

Full Length Research Paper

Transfer of stripe rust resistance from *Aegilops variabilis* to bread wheat

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Accepted 19 October, 2010

In terms of area, the bread wheat producing regions of China comprise the largest area in the world that is constantly threatened by stripe rust epidemics. Consequently, it is important to exploit new adult-plant resistance genes in breeding. This study reports the transfer of stripe rust resistance from *Aegilops variabilis* to bread wheat resulting in resistant line, TKL2(R). Genetic analysis of the segregating populations derived from a cross between TKL2(R) and a susceptible sister line, TKL2(S), indicated that the adult-plant resistance to *Puccinia striiformis* f. sp. tritici in TKL2(R) is conferred by a single dominant gene. This gene provided resistance to physiological races currently endemic to China, thus indicating its potential usefulness in wheat breeding.

Key words: *Aegilops variabilis*, gene transfer, *Puccinia striiformis* f. sp. tritici, *Triticum aestivum*, wide hybridization.

INTRODUCTION

Stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. tritici (PST), is a major disease of common wheat (*Triticum aestivum* L.) in many cool and temperate regions of the world (Stubbs, 1988). In China, more than 20 million ha of wheat is affected by stripe rust, which is considered the most important disease of wheat (Wan et al., 2004, 2007). Stripe rust usually infects bread wheat cultivars during the adult plant stage in early spring. This causes serious yield losses resulting from shriveled grains. Destructive epidemics of stripe rust in China in 1950, 1964, 1990 and 2002 resulted in yield losses of 6.0, 3.2, 1.8 and 1.3 million tonnes, respectively (Wan et al., 2004). The most recent destructive epidemic was caused by a Chinese PST race CYR32.

Resistance breeding is the most economical and environmentally friendly method to reduce damage caused by

stripe rust. Thus, the development of resistant cultivars is a primary breeding objective, especially in southwestern China, where the most severe infections occur. World-wide, a series of stripe rust resistance genes, *Yr1*-*Yr41* and many provisionally designated genes, have been identified (McIntosh et al., 2008). Genes *Yr5*, *Yr10*, *Yr11*, *Yr12*, *Yr13*, *Yr14*, *Yr15*, *Yr24*, *Yr26*, *Yr ZH84* and some other genes are still effective in China whereas *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9* and some others have lost their effectiveness (Wan et al., 2007). This necessitates continued attempts to discover and exploit new adult-plant stripe rust resistance genes. The wild relatives of wheat represent a large reservoir of useful variability that can be used for wheat improvement (Mujeeb-Kazi, 2006). *Aegilops variabilis* Eig [syn. *Aegilops peregrina* (Hack.) Maire and Weiller; 2n = 4x = 28, genomes UUSS (Kimber and Feldman, 1987)] is one of such species that has been exploited effectively in the past. Genes for resistance to root knot nematode (Yu et al., 1990; Coriton et al., 2009), karnal bunt (William and Mujeeb-Kazi, 1996), powdery mildew (Spetsov et al., 1997) and leaf rust

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(Marais et al., 2008) have been transferred from this species. Dhaliwal et al. (1993) found that certain *A. variabilis* accessions exhibited high levels of resistance to stripe rust, yet the transfer of *Yr* genes to bread wheat has not been reported.

The *Ph* gene system that regulates homologous chromosome pairing in wheat can be disrupted to enable introgression of alien genetic material into common wheat (Sears, 1976). Liu et al. (2003) suggested that the Chinese common wheat landrace Kaixian-Luohanmai has a *ph*-like gene, *phKL*, on chromosome 6A. Although it has no detectable effect in wheat itself (Liu et al., 1999), in hybrids between common wheat and alien species such as *A. variabilis*, rye (*Secale cereale* L., $2n = 2x = 14$, RR) and *Psathyrostachys huashanica* Keng ex Kuo ($2n = 2x = 14$, NsNs), the presence of *phKL* significantly enhanced the level of homologous pairing. The effect of the *phKL* gene was similar to (Liu et al., 1999, 2003; Xiang et al., 2005) or stronger (Kang et al., 2008) than that of *ph2* in wheat-alien hybrids.

