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Transfer of disease resistance genes from *Triticum araraticum* to common wheat

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With 5 tables

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Abstract

The wild tetraploid wheat species *Triticum timopheevii* (Zhuk.) Zhuk. var. *araraticum* is a source of pest resistance genes for *Triticum aestivum* L. Our objectives were to describe the breeding behaviour of *T. araraticum* when backcrossed to common wheat, and to transfer resistance to leaf rust (caused by *Puccinia recondita* f.sp. *tritici*) and powdery mildew (caused by *Blumeria graminis* f.sp. *tritici*) to wheat. Crosses were made between five wheat genotypes and 12 *T. araraticum* accessions. Fertility and chromosome numbers of BC_1F_1, BC_1F_2, and BC_2F_1 plants were determined. Resistance to leaf rust was transferred to BC_3-derived families from 10 different *T. araraticum* accessions. Leaf rust resistance genes in nine *T. araraticum* accessions can be assigned to at least four loci. Leaf rust resistance transferred from three accessions was inherited in the hexaploid derivatives as a single, dominant gene in each case. Resistance to powdery mildew was also detected in the *T. araraticum* backcross derivatives. Fertile hexaploid derivatives expressing *T. araraticum*-derived resistance genes can be recovered after two backcrosses to wheat cultivars.

Key words: *Triticum aestivum* — *Triticum araraticum* — interspecific hybrid — introgression — leaf rust — powdery mildew

Two species of wheat are tetraploid: the emmer wheats (*Triticum turgidum* L.) with the genomic constitution AABB and the timopheevi wheats (*Triticum timopheevii* Zhuk.) with the genomic constitution A^tA^tGG. The emmer wheats are progenitor species of common wheat (genomic constitution AABBDD), and the A and B genomes of *T. turgidum* are homologous to the A and B genomes of *T. aestivum* L. The A^t and G genomes of the timopheevi wheats have reduced pairing with the A and B genomes, respectively, and are considered partially homologous to them (Feldman 1966). The timopheevi wheats include the cultivated *T. timopheevii* var. *timopheevii* and its wild progenitor, *T. timopheevii* var. *araraticum* (herein referred to as *T. araraticum*).

Cultivated *T. turgidum* has been an important source of useful traits for the improvement of *T. aestivum*, including resistance to diseases (McIntosh 1991) and insects (Carlson et al. 1978, Stebbins et al. 1983). The timopheevi wheats have been used as sources of resistance genes for common wheat to a lesser degree, although resistance to a number of disease and insect pests of wheat has been detected in *T. timopheevii* and *T. araraticum* (McIntosh and Gyarfas 1971, Gill et al. 1983, Tomar et al. 1988, Brown-Guedira et al. 1996). Cultivated *T. timopheevii* has been used for wheat improvement to a greater extent than has its wild progenitor, *T. araraticum*. Because *T. timopheevii* is a cultivated species, the chances of recovering agronomically acceptable derivatives from crosses with wheat are greater. However, *T. timopheevii* is endemic to the country of Georgia and shows little diversity for karyotype, morphology, or seed storage proteins (Jakobashvili 1989, Badaeva et al. 1994b). *T. araraticum*, on the other hand, is distributed widely in the Middle East and surrounding areas (Tanaka and Ishii 1973). Because the species is found in more diverse ecological regions, chances are greater that it contains more diverse genes useful for wheat improvement.

One faces many problems when attempting to introgress genes into wheat from *T. araraticum*. Viable hybrid seed can be recovered from crosses of *T. araraticum* with hexaploid wheat without performing embryo rescue, but mature F_1 plants are sterile (Shands 1941). Because of reduced recombination between *T. aestivum* and *T. araraticum*, recovery of desirable plant types in the progeny of the interspecific cross may be difficult.

Allard and Shands (1954) isolated two agronomically acceptable wheat lines with *Sr*36 and *Pm*6, genes for resistance to stem rust and powdery mildew, respectively, from an interspecific cross of *T. timopheevii* and wheat after two generations of backcrossing. The only resistance gene transferred to wheat from *T. araraticum* is *Sr*40, which confers resistance to stem rust. It was transferred from two Turkish *T. araraticum* accessions after six backcrosses to a stem rust susceptible hexaploid wheat line (Dyck 1992). Resistant lines were tested for traits associated with quality, and no deleterious effects were observed in the derived lines carrying *Sr*40.

There have been limited attempts to transfer pest resistance genes from *T. araraticum* to common wheat. In this study, a large number of crosses were made between *T. araraticum* and locally adapted hard red winter wheat cultivars and breeding lines in order to: (1) describe breeding behaviour of the species with wheat; (2) transfer pest resistance genes from *T. araraticum* to wheat by backcrossing; and (3) determine recovery frequency and inheritance of resistance to leaf rust and powdery mildew. Attempts were made to determine whether diversity for leaf rust resistance genes occurs in *T. araraticum* and to sample that diversity.

