Plant Prod. Sci. 7 (3) :319 — 323(2004)

Identification of Random Amplified Polymorphic DNA and Simple Sequence Repeat Markers Linked to Powdery Mildew Resistance in Common Wheat Cultivar Brock

Zhenying Wang, Qi Zheng, Yongkang Peng, Chaojie Xie*, Qixin Sun* and Zuomin Yang*

*(Department of Biology, Tianjin Normal University, Tianjin, 300074, P, R China; *Department of Plant Genetics and Breeding, China Agricultural University, Beijing, 100094, P.R. China)*

Abstract : A total of 350 rapid amplified polymorphic DNA (RAPD) primers and 100 simple sequence repeat (SSR) primer pairs were screened to identify polymorphic markers associated with powdery mildew resistance. Only primer OPP15 produced a 900bp reproducible DNA fragment ($OPP15_{900}$) in the resistant parent cv. Brock and most of the resistant individuals, but this DNA fragment was absent in susceptible parent Jing411 and Line 015. The progeny, including 218 resistant and 81susceptible lines, derived from a cross Line 015/Brock//$Jing411^2$ was used for linkage analysis. 209 resistant and 8 susceptible individuals yielded $OPP15_{900}$ products, but 73 susceptible and 9 resistant ones yielded no $OPP15_{900}$ products. One dominant RAPD molecular marker $OPP15_{900}$ linked to powdery mildew resistance gene was identified in Brock with a genetic distance of 6.0 cM. A SSR marker Xgwm114 was also proved to link with the powdery mildew resistance and genetic distance of 9.3 cM. These two new molecular markers are useful for facilitating selection and pyramiding the resistance genes in wheat breeding.

Key words : Powdery mildew resistance, RAPD marker, SSR marker, Wheat.

Powdery mildew is one of the most serious diseases of common wheat in China and many countries of the world. Up to now, although 25 genes (*pm1-pm25*) for resistance to powdery mildew caused by *Erysiphe graminis* DM f. sp. *tritici* have been identified and assigned to specific chromosomes or chromosome arms (Mclntosh et al., 1998), most of the resistance genes has already been overcome by new virulent *Erisiphe graminis* strains. It is therefore necessary to extend the search for new sources of genetic resistance to powdery mildew for cultivated wheat. Molecular marker technology is widely used to find markers linked to target genes (Hu et al., 2001, Hartl et al., 1995, Schachermayr et al.,1995). The molecular marker analysis in wheat appears to be particularly suited for the identification of markers linked to the powdery mildew resistance gene (Devos et al., 1992, Röder et al., 1998a, b, Wang et al., 2000). In this study, we used wheat cultivar Brock carring resistance gene *Pm2* as experiment material. Although most of the wheat cultivars carrying *Pm2* became susceptible, Brock remained resistant. Thus Brock may have a resistance gene different from *Pm2*. In this paper, we report the identification of random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers linked to the powdery mildew resistance gene in common wheat cultivar Brock and their progenies.

Materials and Methods

1. Plant materials

Common wheat cultivars Brock, Jing411, Line 015 and their progenies were used for RAPD and SSR for molecular markers identification. Cultivar Brock, the donor of the resistance gene, was kindly provided by Dr. Ray Johnson. It contained *Pm2* gene, and resistant to powdery mildew, although the other cultivated wheats carrying *Pm2* are susceptible (Wang et al., 2001). Therefore, Brock was thought to carry an unknown resistance gene. Common wheat cultivars Jing411 and Line 015 are fine wheat varieties cultivated widely in North China, and are susceptible to powdery mildew pathogen.

Line 015 was crossed with Brock to obtain F_1 seed, and the F_1 plant was crossed with Jing411. The resistant F_1 (Line 015/Brock //Jing411) was chosen and crossed with Jing411 again. The BC_1F_1 plant that was resistant based on the inoculation test was selfed to produce BC_1F_2 segregated population. From this population, 218 resistant and 81 susceptible progenies were obtained and they were used for linkage analysis. Up to now, we have not seen any reports about the molecular markers linked to powdery mildew resistance in Brock. In this experiment, we used the RAPD technique to identify the molecular markers linked to resistance genes different from *Pm2* in Brock.

Received 31 July 2003. Accepted 25 February 2004.
Corresponding author: Y. K. Peng (pykcell@eyou.com, fax +86- 022-23540057).

