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Journal

Theoretical and Applied Genetics, 111(3)

ISSN

0040-5752

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Publication Date

2005-08-01

DOI

10.1007/s00122-005-2048-y

Peer reviewed

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Molecular characterization of durum and common wheat recombinant lines carrying leaf rust resistance (*Lr19*) and yellow pigment (*Y*) genes from *Lophopyrum ponticum*

Received: 1 March 2005 / Accepted: 15 April 2005 / Published online: 24 May 2005
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Abstract Chromosome 7E from *Lophopyrum ponticum* carries a valuable leaf rust resistant gene designated *Lr19*. This gene has not been widely used in common wheat breeding because of linkage with the yellow pigment gene *Y*. This gene tints flour yellow, reducing its appeal in bread making. However, a high level of yellow pigment is desirable in durum wheat breeding. We produced 97 recombinant chromosomes between *L. ponticum* transfer 7D.7E#1 and its wheat homoeologues, using the *ph1b* mutation that promotes homoeologous pairing. We characterized a subset of 37 of these lines with 11 molecular markers and evaluated their resistance to leaf rust and the abundance of yellow pigment. The *Lr19* gene was mapped between loci *Xwg420* and *Xmwg2062*, whereas *Y* was mapped distal to *Xpsr687*, the most distal marker on the long arm of chromosome 7. A short terminal 7EL segment translocated to 7A, including *Lr19* and *Y* (line 1-23), has been transferred to durum wheat by backcrossing. The presence of this alien segment significantly increased the abundance of yellow pigment. The *Lr19* also conferred resistance to a new durum leaf rust race from California and Mexico that is virulent on most durum wheat cultivars. The new durum lines with the recombinant 7E segment will be useful parents to increase yellow pigment and leaf rust resistance in durum wheat breeding programs. For the

common wheat breeding programs, we selected the recombinant line 1-96, which has an interstitial 7E segment carrying *Lr19* but not *Y*. This recombinant line can be used to improve leaf rust resistance without affecting flour color. The 7EL/7DL 1-96 recombinant chromosome did not show the meiotic self-elimination previously reported for a 7EL/7BL translocation.

Introduction

Chromosome 7E from *Lophopyrum ponticum* (Podp.) Löve carries several interesting genes including the leaf rust resistance gene *Lr19* (Sharma and Knott 1966), the endosperm yellow pigment gene *Y* (Knott 1968), and the segregation distortion factor *Sd1* (Sears 1977). To transfer *Lr19* into wheat, winter wheat ‘Argus’, which includes a substitution of a complete *L. ponticum* chromosome 7E for wheat chromosome 7D, was backcrossed to ‘Thatcher’, and the derivatives were subjected to irradiation. This resulted in the translocation stock T4, later designated ‘Agatha’ (Sharma and Knott 1966). The translocated fragment of 7E does not pair with homoeologous wheat chromosomes during meiosis, causing it to be transmitted as a single, large linkage block (Knott 1980). To reduce the size of the 7E segment, E.R. Sears (1972a, 1972b) used the *ph1b* mutation, which promotes homoeologous recombination. He produced ten new 7D–7E recombinant lines with smaller 7E segments and observed that chromosomes with longer 7E segments were preferentially transmitted through the pollen (Sears 1977). Zhang and Dvorak (1990) later found that the *Sd1* locus was responsible for this preferential transmission and that it was proximal to *Lr19*.

The original 7E chromosome was incorporated into cultivars ‘Agatha’ (Knott 1980), ‘Oasis’, and others (Monneveux et al. 2003; Rajaram 2001). Agronomic characterization of several isogenic lines for this translocation demonstrated that the presence of the 7E

Communicated by P. Langridge

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segment was associated with a yield increase under irrigated conditions (Monneveux et al. 2003; Singh et al. 1998). In spite of its positive contribution to yield and leaf rust resistance, this segment has not been extensively used in agriculture. The reason of its limited use is the presence of the *Y* gene that tints flour yellow, reducing its appeal in countries where white flour is preferred for bread making (Knott 1968).

Different efforts have been undertaken to separate *Y* from *Lr19*. Knott (1980) developed two ethyl-methane-sulfonate mutants with white endosperm, but unfortunately, these mutations were associated with reduced agronomic performance (Knott 1989). The two genes were also separated by a second round of homoeologous recombination (Marais 1992). One line, designated *Lr19-149*, showed the desired combination of white endosperm and leaf rust resistance. In this line, a small segment of the 7EL arm was translocated as an interstitial segment within wheat chromosome arm 7BL (Prins et al. 1997). However, *Lr19-149* and the secondary recombinants developed later (Marais et al. 2001) showed a high rate of self-elimination during male meiosis, in contrast with the preferential transmission of the larger 7EL segment. This characteristic limited the use of these new recombinants in common wheat breeding programs.

