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Molecular tagging of a stripe rust resistance gene in *Aegilops tauschii*

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Abstract Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (PST), is one of the most important diseases of common wheat (*Triticum aestivum* L.). China has the largest stripe rust epidemic areas in the world and yield losses can be large. *Aegilops tauschii* Coss, the D-genome progenitor of common wheat, includes two subspecies, *tauschii* and *strangulata* (Eig) Tzvel. The ssp. *strangulata* accession AS2388 is highly resistant to the prevailing physiological races of PST in China, and possesses a single dominant gene for stripe rust resistance. In order to tag this gene, AS2388 was crossed with the highly susceptible ssp. *tauschii* accession AS87. The parents, F_2 plants, and $F_{2:3}$ families were tested at adult plant stage in field trials with six currently prevailing races. Simple sequence repeat (SSR) primers were used to identify molecular markers linked to the resistance gene. SSR markers *Xwmc285* and *Xwmc617* were linked to the resistance gene on chromosome arm 4DS flanking it at 1.7 and 34.6 cM, respectively. Based on the chromosomal location, this gene temporarily designated as *YrAS2388* is probably novel. The resistance in *Ae. tauschii* AS2388 was partially expressed in two newly developed synthetic hexaploid backgrounds.

Keywords *Puccinia striiformis* · Yellow rust · SSR marker · Molecular mapping · Wheat

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Introduction

Stripe rust (yellow rust) caused by *Puccinia striiformis* Westend. f. sp. *tritici* (PST) is one of the most important disease of common wheat (*Triticum aestivum* L. 2n = 6x = 42, AABBDD). China has the largest stripe rust epidemic areas in the world and large yield losses are sometimes experienced (Wang et al. 1995). Widespread stripe rust epidemics occurred in 1950, 1964, 1990, 2002, 2003 and 2009, and localized epidemics occur in most years (Wan et al. 2004; Kang et al. 2010). During the last 10 years, the areas affected annually by stripe rust were on average about 4 million hectares (Kang et al. 2010).

A total of 68 PST races have been identified in China; of these, 33 are designated as races CYR1

(Chinese Yellow Rust) to CYR33 and 35 are described as pathotypes (Wan et al. 2007; Chen et al. 2009). CYR32 first found in 1994 and CYR33 found in 1997 are the current predominant races (Chen et al. 2009). Resistant cultivars are the most economical and environmentally friendly means to reduce damage caused by stripe rust. Worldwide, stripe rust resistance genes *Yr1–Yr48* and many provisionally designated genes have been identified in wheat and its relatives (McIntosh et al. 2008, 2010; Marais et al. 2009; Cheng and Chen 2010; Herrera-Foessel et al. 2010; Li et al. 2010). Lines possessing genes *Yr5*, *Yr10*, *Yr12*, *Yr13*, *Yr14*, *Yr15*, *Yr16*, *Yr18*, *Yr24/Yr26*, *Yr36*, *Yr39*, *Yr41*, and some temporarily designated genes are still effective in China whereas at least some lines with *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr20*, *Yr21*, *Yr22*, *Yr25*, *Yr27* and *Yr29* are ineffective to currently prevalent races (Wan et al. 2007; Kang et al. 2010). This necessitates continued attempts to discover and exploit new resistance genes.

Aegilops tauschii Coss. (2n = 2x = 14, genome DD) is the D-genome progenitor of common wheat. This species includes two subspecies, viz. ssp. *tauschii* and ssp. *strangulata*. Ssp. *tauschii* has a very wide geographic distribution extending westwards to Turkey and eastwards to Afghanistan and China, whereas ssp. *strangulata* has a narrow distribution occurring only in two disjoined regions, in southeastern Caspian Iran and Transcaucasia (Kihara et al. 1965; Yen et al. 1983; Jakaska 1995). Previous studies indicated that *strangulata* has better resistance than ssp. *tauschii* (Yildirim et al. 1995; Knaggs et al. 2000; Liu et al. 2010). *Yr*28 is the only gene derived from *Ae. tauschii* currently included in wheat gene catalogue (McIntosh et al. 2008, 2010). Singh et al. (2000) located *Yr28* on chromosome arm 4DS using a mapping population of recombinant inbred lines developed from a synthetic hexaploid wheat (*T. turgidum* × *Ae. tauschii*) × *T. aestivum* cv. Opata 85 cross.

A recent study indicated that *Ae. tauschii* ssp. *strangulata* accession AS2388, originally from Iran, was highly resistant to prevailing PST races in China. Resistance for stripe rust in AS2388 was conferred by a single dominant gene (Liu et al. 2010). The objective of this study was to map the resistance gene using SSR markers.

Materials and methods

Plant materials

Ae. tauschii ssp. *strangulata* accession AS2388 is highly resistant to currently prevailing Chinese PST races, whereas ssp. *tauschii* accession AS87 is highly susceptible (Liu et al. 2010). In this study, 150 AS2388 × AS87 F_2 plants and their corresponding $F_{2:3}$ families were used for resistance assessments and gene mapping. The synthetic hexaploids Syn-SAU-86 derived from *T. turgidum* ssp. *turgidum* AS2313 × *Ae. tauschii* AS2388 and Syn-SAU-88 from *T. turgidum* ssp. *turgidum* AS2334 × *Ae. tauschii* AS2388 (Zhang et al. 2010) and their parents were also tested. The susceptible common wheat line SY95-71 was used a spreader.

