Theor Appl Genet (2006) 113:357–360
DOI 10.1007/s00122-006-0302-6

ORIGINAL PAPER

Identification and mapping of *Rf-I* an inhibitor of the *Rf5* restorer gene for Cms-C in maize (*Zea mays* L.)

Y. M. Hu · J. H. Tang · H. Yang · H. L. Xie · X. M. Lu · J. H. Niu · W. C. Chen

Received: 15 March 2006 / Accepted: 21 April 2006 / Published online: 19 May 2006

Abstract The restoration of the C-type cytoplasmic male sterility (Cms) has been a common agriculture practice in the production of hybrid seed for many years. In this study, a series of crosses between select sterile and restorer lines, as well as a backcross population of (Cms-C77 × 6233) × 6233, were used to investigate the restoration of C-type Cms. Our results demonstrated that there was an inhibitor of the *Rf5* restorer gene. This inhibitor gene, *Rf-I*, maps to chromosome 7 and is tightly linked with SSR markers, umc2326 and umc2327, at a genetic distance 4.7 and 3.4 cM, respectively. After analyzing our data combined with previous studies, we propose that the restoration of C-type Cms has two dominant genes, *Rf4* and *Rf5*. *Rf4* has the ability to restore all genotypes of Cms-C lines; however, there exists an inhibitor for the other restorer gene, *Rf5*; thus, it can restore only those genotypes of Cms-C lines lacking the *Rf-I* inhibitor.

Communicated by R. Hagemann

Y. M. Hu · J. H. Tang (✉) · H. L. Xie · X. M. Lu · J. H. Niu · W. C. Chen
College of Agronomy, Henan Agricultural University, 95 Wenhua Road, Zhengzhou, 450002, China
e-mail: Tangjihua1@163.com

H. Yang
National Maize Improvement Center of China, China Agricultural University, Beijing, 100094, China

Introduction

Cytoplasmic male sterility (Cms) has important economic and logistic advantages in maize for the production of hybrid seed. More than 40 sources of Cms have been found and classified into three major groups; these are designated the Cms-T, Cms-S, and Cms-C groups (Beckett 1971). Compared to T- and S-Cms, the fertility restoration of Cms-C has been found to be very complex in previous analyses. Out of them, Duvick (1972) reported that restoration of Cms-C fertility was controlled by a dominant gene, *Rf4*. Khey-Pour et al. (1981) also found this gene to be sufficient for Cms-C restoration. However, Josephson and Morgan (1978) proposed that fertility restoration in Cms-C was conditioned by the complementary action of the dominant allele of two genes, *Rf4* and *Rf5*, which have since been mapped to chromosomes 8 and 5, respectively (Sisco 1991; Tang et al. 2001a, b). Meanwhile, Chen et al. (1979) considered that two dominant restorer genes in Cms-C had duplicate action. Further complicating the system, Vidakovic (1988) demonstrated the existence of three dominant and complementary genes for full restoration of fertility in Cms-C, adding the gene, *Rf6*. Vidakovic et al. (1997a, b) later reported these complementary genes, *Rf4*, *Rf5*, and *Rf6*, were indeed not the sole genetic system for fertility restoration in Cms-C of maize. Thus, the fertility restoration mechanisms of Cms-C remain unresolved, and, in practice, it is difficult to select restorer lines for some genotypic sterile lines. The objective of this study was to resolve the mechanism of Cms-C restoration and map the gene of an inhibitor of the restorer gene, *Rf5*, with SSR markers.

Materials and methods

Plant materials and phenotype evaluation

The inbred line 6233 with C-type male cytoplasm was selected from restorer individuals of a F_2 population derived from a cross between Cms-C Mo17 and Fengke1, and self-pollinated to F_7, which the restorer line, Fengke1, had two dominant duplicate restorer genes, *Rf4* and *Rf5* (Chen et al. 1979; Tang et al. 2001b). The inbred line 6233 was tested for the restorer gene *Rf5* using tightly linked molecular markers and an allelism test. The fertility of some F_1 progeny derived from crosses between the sterile lines Cms-CMo17, Cms-C77, Cms-Es87-1, Cms-Es478, Cms-C237, and Cms-CZong3 and the restorer lines 6233, A619, Guang10-2, Dan958, and Fengke1, as well as from a backcross (Cms-C77 × 6233) × 6233 were evaluated in Zhengzhou and Sayan, China.

