no polymorphisms (data not shown). This can be explained by the low polymorphism between NILs.

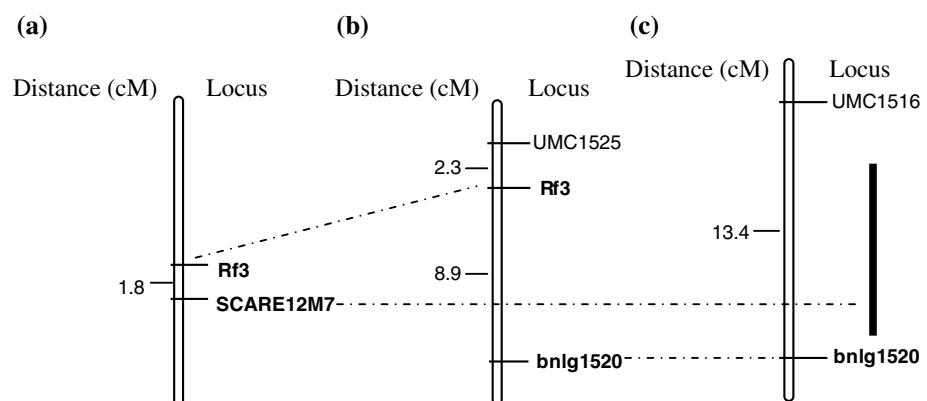
AFLP is an efficient way to screen markers linked closely to the target trait because of its high volume and no sequence information needed (Bentolila and Hanson 2001; Touzet et al. 2004; Hagihara et al. 2005). Previous reports introduced the conversion of AFLP markers into single-locus markers (Meksem et al. 2001; Brugmans et al. 2003; Kusterer et al. 2005). In the study, we identified five AFLP markers linked to *rf3* locus, and converted markers E3P1 and E12M7 into a codominant CAPS marker and a dominant SCAR marker, respectively. During converting E3P1 into CAPSE3P1, flanking sequence for external primers designing were derived from GenBank in NCBI instead of using inverse PCR method in other studies (Wen et al. 2002). Making the best use of available database recourses could save significant labor in laboratory. Unfortunately, the flanking sequence of E7P6 is not available in the database, and thus, the experimental means such as inverse PCR is required for studying unknown sequences in non-modern organism without the whole genomic sequence information, e.g. maize. Additionally, we cannot re-amplify the fragments from marker E10P7, the rate of re-amplification success was 80%, and the corresponding study was nearly 90% (Nicod and Largiadèr 2003). Furthermore, we unprecedentedly employed AFLP analysis with two hexanucleotide-required endonucleases in cutting maize genome DNA. The present data indicated that this modifications displayed less and clearer bands and

higher polymorphism than combination of tetra- and hexa-nucleotide-required endonucleases.

Conventional breeding of restorer lines is laborious and time-consuming, because selected plants should be tested for their ability to restore fertility. The single-locus markers in the study are suitable for efficiently selecting maintainer and restorer plants for S-CMS in maize early at the seedling stage. Especially, CAPSE3P1 can discriminate homozygotes and heterozygotes. Additionally, blastX analysis showed that the sequence of marker SCARE12M7 was high homologous with resistance protein T10rga2-1A in *Triticum aestivum* (E value = $4e-06$). Gene containing this marker segment may encode a resistant protein because pathogen resistant genes have high sequence homology in local region (Hammond-Kosack and Parker 2003). Recently, Shi et al. (2005) identified a QTL resistant to head smut, adjacent to SSR marker bnlg1520 in maize chromosome 2.09bin, on which the *rf3* gene exactly locates (Fig. 4c). It is coincident that marker bnlg1520 is 8.9 cM from *rf3* reported by Tie (2000) (Fig. 4b). Hence, we assumed that marker SCARE12M7 was tightly linked to gene resistant to head smut as well as *rf3*, even cosegregant with this resistant gene. If so, marker SCARE12M7 can be applied to assess and select plants for *Rf3* gene and gene resistant to head smut, simultaneously. However, it is very difficult to select two traits simultaneously using conventional breeding method because both head smut outbreaking and pollen dispersing are at the florescence (Lindsey et al. 1999).

