Evaluation of Israeli *Aegilops* and *Agropyron* Species for Resistance to Wheat Leaf Rust

J. MANISTERSKI, A. SEGAL (deceased), Institute of Cereal Crops Improvement, Tel Aviv University, Tel Aviv, Israel, and A. A. LEVY and M. FELDMAN, Plant Genetics, The Weizmann Institute of Science, Rehovot, Israel

ABSTRACT

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Leaf rust reaction was tested in 274 accessions from 10 *Aegilops* and 2 *Agropyron* species collected from diverse ecogeographical regions in Israel. A high frequency of hypersensitive type resistance was found. Most accessions of *A. speltoides* and *A. variabilis* were resistant, most accessions of *A. bicornis* were susceptible, while other species had different proportions of resistant and susceptible types. Usually, adult plants were more resistant than seedlings at the single-leaf stage. In some species, correlations were found between resistance and ecogeographical parameters such as latitude, altitude, and annual rainfall.

Leaf rust, caused by *Puccinia recondita* Rob. ex Desm. f. sp. *tritici*, is one of the most widespread diseases of wheat in many parts of the world (14). Whereas sources for leaf rust resistance in cultivated wheat are quite limited (10), genes for resistance may be found among the wild relatives of cultivated wheat that have evolved with their parasites and have grown alongside cultivated wheats during the last several thousand years. While the wild progenitor of cultivated wheat, *Triticum turgidum* var. *dicoccoides*, that grows in many parts of Israel, does not possess much of the hypersensitive type of resistance (11,22; Segal, Manisterski, and Wahl, *unpublished*), such resistance was reported in other wild relatives of wheat, e.g., *Aegilops* and *Agropyron* species (7,8,12). Cytogenetic techniques for transferring genes with a qualitative effect from alien species into cultivated wheats have been developed (3–6,9,17). Using these techniques, several genes for leaf rust resistance were transferred into common wheat from *Aegilops* and *Agropyron* species: *Lr*9 from *A. umbellulata* Zhuk. (15), *Lr*19 from *Agropyron elongatum* (Host) P. Beauv. (1,16,19), *Lr*24 from *Agropyron elongatum* (16,20), *Lr*29 from *Agropyron elongatum* (16), and an unidentified *Lr* gene from *A. speltoides* Tausch (2).

In an attempt to identify new sources for resistance to wheat leaf rust, we evaluated a wide array of accessions of wheat relatives that are native to Israel. In addition, disease susceptibility was

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Table 1. Ecogeographical parameters at the sites of collection of *Aegilops* and *Agropyron* species studied[a]

Species	Genome	Ploidy level	Longitude Min.	Longitude Max.	Latitude Min.	Latitude Max.	Altitude (m) Min.	Altitude (m) Max.	Annual rainfall (mm) Min.	Annual rainfall (mm) Max
A. speltoides	S	2×	1,125	1,655	1,195	2,555	15	200	434	619
A. longissima	S^l	2×	1,040	1,750	480	2,755	20	850	95	614
A. sharonensis	S^l	2×	1,205	1,590	1,375	2,540	10	60	530	595
A. searsii	S^s	2×	1,395	2,150	815	2,455	300	850	303	632
A. bicornis	S^b	2×	925	1,555	385	750	20	370	95	233
A. variabilis										
ssp. *euvariabilis*	US^v	4×	1,720	2,250	620	2,955	30	1,200	135	1,042
ssp. *cylindrostachys*	US^v	4×	1,380	2,115	615	2,930	15	900	449	697
A. kotschyi	US^v	4×	1,200	1,790	10	1,515	250	850	78	470
A. ovata	UM^o	4×	1,430	2,245	940	3,015	100	1,800	383	1,373
A. biuncialis	UM^b	4×	1,590	2,250	1,060	3,015	600	1,800	618	1,373
A. triuncialis	UC	4×	2,215	2,250	980	3,015	980	1,600	822	1,373
Agropyron elongatum	E	2×	1,320	1,545	250	2,455	15	450	116	548
Agropyron junceum	J_1J_2E	6×	1,160	1,580	1,355	2,660	2	5	482	552

[a]The longitude and latitude are relative values that correspond to the Israeli net of coordinates (Fig. 1).

