Resistance in *Aegilops squarrosa* to Wheat Leaf Rust, Wheat Powdery Mildew, Greenbug, and Hessian Fly

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**ABSTRACT**


Sixty accessions of *Aegilops squarrosa* (= *Triticum tauschii* Schmal.) were evaluated for resistance to leaf rust (*Puccinia recondita* f. sp. *triticci*) culture PRTUS6; powdery mildew (*Erysiphe graminis* f. sp. *tritici*) composite cultures ABK, 127, YUMA CC, and Asosan; greenbug (*Schizoptera graminum*) biotype E; and Hessian fly (*Mayetiola destructor*) biotype D. Thirty-two accessions were resistant to the leaf rust pathogen, 31 to the powdery mildew pathogen, and 34 to the greenbug; 24 were homozygous and 16 were segregating for resistance to the Hessian fly. Multiple resistances and widespread and six accessions were resistant to both pathogens and both insects. Resistance varied from immune to moderate reactions. Because *A. squarrosa* is the donor of the D genome in common wheat, this species can be readily exploited in breeding wheats for pest resistance.

Common or bread wheat (*Triticum aestivum* L. emend. Thell.) is an allohexaploid (AABBDD) of recent origin (about 8,000 yr) between a tetraploid wheat *T. turdium* L. (AABB) and *Aegilops squarrosa* L. (= *T. tautschii* [DD]) (14). Presumably, initial hybridization was restricted to a narrow range of genotypes of each species, thus the genetic diversity of today's wheats primarily accumulated during the culture and spread of bread wheat into the Mediterranean region, Africa, India, China, and elsewhere. During this expansion, further introgression may have occurred with *T. turdium* but not with *A. squarrosa* because of the latter's strong cross-incompatibility with bread wheat.

A. *squarrosa* is widely distributed in the vast area east of Caucasus, namely, Iran, Afghanistan, Pakistan, and neighboring regions of Turkey, USSR, and China. In the area of its distribution, this species displays tremendous genetic diversity (14). Evaluation of *A. squarrosa* germ plasm collections has revealed good levels of cold hardiness (16), rust and powdery mildew resistance (12,13, 19,21), and insect resistance (6,7). Wheat germ plasm with resistance to leaf and stem rust (12,21,22), Hessian fly (17), and greenbug (11,17) derived from *A. squarrosa* has been developed. Previously, we have reported on disease and insect resistance in wild *Triticum* and *Aegilops* species (5,6). Hatchett and Gill (8,9) reported on Hessian fly resistance in *A. squarrosa*. The objective of the present study was to evaluate 60 accessions of *A. squarrosa* for resistance to leaf rust, powdery mildew, greenbug, and Hessian fly.

**MATERIALS AND METHODS**

The Kansas State University collection of *A. squarrosa* was assembled with donations from J. G. Waines, University of California, Riverside; S. Sakamoto, Kyoto Germplasm Institute, Japan; R. Metzger, Oregon State University; and other miscellaneous sources. Among the 60 accessions evaluated, 35 came from the USSR, 10 from Iran, and one each from Turkey and Afghanistan; 13 were of unknown origin. Plants from 10–20 seeds were used in evaluating each accession for reactions to each pathogen and insect.

Seedlings were tested for reactions to *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* culture PRTUS6 with theuredospore-oil suspension inoculation method and the plant-growing method described by Browder (1). Culture PRTUS6 was selected because it was virulent to lines with several of the known *Lr* genes and many commercial cultivars grown in Kansas. PRTUS6 can be described with the avirulence/virulence formula *Lr* 2a, 9, 16, 18, 19, 24/1, 2c, 2d, 3a, 10, 17. Infection types were produced under growth conditions of 20 ± 2°C and a 12-hr day at about 2,000 lux. Infection types were observed 10–12 days after inoculation and coded according to the system of Browder and Young (2). A line that had an infection type with a sporulation rating (on a scale of 0–9) of 0 was considered immune, 1–3 was considered resistant, 4–6 was considered moderately resistant, and 7–9 was considered susceptible.