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Materials and Methods

Twelve accessions of *T. araraticum*, maintained at the Wheat Genetics Resource Centre (WGRC) at Kansas State University, were used for crossing. Each *T. araraticum* accession was resistant to leaf rust culture PRTUS 25. Eight accessions were resistant to powdery mildew isolate 9 and *Pm*4 Axminster culture. Accessions of *T. araraticum* were used to pollinate emasculated spikes of four wheat cultivars and a germplasm line ('Arlin', 'Karl 92', TAM 107, 'Wrangler', and KS90WGRC10) using the approach method (Curtis and Croy 1988). 'Karl 92', TAM 107, and 'Wrangler' are hard red winter wheat cultivars adapted to the Great Plains region of the USA. 'Arlin' is a hard white winter wheat cultivar, and KS90WGRC10 is a hard red winter wheat germplasm with the *T. tauschii*-derived leaf rust resistance gene *Lr*41 backcrossed into TAM 107. Twenty-six different cross combinations were made between *T. araraticum* accessions and common wheat. Mature F_1 seeds from such crosses usually are viable, but to ensure that hybrids were obtained and to speed generation time, embryos were rescued according to the method of Gill and Raupp (1987).

Germinated F_1 embryos were planted in 5 cm vermiculite-filled pots. Small seedlings were kept in a vernalization chamber at 10 C with an 8 h photoperiod for 7 weeks. Seedlings were transplanted into 3.5 l pots containing a 2:1:1 mixture of soil, peat, and perlite. Plants were grown in a greenhouse at 15–25 C with supplemented lighting to provide a 16 h photoperiod.

All F_1 hybrid plants were male-sterile and were backcrossed as females to their respective common wheat parents. Central florets of each spikelet were removed before pollination, and spikelets were cut just above the top of the stigma; anthers, none of which produced functional pollen, were not removed. Pollination was done by the approach method. Number of spikes crossed and BC_1 seed set for each cross were recorded.

Up to 20 BC_1 seeds were germinated per cross combination on moist filter paper. Root tips from germinated seeds were cut and fixed in a 3:1 mixture of ethanol and glacial acetic acid. Chromosome numbers were determined by the method of Endo and Gill (1984). Germinated BC_1 seeds were planted and vernalized, then transplanted into pots and placed in the greenhouse, as described above. The first emerged spike of each BC_1 plant was covered with a glassine bag and allowed to self-pollinate. Male fertile BC_1 plants were used as males in backcrosses to the respective hexaploid parent; male-sterile plants were used as females for further crossing. Anthers were removed from florets for all crosses, regardless of male fertility.

BC_2 seeds were harvested and planted as described above. Hybrid plants were grown to maturity in the greenhouse to produce BC_2F_1-derived F_2 families. Spikes of BC_2 plants were covered with glassine bags prior to anthesis to prevent outcrossing. Numbers of selfed seed set per spike were recorded.

Leaf rust screening and cytogenetic analysis: Segregating progeny were inoculated with culture PRTUS 25 (avirulence/virulence formula *Lr*24, *Lr*2B, *Lr*2C, *Lr*2D, *Lr*9, *Lr*16, *Lr*17, *Lr*18, *Lr*19, *Lr*1, *Lr*3, 3a, *Lr*10, *Lr*24) of *P. recondita* f.sp. *tritici*. Inoculations were done using a urediniospore oil mixture according to the method of Browder (1971). Infection types were scored according to the system of Browder (1971). The first digit of each code indicates the relative amount of sporulation, and the second digit indicates the relative lesion size, both on a scale of 0–9. A letter following the digits describes the tissue surrounding the lesions (C = chlorotic, P = pale, and X = mixed).

Up to 10 F_2 seedlings from each BC_2F_1 family were inoculated with *P. recondita* at the two-leaf stage. Reaction of plants was scored after 10 days in a 25 C growth chamber. Selected resistant seedlings representing different crosses were vernalized. Plants were grown in the greenhouse and backcrossed to their respective common wheat parents.

Crossed and selfed seeds were harvested from leaf rust-resistant BC_2F_2 plants. Twenty F_3 progeny of each resistant plant were inoculated as above, and their reactions were recorded. BC_2F_2 plants from which only resistant progeny were identified were classified as homozygous resistant. Additional selfed seeds of three homozygous resistant BC_2F_2 families from different crosses were germinated on moist filter paper, and root tips were collected and fixed. Chromosome preparations and C-banding of chromosomes were done according to the method of Gill et al. (1991).

Populations of BC_2F_2 plants from crosses between homozygous leaf rust-resistant BC_2F_2 individuals and the recurrent common wheat parent were inoculated as described above. The resultant infection types were scored after 10 days in the greenhouse. Resistant and susceptible parents were tested with their respective crosses.

The remaining BC_2F_1 seeds from crosses between TAM 107 and BC_2F_2 plants homozygous for leaf rust-resistance genes were germinated on filter paper, and root tips were fixed. Chromosome numbers were determined by the method of Endo and Gill (1984). For meiotic analysis, anthers were collected from young spikes and fixed in a 3:1 ethanol and glacial acetic acid mixture.

Four $BC_2F_{2:3}$ lines homozygous for leaf rust resistance derived from *T. araraticum* accessions TA 28, TA 870, and TA 874, along with their *T. araraticum* and wheat parents, were inoculated with leaf rust culture PRTUS 25 10 days after germination in the greenhouse. Two replications of each line were placed at 16 C, 22 C, and 28 C, and reactions were scored 10, 8, and 7 days after inoculation, respectively.