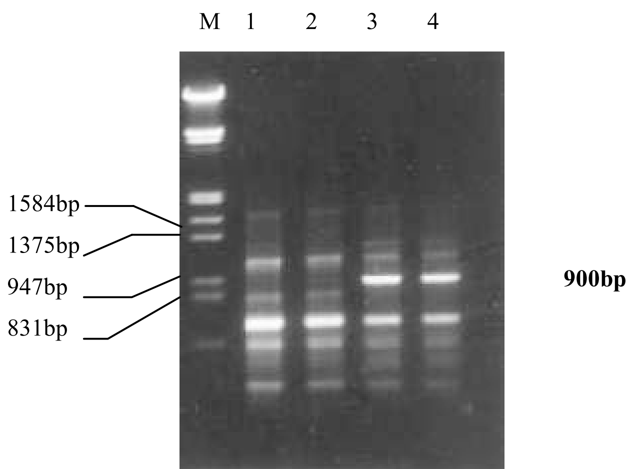

Fig. 1. Amplification pattern of the RAPD primer OPP15. M. λDNA HindIII/EcoRI markers; 1. Jing411; 2.015; 3. Brock; 4. Resistant individual. Arrowheads show the polymorphic fragments.

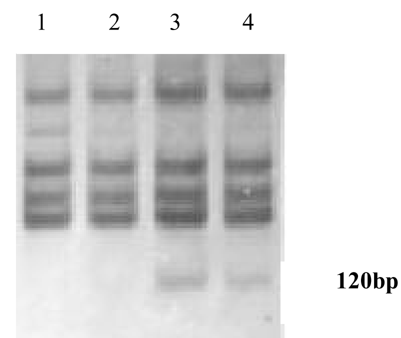

Fig. 2. SSR products amplified by Xgwm114. Jing411; 2. Line 015; 3. Brock; 4. Resistant individual.

2. Powdery mildew infection and tissue sampling

The above-mentioned 299 progenies were inoculated with a local isolate of *E. graminis f. sp. tritici* race No.15 when the first leaves were fully expanded in a greenhouse in China Agriculture University. Jing411 and Line 015 were used as susceptible controls. Inoculations were performed by dusting or brushing conidia from neighbouring sporulating susceptible seedlings onto the seedlings to be tested. Reactions were scored 15 days after inoculation when susceptible Jing411 and Line 015 were heavily infected. The response type of the plants to powdery mildew was evaluated using a scale of 1,2,3 and 4 at the three-leaf stage: 0; represents no visible symptoms, 0, necrotic flecks, and 1,2,3 and 4 highly resistant, resistant, susceptible and highly susceptible reactions, respectively (Liu et al., 1999).

3. RAPD and SSR analyses

Genomic DNA was extracted as described previously (Liu et al., 1999). RAPD analysis was performed according to the method of Wang et al. (2000) with slight modification for the reaction, we used in a total volume of 25 l containing 1×PCR buffer, 20ng genomic DNA, 200nM 10-mer Operon primer, 200 μM each deoxynucleotide and 1unit Taq DNA polymerase. The amplification was performed in a Minicycler PTC-150-25 with the program of the first 4 cycles at 96°C for 1 min, 37°C for 1 min and 72°C for 2 min, followed by 45 cycles at 94°C for 45 sec, 37°C for 1 min and 72°C for 90 sec, with a final extension at 72°C for 10 min. PCR products were separated on 1.4% agarose gel (containing 0.5μg/μl ethidium bromide) and visualized under UV light.

SSR markers were amplified in a total volume of 20 μl containing 1 PCR buffer, 500ng genomic DNA, 200 μM each deoxynucleotide and 1U Taq DNA polymerase and 50ng wheat microsatellite primers synthesized according to the sequence published (Röder et al., 1998b). The amplification was performed in a Minicycler PTC-150-25 with the program at 94°C for 3 min, followed by 45 cycles at 94°C for 1 min, 50°C (55°C or 60°C) for 1 min, 72°C for 2 min, and final extension at 72°C for 10 min. The PCR products were separated on an 8% non-denaturing polyacrylamide gel and detected by silver staining.

4. Linkage analysis

A preliminary screening for polymorphism of the molecular markers among the resistance parent Brock, susceptible parents Jing411 and Line 015, and their progenies, was carried out using 350 random 10 mer primers (Operon) and 100 SSR primer pairs. Linkage relationships among the resistance gene, RAPD and SSR markers were analyzed with MAPMAKER (Lander et al., 1987). The mapping population consists of 299 BC_1F_2 plants, including 218 resistant individuals and 81 susceptible individuals. Recombination values were transformed to linkage distances in centiMorgans (cM) according to Kosambi (1944). A LOD score of 3 was set to determine the significance of linkage.