The accessions were evaluated in greenhouse tests for resistance to powdery mildew caused by *Erysiphe graminis* DC. ex Merat f. sp. *tritici* em Marchal by inoculating seedlings in separate plantings with one composite of cultures ABK and 127 and another composite of cultures YUMA CC and Asosan. The virulent/avirulent formulas (17) of the cultures are as follows: 

- **ABK** = *Pm* 1, 2, 6, 7/3a, 3b, 3c, 4, 5, 8, Ma, Amigo; 127 = *Pm* 3b, 3c, 5/1, 2a, 3a, 4, 6, 7, 8, Ma, Amigo; YUMA CC = *Pm* 2, 3a, 3b, 3c, 4, 5, 6, 7, Ma/1, 8, Amigo; and Asosan = *Pm* 2, 3a, 3c, 4, 5, 6, 7, 8, Ma, Amigo/1, 3b. The two composites possessed most of the virulence genes found in the United States and were maintained on susceptible plants. For inoculation, seedlings were dusted with spores and maintained at 16–19°C with light for 12 hr/day. Reactions to infection were read 7–9 days after inoculation on a scale of 0–9, where 0 = immune, no visible signs of infection; 1–3 = highly resistant, increasing from no necrosis to large necrotic areas, and/or increasing from no mycelium to little mycelium; 4–6 = moderately resistant, necrotic areas changing to chlorotic areas, and/or increasing in amounts of mycelium and conidiospore production; and 7–9 = susceptible, decreasing from chlorotic.

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areas to no necrosis, and/or increasing in amount of mycelium and conidiospore production to complete susceptibility (18,23). For each accession, the disease rating was based on the highest infection type score.

For the greenbug (Schizaphis graminum Rond.) resistance test, 10 biotypes of B. graminum, were placed on each of 10–20 plants at the two- to three-leaf stage. The plants were enclosed in plastic cages in a greenhouse maintained at about 22°C. Resistance was determined 8–10 days later. Susceptible plants began to show generalized chlorosis after 5–7 days and were easily distinguished from the dark green resistant plants. Plants with intermediate resistance showed delayed chlorosis (3–5 days). Resistance involves tolerance as well as antibiosis and/or nonpreference (7).

Accessions were evaluated in a greenhouse for resistance to biotype D of Hessian fly (Mayetiola destructor Say). Biotype D larvae infest wheat carrying H1, H2, H3, h4, H6, H7, and H8 genes but not wheat with genes H5, H9, H10, H11, H12, or H13 (8). Greenhouse temperature was maintained at about 20°C throughout the test. Each accession was seeded in a row in a standard greenhouse flat containing soil (10 rows per flat). Methods of infestation and of determining resistance or susceptibility of individual seedlings were similar to those described by Cartwright and LaHue (3). Adult Hessian flies were allowed to oviposit for 2 days on seedlings in the one-leaf stage. Plants were then examined for eggs and found to be infested with 10–15 eggs per plant. Plant reaction was determined about 15 days after infestation. Accessions were scored as resistant, susceptible, or segregating. Susceptible plants were stunted, dark green, and showed live larvae. Resistant plants were not stunted, were yellowish green, and showed a high level of antibiosis in that all larvae died in the first instar.

RESULTS

Among 60 accessions of A. squarrosa evaluated, more than 50% were intermediate to highly resistant in separate tests against the pathogens of leaf rust or powdery mildew or the insects greenbug or Hessian fly (Table 1, Fig. 1). Nineteen accessions were immune to leaf rust culture PRTUS6, whereas 11 were resistant, two were moderately resistant, and 28 were susceptible. Even though powdery mildew composite ABK and 127 has fewer virulence genes than does the composite of YUMA CC and Asosan,

Table 1. Disease and insect resistance in Aegilops squarrosa accessions.

