Theor Appl Genet (2007) 115:501–508
DOI 10.1007/s00122-007-0583-4

ORIGINAL PAPER

Mapping of genome-wide resistance gene analogs (RGAs) in maize (*Zea mays* L.)

Wenkai Xiao · Jing Zhao · Shengci Fan · Lin Li · Jinrui Dai · Mingliang Xu

Received: 17 January 2007 / Accepted: 25 May 2007 / Published online: 21 June 2007

Abstract Isolation and mapping of genome-wide resistance (*R*) gene analogs (RGAs) is of importance in identifying candidate(s) for a particular resistance gene/QTL. Here we reported our result in mapping totally 228 genome-wide RGAs in maize. By developing RGA-tagged markers and subsequent genotyping a population consisting of 294 recombinant inbred lines (RILs), 67 RGAs were genetically mapped on maize genome. Meanwhile, in silico mapping was conducted to anchor 113 RGAs by comparing all 228 RGAs to those anchored EST and BAC/BAC-end sequences via tblastx search (E-value < 10^{-20}). All RGAs from different mapping efforts were integrated into the existing SSR linkage map. After accounting for redundancy, the resultant RGA linkage map was composed of 153 RGAs that were mapped onto 172 loci on maize genome, and the mapped RGAs accounted for approximate three quarters of the genome-wide RGAs in maize. The extensive co-localizations were observed between mapped RGAs and resistance gene/QTL loci, implying the usefulness of this RGA linkage map in *R* gene cloning via candidate gene approach.

Communicated by T. Lübberstedt.

Wenkai Xiao, Jing Zhao and Shengci Fan have contributed equally to this research.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-007-0583-4) contains supplementary material, which is available to authorized users.

W. Xiao · J. Zhao · S. Fan · L. Li · J. Dai · M. Xu (✉)
National Maize Improvement Center of China,
China Agricultural University, 2 west Yuanmingyuan Road,
Beijing 100094, People's Republic of China
e-mail: mxu@cau.edu.cn

Introduction

So far, map-based cloning (or positional cloning) and transposon tagging are two prevalent approaches in cloning resistance (*R*) genes. More than 50 *R* genes have been isolated from diverse plant species, conferring resistance against a wide range of pathogens, including bacteria, fungi, viruses, pests, oomyetes, and nematodes (Whitham et al. 1994; Collins et al. 1999; Cooley et al. 2000; Milligan et al. 1998; Wang et al. 1999; Hulbert et al. 2001). Intriguingly, only a few conserved domains or motifs were shared among these *R* genes, such as NBS (nucleotide binding site), LRR (leucine-rich repeat), PK (protein kinase), TM (transmembrane domain), LZ (leucine zipper), and TIR (Toll-Interleukin-1) (Meyers et al. 1999, 2003; Liu and Ekramoddoullah 2003; Hulbert et al. 2001). This distinct feature inspires many scientists to isolate resistance gene analogs (RGAs) for the sake of cloning the *R* gene of interest (Collins et al. 1998; Pflieger et al. 1999; Dilbirligi et al. 2004). How to obtain as many RGAs as possible is therefore a key point towards successful *R* gene isolation via this candidate gene approach (Meyers et al. 2003; Monosi et al. 2004). At the beginning, PCR-based isolation of RGAs were attempted in a variety of plant species by using degenerate primers designed on conserved *R* gene motifs to amplify genomic DNA (Collins et al. 1998; Shen et al. 1998). Although a sizable collection of PCR-derived RGAs have been obtained from diverse plant species (Ramalingam et al. 2003), this PCR-based method seems impossible to reveal enough RGAs to cover all potential *R* genes in genome. With the rapid accumulation of EST sequences in

a variety of plant species, a powerful method is adopted by mining EST database using all known *R* gene sequences (Meyers et al. 2003; Dilbirligi and Gill 2003). This data-mining method usually results in more RGAs than PCR-based method, as observed in wheat, sugarcane, and maize (Dilbirligi and Gill 2003; Rossi et al. 2003; Xiao et al. 2006). It is conceivable that an EST database with a good coverage of genomic genes would make it possible to isolate genome-wide RGAs.

As expected, co-linearity is frequently observed between RGA and resistance gene. In flax, one RGA derived from PCR-based amplification was co-segregated with alleles of the *N* locus (Dodds et al. 2001). In *Arabidopsis thaliana*, almost all cloned resistance-gene-like DNA fragments were genetically closely linked to known disease resistance loci (Aarts et al. 1998). Perfect cosegregation was observed between RGA loci and the resistance loci *rp1* and *rp3* in maize (Collins et al. 1998). Two RGAs, pic19 and pic13, were affirmed to be candidates for sugarcane mosaic virus (SCMV) resistance genes *Scmv1* and *Scmv2*, respectively (Quint et al. 2002). The extensive co-linearity implies the highly possibility to identify candidate *R* gene(s) from RGAs.

