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A walk on the wild side: mining wild wheat and barley collections for rust resistance genes

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Abstract. Leaf rust, stem rust, and stripe rust are among the most important diseases of wheat and barley worldwide and are best controlled using genetic resistance. To increase the diversity of rust resistance in wheat and barley, a project was initiated to identify and characterise rust resistance genes from the wild species of *Aegilops sharonensis* (Sharon goatgrass) and *Hordeum vulgare* ssp. *spontaneum* (wild barley), respectively. One hundred and two accessions of Sharon goatgrass from Israel and 318 Wild Barley Diversity Collection (WBDC) accessions from the Fertile Crescent, Central Asia, North Africa, and the Caucasus region were evaluated for resistance to leaf rust, stem rust, and/or stripe rust. Sharon goatgrass exhibited a wide range of infection types (ITs) in response to leaf rust, stem rust, and stripe rust. The percentage of resistant accessions in Sharon goatgrass was 58.8–78.4% for leaf rust, 11.8–69.6% for stem rust, and 46.1% for stripe rust, depending on the race used and the plant growth stage. Genetic studies with Sharon goatgrass revealed oligogenic resistance to leaf rust and stem rust. Wild barley also exhibited a wide range of ITs to leaf rust and stem rust; however, the overall frequency of resistance was lower than for Sharon goatgrass. The percentage of resistant accessions in wild barley was 25.8% for leaf rust and 5.7–20.1% for stem rust, depending on the race used. Resistance to the new virulent stem rust race TTKS (i.e. Ug99), present in eastern Africa, was found in both Sharon goatgrass (70% of accessions) and wild barley (25% of 20 accessions tested). Association mapping for stem rust resistance was applied in the WBDC using Diversity Arrays Technology (DArT) markers. Using the highly conservative *P* value threshold of 0.001, 14 and 15 significant marker associations were detected when the number of subpopulations (K value) was set for 10 and 8, respectively. These significant associations were in 9 and 8 unique chromosome bins, respectively. Two significant marker associations were detected for resistance to the wheat stem rust race MCCF in the same bin as the *rpg4/Rpg5* complex on chromosome 7(5H). The presence of a major stem rust resistance gene in this bin on chromosome 7(5H) was validated in a bi-parental mapping population (WBDC accession Damon × cv. Harrington) constructed with DArT markers. The results from this study indicate that Sharon goatgrass and wild barley are rich sources of rust resistance genes for cultivated wheat and barley improvement, respectively, and that association mapping may be useful for positioning disease resistance genes in wild barley.

Additional keywords: allele mining, linkage disequilibrium, wild species, disease resistance.

Introduction

Leaf rust, stem rust, and stripe rust are among the most important diseases of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) worldwide. Leaf rust (*Puccinia triticina* Eriks.) of wheat is the most common and widely distributed of all the cereal rusts (Kolmer 1996) and accounts for major yield losses every year (Kolmer *et al*. 2004). During the last 20 years in the US, leaf rust has resulted in yield losses of 1–8% (Long and Hughes 2000). Leaf rust (*Puccinia hordei* Otth) of barley is the most common rust disease on the crop and causes sporadic epidemics in localised areas. Yield losses approaching 30% have been reported in some regions (Clifford 1985). Although stem rust (*Puccinia graminis* Pers.: Pers f. sp. *tritici* Eriks. & E. Henn and also f. sp. *secalis* Eriks. & E. Henn) has not been a major problem of wheat and barley in the US for over 4 decades, it is still considered the most destructive cereal rust (Roelfs 1985). Pathologists and breeders must continually be vigilant for virulence changes in the stem rust population. A case in point is TTKS (i.e. isolate Ug99), a new race of *P. g.* f. sp. *tritici*, which was first discovered in Uganda in 1999 (Pretorius *et al*. 2000) and has now spread in east Africa (Wanyera *et al*. 2006). Race TTKS is virulent on many of the wheat and barley cultivars grown throughout the world (Pretorius *et al*. 2000; Jin and Singh 2006; Singh *et al*. 2006; Steffenson and Jin 2006). The introduction of such a race into the US or another major cereal-producing country would have devastating consequences. Stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) is another important rust disease of wheat, particularly in the

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cool and moist regions of the Pacific Northwest. Since 2000, this disease has become increasingly important on wheat in the south-central states and central Great Plains, a region previously considered non-congenial to the pathogen (Chen *et al*. 2002). In 2003, total yield losses to stripe rust in the US were estimated at 86 million bushels (4.8% of wheat production), making it the most important rust disease of wheat over the past few years (Long and Hughes 2000). Stripe rust (*Puccinia striiformis* Westend. f. sp. *hordei* Eriks.) of barley is a relatively new pathogen to the US as it was first found in 1991 in Texas (Brown *et al*. 2001). Since this time, the pathogen has rapidly spread across the western US. Losses approaching 50% were reported in barley due to stripe rust in Mexico (Brown *et al*. 2001).

The cereal rust diseases can be effectively controlled by fungicides (Cook *et al*. 1999; Brown *et al*. 2001). However, the high cost of fungicide treatments and increasing environmental concerns about their use make the deployment of resistant cereal cultivars a more sound control strategy. The use of resistant cultivars to control rust diseases requires the availability of many sources of resistance to counter the continuing evolution of new virulence types in the pathogen populations (Gill *et al*. 1985). Since the time of first domestication, the genetic diversity of cereal crops has been continuously eroded. Thus, it is imperative that their gene pools be expanded by incorporating new resistance genes into breeding programs. In this regard, the wild relatives of wheat and barley offer diverse sources of unique alleles for cultivated cereal improvement (Pasquini 1980). At the University of Minnesota, we initiated a project to identify new rust resistance alleles for wheat and barley from the wild species of *Aegilops sharonensis* Eig (Sharon goatgrass) and *Hordeum vulgare* L. ssp. *spontaneum* (C. Koch) Thell. (wild barley), respectively.

Aegilops is the most closely related genus to *Triticum* (Gill and Friebe 2002) and comprises 23 species that include diploid, tetraploid, and hexaploid genomes (van Slageren 1993). *Aegilops* species such as *Ae. umbellulata*, *Ae. speltoides*, *Ae. squarrosa*, *Ae. longissima*, and *Ae. comosa* are known to be rich sources of resistance to various pathogens and pests (Pasquini 1980; Gill *et al*. 1985; Alam and Gustafson 1988; Manisterski *et al*. 1988; Anikster *et al*. 1992, 2005), and many resistance genes from them have been transferred to wheat. Sharon goatgrass ($2n = 2x = 14$, with a S^{sh} or S^{l} genome formula) is a diploid species that belongs to the section Sitopsis of the genus *Aegilops* (van Slageren 1993). Like all members of the Sitopsis, it belongs to the secondary gene pool of wheat, sharing homoeology with the wheat B genome (Jiang *et al*. 1994; Friebe *et al*. 1996). The species is endemic to the Coastal Plain of Israel and southern Lebanon (Kimber and Feldman 1987; van Slageren 1993; Kutiel 1998; Millet *et al*. 2006), a very limited geographical area that extends for about 200 km from north to south and no more than 15 km east from the Mediterranean Sea. The importance of Sharon goatgrass as a source of leaf rust and stem rust resistance is well documented (Gerechter-Amitai and Loegering 1977; Pasquini 1980; Gill *et al*. 1985; Manisterski *et al*. 1988; Anikster *et al*. 1992, 2005), yet only a few studies have been advanced to introgress this resistance into wheat (Antonov and Marais 1996; Marais *et al*. 2003, 2006). Additional research is warranted to exploit the genetic diversity for rust resistance that is present in Sharon goatgrass.