Results

The resistant cultivar Brock and 218 out of 299 progenies of the hybrid population were resistant to powdery mildew (*E graminis* race No.15) the response type was 0, -1. On the other hand, Jing411, Line 015 and 81 out of 299 progenies were susceptible, the response type was 3-4. The segregation data showed that the resistance was controlled by one dominant gene.

A total of 350 random 10-mer primers were screened to identify polymorphisms of amplified fragments among the resistant parent Brock and susceptible parents Jing411 and Line 015. On the average each primer amplified about 7 fragments ranging from 200bp-3500bp. Of these primers, only one primer, OPP15, yielded reproducible polymorphic amplification patterns in Brock, Jing411 and Line 015.

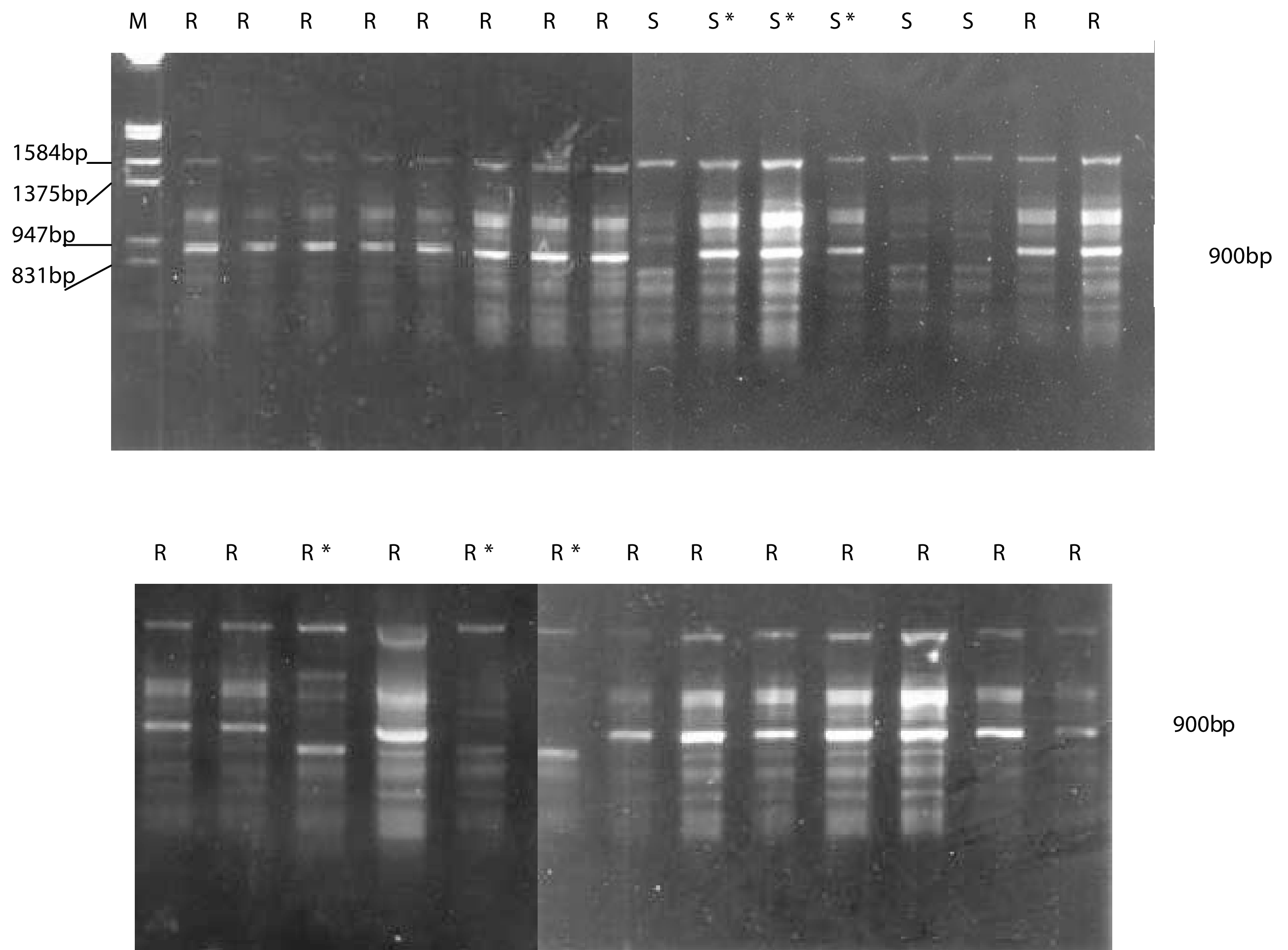

Fig. 3. Amplification pattern of the RAPD primer OPP15.
M: λDNA HindIII/EcoRI marker; R: resistant individual; S: susceptible individuals; R*:lacking amplified product of $OPP15_{900}$ in resistant individuals; S*: present amplified product of $OPP15_{900}$ in the susceptible individual.