In maize, two *R* genes *Hm1* and *Rp-1D*, conferring resistance to the fungus *Cochliobolus carbonum* race 1 and maize common rust (*Puccinia sorghi*), respectively, were cloned via transposon tagging method (Johal and Briggs 1992; Collins et al. 1999). Another *R* gene, *Rxo1*, conditions a resistance reaction to rice bacterial streak disease was recently cloned via candidate gene approach (Zhao et al. 2005). However, many economically important *R* genes have remained obscure thus far, such as those conferring resistances to head smut, stalk rot, virus diseases, and among others. The transposon tagging seems unlikely to become a routine method to clone other maize *R* genes due to rare event to inactivate a functional *R* gene by transposon insertion. Likewise, it is currently very difficult to clone *R* genes via map-based cloning, partly because of highly repetitive sequences and partly because of lacking whole genome sequences in maize. Alternatively, candidate gene approach may be very promising in cloning maize *R* genes due to (1) conserved motifs shared by the known *R* genes (Pflieger et al. 2001) and (2) a very big maize EST database available (Messing and Dooner 2006). Isolation and mapping of maize RGAs have been firstly attempted by Collins et al. (1998). Genome-wide RGAs have been isolated via the modified AFLP, RACE, and data-mining methods (Xiao et al. 2006). On the other hand, tremendous efforts have been made to map resistance genes/QTLs in maize, and this resulted in 17 qualitative and 437 quantitative loci conferring resistant against 11 diseases or disease groups (Wisser et al. 2006). To identify putative candidate *R* genes for all resistance loci, genome-wide maize RGAs need to be isolated and anchored on genome, thus making it possible to find co-localized RGA(s) for any resistance gene/QTL locus.

A total of 228 RGAs have been recovered genome widely from maize, in which 186 *R*-gene-like ESTs and 35 AFLP- and RACE-derived RGAs have been previously reported (Xiao et al. 2006), and another seven *R*-gene-like ESTs have been recently identified (see supplementary data for RGA IDs and sequences). The objective of this research is to anchor these 228 RGAs onto maize genome to construct a RGA linkage map. The map would therefore provide information of candidate genes to accelerate *R* gene cloning in maize.

Materials and methods

Plant materials

Two inbred lines, ‘Zong3’ and ‘87-1’, which make one of the most cultivated maize hybrid ‘Yuyu22’ in China, were used in both developing markers and preparing mapping population. These two lines are highly diverged and show contrasting phenotypes in a number of agronomic traits. RGA-tagged markers were developed based on RGA-sequence differences between ‘Zong3’ and ‘87-1’. The mapping population consisting of 294 recombinant inbred lines (RILs) was developed from the cross of ‘Zong3’ and ‘87-1’ after consecutive self-pollination for more than ten generations (Yan et al. 2006).

Development of RGA-tagged markers

The strategy used to develop RGA-tagged STS and CAPS were described by Xiao et al. (2006). RGA-tagged SNP markers were developed based on the SNPkit method described by Niu and Hu (2004). A non-perfect match primer was synthesized in which a mismatch nucleotide at its 3′-end was artificially introduced. The mismatch nucleotide, together with the nucleotide at the SNP site, would form a restriction recognition site for one allele, but not the other allele in PCR products. Two round PCR amplifications were performed in which a SNP site is firstly amplified using the perfect match PCR primers, followed by the nested PCR amplification using the non-perfect match primer. A SNP site could be detected by digesting PCR products from the second round amplification with the corresponding restriction enzyme.

Genetic mapping based on RGA-tagged markers

All 294 RILs from the mapping population were genotyped at each RGA-tagged marker, and the marker data were

integrated into the existing high-density SSR linkage map (Yan et al. 2006) using the software Mapmaker3.0.

In silico mapping based on anchored EST and BAC/BAC-end sequences

We have collected 10,208 mapped maize ESTs/unigenes from MaizGDB (http://www.maizegdb.org/) and PlantGDB (http://www.plantgdb.org). All 228 maize RGAs were compared to the mapped ESTs via tblastx at *E*-value < 10^{-20} to search for those highly homologous ESTs. A RGA was presumed to locate at the same locus as its highly homologous cognate EST. The SSR markers flanking the mapped RGAs were identified and compared with those presented in the existing SSR linkage map to anchor the RGAs into the SSR linkage map.

A BAC-based whole genome physical map has been established on which 721 FPCs (fingerprint contigs) were assembled to cover 2,149.5 Mb of the B73 genome or about 90.9% of the measured nuclear DNA (Messing and Dooner 2006). Two-thirds of the overlapping BAC clones in the physical map have been sequenced at their ends, yielding a cumulative length of one-eighth of the maize genome (Messing et al. 2004). With the advance of maize genome sequencing, more than 5,000 sequenced BACs were deposited in the database (http://www.plantgdb.org) by the end of 2006. The in silico mapping was therefore conducted by using all 228 maize RGA sequences to compare BAC (sequenced BAC and BAC-end) sequences via tblastx search at *E* value < 10^{-20}. The resultant highly homologous cognate BAC clones were used to find their corresponding FPCs in the website http://www.genome.arizona.edu/fpc/WebAGCOL/maize/WebFPC/. The SSR markers on FPCs flanking the BACs were identified and subsequently used to anchor the corresponding RGAs into the existing SSR linkage map.

Results

Development of RGA-tagged markers

A total of 180 primer pairs were designed based on the RGA sequences and used to amplify genomic DNAs of both parental inbred lines '87-1' and 'Zong 3'. Sequence polymorphisms were observed for most RGAs between two parental lines. Apart from those markers published previously (Xiao et al. 2006), additional 17 markers have been developed in this study (Table 1). This makes a total of 72 RGA-tagged markers, including 19 STS, 51 CAPS, and two SNP markers, for mapping their corresponding RGAs.