Hordeum vulgare ssp. *spontaneum* is the progenitor of cultivated barley (von Bothmer *et al*. 2003). Its natural range spans from Greece, Egypt, Libya, Israel, Lebanon, Syria, and Turkey in the west through Iraq, Iran, Afghanistan, Turkmenistan, and western Pakistan in the east. Wild barley grows over a remarkably wide range of ecological habitats. Not surprisingly, it exhibits a tremendous level of genetic diversity for both abiotic and biotic stresses (von Bothmer *et al*. 2003). The long co-evolution of this subspecies with various pathogens in its natural range makes it one of the most important sources of disease resistance genes for cultivated barley (Fetch *et al*. 2003). In particular, wild barley is considered a rich source of resistance genes to leaf rust, stem rust, and stripe rust (Moseman *et al*. 1990; Fetch *et al*. 2003; B. Steffenson and L. Jackson, unpublished data).

To effectively utilise rust resistance genes from these wild species, additional genetic characterisation is required. This is usually done using standard bi-parental crosses and molecular mapping to determine the number and chromosomal location of rust resistance genes (Ecker *et al*. 1990; Yang *et al*. 2003). An alternative means for mapping and tagging rust resistance genes that does not require bi-parental crosses is association mapping. With association mapping (also referred to as linkage disequilibrium [LD] mapping), statistical assessments are made for associations between genotypes (i.e. molecular markers) and phenotypes in reference germplasm sets (Buntjer *et al*. 2005). This technique can be applied to different sets of germplasm (including raw wild accessions of a crop species) without the need for making crosses or screening progeny. The purpose of this paper is to summarise our research on identifying and characterising rust resistance genes from both Sharon goatgrass and wild barley.

Materials and methods

Plant materials

Sharon goatgrass

One hundred and two accessions of Sharon goatgrass were evaluated for rust resistance (Olivera *et al*. 2007). The accessions were from 11 different collection sites in Israel and covered the entire distribution area of the species within the country (Millet *et al*. 2006). Prior to rust evaluation, all accessions were increased in greenhouse and nethouse.

To investigate the genetics of resistance to leaf rust (races THBJ and BBBB) and stem rust (races TTTT and TPMK) in Sharon goatgrass, a cross was made between the resistant accession 1644 (from Ashdod) and susceptible accession 1193 (from Hefzi Bah). Single F_1 seeds were grown to produce segregating F_2 populations (lots #1 and #2) for the rust evaluations. Then, single F_2 plants were grown to obtain $F_{2:3}$ families for confirmation of F_2 results or for the first determination of inheritance for resistance to other rust cultures. F_2 seed from lot #1 was evaluated sequentially to leaf rust race THBJ and then stem rust race TTTT at the seedling stage. At the adult plant stage, these same F_2 plants were inoculated with leaf rust race THBJ on the flag and flag-1 leaves and with stem rust race TTTT on the upper two internodes of the plants. Care was taken to not overspray the respective pathogens onto the other target tissue. The lot #1 F_2 plants generated ample $F_{2:3}$ seed.

These $F_{2:3}$ families were tested for their seedling reaction to leaf rust races THBJ and BBBB and also stem rust race TTTT in 3 separate experiments. F_2 seed from lot #2 was inoculated with stem rust race TPMK at the seedling stage only.

Wild barley

Three hundred and eighteen wild barley accessions were assembled for the Wild Barley Diversity Collection (WBDC) by Dr Jan Valkoun (Barley Curator, ICARDA, Aleppo, Syria) and B. Steffenson. Most accessions (314) were from the ICARDA genebank, but a few (4) were from the wild barley collection at the University of Minnesota. The accessions for the WBDC were selected based on various ecogeographic characters (e.g. longitude/latitude, elevation, high/low temperature, rainfall, soil type) and were from 19 different countries, mostly in the Fertile Crescent (77.4%), but also Central Asia (15.7%), North Africa (3.8%), and the Caucasus region (2.8%) (Table 1). The origin of accession OUH602 is not known. Single plant selections were made from the raw accessions, selfed twice, and then tested for their reaction to leaf and stem rust.

Rust pathogens for evaluating resistance in Sharon goatgrass

Information about the rust pathogens used in the Sharon goatgrass phenotyping tests is summarised in Table 2. The rust races were selected based on their differential virulence pattern and/or importance in agriculture (Olivera *et al*. 2007). Prior to being used for inoculation, all rust races were verified for virulence phenotype, purified, and then increased on a susceptible wheat cultivar. Urediniospores were then stored in glass ampoules at −80°C until need for inoculation.

Table 1. Number and percentage of wild barley accessions in the Wild Barley Diversity Collection listed by country of origin

Country	No. of accessions	Percentage of accessions
Afghanistan	7	2.20
Armenia	1	0.31
Azerbaijan	7	2.20
Cyprus	3	0.94
Egypt	1	0.31
Iran	19	5.97
Iraq	10	3.14
Israel	41	12.89
Jordan	61	19.18
Kazakhstan	3	0.94
Lebanon	19	5.97
Libya	8	2.52
Pakistan	2	0.63
Russia	1	0.31
Syria	73	22.96
Tajikistan	7	2.20
Turkey	23	7.23
Turkmenistan	18	5.66
Uzbekistan	13	4.09
Unknown	1	0.31
Total	318	100

Rust pathogens for evaluating resistance in wild barley

Information about the rust pathogens used in the wild barley phenotyping tests is summarised in Table 2. Race MCJ (isolate ND8702) of *P. hordei* was used for the leaf rust evaluation. It has been one of the most common leaf rust races in the US for some time and possesses a relatively narrow virulence spectrum. Three races (MCCF, QCCJ, and TTKS) of wheat stem rust (*P. g.* f. sp. *tritici*) and 1 isolate (92-MN-90) of rye stem rust (*P. g.* f. sp. *secalis*) were used in the study. These races were selected for their ability to detect various stem rust resistance genes in cultivated barley and also for their importance in agriculture. Race MCCF differentiates cultivars with and without the stem rust resistance *Rpg1* (Steffenson *et al*. 1993; Sun and Steffenson 2005). Race QCCJ is virulent for *Rpg1*, but is useful for differentiating cultivars with and without *rpg4* (Jin *et al*. 1994; Sun and Steffenson 2005). Race TTKS is not only virulent for wheat cultivars with *Sr31*, but also barley cultivars with the widely used gene *Rpg1*. To assess whether resistance to race TTKS exists in wild barley, a subset of 20 accessions exhibiting resistance to races MCCF and QCCJ were tested to this race. Isolate 92-MN-90 of *P. g.* f. sp. *secalis* was selected for this study because it is virulent for *Rpg1*, but is useful for differentiating barley accessions with and without *Rpg5* (formally designated as *RpgQ*) (Sun and Steffenson 2005). Prior to being used for inoculation, the rust races used for barley were verified for virulence phenotype, purified, and then increased on a susceptible wheat or barley cultivar. Urediniospores were then stored in glass ampoules at −80°C until need for inoculation.

Plant growth conditions, inoculation protocols and disease assessment for Sharon goatgrass

Plants were grown according to the methods given in Olivera *et al*. (2007). All accessions were evaluated to leaf rust, stem rust, and stripe rust at the seedling stage. In addition, adult plant tests were conducted with leaf rust race THBJ and stem rust race TTTT. Inoculation and disease assessment protocols were done as described by Olivera *et al*. (2007) with one exception; in the study of the genetics of resistance, F_2 seedlings were inoculated with stem rust race TTTT at 3–4-leaf stage instead of the 2-leaf stage. For the summary of data, infection types (ITs) from 0 to 1 were considered indicative of resistance, 2 of an intermediate reaction, and 3 to 4 of susceptibility. Accessions were classified as heterogeneous if they clearly contained both resistant and susceptible plants, resistant and intermediate plants, or susceptible and intermediate plants. All experiments were done in a completely randomised design with one replicate and were repeated at least once.