In the field, the crosses between select sterile lines and restore lines were sown in one or three plots respectively; each plot was 4 m in length and contained 15 plants, seeds of the BC_1 populations were sown using the single-seed planting method in nine plots. The percentage restoration was evaluated by monitoring anthesis of each plant daily. The criterion of fertility was then rated on a scale of 0–5 based on the percentage of exserted anthers: (0) no anthers exserted; (1) > 0–5% anthers exserted; (2) 5–25% anthers exserted; (3) 25–50% anthers exserted; (4) 51–75% anthers exserted; (5) more than 75% anthers exserted. At the same time, the dehiscence of anthers was graded on a scale of I to III: (I) no dehiscent anthers; (II) few dehiscent anthers; (III) many normal, dehiscent anthers. To identify the fertility of each plant, a combined score of 4/III or 5/III was regarded as restored, while combined scores of 0/I and 1/I were recorded as sterile (Duvick et al. 1961; Li et al. 1963).

Linkage analysis

Genomic DNA was extracted from young leaves using the cetyltrimethylammonium bromide (CTAB) method (Murrsay and Thompson 1980). Bulked segregant analysis (BSA) was used to detect the linkage of the *Rf-I* gene. Fertile bulk and sterile bulk were made by mixing equal amounts of DNA from ten fertile plants and ten sterile plants, respectively (Michelmore et al. 1991).

In accordance with their bin location, a total of 521 SSR markers were chosen from the maize genome database (http://www.maizegdb.org) to detect polymorphism of the two parents and bulks. The products amplified using SSR primers were separated by electrophoresis on 6% polyacrylamide gels and visualized by silver-staining. Based on the SSR data and the phenotype of the segregating individuals, linkage analysis was performed with JOINMAP version 3.0 (Stam 1993).

Results

Identification the restorer gene *Rf5* in inbred line 6233

The SSR markers, bnlg1711 and bnlg1346, which are tightly linked with the *Rf5* gene, were used to identify the genotype of the restorer lines 6233 (Tang et al. 2001b), A619 (*Rf4Rf4rf5rf5*; Sisco 1991), Fengke1 (*Rf4Rf4Rf5Rf5*; Chen et al. 1979; Tang et al. 2001b), Guang10-2 (*Rf4Rf4rf5rf5*; Tang et al. 2001b), and Dan958, as well as the sterile lines, Cms-C Mo17 and Cms-ES 87-1, which were identified simultaneously as a control. The results from PCR amplification demonstrated that the inbred line, 6233, had one restorer gene, *Rf5* (The figures are not shown).

For further genotypic identification of the inbred line 6233, the F_2 population, including 213 derived from the cross, Cms-C 6233 × A619, were evaluated in the summer of 2004. There were 189 fertile plants and 16 sterile plants in this population, and the segregating ratio of male fertile to male sterile plants fell to the theoretical ratio 15:1 ($\chi^2 = 0.85$). This result also indicated that the inbred line 6233 had a single restorer gene, *Rf5*.

Identification the *Rf5* restorer inhibitor gene, *Rf-I*

The F_1 progeny of crosses between five sterile lines, and the restorer line 6233 were evaluated in the summer of 2003 in Zhengzhou (Table 1). The F_1 progeny of the sterile lines Cms-CMo17, Cms-Es87-1, Cms-Es 478, Cms-C237, and Cms-CZong3 and the restorer line 6233 presented full restoration; however, the F_1 progeny of Cms-C77 and 6233 were sterile. They also exhibited sterility in the winter of 2003 and 2004 in Sanya, as well as in the summer of 2004 at Zhengzhou (Table 2). It was very interesting that only the F_1 progeny between Cms-C77 and 6233 presented sterility.

Samples from the backcross population of (Cms-C77 × 6233) × 6233 were also evaluated, finding 59 sterile plants and 56 fertile plants out of 123 individuals, a ratio of restored to sterile plants following the theoretical ratio 1:1 by the chi-squared test (Table 2). These results demonstrate that the sterile line, Cms-C77, has a dominant inhibitor gene, which is

Table 1 Fertility performance of F_1 progeny derived from crosses between sterile (Cms) and restorer lines

Year	Combinations	Total plants	Restorer plants	Sterile plants
2003	Cms-CMo17 × 6233 (*rf4rf4Rf5Rf5*)	15	15	
	Cms-Es87-1 × 6233 (*rf4rf4Rf5Rf5*)	16	16	
	Cms-Es478 × 6233 (*rf4rf4Rf5Rf5*)	15	15	
	Cms-C77 × 6233 (*rf4rf4Rf5Rf5*)	14		14
	Cms-C77 × Dan958 (*rf4rf4Rf5Rf5*)	15	15	
	Cms-CZong3 × 6233 (*rf4rf4Rf5Rf5*)	13	13	
2004	Cms-C77 × 6233 (*rf4rf4Rf5Rf5*)	15		15
	Cms-C77 × A619 (*Rf4Rf4rf5rf5*)	15	15	
	Cms-C77 × Guang10-2 (*Rf4Rf4rf5rf5*)	15	15	
	Cms-C77 × Fengke1 (*Rf4Rf4Rf5Rf5*)	15	15	
	Cms-C77 × Dan958 (*rf4rf4Rf5Rf5*)	15	15	
	Cms-C77	15		15