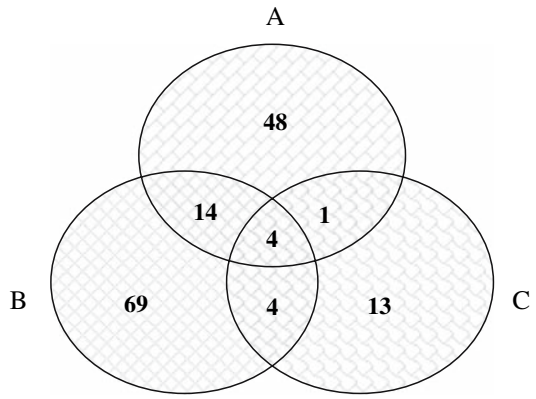

Fig. 1 The *number* of mapped RGAs and their overlapping profiling by using the three different mapping approaches. **a** The 'genetic' mapping approach. **b** The BAC-based in silico mapping approach. **c** The EST-based in silico mapping approach

Genetic mapping based on the RIL mapping population

All 294 RILs from the mapping population were genotyped at the developed 72 RGA-tagged markers. The marker data were integrated into the existing high-density SSR linkage map by using the software Mapmaker3.0. Except for five RGAs, the remaining 67 RGAs could be integrated into the existing SSR linkage map based on their marker data. RGA ZmMla1(2) had two different marker profilies and was therefore mapped onto two different loci. The same phenomenon was observed for another RGA ZmCf5(7) that was also anchored onto two loci. Consequently, 67 out of the 72 RGAs were genetically mapped onto 69 loci on maize genome (Figs. 1, 2 and supplementary Table 1).

In silico mapping based on anchored EST and BAC/BAC-end sequences

Of the 228 maize RGAs, 22 were found to share highly homologous to the mapped ESTs/unigenes at *E*-value < 10^{-20} via tblastx search (Fig. 1). The flanking SSR markers were identified from our collected database and used to anchor these 22 RGAs into the existing SSR linkage map (Fig. 2 and supplementary Table 1). Meanwhile, at the same searching criteria, 91 RGAs were found to show highly homologous to anchored BAC (sequenced BAC and BAC-end) sequences (Fig. 1). The BAC IDs were retrieved and used to search for their FPCs in http://www.genome.arizona.edu/fpc/WebAGCOL/maize/WebFPC/. The flanking SSR markers were identified and subsequently used to locate these 91 RGAs on the existing SSR linkage map (Fig. 2 and supplementary Table 1).