Primer OPP15 produced an amplification fragment of 900bp ($OPP15_{900}$), which was present in Brock and the resistant progenies but absent in the Jing411 and Line 015 (Fig.1).

A total of 100 SSR primer pairs were screened to identify polymorphisms as in the RAPD analysis. Among them the primer pair for Xgwm114 produced a stable polymorphic product of 120bp in resistant parent Brock. The resistant progenies have this fragment named $Xgwm114_{120}$, while susceptible parent Jing411, Line 015 and susceptible progenies did not show this fragment (Fig. 2).

The linkage of the two molecular markers $OPP15_{900}$ and $Xgwm114_{120}$ to the powdery mildew resistance was tested using 299 progenies, including 218 resistant and 81 susceptible ones that had been tested for powdery mildew infection. Among the 218 resistant individuals (96%) 209 had the fragment $OPP15_{900}$, and the remaining 9 did not. Among the 81 susceptible individuals, 8 individuals had the product of $OPP15_{900}$ (Fig. 3). This result suggested that molecular marker $OPP15_{900}$ is linked to a resistant gene of powdery mildew in cv. Brock, and the linkage distance between *Pm* gene and $OPP15_{900}$ is 6.0 cM (Fig. 5).

The primer pair Xgwm114 amplified the fragment of $Xgwm114_{120}$ in 299 individuals (218 resistant individuals and 81 susceptible). This result showed that 197 out of 218 resistant individuals and 5 out of 81 susceptible have $Xgwm114_{120}$ product (Fig. 4). It is suggesting that $Xgwm114_{120}$ is probably linked to a resistant powdery mildew, and linkage distance between the Pm gene and $Xgwm114_{120}$ is 9.3 cM (Fig. 5).

Discussion

Up to now, at least 25 genes conferring powdery mildew (*E. graminis f. Sp. tritici*) resistance are known in wheat germplasm, but most of them have already been overcome by new virulent *E. graminis* strains (Cenci et al., 1999). It is therefore necessary to extend the search for new sources of genetic resistance to powdery mildew to a gene pool of cultivars. Brock is a wheat cultivar resistant to powdery mildew (Liu et al., 1999). RAPD markers are widely used to find markers

linked to powdery mildew resistance genes (Wang et al., 2000). In this study, we used the RAPD technique employing a total of 350 random 10 mer primers (Operon primers) to identify polymorphic markers between resistant and susceptible plants.

The polymorphic fragments of $OPP15_{900}$ were identified in the resistant parent Brock and most of resistant individuals. Linkage analysis showed that the amplified product $OPP15_{900}$ was associated with a powdery mildew resistance in resistant cv. Brock with a genetic distance of 6.0 cM. We have screened 11 wheat cultivars carrying *Pm2*, including Brock, for the resistance to powdery mildew (isolate No.15). However only Brock was resistant and the other cultivated wheats carrying *pm2* were susceptible to the isolate No.15, so that we conclude an unknown powdery mildew resistance gene was existed in Brock. We tested 10 differential wheat varieties with *Pm2* gene using OPP15 primer, but polymorphic fragments of $OPP15_{900}$ have not been identified.

Thus, there is no relationship between the $OPP15_{900}$ and *Pm2*. We conclude that $OPP15_{900}$ molecular marker in this research may be a new molecular marker probably associated with an unknown powdery mildew resistance gene in Brock.

Simple sequence repeat DNA (SSR DNA) called microsatellites are also ubiquitously interspersed in the eukaryotic genome, and SSR is more polymorphic than other marker systems (Röder et al., 1995, 1998a; Tautz et al., 1984). Röder (1995) has successfully constructed a genetic map of wheat genome containing 279 microsatellites located them in seven different chromosome groups, and reported that Xgwm114 was located on chromosome arm 3BL and 3D. In this study, we could not conclude whether or

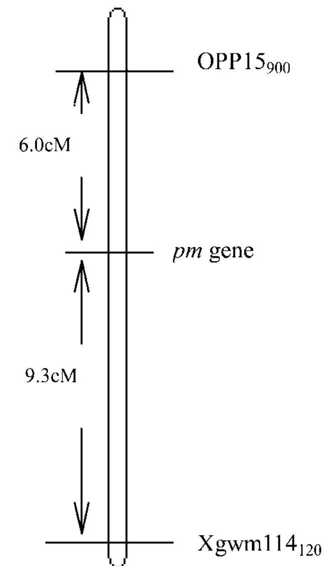

Fig. 5. Linkage distance of $OPP15_{900}$, $Xgwm114_{120}$ and *pm* gene.