Seeds from each of 234 $BC_2F_{2:3}$ lines that were either susceptible or segregating for resistance to leaf rust were sown in a single 1.5 m row in October, 1993 at Manhattan KS, USA, and seed harvested in June, 1994 from selected rows was sown in 3-row, 1.5 m-long plots in October, 1994 at the same location. Leaf rust notes (resistant, segregating, or susceptible) were taken in May of 1994 and 1995, about 2 weeks after heading.

Nine different *T. araraticum* accessions that had donated leaf rust resistance genes to wheat progenies were crossed to one another in 16 different combinations. Populations of F_2 plants from each cross were inoculated with PRTUS25 as above, and responses were recorded. Identities of the progeny of individual F_1 plants were preserved during testing, but data then were pooled. *T. araraticum* parents were tested with their respective crosses, and the leaf rust-susceptible TAM 107 was included in each test as a control. Leaf rust readings were taken after plants were in the greenhouse for 10 days.

Powdery mildew screening: Segregating progenies from crosses between wheat and *T. araraticum* accessions TA 145 and TA 895 were screened at the USDA-ARS facilities at North Carolina State University in Raleigh, NC for reaction to two *Blumeria* (*Erysiphe*) *graminis* f.sp. *tritici* isolates: no. 8 (avirulence/virulence formula *Pm*2, *Pm*3a, *Pm*12, *Pm*16, *Pm*20/*Pm*1, *Pm*3b, *Pm*3a, *Pm*4a, *Pm*4b, *Pm*5, *Pm*6, *Pm*7, *Pm*8, *Pm*17) and WKin91 (avirulence/virulence formula *Pm*3b, *Pm*4a, *Pm*8, *Pm*12, *Pm*13, *Pm*16, *Pm*20/*Pm*1, *Pm*2, *Pm*3a, *Pm*3c, *Pm*4b, *Pm*5, *Pm*7, *Pm*9, *Pm*17, *MA*). In order to test individual F_2 plants for reaction to both isolates, screening was initially done on detached leaves using a method similar to that described by Leath and Heun (1990). Up to 10 F_2 plants were screened from each BC_2F_1 family. Two sections of leaf were taken from each plant when seedlings were 10 days old. Leaf sections were placed on replicate plates of 0.5% DIFCO Bacto-Agar medium containing 50 mg/l of benzimidazole. Forty-two leaf sections were placed on each Petri dish, including 10 individuals from each of four families, the recurrent parent, and the cultivar 'Chancellor' as a susceptible control. Inoculations were done by inverting a cup over each Petri dish to serve as an inoculation chamber, then blowing conidia from diseased leaf sections into the inverted cup. Powdery mildew ratings were made on a scale of 0–9, with 0 signifying complete absence of infection and 9 signifying 100% infection. Reactions of plants scored as resistant (scores less than 4) in the detached leaf test were confirmed by inoculating seedlings with a mixture of the two *B. graminis* f.sp. *tritici* cultures. Disease reaction was scored after 14 days in the greenhouse. Resistant seedlings were vernalized and grown to maturity in the greenhouse, and selfed seeds were harvested. Twelve F_2 progenies from each of 13 resistant BC_2F_2 individuals were screened for powdery mildew resistance as seedlings at Kansas State University. Seeds of two $BC_2F_{2:3}$ lines that appeared to be homozygous for resistance were sent to ARS-NCSU for further testing. Two leaf segments each of the derived lines

Table 1: *Triticum araraticum* accessions, collection sites, and reactions to leaf rust and powdery mildew

T. araraticum accession	Collection site[1]	Leaf rust[2] PRTUS 25	Powdery mildew[3] Isolate 9	*Pm*4 Axminster
TA 28	2 km NW of Salahadin	12C	9	–
TA 30	41 km NW of Sulaimaniya to Surdash	13C	5	–
TA 145	2 km NW of Salahadin	12C	4	0
TA 870	1 km NE of Salahadin	01C–23C	4	2
TA 874	2 km NW of Salahadin	–	5	–
TA 878	4 km NE of Shaqlawa	12C–33C	9	0
TA 895	13 km W of Shaqlawa	02C–22C	4	0
TA 913	1 km NE of Salahadin	12C–23C	4	4
TA 920	1 km NE of Salahadin	12C	4	4
TA 1485	1 km NE of Salahadin	12C–23C	3	0
TA 1520	21 km S of Harir between Rowandus and Shaqlawa	02C–13C	3	1
TA 1538	19 km E of Sulaimaniya to Chuarta	01C–12C	4	0

[1]All collection sites are in north-eastern Iraq
[2]First and second digits indicate relative amount of sporulation and lesion size, respectively. C = chlorosis
[3]Rated on a scale of 0 to 9, where 0 = lowest infection

and parents were screened for reaction to 37 different cultures of *B. graminis* f.sp. *tritici* as previously described.

Results

The selection of *T. araraticum* accessions for crossing was made originally on the basis of resistance to leaf rust, but, after interspecific hybridization had been made, resistance to powdery mildew was detected in nine of the *T. araraticum* parents (Table 1). Accessions were collected from eight different collection sites, all in north-eastern Iraq.