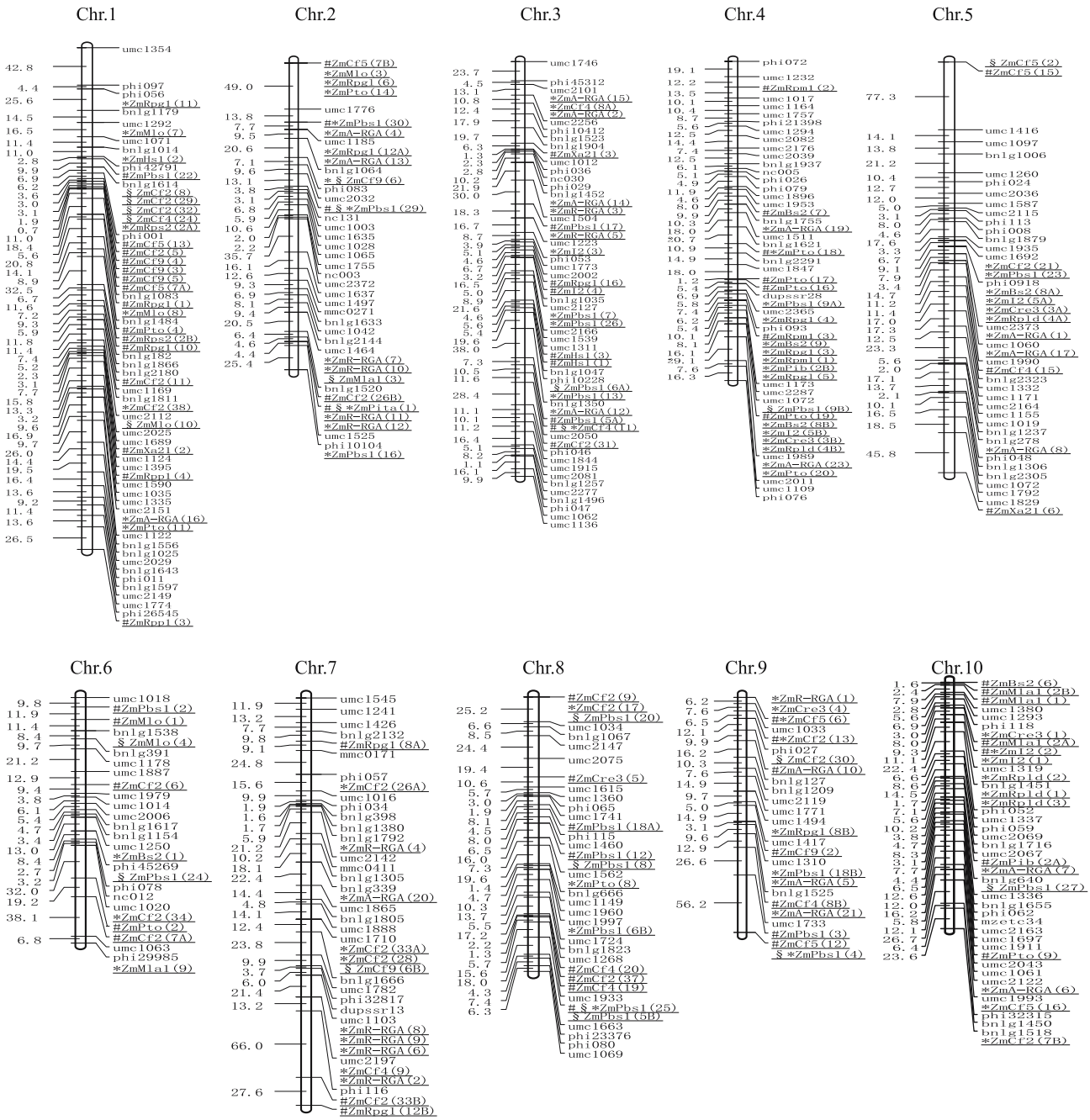

Fig. 2 The RGA linkage map in maize. A total of 153 RGAs were mapped onto 172 loci on maize genome and the mapped RGAs are *underlined*. # These RGAs were mapped by using the 'genetic' mapping method. * These RGAs were mapped by using the BAC-based in silico mapping method. § These RGAs were mapped by using the EST-based in silico mapping method. #*§ These RGAs were mapped at the same locus by using the three different mapping methods. #* These RGAs were mapped at the same locus by using both the 'genetic' and BAC-based in silico mapping methods. #§ These RGAs were mapped at the same locus by using both the 'genetic' and EST-based in silico mapping methods. *§ These RGAs were mapped at the same locus by using both the EST- and BAC-based in silico mapping methods. **A**, **B** indicating the same RGA was mapped onto two different chromosomal regions

The RGA linkage map

As expected, common RGAs were shared among the 'genetic' mapping, BAC-based in silico mapping, and EST-based in silico mapping approaches (Table 2; Fig. 1). Four RGAs, ZmCf4(11), ZmPbs1(25), ZmPita(1), and ZmPbs1(29), were present in all three mapping approaches. Besides these four RGAs, the numbers of common RGAs between the 'genetic' and BAC-based in silico, between the 'genetic' and EST-based in silico, and between the two in silico mapping

Table 1 Primer sequences for the 17 RGA-tagged markers

	RGA IDs	R-gene like ESTs	Primer sequences (5′-3′)		Restriction enzymes
			Forward primers	Reverse primers	
STS	ZmPbs1(12)	ZMtuc03-08-11.16729	GGCTGCTGGTATAGTTGTTGTTGAA	TGGGTATTTGGCACCTGAGTATG	–
	ZmRpg1(12)	ZMtuc02-12-23.10883	TAGTCCACCGTGATCTCAAGGC	CACAGTCGTGCTAGTCCCACTCT	–
	ZmCf2(31)	CB331101	GTCACCCGCCTAGATCTTGGT	AAATGGTCCAGACGTGGGAAT	–
CAPS	ZmCf5(6)	ZMtuc02-12-23.13447	GCTTGGTTACAATCAGTTGACAGG	GTCTTGACAGTACCGTCTGGTCC	*Msp*I
	ZmCf9(2)	ZMtuc03-08-11.10388	TGCACTCCTTCACAGTTCAGGTC	CGCTATTTGGTCAATCTCCGTG	*Xsp*I
	ZmPbs1(25)	ZMtuc03-08-11.21462	CAAGCTCACCAAGATGTCGGAC	TCTGCAGTGTTCCTCCCTTTCC	*Hae*III
	ZmRppl(3)	ZMtuc02-12-23.16870	AACGAGCTCTCCGGCAAGATA	TATTCTCCCACAGCTGCAGCA	*Xsp*I
	ZmPto(9)	ZMtuc03-08-11.13789	GACTGGGAGAAGGTGGTGATCC	CTCGTACGTCAGCTACAGCATGC	*Alu*I
	ZmCf9(3)	CD448503	GGCACGAGGGAACTAGTCTCAAGT	CAGATTGCAAACCTGACAAACCTT	*Msp*I
	ZmCf9(5)	CB815939	TGCCACGGAATAGTACAGCGAC	GCTAAAGAACCTACGTCACCTCGAC	*Msp*I
	ZmCf4(10)	AW067239	AGGAGCTCGAGATCTACAGCAA	TGAAGGCGTTGTCGTTGA	*Alu*I
	ZmCf5(7)	CD980669	GCCGAGGTGTCGTAGGTTCTTT	GGGATTACAGAGTTGTGACGGAA	*Xsp*I
	ZmCre3(5)	CD435787	AAGGTACCATGGCTCGATGATG	AGATCCTGCACATTCCAGAGAAA	*Msp*I
	ZmPto(4)	CD444610	GCGAGATGATCCTGGTGTACGA	GGAGTAGACGTCGGACTTGTTCG	*Msp*I
	ZmXa21(6)	BG321296	CCTTYTGGGAGATTTCGGCATC	TGCGTGCATKAAAGAGCYACTT	*Alu*I
SNP	ZmXa21(3)	AW061999	CCATCTTTGAGACTTTGATCAT	GCTCCAGAGTATGCTGAAGGCT	
			AATACTATCCGTTGACTCTACcTGCA	GCTCCAGAGTATGCTGAAGGCT	*Hin*dIII
	ZmPto(10)	ZMtuc03-08-11.3020	GACTTCTTGGTTGCTGCATTCA	TCTACTACTGGAGATTGTAAGCGGC	
			GACTTCTTGGTTGCTGCATTCA	TCATGAAGATGCTACAAGGAAAgCT	*Eco*RI