Rf the dominant gene that has restorer fertility ability for Cms in nucleolus; *rf* the recessive gene of *Rf* allele

Table 2 Fertility performance of cross Cms-C77 × 6233 and backcross with 6233

Environments	Populations	Total plants	Restorer plants	Partial restoration plants	Sterile plants	Theoretical ratio	Chi-square test	Probability
2003,summer Zhengzhou	P_1	14			14			
	P_2	15	15					
	F_1	14			14			
2004,summer Zhengzhou	P_1	20			20			
	P_2	30	30					
	F_1	45			45			
	BC_1	123	59	8	56	1:1	0.08	0.78
2003, winter Sanya	P_1	15			15			
	P_2	14	14					
	F_1	30			30			
2004, winter Sanya	P_1	15			15			
	P_2	13	13					
	F_1	32			32			

$\chi^2_{0.05} = 3.84$; P_1, Cms-C77, P_2, 6233; F_1, Cms-C77 × 6233; BC_1, (Cms-C77 × 6233) × 6233

named *Rf-I* that can inhibit expression of the restorer gene, *Rf5*. At the same time, the younger leaves of this backcross population were harvested for DNA extraction and linkage analysis.

To further analyze the genetic effect of this inhibitor gene, a set of F_1 progeny from the sterile line Cms-C77, crossed to the restorer lines A619, Guang10-2, Fengke1, Dan958, and 6233, were evaluated in the summer of 2004 in Zhengzhou (Table 1). All of the F_1 progeny, except those from Cms-C77 × 6233, exhibited restoration of fertility. This indicated that the inhibitor gene, *Rf-I*, has no effect on the *Rf4* restorer gene, in contrast to the effect on *Rf5*.

Mapping the dominant inhibitor gene, *Rf-I*

Out of the 521 SSR markers used in this study, the primers umc1978, bnlg1904, umc2326, umc2327, umc1666, bnlg2203, and umc2325 amplified polymorphisms between the two parents and two bulks; all of these primers reside on the chromosomal bin 7.02. To determine the chromosomal location of the *Rf-I* gene, DNA from 115 individuals of the backcross population of (Cms-C77 × 6233) × 6233 was amplified with these SSR primers. The patterns of amplification for some individuals using the SSR marker, umc2327, are shown in Fig. 1 as examples. The linkage map of the SSR markers and *Rf-I* gene were calculated with JOINMAP, and showed in Fig. 2, the genetic distances between the *Rf-I* and the SSR primers umc2326 as well as umc2327 were 4.7 and 3.4 cM, respectively.

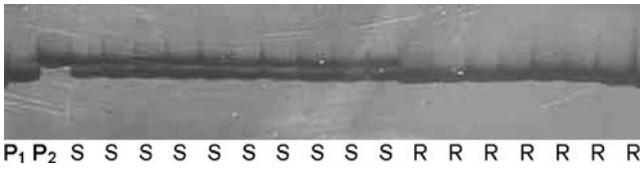

Fig. 1 The SSR products amplified by umc2327 from parents and some BC_1 individuals. P_1: 6233; P_2: Cms-C77; S: some BC_1 sterile individuals; R: some BC_1 restorer individuals