Heretofore, all of the cloned restorer genes, except *Rf2* in T-CMS maize, encode PPR proteins (Bentolila et al. 2002; Koizuka et al. 2003; Brown et al. 2003; Kazama and Toriyama 2003; Komori et al. 2004; Akagi et al. 2004). Due to its functioning gametophytically and large genome size as non-modern organism, the cloning of *Rf3* in S-CMS maize would further demon-

Fig. 4 Comparative linkage relationship of *Rf3* and associated locus. **a** Linkage relationship of *Rf3* and marker SCARE12M7 from the present study; **b** Linkage relationship of *Rf3* and marker bnlg1520 (from Tie 2000); **c** mapping of a putative QTL resistant to head smut in maize. *Black broad line* indicates putative QTL resistant to head smut mapped on the chromosome 2L of maize (from Shi et al. 2005)



strate whether PPR-containing protein is prevalent in fertility restorer. Additionally, the *Rf3* locus segregated with the presence of shorter transcripts of both the CMS-associated *orf355/orf77* region and smaller transcripts of *cob* and *atp6* (Wen and Chase 1999a). Wen et al. (2003) showed that a nuclear fertility restorer mutation disrupts the accumulation of mitochondrial ATP synthase subunit α in the developing pollen of S male-sterile maize. According to the above effects on multiple transcripts, the cloning of *Rf3* will permit detailed explanation on the interaction of mitochondrial and nuclear genomes, which results in the restoration of pollen fertility. This study provides the foundation for map-based cloning of the *Rf3* gene.

Although the markers tightly linked to *Rf3* are necessary for high-efficiency map-based cloning, the relationship between genetic and physical distance is of predominant importance concerning the success of a map-based cloning approach. Recent reports showed that genes are hot spots for recombination event. It is suggested that in gene-rich regions the relation of base-pair to cM, with less than 200 kbp/cM, is similar in all plants, regardless of their overall genome size (Künzel et al. 2000). In maize, Fu et al. (2001) demonstrated that unusually high rate of recombination in *bronze* locus is related to very high gene density of the region. A recent paper reported that 42 *Rf* alleles for S-CMS in Mexican maize and Teosinte were mapped to the long arm of chromosome 2 (2L), and 5 of these were further mapped to the *whp1-rf3* region (Susan et al. 2004). It was supported that the *rf3* region of 2L potentially encodes a complex of linked *rf* genes, which have functions in normal mitochondrial gene expression and restore male fertility in S cytoplasm by chance (Susan et al. 2004). Although fertility restoration is controlled by one dominant restorer gene in the present segregating population, it is assumed that the *rf3* region of the materials here may encode a complex of linked *rf* genes which play a role on normal mitochondrial gene expression and display no polymorphism between the parental NILs. However, all of them is not enough to deduce that high rate of recombination exists in *rf3* region. A detailed analysis of genome structure in this region is needed to study the relationship between genetic and physical distance. Additionally, higher recombination rates in distal than in proximal chromosome regions have been demonstrated (Künzel et al. 2000). Therefore, recombination may occur with high frequency since *rf3* gene is located on the distal region of the chromosome 2 long arm (2.09bin) (Kamps and Chase 1997; Shi et al. 1997; Tie 2000). If so, we can attempt to use present linked markers, especially E7P6, 0.9 cM from *Rf3* locus, as landmarks to screen S-

Mo17^{Rf3Rf3} BAC library for genome walking to identify candidates of *Rf3* gene.

In this study, we identified markers closely linked to the *Rf3* gene of S-CMS in maize. We also converted markers E3P1 and E12M7 into a codominant CAPS marker and a dominant SCAR marker, respectively. Such information will provide a basis for a map-based cloning approach and marker-assisted breeding.