Crossability of *T. araraticum* with hexaploid wheat

Hybrid plants from crosses between wheat and *T. araraticum* were male sterile, and female fertility was low. Overall, the mean number of BC_1 seed per spike was 0.42. The BC_1 seed-set per spike varied among crosses, but differences in mean BC_1 seed-set among the common wheat parents were small. Hybrids having KS90WGRC10 as a recurrent parent set the greatest number of BC_1 seed per spike, with a mean of 0.52. Hybrids having 'Arlin', 'Karl 92', TAM 107, and 'Wrangler' as recurrent parents had means of 0.38, 0.42, 0.43, and 0.37 BC_1 seed per spike, respectively.

Of 114 BC_1 plants grown to maturity, selfed seeds were harvested from 21 plants that were partly male fertile. Female fertility of 91 BC_1 plants that were backcrossed was higher than that of F_1 hybrids, with an average of six BC_2 seed per spike. Male fertility of BC_2 plants was higher than that of BC_1 plants, with 86% setting some selfed seed. However, only 20% of these plants set 10 or more seeds per spike.

Chromosome numbers

With the exception of one 56-chromosome plant, BC_1 plants had chromosome numbers ranging from 36 to 48, with a mean of 41. Of the 21 plants from which selfed seed was obtained, the chromosome number ranged from 39 to 48. One 41-chromosome plant from the cross 'Arlin' × TA 895 was fully fertile.

Chromosome numbers of BC_2 plants varied from 37 to 48, with a mean of 41. After two backcrosses, 66% of plants had chromosome numbers in the range 39–42. Plants with chromosome numbers between 40 and 44 on average set the most selfed seed.

The chromosome numbers of BC_2 plants were related loosely to the chromosome numbers of their BC_1 parents. Forty-four percent of progeny from 42-chromosome BC_1 plants had a chromosome number of 42. Only 3% of progeny from BC_1 plants with chromosome numbers between 36 and 39 had 42 chromosomes. BC_2 plants obtained from BC_1 plants with chromosome numbers in the ranges 36–39, 40–44, and 45–48 had similar mean chromosome numbers of 40.43, 41.33, and 41.28, respectively.

Chromosome numbers of 42 BC_3 plants resulting from crosses between homozygous leaf-rust resistant BC_2F_2 plants and their recurrent parents were determined. Twenty-nine plants had a chromosome number of 42. Three plants had $2n = 43$, seven plants had $2n = 41$ and one plant had $2n = 40$. Telosomic chromosomes were detected in two plants; one plant had 41 chromosomes plus a telosome, and another plant had 40 chromosomes plus a telosome.

Transfer of leaf rust resistance

Seven hundred and thirty-nine BC_2F_1-derived families were screened for reaction to leaf rust. Resistant plants were identified in 66 families, representing 16 of the 26 different *T. aestivum–T. araraticum* cross combinations. These crosses involved 10 different *T. araraticum* accessions and four common wheat genotypes. The majority of lines tested were in a TAM 107 background; 49 of the families in which leaf rust resistance was detected had TAM 107 as a recurrent parent. Small quantities of BC_2F_2 seed were available for leaf rust screening, and segregation ratios within BC_2F_1 families were not determined.

Leaf rust resistance derived from TA 874 was conditioned by a single dominant gene; crosses between TAM 107 and resistant lines derived from TA 874 segregated three resistant to one susceptible (Table 2). The heterogeneity χ^2 value for families of different lineage from this cross (families U3190, U3191, U3193 and U3194) was not significant, and the pooled data of 268 resistant plants: 83 susceptible plants fit a 3:1 ratio ($\chi^2 = 0.01$, $0.95 > P > 0.90$).

Families from crosses between TAM 107 and different homozygous resistant BC_2F_2 plants derived from crosses with TA 28 and TA 870 had segregation ratios that were not homogeneous. For example, observed segregation ratios in the F_2 families designated U3169 and U3170 from the cross TAM 107*4/TA 870 deviated significantly from a 3:1 ratio (Table 2). Both cases had an excess of susceptible plants. However, segregation in the families designated U3166 and U3168 fit a 3:1 ratio. Differences in the transmission of resistance may have been due to different types of transfers (i.e. recombination, translocation, or chro-

Table 2: Numbers of BC_2F_2 or parent plants resistant or susceptible to leaf rust, with χ^2 tests for fit to a 3:1 resistant to susceptible ratio. Plants were inoculated with culture PRTUS 25 of *Puccinia recondita*

BC_2F_2 or parent	Family	Number of plants Resistant	Susceptible	χ^2 (3:1)	P
TAM 107*4/TA 870	U3166	39	11	0.11	0.80–0.70
	U3168	114	39	0.00	0.99
	U3169	58	48	22.19	<0.001
	U3170	65	36	5.55	0.02–0.01
TAM 107*4/TA 28	U3067	177	115	31.45	<0.001
	U3172	63	25	0.32	0.70–0.50
	U3176	43	11	0.40	0.70–0.50
TAM 107*4/TA874	U3190	67	22	0.00	0.99
	U3191	65	19	0.00	0.99
	U3193	81	25	0.05	0.90–0.80
	U3194	64	17	0.49	0.50–0.30
TA 870		40			
TA 28		30			
TA 874		10			
TAM 107			110		

mosome substitution). The BC_2F_2 plants that gave rise to the BC_3F_1 families U3166 and U3168 were descended from different BC_2F_1 and BC_1F_1 plants than those that produced the families U3169 and U3170.