One of the objectives of our study was to develop new recombinant lines with interstitial translocations of the 7EL chromosome (with *Lr19* and without *Y*) to test if the high rate of self-elimination in *Lr19-149* were the result of the presence of a second segregation distortion factor linked to *Lr19* as suggested by Prins et al. (1997), or if it were due to an altered chromosome structure present in that particular line. The second objective of this study was to use this same 7E chromosome segment for durum wheat improvement. The increase of yellow pigment caused by *Y* would be a desirable trait in durum wheat. An additional goal of the incorporation of the 7EL arm segment into durum wheat cultivars was to provide protection to the new leaf rust race present in California and Mexico, which is virulent on a large proportion of the durum wheat cultivars.

Materials and methods

Plant materials

The 7DS·7DL–7EL chromosome translocation no. 1 developed by E.R. Sears (1972a, 1972b) was introgressed into hard white spring wheat cultivar ‘Pavon 76’ by backcrossing, and then crossed and backcrossed with a ‘Pavon’ line carrying the *ph1b* deletion. The homozygous *ph1b*–heterozygous 7DS·7DL–7EL plants were selected and self-pollinated. The resulting progenies were screened by genomic in situ hybridization (GISH) as described below, and plants with recombinant 7E–wheat homoeologue chromosomes were selected, grown, and crossed and backcrossed to ‘Opata’.

For the tetraploid wheat experiments, hexaploid lines with recombinants 1-22 and 1-23 were selected and crossed to durum ‘Aconchi’. In subsequent generations, backcrosses were made to ‘Aconchi’ and breeding lines UC1112 and UC1113. The last two breeding lines have an excellent yield potential but are susceptible to the new leaf rust race from Mexico and have low levels of yellow pigment and unacceptable pasta color. In backcrosses done at University of California (UC) Riverside, 1-22 and 1-23 were backcrossed twice to ‘Aconchi’ and twice to UC1113, with selection in each generation by GISH. At UC Davis, only line 1-23 was used in backcrosses to UC1112 and UC1113, and molecular markers were used to select heterozygous plants in each generation. The BC₄F₂ progeny from UC1113 and BC₃F₂ progeny from UC1112 were analyzed for yellow pigment, whereas BC₃F₂ progeny from UC1113 was used for the leaf rust resistance tests. The BC₄ homozygous lines for the 1-22 and 1-23 recombinants were selected after two backcrosses into ‘Aconchi’, followed by two backcrosses with UC1113 at UCR. Backcrosses of 1-23 into UC1112 and UC1113 will be further advanced to BC₆F₂ to develop isogenic lines for this segment at UC Davis.

Cytology

All analyses were done on squash preparations of root tips collected into ice water from germinating seedlings and fixed in a 3:1 (v/v) mixture of absolute alcohol and glacial acetic acid. The GISH screening for recombinant chromosomes was the same as described before (Lukaszewski et al. 2004), with *L. ponticum* DNA labeled with digoxigenin-11-dUTP, serving as a probe and hybridized to chromosomes with sheared wheat DNA serving as a block, with probe to block ratio of about 1:150. In the last round of screening for recombinant chromosomes, the roles of the probe and block were reversed, and the GISH method used was that of Masoudi-Nejad et al. (2002). In all instances, signals were visualized by anti-digoxigenin-fluorescein and preparations were counterstained with propidium iodide and observed under epi-fluorescence. Labeling of DNA by nick translation and signal visualization were performed using standard kits from Roche Applied Science, following manufacturer’s instructions. Sequential C-banding–GISH experiments to identify wheat chromosomes with 7EL segments were done according to Masoudi-Nejad et al. (2002).

Tests for resistance to *Puccinia triticina*

Three BC₃F₂ families from the UC1113 backcross program were sent to the Cereal Disease Laboratory (St. Paul, Minn., USA) for evaluation with *Puccinia triticina* Eriks. (wheat leaf rust) isolates from California and Mexico. Isolates CA 2.1 and MX 11.1 are highly virulent to durum wheat cultivars and are classified as BBB-10,

14b, 20, 23, 33, 41 (Long and Kolmer 1989), based on virulence to isogenic lines of Thatcher wheat, which differ for single-leaf rust resistance genes. These isolates belong to the same race collected in Mexico and that was previously described by Singh et al. (2004). This new leaf rust race is virulent on most of the current California durum wheat cultivars, including the backcross recurrent parent UC1113.

Nine to 14 plants from each of the tetraploid UC1113 BC₃F₂ families were tested for seedling resistance to each of the leaf rust races, as previously described (Kolmer et al. 2003). Plants were grown in a greenhouse set at 18–21°C, with 8 h of metal halide supplemental lighting at 400–450 $\mu\text{Em}^{-2} \text{s}^{-1}$ at bench level. Seedlings were inoculated with leaf rust races CA 2.1 and MX 11.1. For each race, an oil-spore mixture was atomized onto the seedling plants. The plants were allowed to dry and were then placed in a dew chamber for 18 h, with no light. After incubation, the plants were placed in a greenhouse set at 18–21°C, with supplemental metal halide lighting. Infection types were scored 12 days after inoculation as described before (Long and Kolmer 1989).