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References

- Akagi H, Nakamura A, Yokozeki-Misono Y, Inagaki A, Takahashi H, Mori K, Fujimura T (2004) Positional cloning of the rice *Rf-1* gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondria-targeting PPR protein. *Theor Appl Genet* 108:1449–1457
- Bassam BJ, Caetano Anoll SG, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. *Ann Biochem* 196:80–83
- Bentolila S, Hanson MR (2001) Identification of a BIBAC clone that co-segregates with the petunia restorer of fertility (*Rf*) gene. *Mol Genet Genomics* 266:223–230
- Bentolila S, Alfonso AA, Hanson MR (2002) A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic malesterile plants. *Proc Natl Acad Sci USA* 99:10887–10892
- Brown GG, Formanova N, Jin H, Wargachuk R, Dendy C, Patil P, Laforest M, Zhang J, Cheung WY, Landry BS (2003) The radish *Rfo* restorer gene of Ogura cytoplasmic male sterility encodes a protein with multiple pentatricopeptide repeats. *Plant J* 35:262–272
- Brugmans B, Ron GM, Van Der H, Visser GF, Richard P, Lindhout, van Eck Herman J (2003) A new and versatile method for the successful conversion of AFLP markers into simple single locus markers. *Nucleic Acids Res* 31:e55
- Buchert JG (1961) The stage of the genome–plasmon interaction in the restoration of fertility to cytoplasmically pollen-sterile maize. *Genetics* 47:1436–1440
- Cui X, Wise RP, Schnable PS (1996) The *Rf2* nuclear restorer gene of male-sterile, T-cytoplasm maize. *Science* 272:1334–1336
- Duvick DN (1965) Cytoplasmic pollen sterility in corn. *Adv Genet* 13:1–56
- Feng CD, Stewart JMcD, Zhang JF (2005) STS markers linked to the *rf1* fertility restorer gene of cotton. *Theor Appl Genet* 110:237–243
- Francia E, Tacconi G, Crosatti C, Barabaschi D, Bulgarelli D, Dall’Aglia E, Valè G (2005) Marker assisted selection in crop plants. *Plant Cell Tissue Organ Cult* 82:317–342
- Fu HH, Park W, Yan X, Zheng ZW, Shen BZ, Dooner HK (2001) The highly recombinogenic *bz* locus lies in an unusually gene-rich region of the maize genome. *Proc Natl Acad Sci USA* 98:8903–8908
- Hagihara E, Itchoda N, Habu Y, Mikami T, Kubo T (2005) Molecular mapping of a fertility restorer gene for Owen cytoplasmic male sterility in sugar beet. *Theor Appl Genet* 111:250–255