Resistance assessments in the field

Responses of the F_2 population to stripe rust were assessed at the experimental station of the Triticeae Research Institute at Dujiangyan, and the F_3 families and synthetic hexaploids were tested at Wenjiang. Both sites are near Chengdu. Individual plants were spaced 10 cm apart within a 2-m row, with 30 cm between rows. Each F_3 family was planted in two rows. Spreader rows of SY95-71 were planted perpendicular and adjacent to the rows of tested lines.

Seedlings of SY95-71 and F_2 and F_3 plants were inoculated around 6 weeks after planting, with a urediniospore mixture of races CYR33 (SY11-14), CYR32, CYR31, CYR30, SY11-4, and HY46-8 provided by the Research Institute of Plant Protection, Gansu Academy of Agricultural Sciences. Infection types on individual plants were recorded on a 0–9 scale (McNeal et al. 1971) for three times at 10-day intervals when the susceptible parent *Ae. tauschii* AS87 was fully infected. F_3 families were classified as homozygous resistant or homozygous susceptible when only one phenotypic class was observed, and segregating when both resistant and susceptible plants were observed in the same family.

DNA preparation

Genomic DNA of the parents and F_2 plants of AS2388 × AS87 was extracted from leaves using the

CTAB protocol (Rogers and Bendich 1985). Equal amounts of DNA from 10 highly resistant and 10 highly susceptible plants from the segregating F_2 population were randomly chosen to make bulks for bulked segregant analysis (BSA) (Michelmore et al. 1991).

SSR analysis

A total of 110 pairs of SSR primers covering the D genome were synthesized according to the sequences published in the GrainGenes database (http://www.wheat.pw.usda.gov). SSR analysis followed the procedure of Röder et al. (1998) with minor modifications. PCR was performed in a Gene Amp PCR system 9700 (ABI) in 25 µl reaction volumes containing 1 × buffer, 80 ng of template DNA, 250 nmol of each primer, 1.5 mM of $MgCl_2$, 200 µMof each dNTP and 1U of *Taq* DNA polymerase. The cycling program consisted of 95°C for 5 min, followed by 45 cycles of 94°C for 1 min, 55–61°C (depending on annealing temperature for each primer pair) for 1 min, 72°C for 2 min, and a final extension at 72°C for 7 min. PCR products were separated on 6% denaturing polyacrylamide gels and visualized by silver staining (Tixier et al. 1997) with some modifications (Chen et al. 2008).

Statistical analysis and genetic mapping

Chi-squared (χ^2) tests were used to evaluate the goodness of fit for observed and expected ratios in the F_2 population and among F_3 families. Linkage analysis was conducted with the software Mapmaker 3.0b/EXP (Lincoln et al. 1992). The Kosambi function was used to convert recombination values to map distances. A logarithmic odds (LOD) ratio of 3.0 was used as a threshold for the declaration of linkage. The genetic linkage map was drawn with Mapdraw V2.1 software (Liu and Meng 2003).

Results

Genetic analysis

Ae. tauschii ssp. *strangulata* accession AS2388 was resistant (IT 0–1) and ssp. *tauschii* accession AS87 was susceptible (IT 7–9). The F_2 population from AS2388 × AS87 segregated in 115 resistant (IT 0–1) and 35 susceptible (IT 7–9), fitting a 3R:1S ratio ($\chi^2 = 0.22$, 1 *df*, $P > 0.63$), indicating that the stripe rust resistance was conferred by a single dominant gene temporarily designated as *YrAS2388*. The 150 F_3 families inoculated with the same races were classified as 34 lines homozygous resistant (RR), 81 segregating (Rr) and 35 homozygous susceptible (rr) ($\chi^2_{1:2:1} = 0.97$, 2 *df*, $P > 0.61$), in agreement with the results from F_2 population.

Linkage analysis

Among the 110 pairs of SSR primers used in the study, 51 (46%) gave distinguishable polymorphic patterns between the parents. SSR markers *Xwmc285* and *Xwmc617* on chromosome arm 4DS (Somers et al. 2004) were clearly polymorphic between the resistant and susceptible bulks. The two SSR markers were then used to amplify DNA from all F_2 plants (Figs. 1, 2). Based on genotypes established from the F_3 family data for the two markers (Table 1), *YrAS2388* was linked to *Xwmc285* and *Xwmc617* with genetic distances of 1.7 cM and 34.6 cM, respectively (Fig. 3). The closest SSR marker had a size of 278 bp in AS2388 and 284 bp in AS87 (data not shown).

Expression of resistance gene in synthetic hexaploid wheat

Compared with their *T. turgidum* ssp. *turgidum* parents (AS2313 and AS2334) with infection types (IT) of 6–8, the synthetic hexaploids Syn-SAU-86 and Syn-SAU-88 were more resistant (IT 2–4) at the adult-plant stages. This indicated that the resistance gene in *Ae. tauschii* AS2388 (IT 0–1) was only partially expressed in the synthetic hexaploid backgrounds.

