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Identification and molecular mapping of a leaf rust resistance gene in spelt wheat landrace Altgold

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Abstract Leaf rust, caused by *Puccinia triticina*, is an important disease for wheat production, both in China and worldwide. In laboratory studies spelt wheat (*Triticum aestivum* ssp. *spelta*) landrace Altgold was resistant to *P. triticina* races THT and PHT and genetic analysis indicated that it possessed a dominant leaf rust resistance gene, temporarily designated *LrAlt*. F_6 recombinant inbred lines (RILs) derived from a cross with the susceptible common wheat cultivar Nongda 3338 were used to map *LrAlt* with SSR markers. The resistance gene was distal to SSR loci *Xbarc212*, *Xwmc382*, *Xgwm636*, and *Xwmc407* on the short arm of chromosome 2A. The closest markers *Xbarc212* and *Xwmc382* which co-segregated were 1.8 cM away from *LrAlt*. The relationships of *LrAlt* and other wheat leaf rust resistance genes located on the short arm of chromosome 2A were discussed, suggesting that *LrAlt* might be a new leaf rust resistance gene.

Keywords *Triticum spelta* · Resistance · SSR

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Introduction

Leaf rust, caused by *Puccinia triticina*, is an important foliar disease of wheat (*Triticum aestivum*) in China and elsewhere, causing substantial yield losses annually. The deployment of resistant cultivars has proved the most economic and environmentally safe way to control the disease. Because most of the resistance genes used in wheat resistance breeding are race-specific, the diversity of virulence in the pathogen population and the rapid emergence of races virulent to newly released major resistance genes make it necessary to search for additional effective resistance genes on a continual basis. More than 60 leaf rust resistance genes are formally designated in wheat (McIntosh et al. 2008).

Molecular markers are now widely used in wheat genetics research and breeding. Compared with other marker types, simple sequence repeats (SSRs) or microsatellites are more polymorphic, highly reproducible, and inexpensive to use. Readily available primer sequences and published microsatellite maps (Röder et al. 1998; Stephenson et al. 1998; Pestsova et al. 2000; Gupta et al. 2002; Song et al. 2005; Somers et al. 2004) offer a wealth of choice and reference information for genetic mapping. Leaf rust resistance genes, such as *Lr17a* (Bremenkamp-Barrett et al. 2008; Zhang et al. 2008), *Lr19* (Gupta et al. 2006), *Lr22a* (Hiebert et al. 2007), *Lr52* (Hiebert et al. 2005), and *LrZH84* (Zhao et al. 2008), were mapped with microsatellite markers.

Spelt wheat (*T. aestivum* ssp. *spelta* (*T. spelta*) $2n = 6x = 42$, AABBDD) is a close relative of common wheat, sharing the same genomic structure, and is fully cross-compatible with it. Spelt genotypes are cultivated in small areas in western countries where they are believed to have certain nutritional benefits. Spelt wheats have been reported over many years to have resistance to rusts. Macer (1966) described the presence of yellow rust resistance gene *Yr5* in *T. spelta* var. *album*. Kema (1992) and Kema and Lange (1992) reported high levels of stripe rust resistance among a collection of spelt accessions and identified *Yr5* and *Yr10* in few of them. Dyck and Sykes (1994) reported leaf rust resistance gene *Lr44* on chromosome 1B in spelt accession 7831.

The spelt landrace Altgold (also known as Altgolder Rotkorn), introduced from Europe, is resistant to leaf rust isolates in China. This paper reports the identification and genetic mapping of a new leaf rust resistance gene in this cultivar.

Materials and methods

Plant materials

An F_6 recombinant inbred line population (182 RILs), derived Nongda 3338/Altgold by single seed descent, was used for genetic analysis and mapping of leaf rust resistance. Common wheat cv. Nongda 3338 released from the wheat breeding program of China Agricultural University is susceptible whereas Altgold is resistant to the two leaf rust races inoculated in this study.

In order to validate the presence of a single gene in the resistant RIL'S; as well as to determine if the leaf rust resistance was dominant or recessive, the homozygous resistant line RIL135 was crossed with the susceptible cv. Xuezao. The F_2 offsprings were inoculated and scored for leaf rust response.

Evaluation of leaf rust responses

Seedlings of Nongda 3338, Altgold and the 182 RILs were tested with isolates of the virulent Chinese *P. triticina* races THT (avirulence/virulence formula 9, 24/1, 2a, 2c, 3, 16, 26, 3Ka, 11, 17, 30) and PHT (avirulence/virulence formula 2a, 9, 24/1, 2c, 3, 16, 26, 3Ka, 11, 17, 30) according to the North American system of nomenclature (Long and Kolmer 1989), provided by Drs Wanquan Chen and Taiguo Liu of the Institute of Plant Protection, China Academy of Agricultural Sciences. The isolates were first propagated on the susceptible check Yanda 1817 in the greenhouse. When the first leaves were fully expanded, seedlings were inoculated by brushing urediniospores from the susceptible genotype onto the seedlings to be tested. Inoculated seedlings were placed in plastic-covered cages and incubated at 15°C and 100% relative humidity for 24 h, and then transferred to a greenhouse maintained with 12 h light/12 h darkness at 18–25°C and 70% relative humidity.