Plant growth conditions, inoculation protocols and disease assessment for wild barley

Experiments with all rusts except race TTKS of *P. g.* f. sp. *tritici* were done at the Plant Growth Facility on the St. Paul campus of the University of Minnesota. Three seeds from each WBDC accession were planted in plastic cones filled with a 50 : 50 mixture of soil and Metro Mix 200 (Vermiculite, peat moss, Perlite, and sand) and fertilized with Osmocote 14-14-14 (Scott's Co., Marysville, OH: 1.4 g per cone) and Peters Dark

Table 2. Race, isolate designation, virulence phenotype, and source of rust pathogens used to assess resistance in Sharon goatgrass and wild barley

Pathogen	Race	Isolate	Host evaluated	Virulence/avirulence formula	Source
Puccinia triticina	THBJ	99ND588DLL	Sharon goatgrass	*Lr1*, *2a*, *2c*, *3a*, *16*, *26*, *10*, *14a*, *18/Lr9*, *24*, *3ka*, *11*, *17*, *30*, *B*	J. Kolmer, USDA-ARS, Cereal Disease Lab., St. Paul, MN
P. triticina	BBBB	–[A]	Sharon goatgrass	*Lr*–[B]*/Lr1*, *2a*, *2c*, *3a*, *9*, *16*, *24*, *26*, *3ka*, *11*, *17*, *30*, *B*, *10*, *14a*, *18*	J. Kolmer, USDA-ARS, Cereal Disease Lab., St. Paul, MN
P. hordei	MCJ	ND8702	Wild barley	*Rph1*, *4*, *8*, *10*, *11/Rph2*, *3*, *5*, *6*, *7*, *9*, *12*, *13*, *14*, *15*	B. Steffenson, University of Minnesota, St. Paul, MN
P. graminis f. sp. *tritici*	TTTT	02MN84A-1-2	Sharon goatgrass	*Sr5*, *21*, *9e*, *7b*, *11*, *6*, *8a*, *9 g*, *36*, *9b*, *30*, *17*, *9a*, *9d*, *10*, *Tmp/Sr*–[B]	Y. Jin, USDA-ARS. Cereal Disease Lab., St. Paul, MN
P. graminis f. sp. *tritici*	TPMK	95NE115-A	Sharon goatgrass	*Sr5*, *21*, *9e*, *7b*, *11*, *8a*, *9 g*, *36*, *17*, *9d*, *10*, *Tmp/Sr6*, *9b*, *30*, *9a*	J. Kolmer, USDA-ARS, Cereal Disease Lab., St. Paul, MN
P. graminis f. sp. *tritici*	TTKS	99UGA	Sharon goatgrass and wild barley	*Sr5*, *21*, *9e*, *7b*, *11*, *6*, *8a*, *9 g*, *9b*, *30*, *17*, *9a*, *9d*, *10/Sr36*, *Tmp*	Y. Jin, USDA-ARS, Cereal Disease Lab., St. Paul, MN
P. graminis f. sp. *tritici*	QCCJ	QCC-2	Wild barley	*Sr5*, *21*, *9 g*, *17*, *9d*, *10/Sr9e*, *7b*, *11*, *6*, *8a*, *36*, *9b*, *30*, *9a*, *Tmp*	J. Miller, USDA-ARS, Northern Crop Science Lab., Fargo, ND
P. graminis f. sp. *tritici*	MCCF	A-1	Wild barley	*Sr5*, *7b*, *9 g*, *17*, *10*, *Tmp/Sr21*, *9e*, *11*, *6*, *8a*, *36*, *9b*, *30*, *9a*, *9d*	J. Miller, USDA-ARS, Northern Crop Science Lab., Fargo, ND
P. graminis f. sp. *secalis*	–[A]	92-MN-90	Wild barley	–[A]	A. Roelfs, USDA-ARS, Cereal Disease Lab., St. Paul, MN
P. striiformis f. sp. *tritici*	PST-78	–[A]	Sharon goatgrass	*Yr1*, *3*, *11*, *12*, *16*, *17*, *18*, *19*, *20/Yr2*, *4*, *5*, *6*, *7*, *8*, *9*, *10*, *13*, *14*, *15*	X. Chen, USDA-ARS, Wheat Genetics, Physiology, Quality, and Disease Research Unit, Pullman, WA

[A]Not applicable or not available.
[B]Iindicates no differential in the virulence or avirulence category.

Weather 15-0-15 (Scott's Co., Marysville, OH: 150 g per gallon at 1/16 dilution). Seeds were incubated at 4°C for a week in order to break possible dormancy and then transferred to a growth chamber (20–22°C with 14 h photoperiod provided by 160 W VHO and 60 incandescent lamps). Plants were inoculated with rust when the first leaves were fully expanded, 7 days after being placed in the growth chamber. Inoculation protocols for leaf rust and stem rust were as described by Fetch *et al*. (2003) and Sun and Steffenson (2005), respectively. The only difference in the methodology was that the rust concentration used in the present study was 0.025 mg per plant instead of 0.04 mg (Fetch *et al*. 2003) or 0.035 mg (Sun and Steffenson 2005) per plant.

After the infection period, plants were placed in the controlled environment of the growth chamber. The incubation temperature was 19–21°C for all of the rusts, except race MCCF. For race MCCF, the temperature was set to 22–25°C because this environment is more conducive for the separation of genotypes with and without the gene *Rpg1* (Sun and Steffenson 2005).

Evaluations for resistance to race TTKS were done at the USDA Cereal Disease Laboratory during the winter months. The methods used for this test were as follows. Four seeds from each of the 20 selected WBDC accessions were placed on moist filter paper sealed in Petri dishes at 4°C for 6 days in order to break possible dormancy. The plates were then placed at room temperature for one day. The pre-germinated seeds were then planted in square plastic pots (7 × 7 cm) filled with a 50 : 50 mixture of soil and Metro Mix 200. A water soluble fertiliser (20-20-20, N-P-K) was applied at plant emergence, upon removal from the mist chamber, and again 1 week later. Plants were grown in a greenhouse at 22°C with supplemental lighting provided by 1000 W sodium vapor lamps for 14 h per day. Plants were inoculated with race TTKS when the first leaves were fully expanded, 7 days after being placed in the greenhouse. Inoculation protocols were as described by Sun and Steffenson (2005), except that 0.025 mg of rust was applied per plant instead of 0.035 mg. After the infection period, plants were placed in the controlled environment of the growth chamber set at 20°C with a 14 h photoperiod provided by 160 W VHO fluorescent and 60 W incandescent lamps.

Leaf and stem rust reactions were rated 12–13 days post-inoculation using scales modified from Levine and Cherewick (1952) and Miller and Lambert (1955), respectively. For the summary of data, ITs from 0 to 1 were considered indicative of a resistant host response, 2 of a moderately resistant response, 3–2 of a moderately susceptible response, and 3 to 4 of a susceptible response. All experiments were done in a completely randomised design with 1 replicate and were repeated at least 3 times.

Genotyping wild barley for studies on the feasibility of association mapping of stem rust resistance genes

DNA extraction

DNA was extracted from single-plant selections of each wild barley accession for subsequent genotyping with molecular markers. DNA was extracted from the young tillers of 5-week-old plants using the FASTprep kit (Q-BIOgene, Irvine, CA).