Table 2 The common RGAs identified in the three mapping approaches

Common RGAs between/among	RGA names	No. of RGAs
Between the 'genetic' and BAC-based in silico mapping approaches	ZmPbs1(25), ZmCf4(11), ZmPita(1), ZmPbs1(29), ZmCf2(7), ZmCf2(13), ZmCf2(33), ZmCf2(26), ZmCf4(8), ZmCf5(6), ZmRps2(2), ZmPbs1(18), ZmRpg1(12), ZmPto(18), ZmI2(2), ZmRpg1(8), ZmPib(2), ZmPbs1(30)	18
Between the 'genetic' and EST-based in silico mapping approaches	ZmPbs1(25), ZmCf4(11), ZmPita(1), ZmPbs1(29), ZmPbs1(5)	5
Between the BAC-based and EST-based in silico mapping approaches	ZmPbs1(25), ZmCf4(11), ZmPita(1), ZmPbs1(29), ZmPbs1(4), ZmPbs1(9), ZmPbs1(6), ZmCf9(6)	8
Among the three mapping approaches	ZmPbs1(25), ZmCf4(11), ZmPita(1), ZmPbs1(29)	4

approaches were found to be 14, 1, and 4, respectively (Table 2; Fig. 1). After accounting for redundancy, the total RGA number was reduced to 153 that covered about three quarters of the genome-wide maize RGAs (153 out of 228 RGAs). These 153 RGAs were finally mapped onto 172 loci in maize genome to construct a RGA linkage map (Fig. 2). The known *R* genes and their corresponding maize RGAs, RGA IDs and their domains, RGA locations and their flanking SSR markers, and etc. were summarized for these 228 genome-wide maize RGAs (supplementary Table 1).

Analysis of the RGA linkage map reveals several distinct features concerning RGA locations. First, most RGAs tend to cluster together, taking chr.1 for example, five RGAs are clustered in an interval of bnlg1614/phi001 and another six RGAs located between phi001 and bnlg1083. Second, some RGA clusters are closely located, for instance, the above two RGA clusters are in close vicinity. Third, of the 153 mapped RGAs, 19 RGAs have two separate chromosomal locations (Fig. 2), resulting in totally 172 RGA loci in the RGA linkage map. It is very interesting that a cluster of four RGAs [ZmBs2(8), ZmI2(5), ZmCre3(3), and ZmRpld(4)] was mapped on two chromosomal locations, in the interval of umc1072/umc1989 on chr.4 and in the phi0918/umc2373 region on chr.5 (Fig. 2).

Discussion

Map-based cloning and transposon tagging are currently two prevalent approaches to isolate functional genes. As an

alternative way, the candidate gene approach nowadays is becoming more and more popular (Pflieger et al. 2001). In higher plant species, with the advance of genomic researches and availability of information of ever-increasing functional genes, candidate genes for monogenetic or even quantitative traits could be easily identified and this makes the candidate gene approach practical to clone the target functional genes, especially for resistance genes (Wang et al. 2001; Pflieger et al. 2001). Consequently, many genes including *R* genes have been cloned by candidate gene approach in plant (Bout and Vermerris 2003; Li et al. 2006; Sawers et al. 2006; Feuillet et al. 1997). With an aim to clone genome-wide *R* genes, we attempted to isolate as many RGAs as possible (Xiao et al. 2006), and to map them onto maize genome in the present study. Considering totally 228 RGAs have been identified genome widely in maize and most of them were derived from putative expressed resistance genes (Xiao et al. 2006), this RGA linkage map composed of 153 maize RGAs could provide valuable information to identify candidate *R* genes. The co-segregation between a mapped RGA and a major resistance gene or QTL would lead to identification of the candidate *R* gene or at least the RGA sequence could be used to develop closely-linked markers.