Evaluations of the recombinant hexaploid lines for resistance to leaf rust were done at UC Riverside, with leaf rust isolate BBBQ obtained from Dr. D.L. Long, Cereal Disease Research Unit, USDA. This isolate is virulent on ‘Pavon’, ‘Opata’, ‘Aconchi’, and UC1113 but not on lines carrying *Lr19*.

RFLP and PCR marker identification

The F₂ plants segregating for the recombinant chromosomes selected by GISH were characterized with RFLP markers (Dubcovsky et al. 1996) and STS marker (Prins et al. 2001) previously mapped on homoeologous group seven. The CDO and WG clones were provided by M. Sorrells (Cornell, USA), PSR markers by M. Gale (John Innes, UK), and MWG markers by A. Graner (Gatersleben, Germany). Based on these molecular markers, graphical genotypes were elaborated for each recombinant line. These recombinant lines were also evaluated in progeny tests for segregation distortion of the *L. ponticum* alleles (*Sdl*), for yellow pigment (*Y*, see below), and for resistance to leaf rust (*Lr19*). Each of these traits was mapped as a Mendelian locus relative to the molecular markers in the recombinant lines.

Spectrophotometric determinations of yellow pigment

For the evaluation of the yellow pigment content on the recombinant lines, we used a modified version of the AACC 14-50 procedure (AACC 1997). Briefly, 2 g of grain were ground in a mortar, and pigments were extracted from 0.5 g of integral flour with three volumes of water-saturated *n*-butanol. The spectrophotometric determinations of yellow pigment were carried out at

448 nm and compared among lines carrying the 7EL segment and sister lines without this alien segment.

High-performance liquid chromatography analyses

For the high-performance liquid chromatography (HPLC) analyses of the yellow pigments, 40 mg sodium carbonate was added to 0.4 g of wheat integral flour. Samples were extracted with 0.8 ml methanol/tetrahydrofuran [1:1 (v/v)] by shaking for 2 h, and then centrifuged 2 min at 13,000 rpm. The supernatant was filtered with 0.45 μm nylon filter, and 20 μl of the solutions was injected into PerkinElmer HPLC system (Series 4 liquid chromatograph) equipped with a Shimadzu detector (SPD-6AV UV–Vis spectrophotometric). Separation was done on a 250 $\times$ 4.6 column (Waters Sepherisorb ODS-2), with a particle diameter of 5 μm and 1.0 ml/min of 100% methanol as mobile phase. The eluted pigments were detected using a wavelength of 447 nm.

Individual plants were used as experimental units in both the spectrophotometric quantifications and the HPLC experiments. Plants were grown in separate pots in a greenhouse with average temperatures of 20–25°C and a 16-h photoperiod. Pigment extracts were made from pools of 10–15 seeds per plant.

In hexaploid wheat, the HPLC peak heights were compared using six plants with the 7EL segment from recombinant lines 1-2, 1-6, 1-20, 1-21, 1-22, and 1-23 (Table 1) and six sister lines without the 7EL segment. The data were analyzed in a randomized complete block design, with recombinant families used as blocks. For the durum wheat HPLC experiment, BC₂F₂ seed pools from four 7E/7A heterozygous BC₂UC1112 plants were compared with four homozygous BC₂ sister lines homozygous for the absence of the 7EL segment.

Statistical analyses

SAS software, version 8 (SAS Institute 2001), was used for all statistical analyses. Normality of the residuals was tested using the Shapiro–Wilk test and the homogeneity of variances using Levene’s test (SAS Institute 2001). The Tukey additivity test (SAS Institute 2001) was used in the randomized complete block design to test for the absence of multiplicative effects. Means are reported followed by their standard error.

Results

Molecular characterization of wheat—*L. ponticum* recombinants by C-banding and GISH

A total of 97 recombinant chromosomes carrying *L. ponticum* 7EL segments smaller than the original 7D.7E#1 line were identified among 1,000 plants screened by GISH. Figure 1 shows a subset of the

recombinant chromosomes with shorter terminal 7EL segments that resulted from the elimination of the proximal region of 7EL (1-32, 1-49, 1-64, 1-36, and 1-22) or with interstitial 7EL segments resulting from the elimination of the terminal region of the 7EL segment from 7D.7E#1 (1-43, 1-47, and 1-48).