Infection types (ITs) were assessed 13–16 days after inoculation when the susceptible check was fully infected. Scoring was conducted on a 0–4 scale, with 0 representing immunity (no sign of infection),

0 for necrotic flecks, 1 for small uredinia surrounded by a necrosis, 2 for small to medium uredinia surrounded by chlorosis, 3 for medium uredinia without chlorosis or necrosis, and 4 was large uredinia without chlorosis or necrosis. Plants scored IT 3 or higher were considered susceptible.

Molecular marker analyses

Of the 182 RILs, 173 homozygous (83 resistant and 90 susceptible) RILs were included in molecular marker analyses. Genomic DNA was extracted from leaf tissue samples following the method of Song and Henry (1995). Three plants in each line were taken for DNA extraction. Bulked segregant analysis (Michelmore et al. 1991) was used in a preliminary screen to identify molecular markers likely to be linked to the leaf rust resistance gene(s) in Altgold. Genomic DNA from eight homozygous resistant and eight homozygous susceptible RILs were mixed in equal amounts to form resistant and susceptible pools. Markers showing polymorphisms between the parents and bulked pools were then genotyped across the entire RIL population.

The microsatellite primer pairs were chosen from the GWM, WMC, CFA, CFD, and BARC series, with six markers per chromosome, details for which are available in the GrainGenes database (http://www.wheat.pw.usda.gov).

PCR were performed in volumes of 10 µl containing 10–50 ng of genomic DNA, 1× PCR buffer (10 mM Tris–Hcl, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 20 ng of each primers, and 0.75 U Taq DNA polymerase. The amplification programs were as follows: 94°C for 3 min, followed by 40 cycles of 94°C for 1 min, 50–60°C (depending on the specific primers) 1 min and 72°C for 2 min, and a final extension at 72°C for 10 min. PCR products were separated in 8% non-denaturing polyacrylamide gels (39:1, acrylamide:bisacrylamide), which were then silver-stained and photographed.

Data analysis

Chi-squared (χ^2) tests were used to determine the goodness of fit of observed and expected segregation ratios. MAPMAKER (Lander et al. 1987) version 3.0 was used to calculate the genetic distances between markers and the resistance gene.

Results

Identification of a gene for leaf rust resistance in spelt landrace Altgold

Altgold seedlings were resistant to isolates THT and PHT (IT 0;-1), whereas Nongda 3338 seedlings were susceptible (IT 4). Of the 182 RILs inoculated with isolate THT, 83 were homozygous resistant (IT 0;-2), 90 were homozygous susceptible (IT 3–4), and 9 segregated. A single gene for resistance was apparently segregating. F_2-derived F_6 lines tested in F_7 should segregate 31:2:31. The ratio 83:9:90 was a very close fit to the expected ratio ($\chi^2_{31:2:31} = 1.83$, 2 df, $P > 0.25$). The nine non-homozygous RILs were excluded from the further analysis. Then the tests were repeated with isolates THT and PHT, respectively. The results of these tests were in perfect agreement with the first. When inoculated with isolate THT, the F_2 seedling population of RIL135/Xuezao segregated 189 resistant (IT 0;-2):47 susceptible (IT 3-4), fitting an expected 3:1 ratio ($\chi^2_{3:1} = 3.25$, 1 df, $P > 0.05$), and suggesting a single dominant gene for resistance, temporarily designated *LrAlt*.

Genetic mapping of the leaf rust resistance gene

In the initial BSA using 126 primer pairs, one primer pair, BARC212, yielded a polymorphism between the parents and homozygous leaf rust response DNA pools. Testing of the individual RILs confirmed that *Xbarc212* was linked with *LrAlt* (Table 1; Fig. 1). Since *Xbarc212* had previously been mapped on the short arm of chromosome 2A (Song et al. 2005;

Table 1 Distribution of molecular marker genotypes among homozygous RILs of Nongda 3338/Altgold with and without *LrAlt*

Locus	Genotypes of 83 resistant lines		Genotypes of 90 susceptible lines	
	A[a]	B[a]	A	B
Xbarc212/Xwmc382[b]	82	1	5	85
Xgwm636	78	5	4	86
Xwmc407	78	5	6	84

[a] *A* homozygous for the allele from Altgold, *B* homozygous for the allele from Nongda 3338

