Juvenile resistance to diseases in *Aegilops tauschii* Coss. samples

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Summary

Seedling (juvenile) resistance to 4 diseases was studied in 452 *Ae. tauschii* samples from VIR World Collection. Eight accessions were highly resistant to leaf rust and they likely have gene for resistance Lr41. Rust from their naturally infected leaves was nonpathogenic on 25 wheat varieties that evidently indicates to the existence of the causal agent of leaf rust specialized to *Ae. tauschii.* Two samples possess high resistance to dark-brown leaf spot blotch; level of resistance was not changed after 9 continuous subcultivation of artificial *B. sorokiniana* population on leaves. Six samples were classified as resistant after inoculation with mixture of 7 *Stagonospora nodorum* isolates, but as susceptible to single isolate of the pathogen. All samples were susceptible to common root rot. Usefulness of selected *Ae. tauschii* samples for introgression hybridization to transfer juvenile resistance into bread wheat is discussed.

Index words: *Aegilops tauschii*, leaf rust, dark-brown leaf spot blotch, common root rot, *Septoria nodorum* blotch, juvenile resistance

Introduction

Fungal diseases considerably limit wheat (*Triticum aestivum* L.) yield and quality. The cheapest and ecologically profitable method to control economically important diseases is to breed and grow resistant varieties. First and possible key step in their breeding is identification of new donors for resistance, i.e. samples with high level of the trait expression, easily transferred via crosses, and protected by genes for resistance, that are not present in currently growing cultivars. Wheat samples from Vavilov Institute of Plant Industry (VIR) World Collection with effective juvenile resistance to leaf rust (*Puccinia recondita* Rob. ex Desm.) have only genes Lr 9, 19, 24, 41 now widely used in breeding (Tyryshkin et al., 2004); no one genotype was found to be highly resistant to dark brown leaf spot blotch and common root rot (both caused by *Bipolaris sorokiniana* Shoem.) and septoria glume blotch (*Stagonospora nodorum* Berk.) (Tyryshkin and Tyryshkina, 2003).

One of the possible approaches to create new wheat donors is introgressions of genes for resistance from wild relatives, *Ae. tauschii* being among the most perspective because of complete homology of its genome to genome D of *T. aestivum* (Konarev et al., 1970; Gill and Raupp, 1987). At least 8 wheat genes for resistance to leaf rust were transferred from *A. tauschii* (Rowland and Kerber, 1974; Raupp et al., 1983; Kerber, 1987; Cox et al., 1994), synthetic hexaploid lines were created with resistance to *B. sorokiniana* (Mujeeb-Kazi et al., 1996; Mujeeb-Kazi et al., 2001) and *S. nodorum* (Nicholson et al., 1993) from *A. tauschii.*

VIR World Collection includes more than 450 *Ae. tauschii* samples originated from different regions of Europe and Asia. Numerous works have been performed to evaluate their juvenile and adult resistance to fungal diseases including leaf rust (Migushova and Grigorieva, 1973; Navruzbekov and Chikida, 1993; Mikhailova and Navruzbekov, 1997; Mikhailova., 2003), septoria glume blotch (Yamaleev et al., 1990) and dark-brown leaf spot blotch (Tyryshkin et al., 1997; Strahova et al., 2001). Juvenile resistance usually was studied

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after inoculation with pathogens of detached leaves placed on cotton-wool wetted with water solution of benzimidazole (Mikhailova and Kvitko, 1970). Genotype-dependent induction of resistance to foliar diseases by this chemical was shown in wheat (Tyryshkin et al., 2005), barley (Tyryshkin and Solovieva, 2001) and *Aegilops* (Kolesova and Tyryshkin, 2004) so use of that technique could lead to mistaken description of susceptible samples as resistant; high correlation of disease ratings of intact plants was found with that of leaf segments placed on water.

The purpose of present work is to reevaluate juvenile resistance in *Ae. tauschii* samples to three foliar diseases and common root rot.

Material and Methods

Seedling (juvenile) resistance to diseases was studied in 452 *Ae. tauschii* samples from VIR World Collection.

The seeds were sown into cuvettes on wet cotton wool and kept in the darkness. After seedling emergence cuvettes were placed in light room at 20-22 °C day and night temperature and photoperiod 16:10 (L:D) h. Spring wheat cv. Leningradka was used as susceptible control in all experiments.