The aim of this study was to transfer stripe rust resistance from *A. variabilis* to bread wheat using the *phKL* genotype. When resistant derivatives were obtained, they were characterized by means of cytogenetic and segregation analyses.

MATERIALS AND METHODS

Plant materials

The bread wheat genotypes used in this study included the following: Chinese landrace Kaixian-luohanmai (KL) which has a *ph*-like gene, *phKL* (Liu et al., 2003) and shows high crossability with other species (Liu et al., 1999; Kang et al., 2008); bread wheat breeding line 3854; *A. variabilis* accession 13E provided by Dr. A. Mujeeb-Kazi, CIMMYT, Mexico, and the stripe rust susceptible bread wheat, SY95-71, which has been widely used in China as a highly susceptible stripe rust spreader genotype (Shu et al., 1999).

Alien transfer

The stripe rust susceptible bread wheat landrace KL with many undesirable agronomic characters was pollinated with resistant *A. variabilis* 13E. In order to improve the fertility and agronomic character, resistant F_1 plants were crossed with and backcrossed (female parent) to elite bread wheat breeding line 3854. The back-cross progenies (KL/*A. variabilis* 13E/2/3854) were selfed until the BC_1F_4 generation. During this process, progenies resistant to the natural stripe rust infection in the field was selected. However, only one resistant plant with normal fertility and 42 chromosomes were obtained in BC_1F_4 . Some BC_1F_5 plants derived from the BC_1F_4 were resistant and the others were susceptible. One resistant plant and one susceptible plant were selected and advanced to the BC_1F_6 generation when homozygous resistant (TKL2(R)) and homozygous susceptible (TKL2(S)) lines was selected. To further study the inheritance of resistance, hybrid F_1 and F_2 populations between resistant TKL2(R) and susceptible TKL2(S) lines were produced.

Cytological observation

Root tip chromosome counts and analysis of chromosome pairing

during meiotic metaphase I in pollen-mother-cells (PMCs) of TKL2(R) and TKL2(S) were observed as described by Zhang et al. (2007). In order to judge whether TKL2(R) is a translocation line, it was crossed with wheat parent KL and the chromosome pairing of the F_1 hybrid was observed.

Adult-plant resistance evaluation

Field evaluation for stripe rust resistance was carried out at the Triticeae Research Institute experimental station in Dujiangyan, which has a favorable environment for stripe rust development. Lines TKL2(R) and TKL2(S) and their parents were evaluated during three crop seasons, viz. 2006 - 2007, 2007 - 2008 and 2008 - 2009 and the F_1 and F_2 populations were used for genetic analysis and were evaluated in the 2007 - 2008 and 2008 - 2009 crop seasons. Each experiment consisted of two replicates. The individual plants were spaced 10 cm apart within 2 m rows and the row spacing was 30 cm. The highly susceptible spreader variety, SY95-71, was planted on both sides of each experimental row.

An artificial stripe rust epidemic about six weeks after planting by inoculating both the experimental rows and the SY95-71 spreader rows with mixed urediospores of *P. striiformis* pathotypes CYR32, CYR31, CYR30, SY11-14, SY11-4 and HY46-8, which were provided by the Research Institute of Plant Protection, Gansu Academy of Agricultural Sciences. These pathotypes currently occur in the wheat production regions of China (Wan et al., 2007). Stripe rust infection types were recorded three times at 10-day intervals using a 0 - 9 scale (McNeal et al., 1971). The first disease notes were taken when the susceptibility of the flag leaves of the susceptible check SY95-71 was fully expressed. Chi-square tests were made to analyze the data on inheritance of stripe rust resistance in the F_2 population.