Diversity arrays technology (DArT) markers

Diversity Arrays Technology (DArT) was used to develop DArT markers (Jaccoud *et al*. 2001; Wenzl *et al*. 2004) for the WBDC. Over 6000 clones from 16 plates (each with 384 wells) were used to genotype the WBDC accessions. Of the 16 plates, 4 had wild barley specific clones and 12 had cultivated barley clones (Wenzl *et al*. 2006). The 4 wild barley plates were enriched for wild barley specific clones through suppression subtractive hybridisation using the cultivated barley cv. Harrington as tester. The clones in 2 of 4 wild barley specific plates were constructed from 31 WBDC accessions, selected for maximum genetic diversity based on ten microsatellite markers. This set of 31 included 4 accessions (41-1, Damon, OUH602 and Shechem) used in wild × cultivated barley mapping populations (Baum *et al*. 2003; Yun *et al*. 2005; Alsop *et al*. 2007). The remaining 2 wild barley specific plates were constructed specifically from Damon and Shechem to map multiple disease resistance (Fetch *et al*. 2003) in crosses with susceptible cv. Harrington. The Damon/Harrington and Shechem/Harrington populations are also being used for the validation of association mapping from the WBDC.

Analysis of genotypic data, linkage disequilibrium, and population structure

Scoring of marker data

DARTSOFT, a software package developed by Diversity Arrays Technology Pty Ltd (Canberra, Australia), was used to identify and score DArT markers in a binary format.

Linkage disequilibrium assessment

The squared correlation coefficient (r^2) was used to estimate LD between pairs of DArT marker alleles (Pritchard and Przeworski 2001). LD was estimated using the EMLD program (https://cge.mdanderson.org/~qhuang/Software/pub.htm). Using Microsoft EXCEL software, the estimated r^2 was plotted against the known genetic distance (cM) between markers to determine LD decay in the WBDC.

Population structure analysis

Population structure is an important component in association mapping analyses because it can be source of both type I and type II errors between molecular markers and traits in an autogamous species such as barley (Breseghello and Sorrells 2006; Yu *et al*. 2006). Model-based analyses were performed to study population structure in the WBDC. For these analyses, Bayesian model-based clustering was used to detect population structure using the program STRUCTURE (version 2.0) (Pritchard *et al*. 2000; Falush *et al*. 2003), as described in several previous studies of population-based LD (Thornsberry *et al*. 2001; Rostoks *et al*. 2006). The basis of this clustering method is the allocation of individual genotypes to clusters in such a way that Hardy–Weinberg equilibrium or linkage equilibrium exists within clusters, but is absent between clusters. The Q matrices, an output of this program, were used in association mapping for stem rust resistance genes in wild barley. Several parameters and different ancestry and allelic frequency models were used for estimation of population structure from K = 2 to 20, where K is number of subpopulations assumed (Pritchard *et al*. 2000).

Association mapping for stem rust resistance genes

To explore the feasibility of using association mapping for positioning resistance loci on chromosomes in this suite of wild barley germplasm, we used reaction data from tests with *P. g.* f. sp. *tritici* races MCCF and QCCJ and *P. g.* f. sp. *secalis* isolate 92-MN-90. The GLMM-PQL (Generalized Linear Mixed Model for Penalized Quasi Likelihood) method was used to test associations between binary disease phenotype data (i.e. resistant or susceptible categories) and marker loci distributed throughout the genome (Wolfinger and O'Connell 1993). In this model, the marker data are considered fixed-effect factors and population structure data (Q matrix) as random-effect factors (Kennedy *et al*. 1992). The *R* program was used to fit the model (http://rss.acs.unt.edu/Rdoc/library/MASS/html/glmmPQL.html). Markers exhibiting a *p*-value less than 0.001 for the ODD ratio (>1.0) were considered to have a significant association with stem rust resistance. For association mapping, missing values for DArT markers were replaced using imputation. The imputated data were only used in association mapping, not for LD and population structure estimation.

Results

Sharon goatgrass exhibited a wide range of ITs in response to leaf rust, stem rust, and stripe rust. The highest percentage of resistance identified in the species was to *P. triticina* race THBJ (78.4%) at the adult plant stage (Table 3). At the seedling stage, the frequency of resistance to races THBJ and BBBB was lower at 61.8% and 58.8%, respectively. Most (>73% and >56% for adult and seedling stages, respectively) of the accessions resistant to leaf rust exhibited very low ITs of 0; (data not shown).

The frequency of resistance to stem rust varied markedly depending on the race. The highest percentage found was in response to race TTKS (69.6%) and the lowest to races TPMK (32.4%) and TTTT (11.8%), all at the seedling stage (Table 3). The frequency of resistance to race TTTT was more than 3 times higher at the adult stage (41.8%) than at the seedling stage (11.8%). Of those accessions classified as resistant, 14% and 46% exhibited very low ITs of 0; for race TTTT and race TPMK, respectively (data not shown).

Over 45% of the accessions were resistant to race PST-78 of *P. striiformis* f. sp. *tritici* (Table 3). Of these resistant accessions, 90% were highly resistant giving low ITs of 0; (data not shown).

Accessions with heterogeneous reactions were observed in response to all 3 rusts and ranged from 6.9 to 23.5% for the germplasm collection. Thus, in cases such as with leaf rust race BBBB, the level of heterozygosity for rust resistance genes can be fairly high.

At the seedling stage, F_2 progeny from lot #1 of the 1644/1193 population segregated in an approximate 3 : 1 ratio for resistance : susceptibility to leaf rust race THBJ, indicating that resistance in accession 1644 is controlled by a single dominant gene (Table 4). This result was confirmed in the analysis of the $F_{2:3}$ generation as the number of homozygous resistant, segregating, and homozygous susceptible families approximated a 1 : 2 : 1 ratio. The same lot #1 F_2 plants tested for resistance to race THBJ at the seedling stage were also tested for reaction at the adult plant stage, and the correspondence of reactions

Table 3. Number and percentage of Sharon goatgrass accessions exhibiting resistant, intermediate, susceptible, or heterogeneous reactions to leaf rust, stem rust, and stripe rust of wheat

Pathogen	Resistant	Intermediate	Susceptible	Heterogeneous	Total
Puccinia triticina race THBJ[A]	80 (78.4)	2 (2.0)	9 (8.8)	11 (10.8)	102 (100)
P. triticina race THBJ[B]	63 (61.8)	7 (6.9)	14 (13.7)	18 (17.6)	102 (100)
P. triticina race BBBB[B]	60 (58.8)	2 (2.0)	16 (15.7)	24 (23.5)	102 (100)
P. graminis f. sp. *tritici* race TTTT[A]	41 (41.8)	10 (10.2)	37 (37.8)	10 (10.2)	98 (100)
P. graminis f. sp. *tritici* race TTTT[B]	12 (11.8)	5 (4.9)	72 (70.6)	13 (12.7)	102 (100)
P. graminis f. sp. *tritici* race TTKS[B]	71 (69.6)	12 (11.8)	12 (11.8)	7 (6.9)	102 (100)
P. graminis f. sp. *tritici* race TPMK[B]	33 (32.4)	18 (17.6)	44 (43.1)	7 (6.9)	102 (100)
P. striiformis f. sp. *tritici* race PST-78[B]	47 (46.1)	6 (5.9)	35 (34.3)	14 (13.7)	102 (100)

[A]Adult plant reaction.
[B]Seedling reaction.