[b] Co-segregating

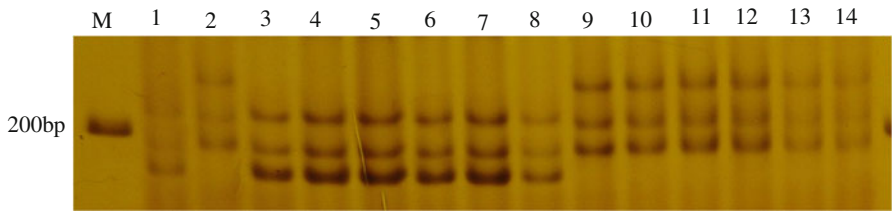

Fig. 1 Amplifications of SSR marker *Xbarc212* in Altgold (*lane* 1), Nongda 3338 (*lane* 2), homozygous resistant RILs (*lanes* 3–8), and homozygous susceptible RILs (*lanes* 9–14) of Altgold/Nongda 3338. *M* 100 bp DNA ladder

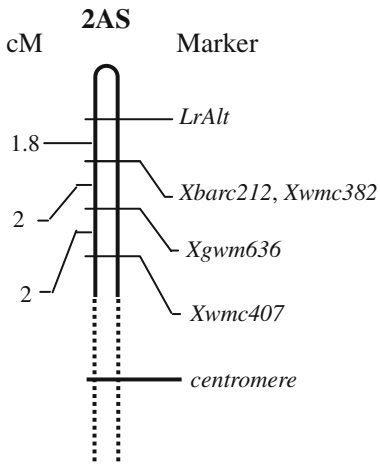

Fig. 2 Linkage map of the leaf rust resistance gene *LrAlt* region on chromosome 2AS

Somers et al. 2004), we assayed additional SSR markers located on 2AS for more polymorphisms. Three primer pairs (WMC407, WMC382, and GWM636) detected polymorphisms between the resistant and susceptible pools and were subsequently found to be linked to *LrAlt* (Table 1). Linkage analysis indicated that *LrAlt* was 1.8 cM distal to the co-segregating markers *Xbarc212* and *Xwmc382* (Fig. 2).

Discussion

We identified a dominant leaf rust resistance gene, *LrAlt*, in the spelt wheat landrace Altgold. The resistance locus was distal to four SSR markers on the short arm of chromosome 2A. The only previously located leaf rust resistance gene in spelt wheat is *Lr44* which is in chromosome 1B (Dyck and Sykes 1994).

The marker order on our genetic map is similar to the high density consensus map developed by Somers et al. (2004) except that *Xbarc212* and *Xwmc382* mapped proximal to *Xgwm636* and *Xwmc407* on the map by Somers et al. (2004), whereas our map indicated that *Xbarc212* and *Xwmc382* were distal to *Xgwm636*. However, the order of markers on our map agreed well with the map by Bremenkamp-Barrett et al. (2008).

Several wheat leaf rust resistance genes are reportedly located on chromosome 2AS, including *Lr11*, *Lr17*, *Lr37*, and *Lr45* (McIntosh et al. 2008). *Lr11* was located on chromosome 2A and reported as a dominant gene in cv. Hussar by Soliman et al. (1964), and Gupta et al. (1984) claimed that it behaved as a recessive allele in Sonalika. Neither report seems to be correct; Sonalika does not carry *Lr11* and the gene is not located on chromosome 2A (McIntosh, personal communication). The *Lr17* locus has two resistance alleles, *Lr17a* and *Lr17b* (Singh et al. 2001; McIntosh et al. 2008). Bremenkamp-Barrett et al. (2008) reported that *Lr17a* was flanked by SSR markers *Xgwm614* and *Xwmc407*, both of which are proximal to *Xgwm636* on the map of Somers et al. (2004). Zhang et al. (2008) also found that *Lr17a* was proximal to *Xgwm636* at a distance of 4.0 cM. Our results showed that *LrAlt* was distal to *Xgwm636*. Gene *Lr37*, derived from wheat wild relative *Aegilops ventricosa*, is located on a 2NS-2AS translocation in the wheat cultivar VPM1 (Bariana and McIntosh 1993). Helguera et al. (2003) developed a PCR marker that is amplified in lines possessing the translocation. When we tested the PCR marker for *Lr37* on Altgold and Nongda 3338, the predicted 259-bp PCR product was absent (data not shown). Gene *Lr45* was located in a large segment of a rye 2R chromosome translocated to wheat chromosome 2A (McIntosh et al. 1995). Because *Lr37* and *Lr45* are located in alien chromosome segments, and cannot be mapped precisely relative to genes in normal wheat chromosomes, they

are named as separate loci in accordance with the rules of nomenclature in wheat (McIntosh et al. 2008). The evidence presented above suggests that *LrAlt* is likely to be a new leaf rust resistance gene.

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