A subset of 37 recombinant lines was characterized with ten RFLP and one STS marker (Table 1). Lines with long terminal 7EL segments, with breakpoints either in the middle or the proximal half of the 7E segment, were not included in this analysis, because these segments were unlikely to have the desired recombination point between *Lr19* and *Y*. Earlier work by Marais et al. (2001) indicated that *Y* was distal to *Lr19*, and both loci were located in the distal part of the chromosome. Eighteen lines were selected with small terminal 7EL segments and 19 lines with interstitial 7EL segments. These last lines were generated by recombination events in the distal region of the 7EL segment from 7DS·7DL–7EL#1. These 19 recombinant chromosomes include 1-28.1, which is a secondary recombinant derived from 1-28 by an additional recombination event between the *Xcdo53* and *Xpsr129* loci.

Most of the recombination events occurred between the targeted 7EL and 7DL segments. However, it was apparent in GISH screening that other group 7 chromosomes of wheat also must have participated in exchanges. A subset of the suspected non-7D recombinants was screened by sequential C-banding-GISH and RFLP markers and showed that approximately 20% of the lines were recombinants between 7EL and 7AL chromosome arms (e.g., 1-1, 1-20, 1-21, 1-22, 1-23, 1-47, and 1-64). No recombinants were found between 7EL and 7BL chromosome arms. Among the recombinant lines where the distal 7EL region was replaced by a 7AL segment, recombinant lines (e.g., nos. 1-1 and 1-47) included fragments from three different chromosomes (7DS·7DL–7EL–7AL). The presence of a duplicated 7AL segments in these lines may limit their use in cultivar development.

Lines with recombination events between the same flanking markers were grouped, resulting in the eleven different patterns presented in Table 1. The number of recombination events observed between markers (Table 1, second line) was roughly proportional ($r=0.75$) to the genetic distances between the same markers in the genetic map of *Triticum monococcum* (Dubcovsky et al. 1996). These results suggest that the recombination rate between wheat and *Lophopyrum* homoeologous chromosomes can be used to estimate the genetic distances among markers and traits in this set of recombinant lines.

Mapping of *Sdl*, *Lr19*, and *Y* genes

Sdl

The original 7DS·7DL–7EL#1 recombinant chromosome showed preferential transmission due to the

presence of the segregation distortion locus *Sdl*. The F_2 progeny from a heterozygous 7DS·7DL–7EL#1 line showed 32 homozygous 7EL lines (36.6%), 49 heterozygous (55.7%), and only seven homozygous for the absence of the 7EL chromatin. This represents a significant departure from the expected 1:2:1 ratio (χ^2 , $P=0.0005$).

The lines carrying the proximal region of the original 7EL#1 segment also showed preferential transmission of the 7E chromatin through the female gametes (30 with 7EL:13 without 7EL; 1:1 ratio χ^2 , $P=0.01$) but not through the male gametes (18 with 7EL:11 without 7EL; 1:1 ratio χ^2 , $P=0.19$). The 17 lines carrying small distal 7EL segments showed normal segregation for the recombinant chromosomes. The composite female transmission rate of these recombinants was not significantly different from the expected 1:1 segregation (48 with 7EL:54 without 7EL; χ^2 , $P=0.55$).

Based on the previous results, we concluded that *Sdl* is located in the proximal half of the 7EL chromatin within the 7DS 7DL–7EL#1 line and proximal to *Xpsr129*. Unfortunately, because of the specific objectives of this study, we only selected lines with recombination events in the distal region of the 7EL segment, which are not the most appropriate for a more precise mapping of *Sdl*.

Lr19

The *Lr19* gene was mapped between RFLP loci *Xwg420* and *Xmwig2062*, completely linked to the dominant PCR marker STSLr19-130 (Table 1). Four recombinants were observed between *Lr19* and *Xwg420*, but only one with *Xmwig2062*, suggesting that *Lr19* is likely located in the distal part of this interval, closer to *Xmwig2062* than to *Xwg420* (Table 1).

Y

The heterozygous recombinant lines were self-pollinated, and the F_2 progeny for each line was classified into positive 7EL and negative 7EL, using different molecular markers depending on the position of the recombination point. The recombinant lines were considered to have the 7E *Y* allele if the spectrophotometer scores of the 7EL positive lines were significantly higher than those from the negative plants. If no significant differences were detected, the recombinant lines were considered to have the wheat allele. All the lines carrying terminal 7EL segments showed increased yellow pigment relative to the corresponding negative controls, indicating that *Y* was located on the distal side of the 7EL segment (Table 1).