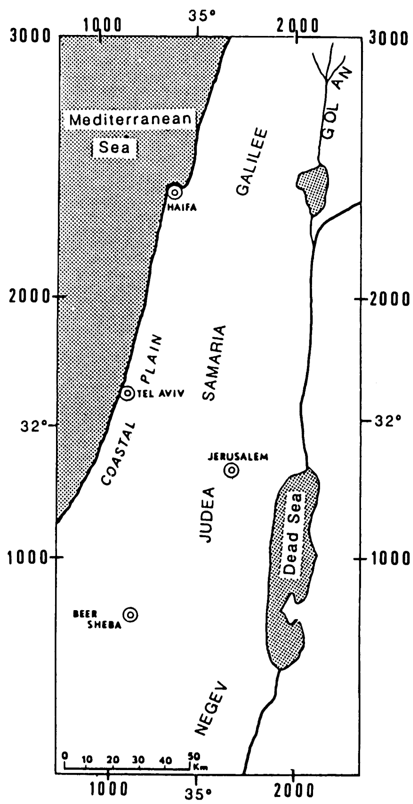

Fig. 1. A map of central and northern Israel indicating the Israeli net of coordinates.

correlated with several environmental factors.

MATERIALS AND METHODS

Two hundred seventy-four accessions from 10 *Aegilops* and 2 *Agropyron* species were collected in Israel from various locations with diverse ecological conditions (Table 1, Fig. 1). These accessions belong to the following species: *A. speltoides*, *A. longissima* Schweinf. & Muschl., *A. sharonensis*, *A. searsii* M. Feldman & M. Kislev, *A. bicornis* (Forsskal) Jaub. & Spach, *A. variabilis* subsp. *euvariabilis* and subsp. *cylindrostachys*, *A. kotschyi*, *A. ovata*, *A. biuncialis* Vis., *A. triuncialis*, *Agropyron elongatum*, and *Agropyron junceum*. Details concerning the genomic formula and ploidy level of the different species (based on Feldman and Sears [6]), as well as the ranges of longitude, latitude, altitude, and annual rainfall in the habitats of these accessions are given in Table 1. Spikelets of all accessions were collected in the wild and might have been derived from a single or from several plants. One accession from each collection site was tested for leaf rust reaction. The number of accessions of each species is given in Table 2. All accessions are kept at the Weizmann Institute of Science.

A naturally occurring isolate from a single pustule of *P. recondita* f. sp. *tritici*, collected in 1980 from *T. t.* var. *dicoccoides* grown in a nursery at Bet-Dagan experimental station, was used. The culture was virulent on nearly isogenic wheat lines (the cultivar Thatcher being the recurrent parent) carrying the following resistance genes: *Lr*1, *Lr*2b, *Lr*2c, *Lr*3a, *Lr*3c, *Lr*10, *Lr*12, *Lr*13, *Lr*14a, *Lr*14b, *Lr*16, *Lr*17, *Lr*18, *Lr*20, *Lr*22a, *Lr*22b, *Lr*23, *Lr*27, *Lr*30, and *Lr*B. It was avirulent on lines carrying: *Lr*2a, *Lr*3ka, *Lr*9, *Lr*11, *Lr*15, *Lr*19, *Lr*21, *Lr*24, *Lr*25, *Lr*26, *Lr*28, and *Lr*29. The isogenic lines were obtained from David Long, Cereal Rust Laboratory, ARS, St. Paul, MN. The culture was increased on seedlings of the susceptible Israeli wheat cultivar Seaon, and was used either immediately after collection or kept for several days at 4 C before use.

To obtain uniform germination, seeds were removed from spikelets, placed in petri dishes on filter paper moistened with distilled water, and kept at 4 C for 2–4 days. Following germination, seedlings were transferred to room temperature for an additional 2 days, and then planted in 10-cm-diameter plastic pots, containing a 1:1 vermiculite:soil mixture. Eight seedlings in two pots were used for each accession. Plants were inoculated 6 days later when the first leaf was fully developed.

Twenty milligrams of urediospores were mixed in 5 g of talcum for a group of 20 pots. Inoculation was carried out in a settling tower, designed by A. Segal (18), that rotates the plants in a cloud of spores-talcum mixture.

Following inoculation, plants were incubated for 16 hr in a dew deposition chamber (13) and kept at 100% relative humidity at 18 C. Plants were then transferred to a Karl Weiss model 5.5 E/I U-PK growth chamber maintained at 18 C and 70% relative humidity under a photoperiod of 12 hr (10,000 lx).

