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Evaluation of Stripe Rust Resistance in Hungarian Winter Wheat Cultivars in China

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Stripe or yellow rust (*Yr*), caused by *Puccinia striiformis* Westend. (*Pst*), is one of the most important wheat diseases worldwide. New aggressive *Pst* races can spread quickly, even between countries and continents. To identify and exploit stripe rust resistance genes, breeders must characterize first the *Pst* resistance and genotypes of their cultivars. To find new sources of resistances it is important to study how wheat varieties respond to *Pst* races that predominate in other continents. In this study we evaluated stripe rust resistance in 53 Hungarian winter wheat cultivars in China. Twenty-four cultivars (45.3%) had all stage resistance (ASR) and 1 (1.9%) had adult-plant resistance (APR), based on seedling tests in growth chambers and adult-plant tests in fields. We molecularly genotyped six *Yr* resistance genes: *Yr5*, *Yr10*, *Yr15*, *Yr17*, *Yr18,* and *Yr36*. *Yr18*, an APR gene, was present alone in five cultivars, and in 'GK Kapos', that also had seedling resistance. The other five *Yr* genes were absent in all cultivars tested.

Keywords: adult-plant resistance, *Puccinia striiformis,* seedling resistance, yellow rust

Introduction

Stripe (yellow) rust, caused by *Puccinia striiformis* Westend. (*Pst*) is one of the most important wheat diseases worldwide. Stripe rust epidemics have become more frequent in warmer regions due to the adaptation of the pathogen (Milus et al. 2009; Wellings 2011). In Hungary stripe rust epidemics were historically rare, being recorded in 1926, 1932, 1933, 1936, 1952, 1954, 2000, and 2001 (Csősz 2007). However, in 2014 a severe epidemic occurred causing 70–85% yield losses in susceptible cultivars. Since then stripe rust epidemics have occurred annually.

Disease resistance is cost-effective, safe and efficient in safeguarding wheat production (Line and Chen 1995). Stripe rust reactions in wheat can be characterized at specific

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growth stages (seedling or all-stage resistance (ASR) or adult plant resistance (APR)), by race specificity, temperature sensitivity and genetic basis (Chen 2005). High-temperature adult-plant resistance (HTAP) is defined as a specific form of APR (Chen 2013). ASR is generally race-specific and qualitatively inherited. Contrarily APR, including HTAP, tends to be partial, non-race-specific, and quantitatively inherited (Chen 2013).

More than 80 *Yr* genes in wheat have been formally named, but most are race-specific (McIntosh et al. 2017; Nsabiyera et al. 2018), meaning that they lack durability. The *Yr18/Lr34* gene confers race non-specific, durable resistance to stripe rust and leaf rust (McIntosh 1992) and is associated with a pleiotropic trait known as leaf tip necrosis (*Ltn1*) (Krattinger et al. 2009). Some *Yr* genes including *Yr5, Yr10, Yr15, Yr17, Yr18,* and *Yr36* are still widely effective (Krattinger et al. 2009). The availability of partially or completely linked markers for these *Yr* genes facilitates their use in breeding programs (Klymiuk et al. 2018; Helguera et al. 2003; Lagudah et al. 2006; Marchal et al. 2018; Yuan et al. 2012, 2018).

In this study we determined the seedling and adult plant stripe rust reactions of 53 Hungarian wheat cultivars with predominant Chinese *Pst* races, and used molecular markers to predict the presence or absence of the above six *Yr* genes.

Materials and Methods

All wheat cultivars tested in this study are held by the Cereal Research Non-profit Company Ltd., Hungary. They were registered from 1970 to 2013 (Table 1). Chinese wheat variety 'HuiXianHong' (HXH) was included as a susceptible control in stripe rust assessments. Six germplasms were included as resistant controls; these were: *Triticum spelta* (*Yr5*), Moro (*Yr10*), Avs/6**Yr15* (*Yr15*), PI 672001 (*Yr17*), 98M71 (*Yr18*), and UC1041+*Yr36* (*Yr36*). PI 672001 (*Yr17*) was provided by the U.S. National Plant Germplasm System (NPGS) (https://www.ars-grin.gov/npgs/) and the others were available within our program (Yuan et al. 2012).