Recombinant line 1-32 showed a very small distal 7EL segment by GISH, which did not retain any of the 7E markers used in this study, and therefore, the F_2 progeny could not be classified into positive and negative lines using molecular markers. However, pigment extracts from pools of ten seeds from three different

Table 1 Molecular characterization of 37 recombinant wheat—*Lophopyrum ponticum* 7L arms

Number of recombination events	CDO 673	CDO 53	PSR 129	PSR 547	WG 420	Lr19	STS Lr19-130	MWG 2062	PSR 148	PSR 680	PSR 121	PSR 687	Y
	1	11	1	16	4	0	1	0	2	0	0	1	Y
1-2	+	+	+	+	+	+	+	+	+	+	+	+	+
1-5, 1-7, 1-10,	-	-	-	-	-	-	-	-	-	-	-	-	+
1-16, 1-21, 1-24	-	-	-	-	-	-	-	-	-	-	-	-	+
1-6, 1-3, 1-9, 1-11,	-	-	-	-	-	-	-	-	-	-	-	-	+
1-15, 1-23	-	-	-	-	-	-	-	-	-	-	-	-	+
1-20, 1-22	-	-	-	-	-	-	-	-	-	-	-	-	+
1-49, 1-64	-	-	-	-	-	-	-	-	-	-	-	-	+
1-32	-	-	-	-	-	-	-	-	-	-	-	-	+
1-96	+	+	+	+	+	+	+	+	+	+	+	+	+
1-3, 1-47	+	+	+	+	+	+	+	+	+	+	+	+	+
1-4, 1-12, 1-19, 1-25,	+	+	+	+	+	+	+	+	+	+	+	+	+
1-26, 1-27, 1-28,	+	+	+	+	+	+	+	+	+	+	+	+	+
1-43, 1-48	+	+	+	+	+	+	+	+	+	+	+	+	+
1-29	+	+	+	+	+	+	+	+	+	+	+	+	+
1-1, 1-13, 1-14, 1-30	+	+	+	+	+	+	+	+	+	+	+	+	+
1-17	+	+	+	+	+	+	+	+	+	+	+	+	+
1-28, 1	+	+	+	+	+	+	+	+	+	+	+	+	+

A plus sign indicates presence of the 7EL segment and a minus sign its absence. The second line indicates the number of recombination events observed between the two flanking markers listed in the upper line (estimate of genetic distance)

homozygous 7EL 1-32 plants selected by GISH showed significantly higher ($P=0.00003$) spectrophotometer scores (0.35 ± 0.01) than four sister plants homozygous for the absence of the 7EL segment (0.16 ± 0.01). The observed values indicate that the presence of the 7EL segment in this line was associated with a 116% increase in the spectrophotometer scores relative to the sister lines without the alien segment, confirming the presence of the *L. ponticum* allele of *Y* in 1-32 and its mapping location distal to *Xpsr687*.

HPLC characterization of lines with and without the *Y* gene

The retention times of the HPLC peaks were the same in the lines carrying the 7E *Y* allele and in the non-7EL sister lines in both the tetraploid and hexaploid wheat experiments (Fig. 2). Peak 4 (retention time: 2 min, 50s; Fig. 2), is a system peak, which was observed in the control samples including only the mobile phase and the extraction buffer. As expected, non-significant differences in the height of peak 4 were detected between the samples carrying the 7EL segment and those without the alien segment in both the hexaploid ($P=0.96$) and tetraploid experiment ($P=0.45$).

In the hexaploid wheat experiment (Fig. 2a, b), no differences were detected among the six different recombinant families (including both 7EL and non-7EL siblings) for any of the peaks analyzed. In addition, no significant multiplicative effects with the 7E segment were detected indicating that the 7E segment has similar effects across recombinant lines. However, when seed extracts from lines with the 7EL segment were compared with those from the sister lines without the alien segment, significant differences in height were detected for peaks 2 ($P=0.003$, 85% increase), 3 ($P=0.03$, 50% increase), 5 ($P=0.002$, 76% increase), and 6 ($P=0.003$, 85% increase). Peak 1 showed no significant differences in height between the isogenic lines ($P=0.64$).

In the tetraploid experiment using the UC1112 line (Fig. 2c, d) the peaks were, in general, higher than in the hexaploid wheat (Fig. 2a, b), as expected by the higher levels of yellow pigment observed in the seeds of durum wheat relative to common wheat. When the durum plants with the 7EL segment were compared with their sister plants without the alien segment, significant differences in height were detected for peaks 3 ($P=0.001$, 36% increase), 5 ($P=0.008$, 72% increase), 6 ($P=0.006$, 87% increase), 7 ($P=0.05$, 239% increase), and 8 ($P=0.05$, 213% increase). As in the hexaploid lines experiment, peak 2 was higher in the durum plants with the 7EL segment relative to the sister plants without the 7EL segment, but the differences were not significant ($P=0.14$, 40% increase). Peak 1 showed no significant differences in height between the durum lines with and without the 7EL segment ($P=0.47$).