For inoculation complex population of *P. recondita* (mixture of isolates collected several times during wheat vegetation period in North-West region, Leningradskaia oblast, and North Caucasus, Derbent district), aggressive isolate T of *B. sorokiniana* (Tyryshkin and Mikhailova, 1993) and mixture of 7 *S. nodorum* isolates (MN 1-7) were used. *S. nodorum* isolates were kindly provided by Dr. A. Buloichik (Institute of Cytology and Genetics, National Academy of Sciences, Belarus).

At use of detached leaf technique leaf segments (0.8 – 1 cm) of seedlings (1 leaf stage, usually 14 days after sowing) were placed in cuvettes on cotton-wool wetted with water and sprayed by hand atomizer with water suspension on the pathogen spores. After inoculation the cuvettes were wrapped into polyethylene sheets, covered with glass, kept in darkness for 24 h. and transferred to light room. Concentrations of suspensions (spores/ml) were 3×10^4 for *B. sorokiniana* and 1×10^6 for *S. nodorum.*

At inoculation of intact plants seedlings were sprayed with water suspension of the pathogens spores, cuvettes were wrapped into polyethylene, kept in the darkness for 24 h. and placed in light room; polyethylene from cuvettes inoculated with *P. recondita* was removed. Concentrations of suspensions (spores/ml) were 3×10^4 for *P. recondita*, 5×10^4 for *B. sorokiniana* and 1×10^7 for *S. nodorum.*

Types of reaction were scored 7-10 days after inoculation with *P. recondita* according to scale of Mains and Jackson (1926); samples with types 0, 0;, 1, were classified as highly resistant.

Disease ratings were scored 5-7 days after inoculation with *B. sorokiniana* or *S. nodorum* according to original scale (Tyryshkin et al., 2004) from 0 (no symptoms) to 6 (death of leaf).); samples with ratings 0 - 3 were classified as resistant.

Samples selected as resistant in intact tests were reevaluated for reactions in at least 4 independent experiments.

All entries with high level of juvenile resistance to leaf rust were assessed for resistance at field conditions (North-West region, Leningradskaia oblast, and North Caucasus, Derbent district), and for reaction to inoculation with the pathogen clone virulent to gene to *Lr*41 at detached leaves test (Tyryshkin et al., 2005).

Samples with high level of resistance to septoria nodorum blotch were additionally inoculated with single aggressive isolate MN2 of the causal agent.

To study possible durability of resistance to dark-drown leaf spot blotch 9 continuous subcultivation of artificial *B. sorokiniana* population (mixture of 50 isolates of different origin) on leaves of resistant samples and comparison of initial and resulting aggressiveness of the pathogen were used (Tyryshkin and Voronkova, 1997).

To study resistance to common root rot germinated seeds were sown in sand infested with conidia of *B. sorokiniana* isolate T (10^4 conidia/g). Disease ratings on roots were scored 15 days after according to scale from 0 (absence of symptoms) to 6 (plant death) (Tyryshkin et al., 2004). Due to problem with germination only 395 samples were evaluated for resistance to the disease.

Results

At 7th day after inoculation of intact plants of *Ae. tauschii* samples under study 65 exhibited low types of reaction (0, 0;) and 19 were heterogeneous for reaction (low and high types in one sample). At 10th day after inoculation only 8 samples were classified as resistant (Table) and 10 (kk-82, 111, 113, 426, 467, 1221, 1227, 1235, 3602, 3604) were heterogeneous. After inoculation with clone virulent to gene *Lr*41, on leaf segments of all 8 resistant samples compatible reaction (type 3) was observed.

All 8 samples identified as resistant were severely infected with leaf rust in fields at Dagestan and Leningradskaia oblast. Rust from these samples was combined and used for laboratory inoculation of the same samples and 25 wheat varieties including known as highly susceptible to leaf rust. *Ae. tauschii* was susceptible to this pathogen, wheat genotypes were immune (type of reaction 0).

At low infection pressure of *B. sorokiniana* (3×10^4 conidia/ml) 28 accessions were classified as resistant and 51 as heterogeneous for resistance to dark-brown leaf spot blotch. Only 2 samples (kk- 1804, 3683) were resistant (disease scoring 2) when inoculated in intact state at high infection pressure (5×10^4 conidia/ml) (Table). After 9 cycles of artificial *B. sorokiniana* population on their leaves no increase of the pathogen compatibility was observed: disease scorings after inoculations with initial population and resulting subpopulations were the same (0-1).

After inoculation of detached leaves with *S. nodorum* (1×10^6 spores /ml) 193 samples were weakly (disease rating 0, 1) and 29 moderately infested (2, 3), 85 had heterogeneous reaction. At intact state under higher inoculation pressure (1×10^7 spores /ml) six samples were classified as resistant (Table) and three (kk-1112, 1249, 3604) as heterogeneous. All of them were susceptible at this inoculum concentration to isolate MN2 (disease scorings 5, 6).