<table>
<thead>
<tr>
<th>Accession informationa</th>
<th>Country of originb</th>
<th>Leaf rustc</th>
<th>Powdery mildewc</th>
<th>Hessian flyd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1654 (G445 [PI 17029])</td>
<td>Turkey</td>
<td>78X</td>
<td>8 8 8 R H</td>
<td></td>
</tr>
<tr>
<td>1654 (G448 [AE236/75])</td>
<td>USSR</td>
<td>88X</td>
<td>8 8 8 S S</td>
<td></td>
</tr>
<tr>
<td>1654 (G432)</td>
<td>Iran</td>
<td>88X</td>
<td>8 8 8 S S</td>
<td></td>
</tr>
<tr>
<td>1654 (G428 [KU2120-1])</td>
<td>Iran</td>
<td>88X</td>
<td>8 8 8 S S</td>
<td></td>
</tr>
<tr>
<td>1654 (G429 [AE2/73])</td>
<td>Iran</td>
<td>88X</td>
<td>8 8 8 S S</td>
<td></td>
</tr>
<tr>
<td>1654 (G429 [KU200-9])</td>
<td>Iran</td>
<td>88X</td>
<td>8 8 8 S S</td>
<td></td>
</tr>
<tr>
<td>1654 (G430 [KU200-10])</td>
<td>Iran</td>
<td>88X</td>
<td>8 8 8 S S</td>
<td></td>
</tr>
</tbody>
</table>

*Kansas State University accession number followed by previous accession numbers; the last number, in most cases, pertains to the original accession number of the collector. G numbers = University of California, Riverstide, collectors/curators B. L. Johnson and J. G. Waines; KU = Kyoto University, Japan, collectors Kihara et al (14); AE = Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, East Germany, collector/curator K. Lehmann; Jenkins = origin traced to B. C. Jenkins; and P = origin traced to E. R. Sears, University of Missouri, Columbia. Accessions 1642, 1643, 1706, 1715 were collected by D. Zohary, Hebrew University, Jerusalem, Israel, but his original accession numbers were not known.

**y = Origin not traceable.

†Scorings based on those of Brower and Young (2). The first code portrays relative sporeulation on a scale of 0–9 and the second code shows relative lesion size on a scale of 0–9, the third (alphanumeric) code describes infection type: X = indefinite, C = chlorotic, P = pale, and N = necrosis.

‡Scoring based on relative sporulation on a scale of 0–9: the first score against composite cultures ABK, 127; the second against composite cultures YUMA CC, Asosan.

R = resistant, S = susceptible, and 1 = intermediate.

S = susceptible, R = resistant, and H = segregating R and S.

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Fig. 1. Frequency of resistance (moderate or intermediate to immune) and susceptibility among 60 accessions of Aegilops squarrosa to pathogens causing leaf rust and powdery mildew and to the greenbug and Hessian fly. For Hessian fly, segregating accessions were considered 50% resistant and 50% susceptible.
there was not much difference in the reactions of the accessions to the two composites. Four accessions showed immune reaction, seven were highly resistant, 20 accessions were moderately resistant to both composites, and 29 accessions were susceptible. The latter included seven accessions that gave a rating of 6 to one composite and a rating of 7 or 8 to the second composite. Twenty-one accessions gave a highly resistant reaction to greenbug biotype E infestations, 13 were moderately (intermediate) resistant, and 26 were susceptible. In reaction to Hessian fly biotype D, 24 accessions were resistant, 16 were segregating for resistance, and 20 were susceptible (Table 1).