Table 4. Segregation for resistance to leaf rust and stem rust in population 1644/1193 of Sharon goatgrass
R, Resistant; S, susceptible; HR, homozygous resistant; Seg, segregating; HS, homozygous susceptible

Pathogen/race	Growth stage evaluated	F_2 plants					$F_{2:3}$ families					
		R	S	Ratio fit	χ^2	Prob> χ^2 value	HR	Seg.	HS	Ratio fit	χ^2	Prob> χ^2 value
					Lot #1							
Puccinia triticina race THBJ	Seedling	88	33	3 : 1	0.33	0.56	33	54	34	1 : 2 : 1	1.41	0.49
P. triticina race THBJ	Adult	92	20	3 : 1	3.05	0.08	–	–	–	–	–	–
P. triticina race BBBB	Seedling	–	–	–	–	–	30	58	34	1 : 2 : 1	0.56	0.76
P. graminis f. sp. *tritici* race TTTT	Seedling	84	42	3 : 1	4.67	0.03	32	56	34	1 : 2 : 1	0.89	0.64
P. graminis f. sp. *tritici* race TTTT	Adult	90	23	3 : 1	1.30	0.25	–	–	–	–	–	–
					Lot #2							
P. graminis f. sp. *tritici* race TPMK	Seedling	114	11	1 : 1	1.39	0.24	–	–	–	–	–	–

between the 2 tests was very close. This suggests that the same dominant gene confers resistance at both the seedling and adult plant stages. Remnant seed of $F_{2:3}$ families from lot #1 were grown and evaluated to race BBBB at the seedling stage. This was done to determine if the single dominant gene conferring resistance to race THBJ was also effective against race BBBB. All families susceptible to race THBJ were also susceptible to race BBBB. The number of resistant and segregating families to the 2 races was in close agreement but varied slightly, probably due to mis-scoring or an insufficient sample size of plants within an $F_{2:3}$ family. Based on the overall results, it appears that the same gene confers resistance to both races of leaf rust. Within segregating families, the ratio of resistant : susceptible plants was approximately 3 : 1 in most cases, thereby confirming that resistance to race BBBB is under the control of a single dominant gene (data not shown).

The F_2 progeny from lot #1 of the 1644/1193 population also were evaluated at both the seedling and adult plant stages to stem rust race TTTT. Segregation at the seedling stage approximated a 3 : 1 ratio for resistance : susceptibility, indicating that stem rust resistance in accession 1644 is controlled by a single dominant gene. This result was confirmed in the $F_{2:3}$ generation with remnant seed of the families. At the adult plant stage, segregation of F_2 progeny to race TTTT conformed to a ratio for a single dominant gene. Moreover, aside from the 13 missing progeny in the adult plant test, the correspondence between the reaction at the seedling stage and adult plant stage was very close. The discrepancies were likely due to miss-scoring or perhaps a greater chance for disease escape with adult plants. This result suggests that the same gene was effective in conferring resistance to race TTTT at both growth stages. Comparison of reactions in the same lot #1 $F_{2:3}$ families revealed no association between leaf rust resistance (i.e. to races THBJ and BBBB) and stem rust resistance (i.e. race TTTT) (data not shown). This indicates that the gene controlling resistance to leaf rust is different from the one controlling resistance to stem rust and that the 2 genes are not linked.

The F_2 seed of lot #2 from the 1644/1193 population was tested for reaction to stem rust race TPMK. The number of resistant : susceptible progeny approximated a 15 : 1 ratio, indicating that resistance in 1644 to race TPMK is controlled by 2 independently segregating dominant genes (Table 4).

In wild barley, a wide range of ITs was observed in response to leaf rust and stem rust. The frequency of resistance to leaf rust was relatively low at 25.8% (Table 5). Of the resistant accessions, 36 (11.3%) were highly resistant giving ITs of 0; (data not shown). These highly resistant accessions were found almost exclusively in the Fertile Crescent, specifically in Israel, Jordan, and Syria. The frequency of resistance to the 2 *P. g.* f. sp. *tritici* races MCCF and QCCJ was very low, ranging from just 5.7 to 10.4%. In contrast, the frequency of resistance to *P. g.* f. sp. *secalis* isolate 92-MN-90 was markedly higher at 20.1%. The number (and percentage) of accessions giving highly resistant ITs of 0; was 4 (1.3%), 9 (2.8%), and 26 (8.2%) for *P. g.* f. sp. *tritici* races MCCF and QCCJ, and *P. g.* f. sp. *secalis* isolate 92-MN-90, respectively (data not shown). These

Table 5. Number (and percentage) of wild barley accessions from the Wild Barley Diversity Collection exhibiting resistant, moderately resistant, moderately susceptible, and susceptible reactions to stem rust and leaf rust of barley

Pathogen	Resistant	Moderately resistant	Moderately susceptible	Susceptible	Total
Puccinia graminis f. sp. *tritici* race MCCF	18 (5.7)	12 (3.8)	7 (2.2)	281 (88.4)	318 (100)
P. graminis f. sp. *tritici* race QCCJ	33 (10.4)	11 (3.5)	14 (4.4)	260 (81.8)	318 (100)
P. graminis f. sp. *secalis* isolate 92-MN-90	64 (20.1)	20 (6.3)	21 (6.6)	213 (67.0)	318 (100)
P. hordei race MCJ	82 (25.8)	9 (2.8)	9 (2.8)	218 (68.6)	318 (100)

highly resistant accessions were from diverse regions across the Fertile Crescent and Central Asia. The highest proportion of highly resistant accessions from the Fertile Crescent was in Israel and from Central Asia in Tajikistan, Turkmenistan, and Uzbekistan. Twenty accessions exhibiting a high level of resistance to races MCCF and QCCJ were subsequently evaluated to race TTKS. Of these 20 accessions, 5 (25%) were resistant (ITs of mostly 21 to 213-), 4 (20%) were intermediate (ITs of 23- to 3-2), 7 (35%) were susceptible (ITs of 3), and 4 (20%) were variable (i.e. plants within the accession showed a range of ITs from 0; to 3 in the 2 experiments). The resistant accessions were from diverse geographic regions that included northern and central Israel, western Tajikistan, and eastern Uzbekistan.

One thousand and ninety DArT markers were genotyped on the WBDC, of which 818 were previously mapped to a consensus barley linkage map (Wenzl *et al*. 2006). These 818 DArT markers were distributed across the entire genome and were used in the estimation of LD because their chromosomal positions were known. LD estimates in wild barley varied greatly across the chromosomes, but in general were very low. For all chromosomes except 4(4H), LD decayed rapidly and was less than 1 cM. With chromosome 4(4H), LD decayed slowly and extended up to 40–50 cM at $r^2 = 0.5$ (see Fig. 1 for examples with 75 and 35 markers for chromosome 5(1H) and 4(4H), respectively).

Using 1088 DArT markers, model-based population structure revealed 10 or 8 subpopulations, depending on whether the minimum individual or mean log-likelihood values were used, respectively. When 10 subpopulations were considered in the analysis, 9 significant marker associations were detected for race MCCF, 4 for race QCCJ, and 1 for isolate 92-MN-90 (Table 6 and Fig. 2). For race MCCF, the significant associations were found in chromosome 2(2H) bin 11, chromosome 3(3H) bin 2, chromosome 5(1H) bin 6, chromosome 6(6H) bin 7, and chromosome 7(5H) bins 1, 2, and 13. Two markers each were found significant for association in bins 1 and 2 on chromosome 7(5H); thus, only 7 of the 9 significant marker associations were in unique regions of the genome. The association found in bin 13 on chromosome 7(5H) also contains the *rpg4/Rpg5* stem rust resistance gene complex (Fig. 2). No associations were found in bin 1 of chromosome 1(7H), the region where the only other mapped stem rust resistance gene (i.e. *Rpg1*) resides. For race QCCJ, significant associations were found in chromosome 2(2H) bin 11 and chromosome 3(3H) bins 5 and 13. Two markers were found significant for bin 11 on chromosome 2(2H); thus, 3 of the 4 significant marker associations were in unique regions. For isolate 92-MN-90, only 1 significant marker association was found and that was in bin 2 of chromosome 3(3H). There were only 2 cases where significant associations were found for more than 1 rust culture: bin 11 of chromosome 2(2H) for MCCF and QCCJ and bin 2 of chromosome 3(3H) for MCC and 92-MN-90.