A mixture of Chinese *Pst* races CRY31, CRY32 and CRY33 was used for inoculation of both seedlings and adult plants. Seedling inoculations were carried out when the first leaves were fully expanded. A water-spore suspension was manually injected into leaf bundles using a 2.5 ml syringe. The inoculated seedlings were incubated at 10 °C and 100% relative humidity in darkness for 24 h and then moved to a cabinet with 16 h light at 15 °C and 8 h darkness at 10 °C. Infection types (ITs) at the seedling stage were recorded 15 days post inoculation when control variety HXH showed fully developed symptoms using a 0–9 scale, where IT 0–3 were considered resistant, 4–6 intermediate, and 7–9 susceptible (Line and Qayoum 1992).

Field tests were conducted at the experimental station of Shandong Agricultural University at Tai'an during two cropping seasons (2014–2015 and 2015–2016). Wheat seeds were sown in one-row plots (1.5 m length, 28 cm apart) in early October. One row of HXH was also sown in every 20th row as a susceptible check and source of inoculum. HXH was also sown in the greenhouse and inoculated in mid-February to obtain fresh urediniospores for field inoculations. Field plots were inoculated at the tillering stage in

Table 1. Seedling infection types, adult plant infection types and severities and marker-predicted *Yr* genes in 53 Hungarian wheat cultivars

Cultivar name	Year of registration[a]	Seedling stage		Adult-plant stage			Marker prediction
		Phenotype	IT	Phenotype	IT (severity%) (2015)	IT (severity%) (2016)	
GK Bagoly	2000	I	5	I	4 (30%)	5 (30%)	0
GK Barna	1990	I	6	R	3 (10%)	3 (1%)	0
GK Békés	2005	R	1	R	2 (30%)	2 (5%)	0
GK Berény	2010	I	6	I	3 (20%)	4 (30%)	0
GK Cinege	2002	R	1	R	2 (1%)	2 (5%)	0
GK Csillag	2005	R	2	R	2 (5%)	2 (5%)	0
GK Csongrád	2001	R	1	R	1 (1%)	1 (1%)	0
GK Csörnöc	1995	R	2	R	2 (10%)	1 (1%)	0
GK Délibáb	1992	I	5	I	5 (50%)	4 (30%)	0
GK Élet	1996	I	5	I	4 (15%)	5 (30%)	0
GK Fény	2006	I	4	R	2 (10%)	2 (10%)	0
GK Futár	2011	R	3	R	2 (5%)	2 (10%)	0
GK Garaboly	1998	I	5	I	4 (15%)	6 (70%)	0
GK Góbé	1992	I	5	I	3 (10%)	4 (10%)	0
GK Göncöl	2009	R	2	R	2 (10%)	2 (5%)	0
GK Hajnal	2010	I	5	R	2 (5%)	2 (5%)	0
GK Hargita	2003	R	2	R	2 (5%)	2 (10%)	0
GK Hattyú	2002	R	1	R	2 (5%)	2 (5%)	0
GK Héja	2001	R	2	R	2 (10%)	2 (5%)	0
GK Holló	2001	I	4	R	1 (1%)	2 (5%)	0
GK Hunyad	2005	I	5	R	1 (1%)	2 (10%)	*Yr18*
GK István	1987	I	6	I	5 (70%)	6 (70%)	0
GK Jászság	1999	R	2	R	3 (5%)	2 (1%)	0
GK Kalász	1996	I	5	R	2 (10%)	1 (10%)	0
GK Kapos	2003	R	1	R	3 (10%)	3 (15%)	*Yr18*
GK Kincső	1983	I	5	I	4 (30%)	3 (15%)	0
GK Körös	2010	R	1	R	2 (10%)	2 (5%)	0
GK Ledava	2002	R	3	R	2 (5%)	2 (5%)	0
GK Marcal	1995	R	3	R	1 (1%)	1 (1%)	0
GK Március	2008	R	1	R	2 (1%)	N/A	0
GK Mentor	2013	I	5	R	2 (5%)	3 (30%)	0
GK Mini Mano	1983*	R	1	R	3 (10%)	2 (10%)	0

Table 1. (cont.)