Leaf rust resistance derived from TA 28, TA 870, and TA 874 in $BC_2F_{2:3}$ lines was expressed at a level comparable to that of the donor parent (Table 3). Whereas the infection type of the donor parent was generally within the range of reactions observed in the derived lines, the line designated U2657-2-28-1 had a lower infection type at 28°C than did its *T. araraticum* parent. This line was derived from a cross between TA 874 and the wheat cultivar 'Karl 92'. Infection types of 'Karl 92' were lower than TAM 107 infection types at all three temperatures when inoculated with PRTUS 25 (Table 3); therefore, a combination of genes from 'Karl 92' and TA 874 may have produced progeny with an infection type lower than that of either parent.

Lines scored as homogeneous or segregating for leaf rust resistance as seedlings were classified similarly in the field in 1994 and/or 1995. Therefore, resistance was expressed at the adult-plant stage. However, few $BC_2F_{2:3}$ lines had overall phenotypes similar to those of their respective recurrent parents, suggesting that further backcrossing will be needed to recover desirable agronomic types.

Table 3: Infection types (C = chlorosis, P = green and X = mixed) of four $BC_2F_{2:3}$ lines carrying leaf rust resistance genes derived from *Triticum araraticum*, their respective donor parents, and recurrent wheat parents when inoculated with leaf rust culture PRTUS 25 and placed at different temperatures

Wheat genotype	16°C	22°C	28°C
U2665-14-5-2 (TAM 107*3/TA 28)	03C–13C	03C–13C	14C–24C
TA 28	04C	12C	23C–23X
U2664-1-10-1 (TAM 107*3/TA 870)	04C	13C	13C–34
TA 870	13C–23C	12C	23X–34X
U2668-1-8-1 (TAM 107*3/TA 874)	03C–14C	03C–13C	13C–23C
U2657-2-28-1 ('Karl 92'*3/TA 874)	03C–13C	03C–14C	14C
TA 874	03C–14C	02C–13C	23C–23X
TAM 107	88P	88P	88P
'Karl 92'	78X–88P	78X	78X

[1]First and second digits indicate relative amount of sporulation and lesion size, respectively

Severe leaf chlorosis of many lines challenged by the leaf rust pathogen in 1995 field plots was the most obvious problem introduced by introgression of *T. araraticum* genes. In 1994, when the leaf rust epidemic was less severe, extensive chlorosis was not observed in plant rows segregating for resistance. A severe epidemic of soilborne mosaic virus also occurred at the Manhattan location in 1995. The chlorosis was most severe in crosses having TAM 107 as the recurrent parent. No evidence of chlorosis was seen in TAM 107 or any of the other recurrent parents. The extent of chlorosis also was related to the severity of soilborne mosaic virus in a plot. Testing of leaf rust-resistant lines in different environments will be done to determine whether the chlorosis observed in resistant lines was caused by the interaction of the *T. araraticum*-derived resistance genes and heavy levels of leaf rust inoculum, an interaction of other *T. araraticum* genes associated with resistance and the wheat background, or the effect of leaf rust in combination with extremely wet soil and soilborne mosaic virus.

Cytogenetic analysis of leaf rust resistant lines

Meiotic analysis of pollen mother cells of BC_3F_1 plants from the lines U3067, U3166, U3172, and U3193 was performed to determine whether abnormalities in chromosome pairing influenced the transmission of leaf rust resistance observed in these lines (Table 2). Overall, a mean of 20 bivalents and one univalent was observed per cell with a low frequency of multivalents. In each line, ring bivalents were more frequent than rod bivalents. A trivalent was observed at a low frequency in all lines except U3193. A quadrivalent was detected in 29% of cells of one line, U3067. Transmission of leaf rust resistance in this line was poor (Table 2).

Pairing was most normal in the line U3193, in which no multivalents were observed. Transmission of leaf rust resistance in this line was normal (Table 2). C-banding of metaphase I chromosomes showed that this line was carrying one copy of chromosome 5G from *T. araraticum*, which failed to pair with chromosome 5B of wheat in approximately one-third of the

cells examined, accounting for the average of 0.67 univalents per cell. These two chromosomes were associated as a rod bivalent in half of the cells analysed and as a ring bivalent in the others.

One plant from U3166 had a chromosome number of $2n = 40$. An average of two univalents was observed in each cell. These univalents were heteromorphic, indicating that the plant was probably monosomic for two chromosomes.

To determine whether resistance was associated with a chromosome substitution or translocation that could be detected by chromosome banding, C-banding was performed on mitotic metaphase chromosomes from root tips of BC_2F_3 plants that were homozygous resistant and homozygous susceptible to leaf rust. Plants from crosses between TAM 107 and each of the accessions TA 28, TA 870, and TA 874 were examined. In lines derived from all three *T. araraticum* accessions, chromosome 2G from *T. araraticum* was substituted for chromosome 2B of wheat. However, this chromosome was not associated with leaf rust resistance; the 2G(2B) substitution was also detected in homozygous susceptible plants. Chromosome 5G of *T. araraticum* substituted for chromosome 5B of wheat in some plants from each of the three crosses, but not all resistant plants carried a copy of chromosome 5G. Other *T. araraticum* chromosome substitutions were not detected. However, both A and A^t genome chromosomes are lightly banded, and chromosome substitutions or rearrangements involving A and A^t genome chromosomes may not have been detected by C-banding.