RESULTS

TKL2(R) and TKL2(S) had similar morphologies; their plant height was about 84 cm, shorter than the KL parent (120 cm) and 3854 (95 cm), yet taller than *A. variabilis* 13E (60 cm). Both TKL2(R) and TKL2(S) had tenacious glumes indicative of a wide cross origin, whereas KL and 3854 are free threshing and *A. variabilis* 13E has tenacious glumes. In *A. variabilis*, tenacious glumes are controlled by genes on chromosome 2U or 2S (Friebe et al., 1996). This suggests that chromosome 2U or 2S, or a fragment thereof, carrying gene(s) for tenacious glumes was present in TKL2(R) and TKL2(S).

Root-tip chromosome counts showed that both TKL2(R) and TKL2(S) had 42 chromosomes. During meiotic metaphase I in pollen-mother-cells (PMCs), most of the 42 chromosomes in each line paired normally to produce 21 ring bivalents (Figure 1A). The mean pairing configuration for TKL2(R) was: 19.8 ring bivalents (II) + 1 rod bivalent + 0.1 univalents (I) + 0.2 trivalents (III).

Mean chromosome pairing in the cross TKL2(R) / KL was 19.4 ring II + 1.2 rod II + 0.3 I + 0.1 III and very similar to that above for TKL2(R) / TKL2(S). Most of the PMCs contained 21 bivalents (Figure 1B). Thus, TKL2(R) was not an alien substitution line. However, it could be a translocation line possessing chromosome segment(s) from the alien parent.

Field evaluation of the material over three crop seasons

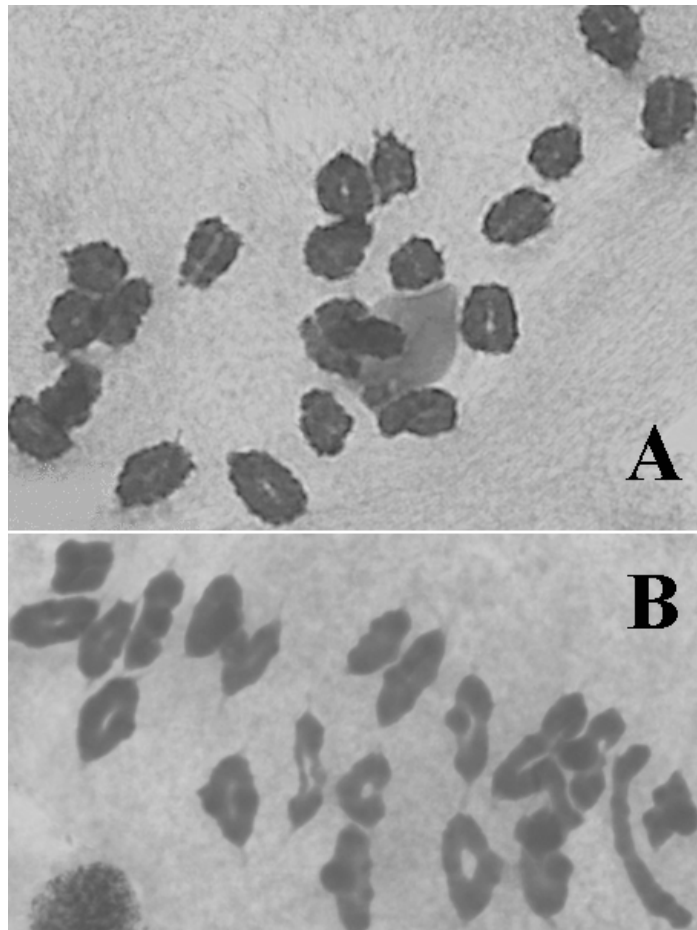


Figure 1. Chromosome pairing in pollen mother cells showing the formation of (A) 21 ring bivalents in the resistant, homozygous bread wheat line TKL2(R) and (B), 20 ring and 1 rod bivalents in a hybrid of TKL2(R) with bread wheat cultivar KL.

Table 1. Infection type (IT) data obtained following the evaluation of parental lines, F₁ and F₂ progeny for adult plant resistance to *Puccinia striiformis*. The numbers of plants that exhibited a particular IT are indicated.