Discussion

Three designated stripe rust resistance genes on chromosome 4D, *Yr22*, *Yr28* and *Yr46*, are listed in the wheat gene catalogue (McIntosh et al. 2008, 2010). *Yr46*, is an APR (adult plant resistance) gene on chromosome 4DL (Herrera-Foessel et al. 2010). *Yr22* identified in common wheat cultivar Lee (Chen

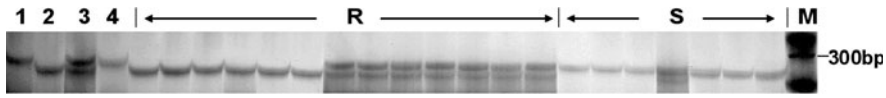

Fig. 1 Electrophoresis of PCR products amplified with SSR primer WMC285 in 6% denaturing polyacrylamide gels. *1* AS87, *2* AS2388, *3* resistant bulk, *4* susceptible bulk, *R* resistant F_2 plants; *S* susceptible F_2 plants, *M* marker

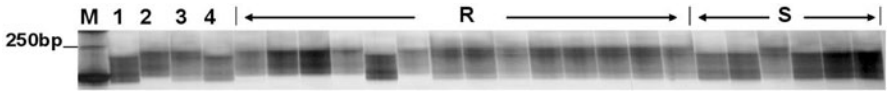

Fig. 2 Electrophoresis of PCR products amplified with SSR primer WMC617 in 6% denaturing polyacrylamide gels. M, marker *1* AS87, *2* AS2388, *3* resistant bulk, *4* susceptible bulk, *R* resistant F_2 plants, *S* susceptible F_2 plants

Table 1 F_2 genotypes inferred from seedling reactions of F_3 families and the corresponding alleles at SSR loci *Xwmc285*-4D and *Xwmc617*-4D

Markers	F_2 Genotype	Allele			Total
		A	H	B	
Xwmc285-4D	RR	32	2	0	34
	Rr	2	79	0	81
	rr	0	1	34	35
	Total	34	82	34	150
Xwmc617-4D	RR	16	16	2	34
	Rr	13	50	18	81
	rr	0	14	21	35
	Total	29	80	41	150

RR homozygous resistant, *Rr* segregating, *rr* homozygous susceptible, *A* homozygous for the AS2388 allele, *B* homozygous for the AS87 allele, *H* heterozygous

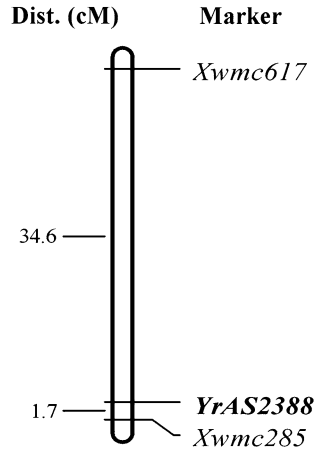

Fig. 3 Genetic linkage map of resistance gene *YrAS2388* and SSR markers on chromosome 4DS. Locus names and corresponding locations on the genetic map are indicated on the right side and map distances (cM) are shown on the left. The position of RFLP marker *Xmwg634* was obtained from a composite map (http://grain.jouy.inra.fr/cgi-bin/cmap/map_details?ref_map_set_acc=Wheat_Composite_2004;ref_map_accs=Wheat-Composite2004-4D;highlight=45233)

et al. 1995) is ineffective against currently prevalent races in China (Kang et al. 2010). *Yr28* derived from *Ae. tauschii* accession W-219 (Singh et al. 2000) was identified in a synthetic hexaploid, but this line is susceptible to the prevalent race SY11-4 (Wan et al. 2004) whereas ssp. *strangulata* accession AS2388 with gene *YrAS2388* is resistant (Liu et al. 2010). Thus *YrAS2388* must be different from *Yr28*. Moreover, *Yr28* is linked to the RFLP marker *Xmwg634-4D* located in the middle region of genetic map of chromosome arm 4DS. *YrAS2388* is closely linked to SSR marker *Xwmc285-4DS* which is 38.0 cM distal to *Xmwg634-4D* (http://wheat.pw.usda.gov/), indicating that *YrAS2388* and *Yr28* are at different loci (Fig. 3).

Several years of assessments have indicated that *YrAS2388* is highly resistant to stripe rust in China (Liu et al. 2010), thus indicating a potential value for wheat improvement. However, *YrAS2388* was not as effective in two synthetic hexaploid backgrounds as it was at the diploid level. This suggests the presence of suppressor(s) in the A/B genomes as reported previously (e.g. Kema et al. 1995; Ma et al. 1995; Yang et al. 2003; He et al. 2007; Rizwan et al. 2007). However, the resistance level of the two synthetics was sufficiently effective for resistance breeding. It will be interesting to determine if some hexaploid wheat backgrounds permit *YrAS2388* to be expressed at the same level as in the diploid genotype.

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