Cultivar name	Year of registration[a]	Seedling stage		Adult-plant stage			Marker prediction
		Phenotype	IT	Pheno-type	IT (severity%) (2015)	IT (severity%) (2016)	
GK Miska	1998	I	5	R	2 (10%)	1 (1%)	0
GK Mura	1998	I	6	R	1 (5%)	2 (5%)	0
GK Nap	2006	I	6	I	4 (10%)	3 (10%)	0
GK Olt	1992	I	6	I	5 (30%)	5 (30%)	0
GK Őrség	1991	I	4	R	3 (10%)	1 (1%)	0
GK Öthalom	1985	R	2	R	3 (10%)	2 (5%)	0
GK Petur	2003	I	5	R	3 (15%)	3 (20%)	0
GK Piacos	1999	I	5	R	3 (10%)	2 (5%)	*Yr18*
GK Pilis	2013	R	1	R	2 (10%)	2 (30%)	0
GK Pinka	1994	I	4	I	4 (15%)	3 (20%)	0
GK Rába	2000	I	5	R	1 (5%)	1 (1%)	*Yr18*
GK Rozi	2010	R	1	R	2 (1%)	1 (1%)	0
GK Smaragd	2002	I	5	R	3 (15%)	3 (15%)	0
GK Szala	2005	I	5	R	2 (5%)	2 (5%)	*Yr18*
GK Tisza	2003	S	7	R	2 (10%)	2 (10%)	*Yr18*
GK Tündér	2001	R	1	R	3 (5%)	1 (1%)	0
GK Véka	1996	R	1	R	2 (10%)	1 (1%)	0
Gk Vitorlás	2010	S	7	I	6 (50%)	N/A	0
GK Zombor	1985	R	1	R	2 (5%)	2 (10%)	0
GK Zugoly	1993	R	1	R	2 (10%)	N/A	0
Yubileynaja 50	1970	I	5	I	5 (30%)	5 (20%)	0
HXH	N/A	S	8	S	8 (80%)	8 (80%)	0
Triticum spelta	N/A	N/A	N/A	N/A	N/A	N/A	*Yr5*
Moro	N/A	R	1	R	1 (1%)	1 (2%)	*Yr10*
Avs/6**Yr15*	N/A	N/A	N/A	N/A	N/A	N/A	*Yr15*
PI672001	N/A	N/A	N/A	N/A	N/A	N/A	*Yr17*
98M71	N/A	I	4	R	1 (1%)	3 (5%)	*Yr18*
UC1041+*Yr36*	N/A	N/A	N/A	N/A	N/A	N/A	*Yr36*

R – resistant; S – susceptible; I – intermediate responses; N/A – not applicable or not tested in this study; [a]Year of test in Hungarian National Variety Trials; * variety candidate.

late March, and the procedure was repeated three times at weekly intervals. ITs were recorded 4–6 weeks after inoculation when rust severities on the susceptible HXH control reached 60–80%. Two to three sequential scorings were conducted and the highest ITs were recorded for each line. Disease severities were evaluated at the flowering stage using the modified Cobb scale (Peterson et al. 1948), which expresses the percentage infected leaf area with disease symptoms.

Genomic DNA was isolated from seedlings at the two-leaf growth stage by the Sarkosyl method (Yuan et al. 2012). DNA samples were diluted to 50 ng/µl with ddH_2O. PCR amplification was done in 20 µl reaction volumes, with 10 µl 2×Es Taq MasterMix (CoWin, Biosciences, Beijing), 0.5 µl of each forward and reverse PCR primer (each 0.4 µmol/L), 100 ng template DNA, and ddH_2O to reach the final volume. PCR primers for genotyping *Yr5*, *Yr10*, *Yr15*, *Yr17*, *Yr18,* and *Yr36* are listed in Table 2. Nested PCR were required for the *Xsdauw79* marker. PCR and *Yr* gene identification were performed following published methods (Table 2).

Results

HXH was susceptible at the seedling stage (IT 8). Among the 53 Hungarian cultivars, 24 were resistant (IT 1-3), 27 intermediate (IT 4–6), and 2 susceptible (IT 7) (Table 1). Among the selected reference lines, 'Moro' containing gene *Yr10* was resistant (IT 1) and '98M71' containing *Yr18* gave an intermediate response (IT 4).