Combinations and their parents	IT* in 2006-2007			IT in 2007-2008			IT in 2008-2009		
	0-2	7-9	Total	0-2	7-9	Total	0-2	7-9	Total
<i>A. variabilis</i> 13E	40	0	40	40	0	40	40	0	40
KL	0	40	40	0	40	40	0	40	40
3854	0	40	40	0	40	40	0	40	40
TKL2(R)	40	0	40	40	0	40	40	0	40
TKL2(S)	0	40	40	0	40	40	0	40	40
TKL2(R) × TKL2(S) F ₁	-	-	-	40	0	40	40	0	40
TKL2(R) × TKL2(S) F ₂	-	-	-	137	43	180	58	19	77

*This scoring was done according to McNeal et al. (1971).

with mixed inoculum of six stripe rust races consistently showed that *A. variabilis* 13E and TKL2(R) were resistant (infection type 0 - 2), whereas bread wheat cultivar KL and the breeding lines 3854 and TKL2(S) were susceptible (infection types 7 - 9) (Table 1). The F₁: TKL2(R) /

TKL2(S) was also resistant (2007 - 2008 and 2008 - 2009) showing that resistance was dominant. Among the 180 F₂, plants from this cross, 137 were resistant (infection type 0 - 2) and 43 were susceptible, fitting a 3R :1S ratio ($\chi^2 = 0.07$, $P > 0.75$) and indicative of a single

dominant gene for resistance. In 2008 - 2009 (Table 1) segregation in a further population of F₂ plants was 58 resistant: 19 susceptible, again a 3R : 1S ratio ($\chi^2 = 0.004$, $P > 0.90$).

DISCUSSION

In this study, a dominant gene for resistance to stripe rust was apparently transferred from *A. variabilis* 13E to bread wheat breeding line TKL2(R). Both TKL2(R) and a susceptible sister line, TKL2(S), have tenacious glumes presumably derived from an *A. variabilis* group 2 chromosomes. This trait of tenacious glumes did not segregate in F₂ of TKL2(R) x TKL2(S) cross. It could be that TKL2(R) acquired foreign chromatin carrying the resistance gene, but this line differed from TKL2(S) in having a larger translocated segment carrying the rust resistance gene, or a second translocation. Further works are trying to characterize the alien genetic material in TKL2(R).

The dominant stripe rust resistance gene, believed to be derived from *A. variabilis* 13E, provided protection against a population *P. striiformis* races known to occur in Sichuan province. Similar results were observed in Xining (Qinghai province). An attempt to tag the resistance gene was in progress while it was being transferred into the wheat cultivars Sichuan and Qinghai.

It will also be worthwhile to further evaluate the utility of the *phKL* gene in alien gene transfer. In previous studies (Liu et al., 2003; Xiang et al., 2005), the haploid F₁ hybrid between bread wheat cultivar KL and *A. variabilis* (5x = 35, genomes ABDUS) showed a high level of homologous chromosome pairing that was also evident in its hybrids with cereal rye (4x = 28, genome ABDR) (Liu et al., 1999, 2003; Xiang et al., 2005) and *P. huashanica* (4x = 28, genome ABDNs) (Kang et al., 2008). However, it is not clear whether translocations were produced by homologous pairing in these F₁ hybrids and then transmitted to their progenies. To answer this question, it will be necessary to testcross the primary hybrid and analyze the progeny for translocations.

ACKNOWLEDGMENTS

This project was funded through the following: the 973 Program (2009CB118300), the "100-Talent Program" of the Chinese Academy of Sciences (CAS), Key Project of Knowledge Innovation Engineering by CAS (KSCX1-YW-03, KSCX2-YW-N-052), the Ministry of Agriculture of China (2008ZX08002-004) and the Science and Technology Bureau of Sichuan Province. The authors are grateful to Dr. Mujeeb-Kazi A. for kindly providing seeds of *A. variabilis* 13E.

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