Diversity of leaf rust resistance genes transferred from *T. araraticum*

T. araraticum accessions were intercrossed to determine how many loci were involved in the leaf rust resistance transferred to wheat. TA 145, TA 870, and TA 1520 had a leaf rust resistance gene at the same locus (Table 4). No susceptible plants were observed in the F_2 of the cross between TA 145 and TA 870. One small F_2 plant from the cross between TA 145 and TA 1520 was susceptible to leaf rust. Four different translocation types are present in the nine *T. araraticum* accessions that were intercrossed (Badaeva et al. 1994a); therefore, a low frequency of progeny that were aneuploid or had deficiencies and/or duplications of chromosome regions was expected in the F_2 populations from many of these crosses. These could have produced rare susceptible plants from crosses between accessions with the same leaf-rust resistance gene.

TA 28 and TA 1538 probably have a common gene for leaf rust resistance, despite the occurrence of two small susceptible plants (Table 4). No susceptible plants were detected in the F_2 of the cross between TA 1538 and TA 913. In this test, the TA 1538 parent had a low infection type, whereas the TA 913 parent was characterized by a mixed reaction. In TA 913 seedlings, the tissue around some lesions was chlorotic, whereas the tissue around other lesions on the same leaf was not. Plants with reactions similar to those of both parents were observed in the progeny. F_2 plants more resistant than either parent were also observed. TA 913 and TA 1538 may have different alleles for leaf rust resistance at the same locus, or they could have linked resistance genes, with no susceptible recombinants detected in the F_2 population.

The leaf rust resistance gene in TA 28 and TA 1538 apparently is linked to that in TA 145, TA 870, and TA 1520, as indicated by a segregation ratio of 32:1 in the cross between TA 145 and TA 1538. The gene in TA 145, TA 870 and TA 1520 segregates independently of the genes in TA 30 and TA 895. Segregation ratios observed in the crosses between TA 145 and TA 30 and between TA 1520 and TA 895 fit a 15:1 ratio.

The genes in TA 30 and TA 895 are linked (Table 4), and they condition different reactions to *P. recondita*. Although some sporulation occurred on seedlings of TA 30, lesions frequently became necrotic, approximately 10 days after inoculation, without sporulating. This necrosis was not observed in TA 895 or other accessions, although TA 895 consistently had a low infection type (Table 4). The gene in TA 28 and TA 1538 segregates independently of the linked genes in TA 30 and TA 895 (Table 4).

The genetic relationship of the resistance in TA 920 to resistance in other accessions was difficult to determine. The gene in TA 920 segregates independently of the gene in TA 145, TA 870, and TA 1520 (Table 4). Segregation for resistance occurred in the cross TA 920/TA 30, although observed ratios in two runs of the test differed (48:1 in run 1 and 15:1 in run 2). Conflicting results were obtained in two different tests of F_2 from the crosses of TA 920 with TA 28 and TA 1538. In both cases, segregation that fitted a 15:1 ratio was observed in one run, and no segregation was observed in the other run. The infection type of TA 920 varied between 01C and 34X in different tests. When the infection type of TA 920 was relatively high, plants with an infection type similar to that of TA 920 and the high infection types of susceptible plants were difficult to distinguish.

The *T. araraticum* accessions that were intercrossed can be placed into four groups having leaf rust resistance genes at different loci: (1) TA 145, TA 870, and TA 1520; (2) TA 28, TA 1538, TA 913 (and possibly TA 920); (3) TA 30; and (4) TA 895. Linkage occurs between groups (1) and (2) and between groups (3) and (4). It is possible that TA 913 has an allele that differs from the other accessions in group (2). The linkage relationship of the gene in TA 874 that donated leaf rust resistance to wheat was not determined. Therefore, at least four, and possibly as many as six, unique leaf rust resistance genes exist among the accessions and their hexaploid progeny.

Transfer of resistance to powdery mildew

A total of 32 BC_2F_1 families from crosses of 'Arlin'*3/TA 895, 'Karl 92'*3/TA 145, and 'Wrangler'*3/TA 145 were screened for reaction to powdery mildew. Fifteen plants were scored as resistant when screening was performed on detached leaves and on seedlings. All but one of these plants were from the cross 'Arlin'*3/TA 895. One seedling from the cross 'Wrangler'*3/TA 145 was identified as resistant, but no resistant F_3 progeny were recovered from this plant.

Segregation ratios within BC_2F_1 families were not determined. When results from nine different BC_2F_1 families from the cross 'Arlin'*3/TA 895 were combined, 75 plants were susceptible and 13 were resistant. Progeny of nine of the 13 resistant BC_2F_2 plants were segregating for powdery mildew resistance, and four lines were homozygous resistant. The infection types of the homozygous resistant BC_2F_2 plants were lower than those of plants that gave segregating progeny. Therefore, powdery mildew resistance may be conditioned by complementary loci or by an incompletely dominant gene that was transmitted at a lower-than-expected frequency.