Twelve and 14 days after inoculation, seedlings were evaluated for infection type on a scale of 0–4, according to Stakman and Harrar (21), where 0–2 = resistant, and 3–4 = susceptible. Accessions that consisted of both sensitive and resistant plants were scored as having a mixed reaction.

After evaluation of infection type, seedlings were transferred to 15-cm-diameter pots containing a mixture of soil:compost:vermiculite (1:1:1) and kept in a greenhouse at 20 ± 2 C until maturity. Plants were sufficiently fertilized and inoculated at the heading stage. Disease evaluation was as described above. Some accessions failed to grow to maturity and many others did not flower (because of cold requirement) and could not be tested at the adult stage.

For the field test, the different accessions were sown in a field nursery at Bet-Dagan Experimental Station in 1 m rows (about 8–10 plants per row), surrounded by spreader rows of the susceptible cultivar Seaon. A water suspension of urediospores was injected into tillers of Seaon in the spreader rows when plants were at the stage of stem extension. Disease evaluation, done when tested accessions reached "milk" stage, was on a whole row of 8–10 plants per accession, as previously described. Any accession that exhibited both types of reaction was defined as having a mixed reaction.

A quantitative rank was given to the reaction of each tested seedling, ranging from 0 (resistant) to 4 (susceptible). A correlation was run between the mean reaction of each accession and the ecogeographical parameters of its habitat (Table 3). To obtain a meaningful correlation, only species represented by at least 20 accessions were included.

RESULTS AND DISCUSSION

All species tested had resistant accessions, both as seedlings and adult plants in the greenhouse and field trials, with the exception of *A. bicornis* at the adult stage. Obviously, these species comprise a rich source of resistant genes. The different species exhibited different proportions of resistant and susceptible accessions. Most accessions of *A. speltoides* and *A. variabilis*, as well as *Agropyron elongatum*, showed mostly hypersensitive resistance, whereas those of *A. bicornis* were susceptible. The remaining species had similar proportions of susceptible and resistant types. Comparing the reaction of the same plants as seedlings and adults (Table 2) revealed several cases in which plants exhibited a higher resistance at the adult stage. This was particularly noticeable in *A. sharonensis*, *A. variabilis*, and *Agropyron junceum*. A higher resistance at the adult stage was also apparent in the field trials (Table 4). The higher resistance of adult plants may be due to the following: 1) under growth chamber conditions, the level of inoculation at which seedlings are infected is usually high; 2) in the field, some plants might escape the disease because of different rates of growth, i.e., "late" types might encounter the pathogen at high temperatures that are unfavorable for disease development; and 3) plants might have 'adult plant resistance'.

The species that contained a large number of resistant accessions, e.g., *A. speltoides* and *A. variabilis*, normally grow on heavy soils in areas with relatively higher humidity and are late in their development. The pathogen is quite prevalent in these habitats. In contrast, *A. bicornis* (most accessions susceptible) is an early type that grows on light soils in the southern parts of the country where the presence of the pathogen is limited. Other such species, like *A. kotschyi* and several accessions of *A. longissima*, grow in the Negev—a relatively dry region where the presence of the pathogen is limited. *Aegilops searsii*, which also

Table 2. Reaction of various Israeli accessions of *Aegilops* and *Agropyron* species, tested both at seedling and adult stages, to *Puccinia recondita* f. sp. *tritici*

Species	No. of accessions	Seedlings[a] R	Seedlings[a] S	Seedlings[a] M	Adults[a] R	Adults[a] S	Adults[a] M
A. speltoides	8	6	1	1	7	0	1
	2	2	0	0	...	...	...
A. longissima	2	1	1	0	2	0	0
	17	8	8	1	...	...	...
	2	...	...	...	2	0	0
A. sharonensis	7	5	2	0	7	0	0
	4	0	2	2	...	...	...
A. searsii	5	0	4	1	2	2	1
	17	1	13	3	...	...	...
A. bicornis	4	0	3	1	0	4	0
	8	2	6	0	...	...	...
A. variabilis ssp. *euvariabilis*	47	35	4	8	43	1	3
	24	21	1	2	...	...	...
A. variabilis ssp. *cylindrostachys*	2	1	0	1	2	0	0
	5	5	0	0	...	...	...
A. kotschyi	21	3	10	8	10	10	1
	9	5	2	2	...	...	...
A. ovata	22	5	7	10	13	7	2
	13	9	4	0	...	...	...
A. biuncialis	1	1	0	0	1	0	0
	21	12	6	3	...	...	...
A. triuncialis	3	3	0	0	3	0	0
	15	9	1	5	...	...	...
Agropyron elongatum	1	1	0	0	1	0	0
	4	3	0	1	...	...	...
Agropyron junceum	1	0	0	1	1	0	0
	9	3	1	5	...	...	...