Multiple resistance to the two pathogens and the two insects was widespread as determined in separate tests on plants of a single accession. Six accessions, including four from Iran (1647, 1706, 1715, and 2452) and one each from the USSR (1664) and an unknown country (1649), were resistant to leaf rust, powdery mildew, greenbug, and Hessian fly (Tables 1 and 2). Nineteen accessions were resistant to three of the diseases and insects; nine of these accessions were resistant to leaf rust, greenbug, and Hessian fly. Another 19 accessions had resistance to two diseases and insects, and 13 accessions were resistant to one disease or one insect. Only three accessions were susceptible to leaf rust, powdery mildew, greenbug, and Hessian fly.

**DISCUSSION**

Wild relatives of wheat comprise two groups: one includes immediate progenitors of the cultivated wheats; the second includes more distant relatives not directly involved in the evolution of wheat. The gene pool within the former group is more readily available for use in wheat breeding. The progenitor group of wild species includes tetraploid wheats (*T. dicoccoides* Kärn. [AABB] and *T. araraticum* Jakubz. [AAAG]); diploid wheats (*T. boeoticum* Boiss. [AA] and *T. urartu* Tum. [AA]); and goatgrass (*A. squarrosa* [DD]). Our previous report on multiple disease and insect resistance in wild wheats indicated little resistance to leaf rust, greenbug, and Hessian fly in *T. dicoccoides* and to greenbug and Hessian fly in *T. urartu* and *T. boeoticum* (5). *T. araraticum* showed a high frequency of resistance to leaf rust and Hessian fly but was susceptible to greenbug. As documented here, *A. squarrosa* is the only progenitor species that has shown a high frequency of multiple resistance to the two diseases and the two insects. This and the previous reports demonstrate rich genetic diversity in *A. squarrosa*; thus this species should be considered the most important source among the progenitor species for the improvement of common wheat.

Although the evaluation showed a high frequency of disease and insect resistance, it did not reveal whether resistant accessions carry the same or different gene(s) for resistance to a specific pest. However, this question can be resolved by genetic analysis in the host plant. Preliminary work in Hessian fly showed that resistance genes in accessions 1642, 1644, 1645, and 1647 conditioning resistance to Hessian fly biotype D were different from the *H13* gene present in *A. squarrosa* accession KU2076 (9). Such genetic analysis on other resistant accessions is in progress.

For *A. squarrosa* to be a useful source for disease and insect resistance, information is needed on the genetic transfer and expression of *A. squarrosa* resistance genes in common wheat. In one method of genetic transfer, *A. squarrosa* is hybridized with a tetraploid wheat. The F₁ hybrids are generally self-fertile and some produce a synthetic hexaploid common wheat (13, 14). In the second method, *A. squarrosa* is hybridized directly with common wheat and the F₁ hybrids obtained by embryo rescue technique are backcrossed with common wheat (21). Whereas genetic transfer is usually successful with either method, the expression of the transferred gene is not always predictable and depends on the specific pathogen or insect resistance factor. For example, it has been shown that the expression of rust and powdery mildew resistance genes may be altered or suppressed in some genetic backgrounds (12, 14, 21). However, common wheat cultivars with specific suppressor genes and those that lack them have been identified (4). This suggests that cultivars lacking suppressor genes may eventually be used to overcome problems associated with expressivity of transferred genes. Fortunately, *A. squarrosa* genes that condition greenbug and Hessian fly resistance have been fully expressed in common wheat in all cases (7, 10, 11).

Because of the richness of genetic diversity in *A. squarrosa* for disease and insect resistance, it is mandatory that conservation and utilization of this genetic resource receive the highest priority. As previously noted, we have consolidated much of the existing world collections, and the Kansas State University germ plasm collection now contains 353 accessions of *A. squarrosa*. Research is under way to complete the evaluation of the collection for disease and insect resistance and other useful traits. We are developing genetic stocks for rapid and efficient transfer of genes from *A. squarrosa* into wheat. New genes are being identified and characterized by genetic analysis and chromosome mapping. The library of new genes will be valuable in meeting the long-term needs of wheat breeding.

**LITERATURE CITED**

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