In summary, no new peaks were detected in the lines with the 7EL segment, and most of the peaks showed

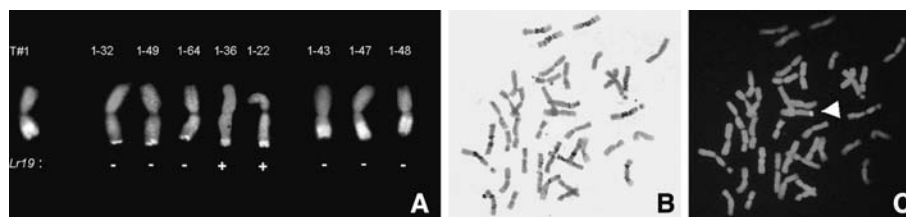


Fig. 1 The E.R. Sears' *Lophopyrum ponticum* transfer 7D.7E#1 (T#1, left) and its selected *ph1b*-induced recombinants visualized by genomic in situ hybridization (GISH). *L. ponticum* DNA served as a probe and wheat DNA as a block (a). The *Lr19* gene is absent (–) in the three recombinants with shortest terminal 7E segments (1-32, 1-49, and 1-64). None of the three recombinants with terminal wheat segments (1-43, 1-47, and 1-48) has *Lr19*; Sequential C banding (b) and GISH (c) on a bread-wheat homozygote for transfer 1-23 identifies chromosome 7A as the recipient of the *L. ponticum* segment. The 7EL segment in 1-23 has the *Lr19* and *Y* genes

increased height when the alien segment was present relative to the sister lines without the alien segment.

Selected lines for pasta-wheat breeding

Two 7EL.7AL recombinant lines carrying short 7EL distal segments including *Lr19* and *Y* were selected for introgression into durum wheat to improve leaf rust resistance and yellow pigment. Recombinant chromosome 1-22 had the shortest 7EL segment that still carried *Lr19* among the 7EL.7AL translocations, with a recombination event between *Xwg420* and *Lr19*. Recombinant line 1-23 had a slightly larger 7EL segment translocated to 7A resulting from a recombination event between RFLP loci *Xpsr547* and *Xwg420*.

Both recombinant chromosomes have been introgressed into durum wheat by four backcrosses, followed by self-pollination and selection of homozygous lines.

The first two backcrosses were to 'Aconchi', the last two to UC1113. Isogenic lines of recombinant 1-23 are being developed for the UC breeding lines UC1112 and UC1113 by six backcross generations for future studies on the effect of this 7EL chromosome segment on yield.

Pigment extracts from pools of 10–15 BC₃F₂ seeds from three different BC₃ UC1112 plants heterozygous for the 7EL segment showed spectrophotometer scores (0.39 ± 0.03) 56% higher ($P=0.002$) than those from four sister lines without the 7EL segment (0.25 ± 0.01). A similar result was observed for UC1113. Pigment extracts from BC₄F₂ seeds from four BC₄ UC1113 plants heterozygous for the 7EL segment showed spectrophotometer scores (0.41 ± 0.01) 52% higher ($P=0.0001$) than those from four sister lines without the alien segment (0.27 ± 0.01).

Resistance to leaf rust isolates CA 2.1 and MX 11.1 was tested for UC1113 BC₃F₂ seeds from two 1-23 BC₃ families segregating for the 7EL segment (4654-3 and 4654-9) and one homozygous for the absence of the alien segment (4654-2) (Table 2). All plants from the 4654-2 BC₃ family homozygous for the absence of the 7EL were susceptible to both leaf rust isolates. However, both BC₃F₂ families segregating for the 7EL segment also segregated for resistance to isolates CA 2.1 and MX 11.1 (Table 2). This result indicates that *Lr19* is effective against this race of leaf rust.

The 7EL segment in 1-23 has normal transmission in durum wheat. This was confirmed in 59 backcross

Fig. 2 High-performance liquid chromatography profiles of methanol/tetrahydrofuran (1 + 1, v/v) extracted pigments from seeds of common wheat 'Pavon' (a, b) and durum wheat UC1112 BC₂F₂ lines (c, d) recombinant substitution backcross lines. a, c Lines without the distal 7EL segment. b, d Lines with the distal 7EL segment carrying the *Y* gene. Peak number 4 is a system peak observed in the control solvent sample