The phenotype at adult-plant stage for most cultivars was similar in IT and disease severities across years (Table 1). Among the 24 cultivars with seedling resistance, 22 were also resistant (IT <3) at the adult-plant stage in both years, 2 ('GK Március', 'GK Zugoly') were resistant on the basis of one year of data (2015). Therefore, these 24 cultivars were classified as having ASR.

Of the 27 cultivars with intermediate seedling responses (IT 4-6), 15 were resistant (IT 1-3) at the adult plant stage (Table 1). Their increased resistance may be conferred by same seedling resistance gene or additional APR genes. Seven of the remaining 12 cultivars were evaluated in the same category (IT 4-6) at the adult-plant stage in both years, and 5, viz. 'GK Góbé', 'GK Berény', 'GK Nap', 'GK Pinka', and 'GK Kincső', were more resistant, and considered to have an intermediate level of resistance to *Pst*. 'GK Tisza' differed greatly in seedling (IT 7) and adult-plant (IT 2) responses, and was therefore considered to have APR (Table 1). 'GK Vitorlas' had inconsistent IT scores and requires further testing (Table 1). Twenty-five of the tested cultivars were released from 1970 to 2000, and the remaining 28 cultivars were more recent. Resistant cultivars (both ASR and APR) were more frequent among the more recent materials.

Disease severities were also scored during these experiments. The severity for HXH was 70%, 50–70% for cultivars with intermediate reactions (IT 4–6), and less than 20% for the majority of seedling resistant cultivars (IT 1–3). Cultivars with intermediate ASR responses generally had reduced disease severity at the adult-plant stage (Table 1).

Table 2. PCR primers for selected *Yr* genes

Yr gene	Primer ID	Primer sequence	Distance from the target gene (cM)	Reference
Yr5	Yr5-Insertion	F: 5'-CTCACGCATTTGACCATATACAACT-3'	Gene-specific	Marchal et al. (2018)
		R: 5'-TATTGCATAACATGGCCTCCAGT -3'		
Yr10	Xsdauw79	F: 5'-TTGCTCTAAGCTGTGGCCT-3' R: 5'-GAGTTCAACCCCGAACACT-3' Nested primer F: 5'-AGAGCCTAAGCGCCTAAGG-3' Nested primer R: 5'-TTAAAATCTCCCAAGTACGCA-3'	Complete linkage (in a population of 7,177 $F_{2:5}$ plants)	Yuan et al. (2018)
Yr15	KinI	F: 5'-GGAGATAGAGCACATTACAGAC-3'	Gene-specific	Klymiuk et al. (2018)
		R: 5'-TTTCGCATCCCACCCTACTG-3'		
Yr17	URIC/LN2	F: 5'-GGTCGCCCTGGCTTGCACCT-3' R: 5'-TGCAGCTACAGCAGTATGTACACAAAA-3'	Complete linkage (specific to chromosome 2N of *Ae. ventricosa*)	Helguera et al. (2003)
Yr18	CsLV34	F: 5'-GTTGGTTAAGACTGGTGATGG-3'	0.4 cM	Lagudah et al. (2006)
		R: 5'-TGCTTGCTATTGCTGAATAGT-3'		
Yr36	Yr36E1a	F: 5'-AAGGCAAAGGCAAAGTGG-3'	Gene-specific	Yuan et al. (2012)
		R: 5'-TGATCTTTACCAAGCATTCG-3'		