- Hammond-Kosack KP, Parker JE (2003) Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. *Curr Opin Biotechnol* 14:177–193
- Kamps TL, Chase CD (1997) RFLP mapping of the maize gametophytic restorer of fertility locus (*rf3*) and aberrant pollen transmission of the nonrestoring *rf3* allele. *Theor Appl Genet* 95:525–531
- Kazama T, Toriyama K (2003) A pentatricopeptide repeat-containing gene that promotes the processing of aberrant *atp6* RNA of cytoplasmic male-sterile rice. *FEBS Lett* 544:99–102
- Koizuka N, Imai R, Fujimoto H, Hayakawa T, Kimura Y, Kohno Murase J, Sakai T, Kawasaki S, Imamura J (2003) Genetic characterization of a pentatricopeptide repeat protein gene, *orf687*, that restores fertility in the cytoplasmic male-sterile Kosena radish. *Plant J* 34:407–415
- Komori T, Yamamoto T, Takemori N, Kashihara M, Matsushima H, Nitta N (2003) Fine genetic mapping of the nuclear gene, *Rf-1*, that restores the BT-type cytoplasmic male sterility in rice (*Oryza sativa* L.) by PCR-based markers. *Euphytica* 129:241–247
- Komori T, Ohta S, Murai N, Takakura Y, Kuraya Y, Suzuki S, Hei Y, Imaseki H, Nitta N (2004) Map-based cloning of a fertility restorer gene, *Rf-1*, in rice (*Oryza sativa* L.). *Plant J* 37:315–325
- Künzel G, Korzun L, Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. *Genetics* 154:397–412
- Kusterer B, Horn R, Friedt W (2005) Molecular mapping of the fertility restoration locus *Rf1* in sunflower and development of diagnostic markers for the restorer gene. *Euphytica* 143:35–42
- Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for construction primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181
- Laughnan JR, Gabay SJ (1978) Nuclear and cytoplasmic mutations to fertility in S male sterile maize. In: Walden DB (ed) *Maize breeding and genetics*. Wiley, New York, pp 427–447
- Lindsey J, Toit D, Pataky JK (1999) Effects of silk maturity and pollination on infection of maize ears by *Ustilago maydis*. *Plant Dis* 83:621–626
- Maureen RH, Bentolila S (2004) Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell* 16:S154–S169
- Meksem K, Ruben E, Hyten D, Triwitayakorn K, Lightfoot DA (2001) Conversion of AFLP bands into high-throughput DNA markers. *Mol Genet Genomics* 265:207–214
- Negi MS, Devic M, Delseny M, Lakshmikumaran M (2000) Identification of AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to a SCAR marker for rapid selection. *Theor Appl Genet* 101:146–152
- Nicod JC, Largiadèr CR (2003) SNPs by AFLP (SBA): a rapid SNP isolation strategy for non-model organisms. *Nucleic Acids Res* 31:e19
- Schnable PS, Wise RP (1998) The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends Plant Sci* 3:175–180
- Shi YG, Zheng YL, Li JS, Liu JL (1997) Mapping CMS-S restorers gene *Rf3* with RFLP and RAPD. *Acta Agron Sin* 23:1–6
- Shi HL, Jiang YX, Wang ZH, Li XH, Li MS, Zhang SH (2005) QTL identification of resistance to heat smut in maize. *Acta Agron Sin* 31:1449–1454
- von Stackelberg M, Lindemann S, Menke M, Riesselmann S, Jacobse HJ (2003) Identification of AFLP and STS markers closely linked to the *def* locus in pea. *Theor Appl Genet* 106:1293–1299
- Susan GL, Chase CD, Ortega VM, Zhao LM (2004) Molecular-genetic characterization of CMS-S restorer-of-fertility alleles identified in Mexican maize and teosinte. *Genetics* 166:959–970
- Tie SG (2000) Genetic characterization and gene mapping of S-CMS in maize. Ph. D. Dissertation. Huazhong Agricultural University, Wuhan
- Touzet P, Hueber N, Bürkholz A, Barnes S, Cuguen J (2004) Genetic analysis of male fertility restoration in wild cytoplasmic male sterility G of beet. *Theor Appl Genet* 109:240–247
- Vos P, Hoigers R, Bleeker M, Reijmans M, Van De Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new concept for DNA fingerprinting. *Nucleic Acids Res* 23:4407–4414
- Wen L, Chase CD (1999) Pleiotropic effects of a nuclear restorer-of-fertility locus on mitochondrial transcripts in male-sterile and S male-sterile maize. *Curr Genet* 35:521–526
- Wen L, Tang HV, Chen W, Chang R, Pring DR, Klein PE, Childs KL, Klein RR (2002) Development and mapping of AFLP markers linked to the sorghum fertility restorer gene *rf4*. *Theor Appl Genet* 104:577–585
- Wen LY, Ruesch KL, Ortega VM, Kamps TL, Laughnan SG, Chase CD (2003) A nuclear restorer-of-fertility mutation disrupts accumulation of mitochondrial ATP synthase subunit α in developing pollen of S male-sterile maize. *Genetics* 165:771–779
- Wise RP, Pring DR (2002) Nuclear-mediated mitochondrial gene regulation and male fertility in higher plants: light at the end of the tunnel? *Proc Natl Acad Sci USA* 99:10240–10242
- Xia JH, Zheng YL (2002) Molecular marker-assisted backcross breeding of maize *Rf3* NIL and its efficient analysis. *Acta Agron Sin* 28:339–344
- Zabala G, Laughnan SG, Laughnan JL (1997) The nuclear gene *rf3* affects the mitochondrial chimeric sequence R implicated in S-type male sterility in maize. *Genetics* 147:847–860
- Zhang Q, Gao YJ, Yang SH, Ragab RA, Saghai Maroof MA, Li ZB (1994) A diallel analysis of heterosis in elite hybrid rice based on RFLPs and microsatellites. *Theor Appl Genet* 89:185–192
- Zhu J, Gale MD, Quarrie S, Jackson MT, Bryan GJ (1998) AFLP markers for the study of rice biodiversity. *Theor Appl Genet* 96:602–611