Generally, an *R*-gene superfamily (haplotype) consists of an array of tandem repeated members (paralogs) and only one or a few of them is responsible for disease resistance (Michelmore and Meyers 1998; Dixon et al. 1996). Apart from the resistance *Pto* gene, the *Pto* complex resistance locus contains five to seven *R*-gene members and the *FEN* member shows up to 80% identity with *Pto* (Martin et al. 1994). The *R* gene *Gro1-4* cloned recently was a member of a gene family consisting of 15 tightly-linked highly homologous members (Paal et al. 2004). In maize, the complex rust resistance *rp1* locus contains nine family members, seven of them, including the truncated gene, are transcribed (Sun et al. 2001); while, the *rp3* locus contains at least nine family members in most *Rp3*-carrying lines and at least five of these are transcribed in the *Rp3-A* haplotype (Webb et al. 2002). Furthermore, different *R*-gene families tend to cluster on genome in many plant species (Wei et al. 1999; Chin et al. 2001; Ashfield et al. 2003). The distinct feature of multiple members in *R* locus is a double-edged sword in *R* gene cloning. On one hand, isolation of BAC clones covering the *R* locus becomes easy by using common primers designed on conserved regions shared by all *R* members. On the other hand, identification of the candidate *R* gene becomes difficult due to interferences from the other non-functional members. For example, development of allele-specific marker for a given *R* member is very difficult since it needs to show polymorphisms not only between resistant and susceptible alleles but also among different members at the same locus.

Hitherto, many resistance genes or QTLs have been mapped on maize genome. Li et al. (2001) recorded 22 qualitative and 68 quantitative *R* loci across maize genome. Wisser et al. (2006) collected 437 *R* QTLs and 17 *R* genes after synthesizing 50 publications related to mapping of maize resistance loci. Visual inspection allows us to find high co-linearity between RGAs and the *R* gene regions. For almost all *R* gene/QTL loci, there are corresponding RGAs present. Just like *R* genes, the mapped RGAs are not evenly distributed within and across ten chromosomes. For example, chromosome 1 contains the most resistance loci, 16 gene/QTL loci for resistance to 10 diseases/pests (Li et al. 2001). This is consistent with RGA distribution that as many as 28 RGAs were mapped on chr.1 (Fig. 2). In bin10.01, there clustered four *R* loci (*rp1*, *rp5*, *rp1-G*, and *rpp9*) (Li et al. 2001). Accordingly, seven RGAs were mapped at the same region. In bin6.01, five *R* genes against southern corn leaf blight, maize dwarf mosaic virus, sugarcane mosaic virus, wheat streak mosaic virus, and high plains virus were detected. Accordingly, three RGAs were mapped in this region. In another closely-linked *R* gene cluster in bin3.04/3.05 (Wisser et al. 2006), we identified nine RGAs in that region (Fig. 2). In our laboratory, QTL mappings were conducted for maize resistance to head smut and stalk rot, and the major resistance QTLs were detected on bin2.09 and bin 10.04, respectively. When compared to the RGA linkage map, we found seven RGAs in bin2.09 and three RGAs in bin 10.04 (Fig. 2). To identify candidate *R* genes from co-localized RGAs, RGA-tagged markers should be firstly developed and then genotype large mapping population to confirm co-segregation between RGA(s) and resistance. This work is currently underway in our lab to find candidate *R* genes for head smut and stalk rot.

First we depended on 'genetic' mapping, since it is the most reliable way to map RGAs. However, it is very costly and time-consuming to develop markers and to genotype mapping population. To make things even worse, no marker could be successfully developed for most RGAs, partly because of unsuitable RGA sequences for primer design and partly because of multiple copies in *R* loci. We almost exhausted our resources to finally develop 72 RGA-tagged markers and mapped 67 of them on maize genome. The RGA-tagged markers developed in our lab may also work or at least make it easy to develop similar markers in other mapping populations. On contrary, in silico mapping strategy is rather convenient, labor-saving, and almost costs nothing. However, this in silico mapping cannot be successful until sufficient sequence databases, like EST/unigene, sequenced BAC, and BAC-end sequences, are available. When we started this mapping study, mapped ESTs/unigenes and BAC sequences were far from enough. At present, however, maize sequences have been rapidly

accumulated, thus making it feasible to in silico map all RGAs.

Four RGAs, ZmCf4(11), ZmPbs1(25), ZmPita(1), and ZmPbs1(29), were present in all three mapping approaches (Table 2; Fig. 1). Furthermore, each of them was mapped at the same locus by using the three different mapping approaches (Fig. 2). Apart from these four RGAs, other 19 common RGAs were present either between the 'genetic' and in silico mapping or between two in silico mapping efforts. Five common RGAs were mapped at the same loci by using different mapping approaches (Fig. 2). It is conceivable that PCR-based 'genetic' mapping is more sensitive and reliable than tblastx-based in silico mapping method. The discrepancy among map locations for the same RGA mapped by using different methods were observed for 14 RGAs (Fig. 2), this discrepancy could be attributed to incompletion of maize genome sequence. If RGA loci were covered by the mapped ESTs/unigenes or sequenced BACs/BAC-ends, RGA locations would be comparable between the 'genetic' and in silico mapping efforts. Otherwise, tblastx-based in silico mapping may anchor RGAs onto their highly homologous EST/unigene or BAC/BAC-end sequences located on other chromosomal regions. Even for the two in silico mapping methods, three of four common RGAs were mapped onto different chromosomal locations, suggested that some mapped ESTs/unigenes could not be found in BAC/BAC-end sequences. In 'genetic' mapping, two RGAs [ZmMla1(2) and ZmCf5(7)] were mapped on two loci; while, in in silico mapping, a cluster of four RGAs [ZmBs2(8), ZmI2(5), ZmCre3(3), and ZmRpld(4)] was mapped on two different chromosomal regions. This phenomenon reveals: (1) chromosomal duplications may exist in maize genome since maize is evolved from a tetraploid ancestor; (2) highly homologous *R* sequences are present in different chromosomal regions.