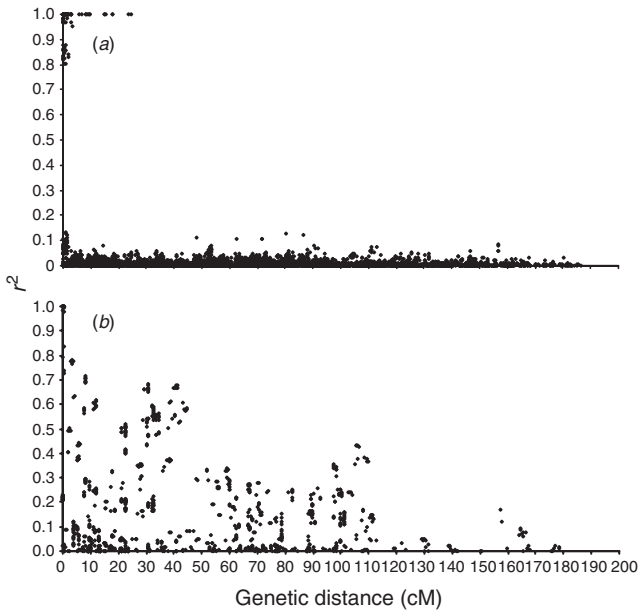

Fig. 1. Linkage disequilibrium (LD) plots for chromosome (*a*) 5(1H) and (*b*) 4(4H) based on DArT markers genotyped on 314 wild barley accessions. LD was estimated using the r^2 value as suggested by Pritchard and Przeworski (2001). LD (r^2) is plotted against genetic distance in pairwise comparison of 75 DArT markers for chromosome 5(1H) and 35 DArT markers for chromosome 4(4H).

When 8 subpopulations were considered in the analysis, 6 significant marker associations were detected for race MCCF, 3 for race QCCJ, and 6 for isolate 92-MN-90 (Table 6 and Fig. 2). For race MCCF, all 6 significant associations were found in the same bins as described for K = 10, namely chromosome 3(3H) bin 2, chromosome 5(1H) bin 6, chromosome 6(6H) bin 7, and chromosome 7(5H) bins 1 (with 2 significant marker associations) and 13. Bin 13 of chromosome 7(5H) is where the *rpg4/Rpg5* stem rust resistance gene complex is located (Fig. 2). For race QCCJ, significant marker associations also were found in the same positions as described for K = 10, namely chromosome 2(2H) bin 11 (with 2 significant marker associations) and chromosome 3(3H) bin 13. For isolate 92-MN-90, 3 significant marker associations each were found

Table 6. Diversity Array Technology (DArT) markers showing significant association ($P < 0.001$) with wheat and rye stem rust resistance in wild barley

Chrom.	Bin no.[A]	Position along chrom. (cM)	DArT marker designation[B]	Markers showing association: K = 10 (A) and 8 (B)[C]		
				MCCF	QCCJ	92-MN-90
2 (2H)	11	101.3	bPb-7991	–	A,B	–
	11	102.4	bPb-3563	–	A,B	–
	11	102.4	bPb-6194	A	–	–
3 (3H)	2	13.7	bPb-6127	A,B	–	B
	2	13.7	bPb-7199	–	–	A,B
	2	13.7	bPb-9402	–	–	B
	5	50.4	bPb-4660	–	A	–
	13	148.3	bPb-6383	–	A,B	–
5 (1H)	6	58.7	bPb-9717	A,B	–	–
6 (6H)	7	77.4	bPb-4783	A,B	–	–
	13	136.1	bPb-2304	–	–	B
	13	137.8	bPb-0403	–	–	B
	13	137.8	bPb-7146	–	–	B
7 (5H)	1	1.7	bPb-0085	A,B	–	–
	1	1.7	bPb-6485	A,B	–	–
	2	18.7	bPb-2460	A	–	–
	2	18.7	bPb-8072	A	–	–
	13	163.6	bPb-5854	A	–	–
	13	163.7	bPb-2314	B	–	–

[A]Kleinhofs and Graner (2001) subdivided each barley chromosome into segments of ~10 cM intervals called bins.
[B]DArT marker nomenclature according to Wenzl *et al*. (2006).
[C]K is the number of subpopulations assumed in population structure analysis using the STRUCTURE program (Pritchard *et al*. 2000).

in bin 2 of chromosome 3(3H) and in bin 13 of chromosome 6(6H). One of the significant marker associations in bin 2 of chromosome 3(3H) also was found in the K = 10 analysis; however, the bin 13 association on chromosome 6(6H) was unique for the K = 8 analysis. In the K = 8 analysis, no marker associations were found in bin 1 of chromosome 1(7H), the region where *Rpg1* resides.

Discussion

The results of this study clearly demonstrate that Sharon goatgrass and wild barley are highly diverse in their response to the major rust pathogens of wheat and barley, respectively. In general, the frequency of leaf and stem rust resistance was much higher in Sharon goatgrass (range 13.1–77.4%, but most over 59%) than in the wild barley (range 5.7–25.8%) germplasm (Tables 3 and 5). Nevertheless, even in wild barley, there are sufficient numbers of resistant accessions to increase the diversity for rust resistance genes in cultivated barley. The level and diversity for rust resistance found in Sharon goatgrass by Olivera *et al*. (2007) as summarised here is in agreement with previous studies on the species in reaction to both leaf and stem rust (Gerechter-Amitai and Loegering 1977; Anikster *et al*. 1992, 2005; Antonov and Marais 1996; Snyman *et al*. 2004). The low percentage of wild barley accessions found resistant to stem rust in this study (Table 5) is in agreement with the study by Fetch *et al*. (2003), who evaluated a collection of 116 wild barley accessions from Israel and Jordan and also Legge *et al*. (1996) and Metcalfe *et al*. (1977) who evaluated accessions from the Middle East. The overall percentage of resistance to leaf rust was only 25.8% in the WBDC, whereas Fetch *et al*. (2003) found 70% and 90% resistance for accessions from Israel and Jordan, respectively. This same trend was found when WDBC data from Israel (51.2%) and Jordan (24.6%) only were considered.

Stem rust race TTKS is a serious threat to wheat and barley production worldwide (Pretorius *et al*. 2000; Singh *et al*. 2006; Steffenson and Jin 2006). Since 1999, this race has the spread from its initial detection site in southern Uganda into Kenya, Ethiopia, and more recently Yemen. Race TTKS is likely to spread quickly into other parts of the Middle East and beyond. To combat this rust threat, immediate action is needed to identify effective resistance and transfer it to wheat and barley. In this study, resistance to race TTKS was found in both Sharon goatgrass and wild barley. Nearly 70% of the Sharon goatgrass accessions tested exhibited resistance to race TTKS (Table 3), and many were highly resistant giving infection types of 0. Only 5 of the 20 wild barley accessions evaluated were resistant to race TTKS, and all of them exhibited moderate resistance. These resistant accessions came from widely separated geographic areas (Israel, Tajikistan, and Uzbekistan) and may therefore contain different resistance genes that may be useful in diversifying resistance to race TTKS in cultivated barley. Work is under way to determine the inheritance of resistance to race TTKS in both Sharon goatgrass and wild barley.