Resistance to powdery mildew in two $BC_2F_{2:3}$ lines (U2659–1-7-M1 and U2659–2-9-M2) derived from the cross 'Arlin'*3/TA895 was expressed at a lower level than that of the

Table 4: Infection types of leaf rust resistant *Triticum araraticum* parents and numbers of F_2 plants with low (01C–13C), intermediate (23X–56X), and high (78X–88P) infection types, with the observed (low + intermediate):(high) ratio and χ^2 tests for fit to a 15:1 ratio. Positions of the columns 'Parent 1' and 'Parent 2' do not indicate male and female parents. Plants were inoculated with culture PRTUS25 of *Puccinia recondita*

Parent 1 (P1)	P1 IT[1]	Parent 2 (P2)	P2 IT		Infection type (number of plants) 01C–13C	23X–56X	78X–88P	Observed ratio (01C–56X): (78X–88P)	χ^2 (15:1)
TA 28	02C–03C	TA 30	01C–03C		156	10	12	13.8:1	0.01
	01C	TA 920	01C–11X	run 1	118	39	9	17.4:1	0.08
	13C–33X		01C–23X	run 2	56	139	0	–	11.96**
	01C	TA 1538	02C–34X	run 1	194	24	2	109.0:1	9.86**
	03C–23X		01C–23X	run 2	126	98	0	–	44.75**
TA 30	–	TA 145	–		83[2]	–	6	13.8:1	0.00
	–	TA 895	–		180	7	5	37.5:1	3.76
	02C–04C	TA 920	01C–12C	run 1	126	17	3	47.7:1	3.81
	04C–13C		03C–13X	run 2	46	24	4	15.0:1	0.07
TA 145	0–13C	TA 870	13C–56X		139	2	0	–	8.23**
	0–02C	TA 920	0–22C		87	42	11	11.7:1	0.37
	23X	TA 1520	23C–34X		–	100	1	–	3.91*
	13C	TA 1538	56X		114	44	5	31.6:1	2.30
TA 870	13C–34C	TA 920	13C–34X		11	56	4	16.8:1	0.00
TA 895	03C–13C	TA 1520	13X–56X		31	130	11	14.6:1	0.01
	01C	TA 1538	02C–34X		116	11	7	18.1:1	0.10
TA 913	23X–34X	TA 1538	13C–23C		63[3]	97[4]	0	–	9.63**
TA 920	23C–77X	TA 1520	23C–56X		0	45	4	11.0:1	0.07
	01C–12C	TA 1538	01C–04C	run 1	207	37	0	–	15.22**
	02C–23X		03C–13C	run 2	109	16	6	20.8:1	0.37

*,**Significantly different from zero at P = 0.05 and P = 0.01, respectively
[1]Infection type
[2]Plants were scored as resistant or susceptible
[3]Number of plants with infection types similar to that of TA 1538
[4]Number of plants with infection types similar to that of TA 913

T. araraticum parent. When inoculated with 37 diverse isolates of powdery mildew, TA 895 consistently had few or no disease symptoms (rating = 0–4) and 'Arlin' was susceptible (rating = 7–9) to 35 isolates (Table 5). Both derived lines gave intermediate reactions when inoculated with 25 isolates. Low reaction types (rating = 0–3) resulted from inoculation of the derived lines with five isolates (9, 127, 146–2a, 209a_2, and Asosan). The Asosan isolate produced no disease symptoms on leaves of U2659-1-7-M1 and U2659-2-9-M2. This isolate gave an intermediate reaction type on the recurrent parent. The derived lines may not be homozygous, because susceptible plants were detected in both lines (Table 5).

Discussion

Whereas production of the initial interspecific hybrid between wheat and *T. araraticum* is not problematic, the production of inadequate numbers of backcrossed seed is a potential barrier to successful introgression from *T. araraticum* in a backcrossing programme. Gill and Raupp (1987) and Cox et al. (1991) observed that, in direct crosses of wheat with the diploid species *T. tauschii* and *T. monococcum*, a potential limiting factor for gene transfer was low production of BC_1 seed. In crosses between *T. araraticum* and wheat, production of an adequate number of BC_2 seed is also important. In this introgression study, no selection for pest resistance was done on BC_1F_1 plants because we were interested in transferring resistance to more than one pest and we needed to keep plants as healthy as possible in order to obtain selfed or backcrossed seed. Because most BC_1F_1 plants were male-sterile, selfed seed for screening could not be obtained. Therefore, randomly backcrossing as many BC_1F_1 plants as possible was necessary. Because female fertility of BC_1F_1 plants is high, production of sufficient numbers of BC_2F_1 seed is simply a matter of making enough pollinations.

We detected resistance to powdery mildew in 38% of the BC_2F_1 families tested; however, resistance to leaf rust was observed in only 9% of the BC_2F_1 families screened. The latter frequency is lower than expected, indicating that there may be selection against chromosomes or chromosomal regions from *T. araraticum*. Evidence of selection both for and against transfer of certain chromosomes of *T. timopheevii* and *T. araraticum* in backcrosses to *T. aestivum* has been reported (Allard 1949, Gill et al. 1988, Friebe et al. 1994, Brown-Guedira 1995).