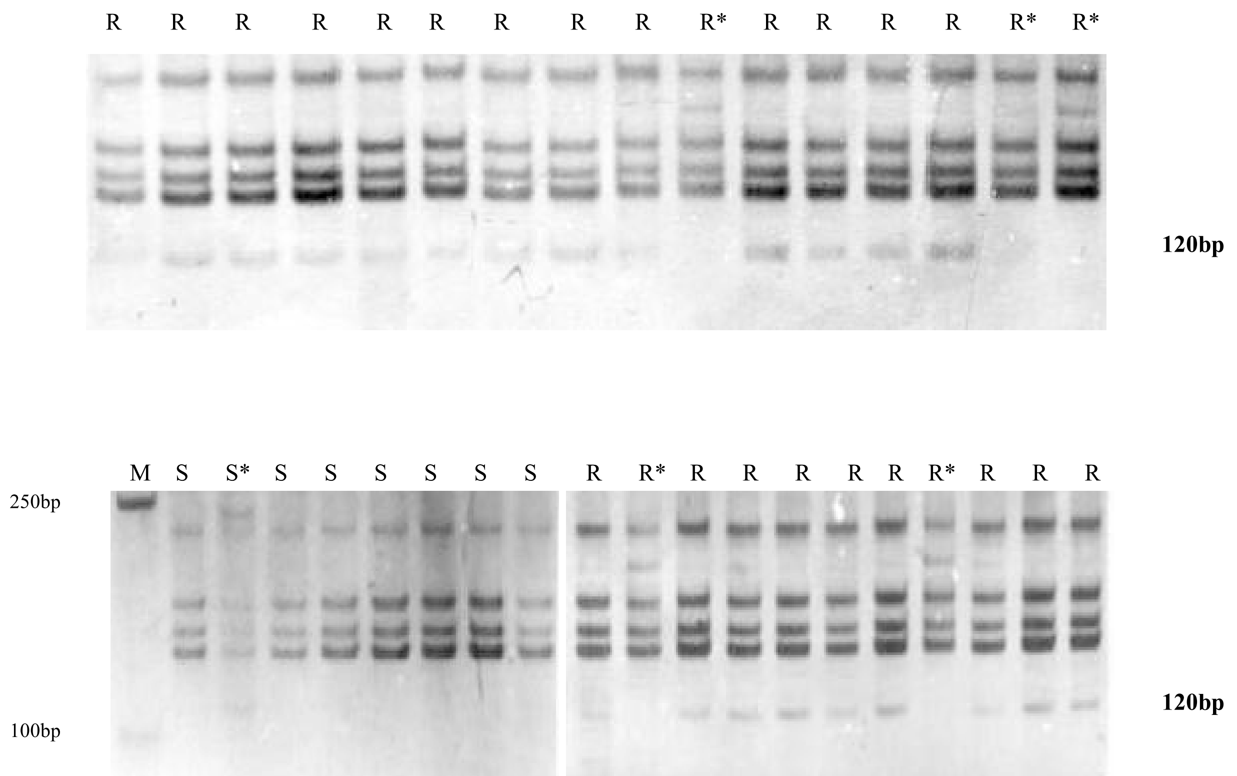

Fig. 4. Amplification pattern of the SSR primer pair Xgwm114.
M: DL2000 marker; R: resistant individuals; S: susceptible individuals; R*: lacking amplified product of $Xgwm114_{120}$ in the resistant individuals.

not $Xgwm114_{120}$ is located on chromosome 3BL/3D, but $Xgwm114_{120}$ was identified to be associated with powdery mildew resistance gene in common wheat Brock from the linkage analysis between $Xgwm114_{120}$ and resistant to powdery mildew in cv. Brock. Further research is necessary to prove this supposition.

The use of RAPD and SSR analyses in wheat appears to be suitable for the identification of the powdery mildew resistance gene. In the present study, we found two new molecular markers, RAPD marker $OPP15_{900}$ and SSR marker $Xgwm114_{120}$. These two molecular markers were shown to be associated with an unknown powdery mildew resistance gene in Brock, and should prove useful for facilitating selection and pyramiding of resistance genes in wheat breeding.

Acknowledgements

This research was supported by the special pre-project of state key basic research in P.R.China (2003CCC00200) and a grant from Tianjin Science and Technology Committee (003701111). We thank Dr. James Titchener (Hillary College in New Zealand) for critical reading of English manuscript.

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