The question mostly concerned about the RGA linkage map may be its usefulness in finding candidate *R* genes, or in other word, how many *R* genes are covered by the mapped RGAs. Most RGAs were derived from the public EST database (MaizeGDB) which has been amounted to 600,000 entries by 10 December 2006 when we performed the last screening. We assumed that the expressed RGAs isolated could cover most *R* genes in maize genome. However, it is also possible that some *R* gene transcripts may not be included in the EST database due to their low expression levels. Of the 228 maize RGAs, only 153 were mapped on maize genome, this means that at least one quarter *R* genes cannot find their corresponding RGAs yet. As more maize sequences are available, more RGAs will be mapped. At the point when maize genome sequence is completed, all RGAs could then be identified by searching for whole genome sequence.

Acknowledgments This study was financially supported by the national outstanding youth foundation of China, 'Construction of a linkage map for genome-wide resistance gene analogs and isolation of important genes in maize', Grant No. 30525035; the national '863' high-tech program of China, 'Functional genomics on resistance to diseases, tolerance to abiotic stress, and grain quality in maize', Grant No. 2006AA10A107; and the natural scientific foundation of Beijing, Grant No. 5060001.

References

Aarts MGM, Hekkert B Te Lintel, Holub EB, Beynon JL, Stiekema WJ, Pereira A (1998) Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. Mol Plant Microbe Interact 11:251–258

Ashfield T, Bocian A, Held D, Henk AD, Marek LF, Danesh D, Penuela S, Meksem K, Lightfoot DA, Young ND, Shoemaker RC, Innes RW (2003) Genetic and physical localization of the soybean *Rpg1-b* disease resistance gene reveals a complex locus containing several tightly linked families of NBS-LRR genes. Mol Plant Microbe Interact 16:817–826

Bout S, Vermerris W (2003) A candidate-gene approach to clone the sorghum Brown midrib gene encoding caffeic acid O-methyltransferase. Mol Genet Genomics 269:205–214

Chin DB, Arroyo-Garcia R, Ochoa OE, Kesseli RV, Lavelle DO, Michelmore RW, 2001 Recombination and spontaneous mutation at the major cluster of resistance genes in lettuce (*Lactuca sativa*). Genetics 157:831–849

Collins NC, Webb CA, Seah S, Ellis JG, Hulbert SH, Pryor A (1998) The isolation and mapping of disease resistance gene analogs in maize. Mol Plant Microbe Interact 11:968–978

Collins N, Drake J, Ayliffe M, Sun Q, Ellis J, Hulbert S, Pryor T (1999) Molecular characterization of the maize *Rp1-D* rust resistance haplotype and its mutants. Plant Cell 11:1365–1376

Cooley MB, Pathirana S, Wu HJ, Kachroo P, Klessig DF (2000) Members of the *Arabidopsis* HRT/RPP8 family of resistance genes confer resistance to both viral and oomycete pathogens. Plant cell 12:663–676

Dilbirligi M, Gill KS (2003) Identification and analysis of expressed resistance gene sequences in wheat. Plant Mol Biol 53:771–787

Dilbirligi M, Erayman M, Sandhu D, Sidhu D, Gill KS (2004) Identification of wheat chromosomal regions containing expressed resistance genes. Genetics 166:461–481

Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JDG, C Lane (1996) The tomato Cf-2 disease resistance locus comprises two functional genes encoding leucine-rich repeat protein. Cell 84:451–459

Dodds PN, Lawrence GJ, Ellis JG (2001) Contrasting modes of evolution acting on the complex N locus for rust resistance in flax. Plant J 27:439–53

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. Plant J 11:45–52

Hulbert SH, Webb CA, Smith SM, Sun Q (2001) Resistance gene complexes: evolution and utilization. Annu Rev Phytopathol 39:285–312

Johal GS, Briggs SP (1992) Reductase activity encoded by the *Hm1* disease resistance gene in maize. Science 258:985–987

Li Y, Wang TY, Jia JZ (2001) Advances in molecular makers studies on disease/pest resistance in maize. Adv Bioeng 21:42–49

Li C, Zhao J, Jiang H, Wu X, Sun J, Zhang C, Wang X, Lou Y, Li C (2006) The wound response mutant suppressor of prosystemin-mediated responses 6 (spr 6) is a weak allele of the tomato homolog of CORONATINE-INSENSITIVE1 (COI1). Plant Cell Physiol 47:653–663

Liu JJ, Ekramoddoullah AK (2003) Isolation, genetic variation and expression of TIR-NBS-LRR resistance gene analogs from western white pine (*Pinus monticola Dougl. ex. D. Don.*). Mol Genet Genomics 270:432–441

Martin GB, Frary A, Wu TY, Brommonschenkel S, Chunwongse J, Earle ED, Tanksley SD (1994) A member of the tomato Pto gene family confers sensitivity to fenthion resulting in rapid cell death. Plant Cell 6:1543–1552

Messing J, Dooner HK (2006) Organization and variability of the maize genome. Curr Opin Plant Biol 9:157–163