Using several accessions of the wild parent and different wheat genotypes when attempting to transfer genes from *T. araraticum* has a number of advantages. In this study, the number of seed obtained at the first backcross varied with different combinations of *T. araraticum* and wheat parents. Although information about resistance to pests other than leaf rust was not available when the initial crosses were made, resistance to powdery mildew and other pests was subsequently detected in the 12 *T. araraticum* accessions that had been crossed to wheat (Brown-Guedira et al. 1996). In addition, although nothing was known about the diversity of resistance to leaf rust in these *T. araraticum* accessions, at least four leaf rust resistance genes were apparently transferred to wheat. Some parental com-

Table 5: Infection types of *Triticum araraticum* parent (TA 895), recurrent wheat parent (Arlin), and two $BC_2F_{2:3}$ lines derived from a cross between wheat and *Triticum araraticum* when inoculated with 37 isolates of *Blumeria graminis* f. sp. *tritici*

	Line			
Isolate	TA 895	'Arlin'	U2659-1-7-M1	U2659-2-9-M2
2	1[1]	9	7	1/5
3a	0	7	4	5
4	0	9	4	4
5	1	9	9	6
6	0	7	4	5
8	0	9	4	3
9	0	7	0	2
10	0	9	5	4
$42a_1$	0	9	4	6
$43a_2$	0	9	3	6
$73b_2$	0	8	5	5
$93b_2$	0	9	0/9	1
95	0	9	4	4
$101a_2$	0	9	0/6	5
$121a_1$	0	8	4	2
127	0	9	2	1
$137a_1$	0	8	4	4
144	0	8	5	4
146-2a	0	6	1	2
153-c	0	9	5	4
$153a_2$	1	9	6	9
$156b_1$	0	9	4	7
169-16	0	9	4	7
$184a_2$	0	9	4	6
$209a_2$	0	8	1	2
$216a_1$	0	9	1/4	4
85063	0	9	8	0
'Asasan'	0	4	0	0
E314	0	8	4	4
E325	0	8	4	4
Fla^+7-12	0/4	9	4	4
M_{1010}	0	9	4	6
Pm4	0	9	4	4
W72-27	0	8	4	4
Wkin91	0	9	4	5

[1]Rated on a scale of 0 to 9 where 0–3 = resistant, 4–6 = intermediate and 7–9 = susceptible

binations of *T. araraticum* and wheat may also produce more desirable progeny than others.

Browder (1980) noted temperature sensitivity of the *T. timopheevii*-derived resistance gene *Lr*18, which becomes ineffective at a temperature of 25°C. In our study, infection types of seedlings carrying *T. araraticum*-derived resistance increased at 28°C, but resistance remained effective in the tested temperature range. The amount of variation in infection type observed at different temperatures was dependent on the wheat background. The leaf rust resistance gene derived from TA 874 gave lower infection types at 28°C in a 'Karl 92' background than in a TAM 107 background.

The chromosomal locations of the transferred leaf rust resistance genes were not determined. Cytogenetic analysis did reveal the presence of chromosomes 2G and 5G in different resistant lines, although these chromosomes did not appear to be related to resistance. McIntosh (1983) noted that the *T. timopheevii*-derived genes *Lr*18 and *Sr*36, located on chromosomes 5BL and 2B, respectively, were transferred to wheat in various independent programmes. He suggested that wheat chromosomes 2B and 5B may have close homology with their *T. timopheevii* homoeologues, making for rapid and repeatable transfers.

Powdery mildew resistance transferred from TA 895 to 'Arlin' is conditioned by a gene other than the *Pm*6 gene already transferred to wheat from *T. timopheevii*, because isolates used in screening were virulent to *Pm*6. In aggregate, the 37 isolates of *B. graminis* f.sp. *tritici* used to screen the two $BC_2F_{2:3}$ lines carrying powdery mildew resistance derived from TA 895 are virulent to all of the named powdery mildew resistance genes with the exception of *Pm*16. That gene was transferred to hexaploid wheat from the wild tetraploid species *T. diccocoides* (McIntosh et al. 1995). Possibly, a new gene for powdery mildew resistance has been transferred to wheat from *T. araraticum*. Wheat lines carrying this gene had intermediate infection types when inoculated with most of the isolates tested, whereas the *T. araraticum* parent was highly resistant to all isolates. The *T. araraticum*-derived resistance gene may have reduced expression in a hexaploid wheat background, or TA 895 may have additional factors conferring powdery mildew resistance that were not transferred.

In summary, direct hybridization of locally adapted wheat cultivars with *T. araraticum* followed by backcrossing to the wheat parent was an effective way of transferring pest resistance from this species. Plants that are fertile and euploid can be obtained after two backcrosses to the wheat parent, but development of germplasm with acceptable agronomic traits may require additional backcrossing. Although transfer of resistance genes from *T. araraticum* to wheat is labour-intensive, the effort is worthwhile when considering the resistance to a wide range of pests and the diversity of leaf rust resistance genes available in *T. araraticum*.

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