Mapping of the nuclear fertility restorer gene for HL cytoplasmic male sterility in rice using microsatellite markers

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Abstract Bulked segregant analysis (BSA) of a BC_1 population derived from Congguang 41A//Miyang 23/Congguang 41B was used to map the nuclear fertility restorer gene for Honglian (HL) cytoplasmic male sterility. One hundred and fifty-nine microsatellite primer pairs were screened for polymorphisms between the parents and between two bulks representing fertile and sterile plants. One microsatellite marker RM258 produced polymorphic products. The nuclear fertility restorer gene for HL cytoplasmic male sterility was mapped on chromosome 10, 7.8cM from RM258. The restorer gene may be clustered on chromosome.

Keywords: rice, HL cytoplasmic male sterility, restorer gene, microsatellite marker.

Cytoplasmic male sterility (CMS) is the foundation of utilization of crop heterosis. In China, hybrid rice breeding based on CMS has achieved great success. There are three types of CMS, that is wild abortive (WA), Baotai (BT) and Honglian (HL) popularly applied in commercial hybird rice seed production. The inheritance of fertility restoration in the WA type CMS has been extensively investigated. Most of the investigators tended to agree that the restoration of WA type CMS is controlled by two nuclear genes, and their chromosomal locations have resolved[1—5]. The BT type CMS is restored by the nuclear fertility restorer gene Rf-1, which was mapped on chromosome 10[6—9]. Our study on the inheritance of fertility restoration of HL type CMS indicated that one nuclear gene controlled the trait[10]. The study reported in this note was undertaken to locate the nuclear fertility restorer gene for HL type CMS using microsatellite markers.

1 Materials and methods

(i) Mapping population development and fertility scoring. A BC_1 population, developed from the cross Congguang 41A//Miyang 23/Congguang 41B, was used as the mapping population. Congguang 41A is a male sterile line of HL type and Miyang 23 is the corresponding restorer line. Pollen fertility was investigated at flowering time. The seed setting rates of bagged panicles were evaluated at maturity. Sterile plants contained less than 5% stainable pollen and produced no fertile seed. All the others were treated as the fertile plants. The segregation of fertile plants (Rfrf) and sterile plants (rfrf) in the BC_1 population was in the ratio of 1:1 (75:98).

(ii) DNA extraction. About 4 g of leave was ground in liquid nitrogen to a very fine powder and incubated with 25 mL of extraction buffer (100 mmol/L Tris HCl (pH 8.0), 50 mmol/L EDTA, 500 mmol/L NaCl, 1.25% SDS (*W/V*), 0.38 g/L $NaHSO_4$) at 65℃ for 20 min. Then 10 mL of 5 mol/L KAc was added and incubated on ice for 20 min. After centrifugation the supernatant was collected. Two thirds volume of pre-chilled isopropanol was added to precipitate DNA. The DNA pellet was washed with 70% ethanol and dissolved in TE buffer. 15 μL of RNase (10 mg/mL) was added in and incubated at 37℃ for 30 min. DNA was re-extracted with 2 volumes of chloroform: isoethymol (24:1) and precipitated by absolute ethanol. After washing twice with 70% ethanol, DNA was redissolved in TE buffer for use.

(iii) Construction of the bulks. Equal amounts of DNA from 15 fertile and 15 sterile individuals were pooled to constitute the fertile and sterile bulks, respectively.

(iv) Microsatellite analysis. One hundred and fifty-nine microsatellite primer pairs used in this

study were kindly provided by Zhu Lihuang, plant biotechnology laboratory, Institute of Genetics, Chinese Academy of Sciences. Polymerase chain reaction (PCR) was performed in 25 μL reactions containing 0.2 μmol/L of each primer, 200 μmol/L deoxyribonucleotides, 50 mmol/L KCl, 10 mmol/L Tris-Cl, pH 8.3, 1.5 mmol/L $MgCl_2$ and 1 unit of Taq polymerase. The PCR profile was 94℃ for 5 min (denaturation), followed by 40 cycles of 94℃ for 1 min, 55℃ for 1 min, 72℃ for 1 min, and finally by 5 min at 72℃ for final extension. The PCR reaction was performed on a Perkin Elmer DNA Thermal Cycler 480. Amplification products were resolved by electrophoresis in 3.5% agarose gels containing 0.5 μg/mL of ethidum bromide.