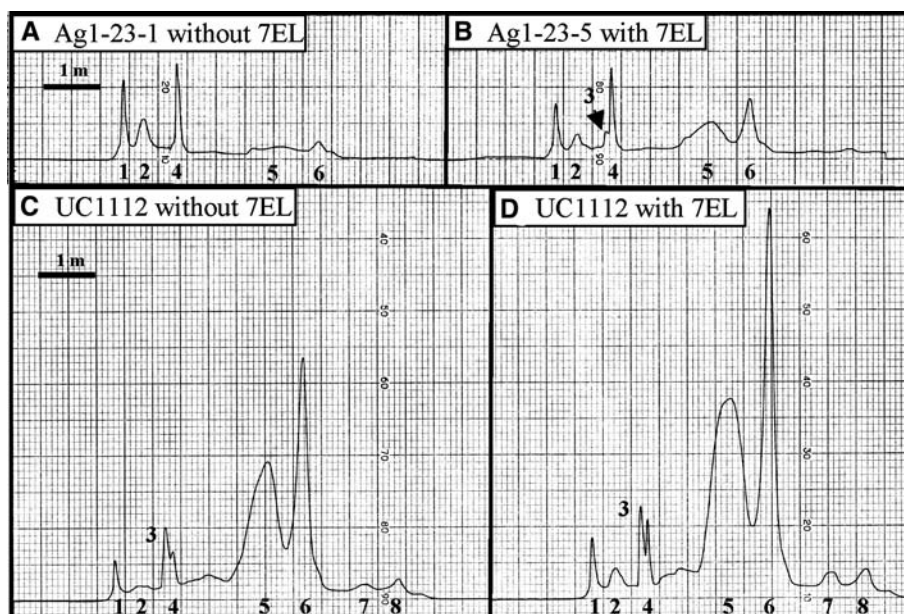


Table 2 Leaf rust resistance to isolates CA 2.1 and MX11.1 in a durum genetic background

Genotype	Isolate CA 2.1 ^a	Isolate MX11.1 ^a
Thatcher (6x)	3+	3+
Thatcher- <i>Lr19</i> (6x, 7EL)	0;	0;
UC1113 (4x)	3+	3+
UC1113 BC ₃ F ₂ ^b (not 7EL) 4645-2	3+ (13 plants)	3+ (14 plants)
UC1113 BC ₃ F ₂ (7EL) 04654-3	0; (6 plants), 3+ (7 plants)	0; (6 plants), 3+ (3 plants)
UC1113 BC ₃ F ₂ (7EL) 04654-9	0; (7 plants), 3+ (3 plants)	0; (7 plants), 3+ (4 plants)

^a0 Immune, ; necrotic flecks, 3 moderate size pustules without chlorosis necrosis, + large uredinia

^bBC₃F₂ plants from the cross UC1113*3/recombinant line 1-23

progeny generated using female gametes (29 with 7EL:30 without 7EL; 1:1 ratio χ^2 , $P = 0.90$) as in 75 BC₃F₂ lines (18 homozygous 7EL:38 heterozygous:19 homozygous for the absence of 7EL; *Xpsr687* 1:2:1 ratio χ^2 , $P = 0.98$). Segregation rates of recombinant 1-22 in durum wheat also appear normal. The female transmission rate was 50% among 30 backcross progeny and the segregation among ten BC₄F₂ plants was two homozygous 7EL, five heterozygous, and three homozygous wheat 7AL plants.

Selected line for bread-wheat breeding

Common wheat line 1-96 showed a 7EL segment including *Lr19* but not *Y*. This was the desired combination to improve leaf rust resistance in bread wheat without negatively effecting flour color. Line 1-96 has an interstitial 7EL segment resulting from a 7EL—7DL recombination event between *Lr19* and RFLP locus *Xmwg2026* (Table 1).

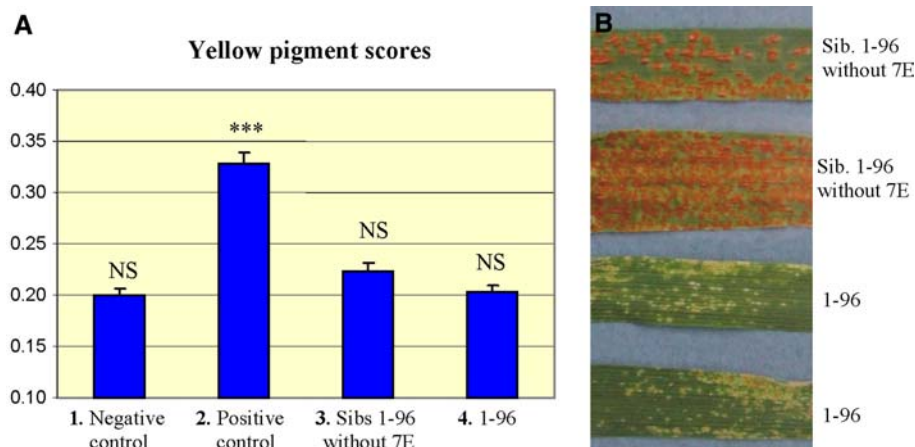
Seed pool spectrophotometer scores from eight BC₁F₂ plants carrying the recombinant 1-96 chromo-

some were not significantly higher than those from seven sister lines without the 7EL segment. In addition, the eight 1-96 plants were not significantly different from an additional negative control line without the 7EL segment, but showed significantly lower levels of yellow pigment than the positive control line with the distal 7EL segment ($P < 0.0001$, Fig. 3a). These results confirmed that 1-96 did not carry the *Y* gene, as expected by the distal location of *Y* and the location of the recombination event in 1-96 (Table 1).