Messing J, Bharti AK, Karlowski WM, Gundlach H, Kim HR, Yu Y, Wei F, Fuks G, Soderlund CA, Mayer KFX, Wing RA (2004) Sequence composition and genome organization of maize. Proc Natl Acad Sci USA 101:14349–14354

Meyers BC, Dickerman AW, Michelmore RW, Sivaramakrishnan S, Sobral BW, Young ND (1999) Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant J 20:317–332

Meyers BC, Kozik A, Griego A, Kuang H, Michelmore RW (2003) Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. Plant cell 15:809–834

Michelmore RW, Meyers BC (1998) Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res 8:1113–1130

Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VA (1998) The root knot nematode resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant cell 10:1307–1319

Monosi B, Wisser RJ, Pennill L, Hulbert SH (2004) Full-genome analysis of resistance gene homologues in rice. Theor Appl Genet 109:1434–1447

Niu TH, Hu ZJ (2004) SNPicker: a graphical tool for primer picking in designing mutagenic endonuclease restriction assays. Bioinformatics 20:3263–3265

Paal J, Henselewski H, Muth J, Meksem K, Menéndez CM, Salamini F, Ballvora A, Gebhardt C (2004) Molecular cloning of the potato Gro1-4 gene conferring resistance to pathotype Ro1 of the root cyst nematode *Globodera rostochiensis*, based on a candidate gene approach. Plant J 38:285–297

Pflieger S, Lefebvre V, Caranta C, Blattes A, Goffinet B, Palloix A (1999) Disease resistance gene analogs as candidates for QTLs involved in pepper–pathogen interactions. Genome 42:1100–1110

Pflieger S, Lefebvre V, Causse M (2001) The candidate gene approach in plant genetics: a review. Mol Breed 7:275–291

Quint M, Mihaljevic R, Dussle CM, Xu ML, Melchinger AE, Lübberstedt T (2002) Development of RGA-CAPS markers and genetic mapping of candidate genes for sugarcane mosaic virus resistance in maize. Theor Appl Genet 105:355–363

Ramalingam J, Cruz CMV, Kukreja K, Chittoor JM, Wu JL, Lee SW, Baraoidan M, George ML, Cohen MB, Hulbert SH, Leach JE, Leung H (2003) Candidate defense genes from rice, barley, and maize and their association with qualitative and quantitative resistance in rice. Mol Plant Microbe Interact 16:14–24

Rossi M, Araujo PG, Paulet F, Garsmeur O, Dias VM, Chen H, Van Sluys MA, D'Hont A (2003) Genomic distribution and characterization of EST-derived resistance gene analogs (RGAs) in sugarcane. Mol Gen Genomics 269:406–419

Sawers RJH, Viney J, Farmer PR, Bussey RR, Olsefski G, Anufrikova K, Hunter CN, Brutnell TP (2006) The maize Oil yellow1 (Oy1) gene encodes the I subunit of magnesium chelatase. Plant Mol Biol 60:95–106

Shen KA, Meyers BC, Islam-Faridi MN, Chin DB, Stelly DM, Michelmore RW (1998) Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. Mol Plant Microbe Interact 11:815–823

Sun Q, Collins NC, Ayliffe M, Smith SM, Drake J, Pryor T, Hulbert SH (2001) Recombination between paralogues at the rp1 rust resistance locus in maize. Genetics 158:423–438

Wang ZX, Yano M, Yamanouchi U, Iwamoto M, Monna L, Hayasaka H, Katayose Y, Sasaki T (1999) The *Pib* gene for blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. Plant J 19:55–64

Wang Z, Taramino G, Yang D, Liu G, Tingey SV, Miao GH, Wang GL (2001) Rice ESTs with disease-resistance gene- or defense-response gene-like sequences mapped to regions containing major resistance genes or QTLs. Mol Genet Genomics 265:302–310

Webb CA, Richter TE, Collins NC, Nicolas M, Trick HN, Pryor T, Hulbert SH (2002) Genetic and molecular characterization of the maize *rp3* rust resistance locus. Genetics 162:381–394

Wei FS, Gobelman-Werner K, Morroll SM, Kurth J, Mao L, Wing R, Leister D, Schulze-Lefert P, Wise RP (1999) The Mla (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153:1929–48

Whitham S, Dinesh-Kumar SP, Choi D, Hehl R, Corr C, Baker B (1994) The product of the tobacco mosaic virus resistance gene N: similarity to toll and the interleukin-1 receptor. Cell 78:1101–1115

Wisser RJ, Balint-Kurti PJ, Nelson RJ (2006) The genetic architecture of disease resistance in maize: a synthesis of published studies. Phytopathology 96:120–129

Xiao WK, Xu ML, Zhao JR, Wang FG, Li JS, Dai JR (2006) Genome-wide isolation of resistance gene analogs in maize (*Zea mays* L.). Theor Appl Genet 113:63–72

Yan JB, Tang JH, Meng YJ, Ma XQ, Teng WT, Subhash C, Li L, Li JS (2006) Improving QTL mapping resolution based on genotypic sampling-a case using a RIL population. Acta Genet Sin 33:617–624

Zhao B, Lin X, Poland J, Trick H, Leach J, Hulbert S (2005) A maize resistance gene functions against bacterial streak disease in rice. Proc Natl Acad Sci USA 102:15383–15388