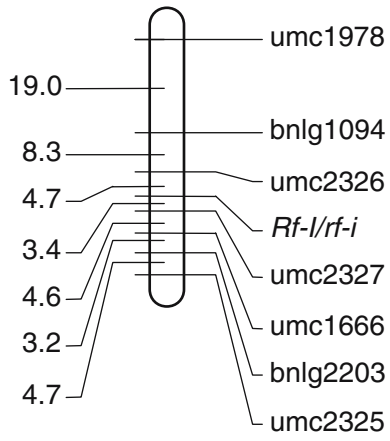

Fig. 2 Location of *Rf-I/rf-i* on chromosome 7

Discussion

Vidakovic et al. (1997b) reported that C-group Cms had perhaps two different restoration mechanisms, one restoration system was the three dominant complementary genes (*Rf4*, *Rf5*, *Rf6*), and the second one was a duplication or a parallel and independent genetic device for fertility restoration in Cms-C. In this study, our results revealed that the restorer gene, *Rf5*, has a corresponding inhibitor gene, *Rf-I*, but this inhibitor has no effect on the *Rf4* restorer gene. Our results are similar to those of Qin et al. (1990), who used C-type and Y-type Cms to investigate the fertility restoration of two Cms, they demonstrated that the Y-type Cms had an inhibitor gene called *Rf7* that most likely inhibits expression of the *Rf5* gene. Moreover, the restorer genes, *Rf4* and *Rf5*, were dominant to both C-group and Y-type Cms, and there is little difference between these two types of Cms (Qin et al. 2001). It is possible the inhibitor genes *Rf-I* and *Rf7* are indeed the same gene, but this remains to be seen. Combining the previous studies, we propose that the restoration of C-Cms has two dominant genes (Chen et al. 1979; Sisco 1991; Tang et al. 2001b), one is the major restorer gene, *Rf4* (Duvick 1972; Khey-Pour et al. 1981; Sisco 1991), which has restorer activity over all types of C-Cms; the other restorer gene, *Rf5*, has an inhibitor, so it can restore only some genotypes of C-Cms—those without the inhibitor gene, *Rf-I*.

In the utilization of C-Cms, many maize breeders have faced the difficulty that some C-group sterile lines are not restored. The reason for this problem is perhaps that the sterile line harbors the inhibitor gene *Rf-I*, and the restorer line being used has the *Rf5* gene. To overcome this problem, restorer lines with *Rf4*, such as A619 (Sisco 1991; Tang et al. 2001a) and Guang10-2 (Tang et al. 2001b), should be used as the donor for selecting new restorer lines, because the *Rf4* restorer is effective in any genotype of C-Cms.

Acknowledgements This work was supported by the National High-technology Project of China and National Natural Science Foundation of China.

References

Beckett JB (1971) Classification of male sterile cytoplasms in maize (*Zea mays* L.). Crop Sci 11:724–726

Chen WC, Luo FH, Ji LY (1979) Some genetic aspects of the C-type cytoplasmic male-sterility in maize and its use in breeding. Acta Agronom Sin 5(4):21–28

Duvick DN (1972) Potential usefulness of new cytoplasmic male sterile and sterility system. In: Proceedings of the 27th annual corn and sorghum research conference, pp 197–201

Duvick DN, Snyder RJ, Anderson EC (1961) The chromosomal location of *Rf1*, a restorer gene for cytoplasmic pollen sterile maize. Genetics 46:1245–1252

Josephson LM, Morgan TE (1978) Genetics and inheritance of fertility restoration of male sterile cytoplasms in corn. In: Proceedings of the 33rd corn and sorghum research conference 7:13

Khey-Pour A, Grace VE, Everett HL (1981) Genetics of fertility restorations in the C-group of cytoplasmic male sterility in maize. Genetics 98:379–388

Li JX, Dai JR, Xu QF (1963) Study on the inheritance of cytoplasmic male-sterile and fertility restoration in maize. Acta Agronom Sin 2:339–362

Michelmore RW, Paran I, Kesseli V (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic region is by using segregating population. Pro Natl Aca Sci USA 88:9828–9832

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Qin TC, Xu ML, Deng DX (1990) Cytoplasmic male-sterility: identification of the number of restorer genes. Maize Genet Coop News Lett 64:124

Qin TC, Xu ML, Deng DX, Chen RL, Bian YL (2001) Study on male-sterility in *Zea mays* VIII. RFLP analysis of mtDNA of Y_{II-1} type maize male-sterile cytoplasm. Acta Agronom Sin 27:185–189

Sisco PH (1991) Duplication complicates genetic mapping of *Rf4* a restoring gene for cms-C cytoplasmic male sterility in corn. Crop Sci 31:1263–1266

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Tang JH, Ji HQ, Huang ZW, Ji LY, Wang CH (2001a) Mapping of the main restorer gene (*Rf4*) of C-cytoplasmic male sterility in maize with RFLP. J Henan Agric Univ 35:99–102

Tang JH, Liu ZH, Chen WC, Hu YM, Ji LY (2001b) Mapping the main restorer genes of C-cytoplasmic male sterility using SSR method. Acta Agronom Sin 34:592–596

Vidakovic M (1988) Genetics of fertility restoration in cytoplasmic male sterility of the C-type (Cms-c) in maize. Maydica 33:51–64

Vidakovic M, Vancetovic J, Vidakovic M (1997a) Complementary genes *Rf4*, *Rf5* and *Rf6* are not the unique genetic system for fertility restoration in cmsC of maize (*Zea mays* L.). Maize Genet Coop News Lett 71:10

Vidakovic M, Vancetovic J, Vidakovic M (1997b) The existence of a duplicated or parallel genetic system for fertility restoration in cmsC of maize (*Zea mays* L.). Maydica 42:313–316