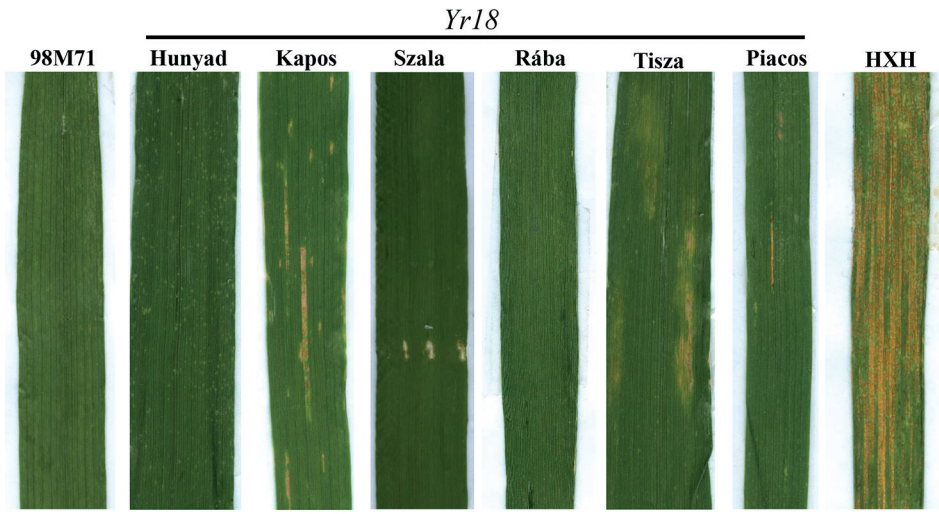

Figure 1. Characteristic stripe rust infection types (ITs) of cultivars positive for *Yr18*. 98M71 and HXH were used as sensitive controls with and without *Yr18* gene, respectively

Marker analysis was carried out for the six selected *Yr* genes. Each PCR marker was confirmed in specific controls and was negative in HXH (Table 2, Fig. S1*). The *Yr18* marker was positive in six cultivars ('GK Hunyad', 'GK Kapos', 'GK Piacos', 'GK Rába', 'GK Szala', and 'GK Tisza') whereas the other *Yr* markers gave negative results for all Hungarian wheats (Table 1).

Five of the *Yr18*-positive cultivars were resistant at the adult-plant stage (Table 1) whereas the sixth line, 'GK Kapos', was also resistant at the seedling stage. Four of the *Yr18*-positive cultivars had adult-plant IT 1-2 without sporulation, and 'GK Kapos' and 'GK Piacos' had IT 3 and traces of sporulation (Table 1, Fig. 1). 'GK Tisza' developed some large chlorotic flecks, but without sporulation (Fig. 1). Leaf tip necrosis, a pleiotropic manifestation of *Yr18* (Singh 1992), was present in all six *Yr18*-positive cultivars.

Discussion

In this study we tested the seedling and adult-plant stripe rust responses of 53 wheat cultivars registered in Hungary between 1970 and 2013 to a mixture of Chinese *Pst* races. About half of the lines had seedling resistance, and an additional 16 (30.2%) were susceptible or intermediate resistant at the seedling stage but were resistant at adult-plant stage. The occurrence of such a high frequency of resistance in Hungarian cultivars was unexpected because stripe rust epidemics were infrequent before 2014. Wheat stripe rust epidemics develop at lower temperatures than other rusts (Stubbs 1985). The Hungarian climate is continental with cold winters and warm summers with low precipitation.

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

Hence, selection for stripe rust resistance by breeders was given relatively low priority. The high frequency of resistance in Hungarian wheat cultivars observed in the current study using Chinese *Pst* races was unexpected and could be caused by resistance genes specifically effective in China or by linkage to other traits.

We molecularly genotyped the Hungarian wheat cultivars for six *Yr* genes (*Yr5*, *Yr10*, *Yr15*, *Yr17*, *Yr18,* and *Yr36*) using well established markers. Only *Yr18* was identified in six cultivars. *Yr18* is recognized as a "slow rusting" gene providing race non-specific, durable, adult-plant resistance (Krattinger et al. 2009). The *Yr18* locus also provides broad-spectrum resistance to leaf rust and powdery mildew (Spielmeyer et al. 2005) and its presence in Hungarian wheats might be indicative of selection for resistance to other diseases. Nevertheless, the six *Yr18*-positive wheat cultivars, especially 'GK Kapos' and 'GK Tisza', could be useful sources of stripe rust resistance for Chinese breeding programs. These last two cultivars might also have additional useful resistance genes.

Since most of the tested *Yr* genes were not present in the Hungarian wheat cultivars it could be worthwhile to incorporate them into on-going wheat breeding programs, preferably by combining them using marker assisted selection.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Figure S1*. PCR amplification of selected *Yr* gene markers