[a] R = resistant, S = susceptible, M = mixed reaction (accessions consisting of both resistant and susceptible plants).

Table 3. Correlations between wheat leaf rust susceptibility at the seedling stage and ecogeographical parameters in several Israeli *Aegilops* species

Species	No. of accessions	Correlation coefficients of susceptibility: Longitude	Latitude	Altitude	Annual rainfall
A. longissima	20	0.284	−0.416*[a]	0.480*	−0.534*
A. searsii	23	0.012	0.132	−0.232	0.094
A. variabilis ssp. *euvariabilis*	72	−0.178	−0.363**	−0.198	−0.336**
A. kotschyi	31	−0.104	−0.241	0.135	−0.121
A. ovata	35	0.303	0.352**	0.173	0.381**
A. biuncialis	22	−0.262	−0.285	0.133	0.072

[a] * = Significant at the 0.05 level, ** = significant at the 0.01 level.

Table 4. Field reaction of Israeli *Aegilops* or *Agropyron* species to *Puccinia recondita* f. sp. *tritici*

Species	No. of accessions	Resistant	Susceptible	Mixed reaction[a]
A. speltoides	9	9	0	0
A. longissima	18	7	7	4
A. sharonensis	7	6	0	1
A. searsii	23	19	2	2
A. bicornis	7	0	7	0
A. variabilis ssp. euvariabilis	59	56	1	2
A. variabilis ssp. *cylindrostachys*	1	1	0	0
A. kotschyi	27	17	7	3
A. ovata	28	28	0	0
A. biuncialis	14	12	1	1
A. triuncialis	...	...	...	...
Agropyron elongatum	1	1	0	0
Agropyron junceum	9	9	0	0

[a] Accessions consisting of both resistant and susceptible plants.

contains many susceptible accessions, grows in mountainous areas and is relatively late. In this respect, it resembles the wild emmer (*T. t.* var. *dicoccoides*), with which it grows sympatrically in many habitats. These two species presumably developed a different mode of protection than those exhibiting hypersensitive resistance, i.e., a slow-rusting type of resistance as found in some populations of *T. t.* var. *dicoccoides* (Segal, Manisterski, and Wahl, *unpublished*).

In a few cases, plants originating from a single accession contained sensitive and resistant plants (mixed reaction). Such a phenomenon could result from incomplete inoculation, seed mixture, or genetic segregation. It is assumed that inoculation was efficient, as previously demonstrated (13,18). However, since seeds of a given accession were often collected from several plants, it seems most probable that different plants of one accession represent different genotypes for this trait. Yet, in very few cases (less than 5%) we found that plants originating from a single spike of *A. longissima* segregated into resistant and sensitive types (*unpublished data*).

Those *Aegilops* and *Agropyron* species, particularly those that possess accessions containing both resistant and susceptible plants, should be studied on the population level in order to estimate the intrapopulation vs. the interpopulation variation. Such a study may indicate if the intraspecific variation can be explained on the basis of ecological factors.

Gill et al (7) suggested that most of the resistance in the genus *Aegilops* was in species with the S genome. Our results show that sources for resistance occur not only in this genome but also in the other genomes tested, i.e., U, M, and C.

Significant correlations were found between susceptibility and certain ecologic parameters (Table 3). In *A. longissima*, susceptibility was correlated with annual rainfall and altitude. In *A. longissima* and *A. variabilis*, susceptibility was negatively correlated with latitude. A similar trend was found for *A. biuncialis* and *A. kotschyi*. It is suggested that in the northern, and thus cooler and more humid habitats, species accumulated more resistance because of the stronger pressure of the pathogen. An exception was *A. ovata*, whose susceptibility was positively correlated with latitude. This indicates that different species may respond in a different way to similar selection pressures by developing different levels of resistance.

Finding the correlation between resistance and ecogeographical parameters might help in defining regions where resistant species have evolved, thus facilitating the strategy for the collection of suitable germ plasm.

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