On a global scale, stripe rust has increased significantly on wheat and barley over the past 2 decades, and was therefore

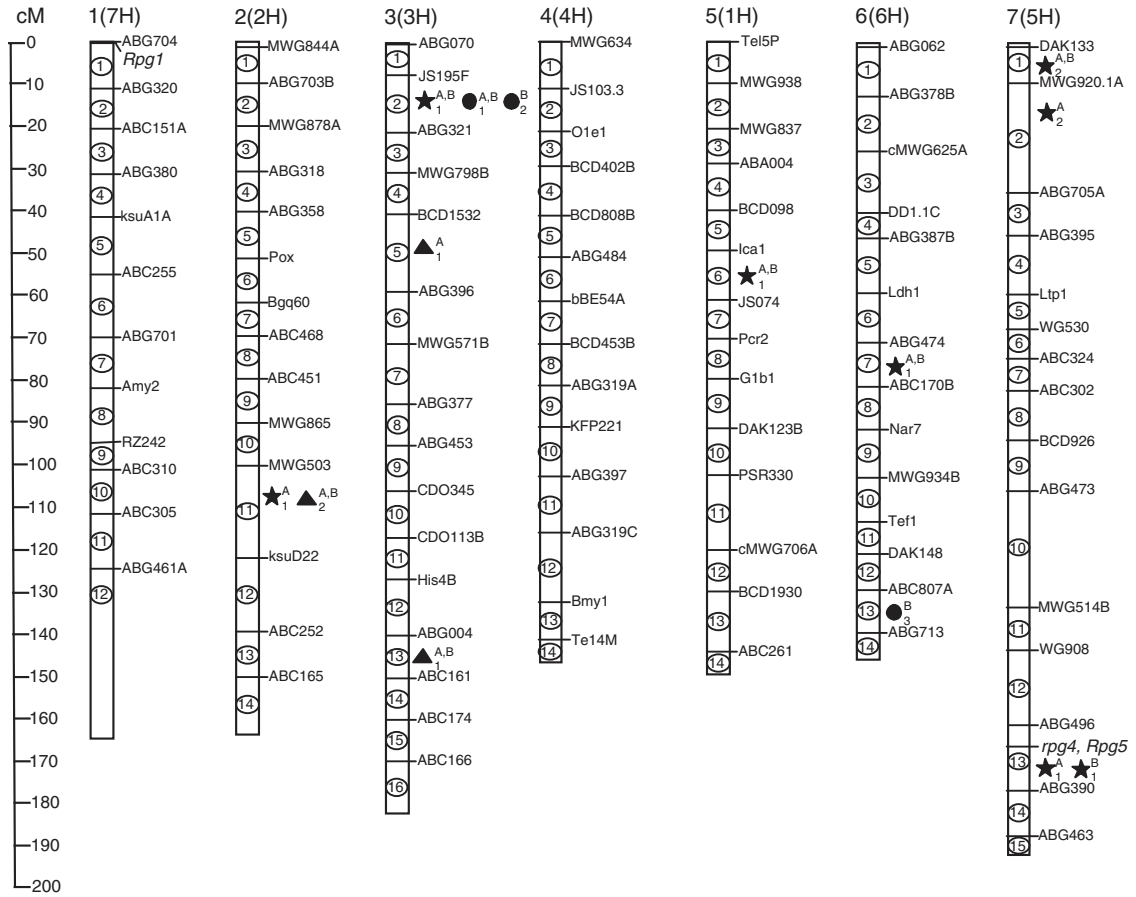

Fig. 2. Barley bin map showing significant associations of Diversity Array Technology (DArT) markers with resistance to wheat stem rust and rye stem rust in the Wild Barley Diversity Collection (WBDC). Each barley chromosome was divided into approximately 10 centiMorgans (cM bins) (designated with a circled number) flanked by molecular markers shown to the right as given by (Kleinhofs and Graner 2001). The relative length of chromosomes is given in cM by the scale at the left. The solid black star, triangle, and circle represent chromosomal regions showing a significant marker association ($P < 0.001$) with resistance to wheat stem rust races MCCF and QCCJ and rye stem rust isolate 92-MN-90, respectively. The superscript letters above the black symbols indicate the significant association was found when the number of subpopulations (i.e. K value) in the WBDC was set to 10 (A) or 8 (B). The number given below the black symbols indicate the number of DArT markers that were significantly associated with stem rust resistance within the respective bins. The previous mapped stem rust resistance genes of *Rpg1* and *rpg4/Rpg5* are given in bold italics on the telomeric region of the short arm of chromosome 1(7H) and the subtelomeric region of the long arm of chromosome 7(5H).

considered in this evaluation study. About half of the Sharon goatgrass accessions evaluated exhibited high (45%) and intermediate (5.6%) resistance to *P. striiformis* f. sp. *tritici* (Table 3). A similar result was reported by Anikster *et al*. (2005) in field tests done at the adult plant stage. Thus, Sharon goatgrass may be a valuable source of resistance for wheat breeding programs as was found for the other 2 rusts. Only a small percentage of accessions from the WBDC have been screened to stripe rust in the field; however, preliminary results show that high levels of resistance are present in some accessions (B. Steffenson and L. Jackson, unpublished data). One of the most resistant accessions found to date is Damon from Israel. The inheritance of stripe rust resistance is currently being investigated in the Damon/Harrington mapping population.

Sharon goatgrass exhibited a high percentage and level of resistance to the 3 rust pathogens summarised in this study (Table 3). These results are similar to other investigations made at both the seedling and adult plant stage (Pasquini 1980; Manisterski *et al*. 1988; Anikster *et al*. 1992; Snyman *et al*. 2004). Sharon goatgrass, like other wild grasses, has likely coevolved in association with the cereal rusts, being exposed to reciprocal selection pressure (Wahl *et al*. 1984). This long-term process occurring in the centre of origin and diversity (Coastal Plain of Israel) has resulted in the development of different levels of resistance in the host and a broad spectrum of virulence in the pathogen (Segal *et al*. 1980). Wild barley has undergone similar reciprocal selection pressure with leaf rust in this same region. The alternate host of barley leaf rust is *Ornithogalum* spp. (Star of Bethlehem Lily), which is endemic to Israel and co-exists with wild barley. In Israel, the percentage of leaf rust resistance was much higher (51.2%) than in other countries of the Fertile Crescent (range 5.3–24.6%) or Central Asia (0%) where a sufficiently large sample was tested.

The value of the wild genetic resources of Sharon goatgrass and wild barley will only be realised when their resistance genes

are transferred into cultivated wheat and barley, respectively. For Sharon goatgrass, the transfer of such genes into wheat is possible, but not routine. Antonov and Marais (1996) and Marais *et al*. (2003) successfully introgressed from Sharon goatgrass 2 genes for leaf rust and stripe rust resistance that were fully expressed in a hexaploid wheat background. Accessions exhibiting high resistance to the 3 rusts would be ideal candidates for an introgression project involving Sharon goatgrass. In contrast to Sharon goatgrass, the transfer of resistance genes from wild barley to barley is routine as no hybridisation barriers are known. Several wild × cultivated barley crosses have been developed to simultaneously identify resistance loci and transfer them into adapted germplasm (Yun *et al*. 2005, 2006; Alsop *et al*. 2007).