2 Results

DNA from Congguang 41A, Miyang 23, fertile bulk and sterile bulk was used as a template in polymerase chain reactions (PCRs) with each of 159 microsatellite primer pairs. Thirty six primer pairs (22.6%) generated polymorphic bands between parents. The microsatellite primer pairs RM258 located on chromosome 10 demonstrated polymorphic amplifications between the sterile and fertile bulks (fig. 1). This indicated that the nuclear fertility restorer gene for HL type CMS was situated on chromosome 10.

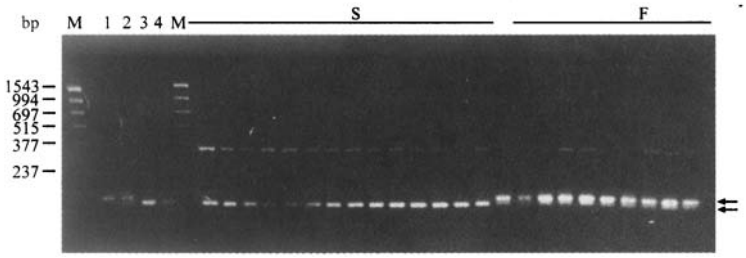

Fig. 1. Amplification profiles obtained with the microsatellite prime pairs RM258 on the two parents, two bulks and 24 individuals of BC_1 population. The arrows indicate the polymorphic band. M, molecular weight marker; 1, Miyang 23; 2, fertile bulk; 3, sterile bulk; 4, Congguang 41A; S, sterile plant; F, fertile plant.

In order to validate the results of the BSA and estimate the exact genetic distance between the microsatellite marker RM258 and the nuclear fertility restorer gene for HL type CMS, 173 BC_1 plants derived from Congguang 41A//Miyang 23/Congguang 41B were analyzed by PCR using the primer pair RM258. In 75 fertile plants 8 plants were recombinant and in 98 sterile plants 5 were recombinant. The linkage analysis with Mapmarker 3.0 revealed that the nuclear fertility restorer gene was linked to RM258. The genetic distance between them was 7.8 cM.

3 Discussion

This study has located the nuclear fertility restorer gene for the HL type CMS on chromosome 10. To our knowledge this is the first report on the HL type CMS nuclear fertility restorer gene mapping. Interestingly, Akagi et al.[8] have located the nuclear fertility restorer gene for the BT type CMS on chromosome 10, 3.7 cM from OSR33. Yao et al.[5] also mapped the nuclear fertility restorer gene Rf4 for the WA type CMS to chromosome 10, 3.3 cM from G4003. Comparison of the various molecular linkage maps suggested that three restorer genes are located in the adjacent region on chromosome 10. Recently, Borner et al.[11] found that Rfg1 determining the restoration of cytoplasmic genic male sterility caused by the G-type cytoplasm may be allelic to the gene determining the restoration of rye CMS caused by the P-type cytoplasm and to Rfc4 that on rye addition lines of chromosome 4RL restores male fertility of hexaploid wheat with T. *timopheevi* cytoplasm, Jia et al.[12] mapped four nuclear fertility restorer genes (Fr, Fr2, $Fr_{PI207228}$ and Fr_{XR235}) to the same linkage group. Singh et al.[13] found that nuclear genes associated with a single Brassica CMS restorer locus influence transcripts of three different mitochondrial gene regions. These results suggests that, just like the disease resistant gene, the restorer genes may also be clustered on chromosome.

Microsatellite markers have the advantages of both the rapidity, straight and, simplicity of RAPD and the stability, reliability and repeatability of RFLP. Although the difference of microsatellite between

genotypes is small (usually a few bps), 36 of 159 microsatellite markers generated polymorphism between parents by high concentration (3%—4%) agarose gel electrophoresis. Our results suggust that the substitution of agarose gel electrophoresis for polyacrylamide gels is feasible. As the density of microsatellite markers, map increased[14], it is expected that microsatellite markers will play a greater role in gene mapping.

In this study, the authors found one microsatellite marker RM258 linking to the nuclear fertility restorer gene for HL type CMS at a distance of 7.8 cM. Searching for markers more tightly linked to the Rf locus and construction of a relatively dense regional map and the physical map encompassing the Rf locus are underway. Our ultimate goal is the isolation of the Rf gene through map-based cloning.

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