All samples under study were susceptible to common root rot.

Discussion

Studying juvenile reaction to leaf rust, in 321 *Ae. tauschii* samples from VIR World Collection Mikhailova and Navruzbekov (1997), Mikhailova (2003) found 75 to be highly resistant (type of reaction 0, 1). In our work from 452 samples 65 were without signs of infection at 7th day after inoculation, but most of them (57) were susceptible or heterogeneous at 10th day. Evidently they possess some genes for resistance expressed as

Table: Characteristics of *Ae. tauschii* Coss.samples from VIR World Collection identified as resistant to one of foliar disease after inoculation of intact seedlings

VIR catalogue No.	Origin	Subspecies, varietas	Disease scores after inoculation with causal agent of		
			leaf rust (type of reaction)	Septoria nodorum blotch	dark-brown leaf spot blotch
79	Russia	typica	0;	6	6
249	Turkmenistan	strangulata	0;	6	6
427	Turkmenistan	meyeri	0;	6	6
428	Turkmenistan	meyeri	0;	6	6
624	Azerbaijan	strangulata	0	6	6
729	Azerbaijan	strangulata	0;	6	6
1959	Iran	strangulata	0;	6	6
3299	Turkey	typica	0;	6	6
3683	Iran	strangulata	4	6	2
1804	Uzbekistan	strangulata	4	2, 3	2
1202	Armenia	strangulata	4	2, 3	6
296	Armenia	strangulata	4	1, 3	6
1954	Iran	typica	4	2	6
285	Azerbaijan	typica	4	1	6
1124	Azerbaijan	strangulata	4	1	6

slow-rusting but they are possibly of little value for gene introgression because of high type of reaction and disease severity at ordinary time of evaluation. All 8 identified resistant samples were resistant as in intact plant test as in test when leaf segments were placed on water (data not shown) so we suppose that this technique can be successfully used to study *Ae. tauschii* resistance to leaf rust. Resistant accessions were susceptible to clone of *P. recondita* virulent in detached leaves test to wheat sample KS90WGRC10 (gene Lr 41); very little frequency of such clones in the pathogen populations (Tyryshkin et al., 2005) permits to suppose presence of the gene in all selected samples and so they are not of interest for gene introgression. Earlier with use of hybridological and molecular analyses it was noted that Lr41 (=Lr39) is common in gene pool of *Ae. tauschii* and present in *Ae. cylindrica* (Singh et al., 2004). Besides Lr41 at least 7 genes for resistance were transferred into wheat genome from *Ae. tauschii* (McIntosh et al., 1998). Genes Lr21, 22a, 32, 42 were not effective against population we used (data not shown) so if samples possessed these genes they were not classified as resistant. Studying of genetic of resistance in components of heterogeneous samples is planned.

Surprisingly all samples identified as possibly possessing gene Lr41 were severely rusted at field conditions. Inoculum from their naturally infected leaves was nonpathogenic on 25 wheat varieties including known as highly susceptible to leaf rust (Leningradka, Vera, Saratovskaja 29, Vera, Luba, Thatcher) and virulent to seedlings of *Ae. tauschi* samples. This evidently indicates to the existence of the causal agent of leaf rust specialized to *Ae. tauschii.* From practical viewpoint, evaluation of resistance to leaf rust at field conditions with the aim to identify sources of resistance for gene introgression to wheat is not advisable: samples classified as susceptible can be affected by this forma specialis.

All 128 *Ae. tauschii* samples studied were highly or moderately resistant to *B. sorokiniana* after inoculation of leaf segments placed on benzimidazole (Tyryshkin et al., 1997). In present study after inoculation of leaf segments placed on water with the same fungi isolate and spore concentration only 28 samples were classified as resistant. It confirmed our data on influence of the chemical to the disease development (Kolesova and Tyryshkin, 2004).

Significant variability for juvenile resistance to spot and septoria nodorum blotches was found in *Ae. tauschii* after inoculation with low concentrations of causal agents spores, but only 2 were resistant to *B. sorokiniana* and 6 to *S. nodorum* after use of high infection pressures. Usefulness of septoria resistant samples for introgression hybridization to transfer juvenile resistance into bread wheat is under question because of their susceptibility to 1 isolate of the pathogen from 6 being in the work.

Two samples kk- 1804, 3683 are of interest for practicial purposes because of high level of juvenile resistance to dark-brown leaf spot blotch that is possibly durable.

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