To effectively utilise the rust resistance present in Sharon goatgrass and wild barley, genetic characterisation is needed. Standard bi-parental crosses have been made to determine the number and chromosomal location of rust resistance genes in these species. Preliminary genetic studies of Sharon goatgrass indicate single gene control of leaf and stem rust resistance in cross 1644/1193 (Table 4). We also found evidence for more than 1 stem rust resistance gene (i.e. two independently segregating genes) in the same cross 1644/1193 when race TPMK was used instead of race TTTT (Table 4). Gerechter-Amitai and Loegering (1977) used 20 cultures of *P. graminis* f. sp. *tritici* and postulated 12 to 13 different stem rust resistance genes in 44 selected accessions of *Ae. sharonensis* and *Ae. longissima*. These data suggest that Sharon goatgrass may possess several stem rust resistance genes. The rust resistance genes identified in this species will enhance the diversity for rust resistance in cultivated wheat; however, they may not necessarily be more durable as the effectiveness of some major effect resistance genes derived from diploid wild species has been short-lived (Kolmer 1996). Still, combining effective resistance genes from various sources may increase the longevity of rust resistance in cultivation. Leaf rust resistance in wild barley is often simply inherited, being controlled by 1 or 2 genes (Jin and Steffenson 1994). Preliminary results from the Damon/Harrington population also indicate simple genetic control to wheat stem rust races MCCF and QCCJ (Alsop *et al*. 2007).

In addition to using standard bi-parental crosses, we also investigated the possibility of using association mapping for positioning stem rust resistance genes in the WBDC. Several key factors to consider as a prerequisite for association mapping are LD and population structure. LD is critical because it dictates the strategy and potential success of the analysis. For example, if LD is high in a species, the whole-genome scan method is most appropriate and will only require a low to medium density of markers to have a reasonable chance of identifying significant associations (Rafalski 2002). The drawback, of course, would be lower resolution of the target locus. If LD is low, the candidate gene strategy may be more appropriate, and the number of molecular markers (in this case SNPs as resequencing will be employed) needed would be high. In this latter case, resolution would be high as the actual sequence polymorphism of the gene conferring the phenotype may be identified. In spite of wild barley being a self-pollinated crop, LD in the WBDC accessions was very low, less than 1 cM for 6 of the 7 barley chromosomes (Fig. 1). Morrell *et al*. (2005) found similar low levels of LD for wild barley using nucleotide sequences of 18 nuclear genes. In cultivated barley, LD is usually much greater extending from 10–60 cM (Kraakman *et al*. 2004; Malysheva-Otto *et al*. 2006; Rostoks *et al*. 2006). Caldwell *et al*. (2006) investigated 4 gene regions surrounding the hardness locus in *Hordeum* germplasm and found LD to extend to at least 212 kb in elite barley cultivars, but decay rapidly in landrace and wild barley accessions. The LD in chromosome 4(4H) was very different than the other 6 chromosomes and extended to 40–50 cM at $r^2 = 0.5$ (Fig. 1). The extended LD on this chromosome indicates that it may have undergone low recombination during evolution. The overall low LD found in WBDC accessions indicates that many molecular markers will be required if a whole-genome scan approach is to have success for association mapping. Nevertheless, we conducted our association mapping analysis based on the available 1088 DArT markers as a 'proof of concept' investigation for stem rust resistance mapping. An additional 3000 SNP markers will be added on the WBDC accessions in the near future and should greatly increase the resolution for association mapping of target genes.

Another important factor in association mapping is population structure. Standard bi-parental crosses typically used in breeding consist of a single population. In contrast, natural populations have structure and often comprise subpopulations with different gene frequencies. Association mapping using natural populations generally results in a higher false positive rate (i.e. association in the absence of linkage) than traditional mapping populations derived from bi-parental crosses. Thus, a detailed analysis of population structure was performed. Several methods and software packages have been developed to assess structure in populations (Labate 2000; Pritchard *et al*. 2000; Huelsenbeck and Andolfatto 2007); however, there are no firm rules for deciding on the final number of subpopulations (i.e. K value) that can account for most of the structure present in a germplasm sample. In this study, we used the software program Structure (Pritchard *et al*. 2000) because it is more robust and powerful than other available programs for analysing population structure. Population structure analyses were conducted 5 separate times for $K = 2$ up to $K = 20$, since the WBDC accessions originated from a very wide geographic area. Pritchard *et al*. (2000) suggested that the K value with the lowest individual log-likelihood value may capture the greatest amount of structure present in a sample. For the WBDC, the lowest individual log-likelihood value found was for $K = 10$. The lowest average log-likelihood value for all five runs of each K value was $K = 8$. Thus, in our association analysis we used both K values.

Using the highly conservative *P* value threshold of 0.001, 14 and 15 significant marker associations were detected when K was set for 10 and 8, respectively (Table 6 and Fig. 2). These significant associations were in 9 and 8 unique chromosome bins, respectively. In general, the results for the 2 analyses were very similar with respect to what bins had significant marker associations for stem rust resistance. The only differences between the analyses were that when $K = 10$, no significant association was detected in bin 13 of chromosome 6(6H), and when $K = 8$, no significant associations were detected in bin 5 of chromosome 3(3H) or bin 2 of chromosome 7(5H). The significant associations found in the bins were almost always

to a single culture of stem rust. The only exception was for bin 2 of chromosome 3(3H), where significant associations were found for both wheat stem rust race MCCF and rye stem rust isolate 92-MN-90. This result may reflect the unique virulence specificities of the 3 rust cultures used in the study. With respect to the different stem rust cultures, the highest number of significant associations was to race MCCF with 9 and 6 marker associations for $K = 10$ and $K = 8$, respectively. This result was surprising because the percentage of WBDC accessions exhibiting resistance to race MCCF was lower (5.7%) than either race QCCJ (10.4%) or isolate 92-MN-90 (20.1%) (Table 5). In barley, 7 stem rust resistance genes have been described (*Rpg1–5*, *RpgBH*, and *RphU*) (Sun and Steffenson 2005), but only 3 have been mapped on the barley genome. *Rpg1* lies at the telomeric region of the short arm of chromosome 1(7H) (i.e. bin 1), whereas *rpg4* and *Rpg5* are closely linked and lie at the subtelomeric region of chromosome 7(5H) (bin 13) (Fig. 2). An important result from the association mapping analysis in this study was that 2 significant marker associations were detected for resistance to wheat stem rust race MCCF in the same bin as the *rpg4/Rpg5* complex. *Rpg5*, or *rpg4* together with *Rpg5*, can confer resistance to race MCCF (Sun and Steffenson 2005; B. Steffenson, unpublished data). If 1 of these 2 genes or another with the same resistance spectrum exists in the WBDC, the association analysis detected it. The WBDC accession Damon is resistant to wheat stem rust races MCCF and QCCJ and rye stem rust isolate 92-MN-90. It was crossed with the susceptible barley cultivar Harrington to develop a double haploid mapping population (based on DArT markers) for validation of association mapping. In this population, a single major gene conferring resistance to wheat stem rust races MCCF and QCCJ was identified in bin 13 of chromosome 7(5H), the same bin as *rpg4/Rpg5*. In fact, the same DArT markers found significantly associated with MCCF resistance in bin 13 of this study were found to be closely linked with the major Mendelian resistance locus in Damon/Harrington population (Alsop *et al*. 2007). Thus, association mapping may still be useful for precisely positioning disease resistance loci in a wild progenitor of a crop species with a seemingly unfavourable LD pattern. No significant marker associations were found in bin 1 of chromosome 1(7H) where *Rpg1* resides. The other significant associations found for stem rust resistance may represent unmapped stem rust resistance genes, new genes, or spurious associations. It is important to resolve whether the identified marker associations represent true underlying disease resistance genes or are simply false positives. In this regard, we are validating our association mapping results in four wild × cultivated barley mapping populations that include, besides Damon, the WBDC accessions of 41-1, OUH602, and Shechem.

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