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Identification of resistance gene analog markers closely linked to the powdery mildew resistance gene *MlG*, derived from wild emmer wheat, *Triticum dicoccoides*.

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ABSTRACT

Wild emmer wheat, *Triticum dicoccoides*, the tetraploid progenitor of cultivated wheat, is a valuable source for resistance to powdery mildew. *MlG* is a novel powdery mildew resistance gene transferred from wild emmer G-305-M accession into Chinese elite wheat cultivar Jing 411. This gene was previously mapped on chromosome 6AL with microsatellite markers. In order to identify closely linked markers for marker-assisted selection and map-based cloning, degenerated primers based on the conserved domains of cloned plant disease resistance genes were used to screen for polymorphism between resistant and susceptible DNA bulks from the cross 'G-305-M/781//Jing 411*3'. Seven resistance gene analog (RGA) markers were identified to have linkage with *MlG* at a distance of 0.6 cM distal to the gene. All seven RGA markers were cosegregating together, indicating the existence of either a cluster of resistance gene structure or suppressed recombination around the *MlG* region. These closely linked RGA markers can be used in marker-assisted breeding to tag the *MlG* gene. The closely linked RGA markers are now in the process of cloning and sequencing to see if they contain any R gene structures.

INTRODUCTION

Wild emmer wheat, the tetraploid progenitor of cultivated wheat, is a valuable source for resistance to powdery mildew (Nevo et al. 2002; Liu et al. 2002). *MlG* is a novel powdery mildew resistance gene transferred from wild emmer into Chinese elite wheat cultivar Jing 411. *MlG* was previously located on chromosome 6AL by using 4 microsatellite markers (Xie et al. 2003). Molecular markers closely linked to resistance genes are prerequisites for marker-assisted selection and map-based cloning of these genes. More than 30 cloned plant resistance genes (R-genes) show a high degree of similarity in domains of nucleotide binding site (NBS) and leucine-rich repeat (LRR) or kinase (Dangl and Jones, 2001). The conserved domains of R- genes allow the isolation of similar sequences in other plant species by PCR amplification using degenerated primers. This approach has been widely used to isolate resistance gene candidates and develop molecular markers such as in potato (Leister et al. 1996), soybean (Yu et al. 1996), and wheat (Feuillet et al. 1997; Chen et al. 1998). The objective of this study is to use the resistance gene analog (RGA) approach to develop closely linked molecular markers for marker-assisted selection and map-based cloning of *MlG*.

MATERIALS AND METHODS

Bulks of DNA were made with lines either resistant or susceptible to powdery mildew from the cross 'G-305-M/781//Jing 411*3' (Xie et al. 2003). A total of 180 RGA primer pairs were used to screen the two DNA bulks. A BC2 F3 segregating population of 162 individuals was used to test the linkage of the polymorphic markers with *MlG*. Both 162 F3 individuals and 20 F4 progenies of each F3 line were tested on detached leaves with culture No. 15. This culture, which was collected from *T.* durum in Israel, is avirulent to *MlG*. PCR amplification was performed as described by Chen et al. (1998). PCR products were electrophoresed using an automated laser fluorescence (ALF) sequencer (Pharmacia). Linkage analyses and map construction were performed with the computer program MULTIPOINT (Mester et al. 2003).

RESULTS AND DISCUSSION

Nearly 8000 fragments from 180 primer pairs were compared between the resistant and susceptible bulks. Nineteen primer pairs revealed polymorphisms between the two opposite bulks. After testing the segregating population (162 individuals), 7 of the 19 fragments showed linkage to MlG. All 7 RGA markers (PG-312, RR-513, SR-250, XX-106, PC-200, PR-375, RX-169/163) were clustered together at a distance of 0.6 cM from MlG. Three SSR markers, Xwmc163, Xwmc256 and Xgwm570, were also mapped using this mapping population (Figure 1). The cosegregation of these seven RGA markers indicates the existence of either a cluster of resistance gene structure or suppressed recombination around MlG region. These seven primer pairs were used to amplify other families of the cross 'G-305-M/781//Jing 411*3', the susceptible parents '781' and 'Jing411', and Chinese Spring. The results showed that all 6 dominant RGA markers were present in the resistant samples and absent in the susceptible samples. The codominant marker RX-169/163 can distinguish between the homozygous and the heterozygous resistant individuals. The susceptible parents '781' and 'Jing411', and Chinese Spring showed no specific bands of these primer pairs. Previously mapped SSR markers are not applicable for identifying *MlG* due to either large genetic distance from the gene or similar size of amplification fragment in susceptible cultivars (Xie et al. 2003), whereas the closely linked RGA markers identified in this study can be used as diagnostic markers for marker-assisted selection of *MlG*. Cloning and sequencing of those closely linked markers are underway.

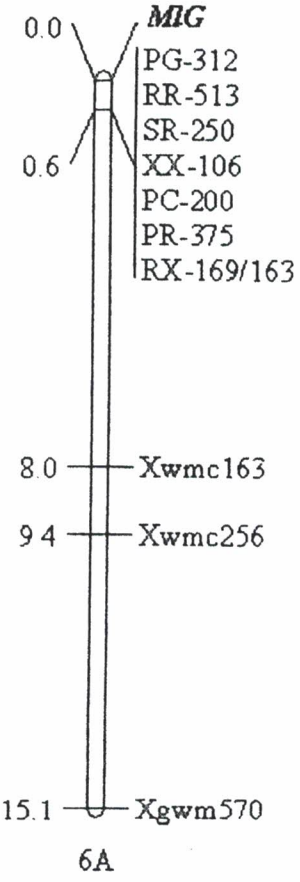

Figure 1. Genetic map of *MlG* tagged with RGA markers.

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