All BC₂ plants carrying the 1-96 recombinant chromosome were resistant to leaf rust isolate BBBQ, whereas their sister plants without the 7EL chromosome segment were uniformly susceptible (Fig. 3b). These results confirmed that the recombinant chromosome 1-96 has the *Lr19* gene

Analysis of the recombinant chromosome 1-96 segregation using RFLP probe PSR129 confirmed the presence of the *Sdl* allele within the 7EL segment in this line. The 7EL segment was present in 29 out of 32 F₂ plants from the self-pollination of a heterozygous 1-96 plant, indicating that this alien segment was preferentially transmitted (3:1 ratio χ^2 , $P = 0.04$).

Fig. 3 a Yellow pigment spectrophotometer scores from seed pool extract. 1 Negative control Average of eight lines without the distal 7EL segment, 2 Positive control average of eight lines with a distal 7EL segment, 3 Sibling lines average of seven plants homozygous for the absence of the 1-96 recombinant chromosome, 4 1-96 average of eight plants carrying recombinant chromosome 1-96 (distal 7EL segment). Plants used for lanes 3 and 4 are selections from the progeny of the same heterozygous plant. **b** Reactions of the same lines to leaf rust race BBBQ



Discussion

Leaf rust resistance

The per-acre value of wheat is low, and to be profitable, it must be grown efficiently with minimum applications

of pesticides. The use of resistance genes provides an efficient and environmentally friendly way to achieve this objective. Unfortunately, the effectiveness of resistance genes in the cultivated wheat gene pool is limited due to the genetic variation and dynamic nature of pathogen populations. This is particularly true for leaf rust populations that can respond very quickly to the introduction of resistance genes in wheat.

The list of the leaf rust resistance genes available for breeding can be expanded by the use of wild *Triticeae* species. These genes can be transferred to wheat by the *ph1b* mutation, which induces homoeologous recombination of alien chromosome with wheat (Sears and Okamoto 1958). The leaf rust resistance gene *Lr19* from *L. ponticum* was an early example of this strategy (Sears 1972a; Sharma and Knott 1966).

Virulence to the *Lr19* gene is rare (McIntosh et al. 1995), but an *Lr19*-virulent leaf rust isolate was found in Mexico in the bread-wheat cultivar 'Oasis 86', which carries the 7EL translocation (Huerta-Espino and Singh 1994). However, results from this study showed that *Lr19* is still effective against the new leaf rust race detected in Mexico and California (Singh et al. 2004), which is highly virulent to durum wheat. Introgression of *Lr19* into durum wheat germplasm might still be a valuable breeding objective for durum breeding programs in this region, at least in the near future.

The new durum leaf rust race that appeared in northwestern Mexico has caused estimated losses of US \$32 million between 2001 and 2003 (Singh et al. 2004). Virulence tests of isolates from 243 fields determined that the same race was present on all the susceptible durum wheat analyzed during these three years (Singh et al. 2004). A major concern about this new race is that most of the durum cultivars from the United States, Canada, and several other countries are highly susceptible. In addition, most of the CIMMYT elite durum germplasm (93% of 1,160 accessions) were also highly susceptible to this leaf rust race (Singh et al. 2004).

Lr19 is a seedling resistance gene and if introduced alone, it would likely have limited durability. To extend the usefulness of this gene, it would be better to combine it with other seedling resistance genes or with slow rusting genes such as the ones present in 'Yavaros C79' (Singh et al. 2004). Slow rusting genes, such as *Lr34* (Dyck 1987; Schnurbusch et al. 2004) or *Lr46* (William et al. 2003), have been used successfully in common wheat to provide durable resistance.

Seed yellow pigment

In spite of its broad resistance spectrum, the *Lr19* gene has been used only in a small number of commercial wheat cultivars. The linkage with the yellow pigment gene *Y* has limited its use in commercial bread-wheat cultivars, in which the white color of the flour is a required characteristic.

The opposite is true for durum wheat cultivars, where a bright yellow color is a very important criterion in the evaluation of the quality for pasta production. The final yellow color of the pasta is the result of the accumulation of a mixture of natural carotenoid pigments present in the seeds and of their oxidative degradation by lipoxygenase (*Lox*) activity (Troccoli et al. 2000). It was also suggested that, in addition to the carotenoids, some other compounds contribute to the yellow pigments present in the durum wheat seeds (Hentschel et al. 2002).

The carotenoid pigments of durum wheat include free lutein ($\approx 85\%$), lutein monoester ($\approx 10\%$), and lutein diester ($\approx 5\%$) (Lepage and Sims 1968). These pigments are antioxidants, which reduce the oxidative damage to biological membranes by scavenging peroxyradicals such as those involved in certain human diseases, in the aging processes, and in the degradation of food quality. Therefore, carotenoid pigments contribute to an increased nutritional value of the pasta products (Bast et al. 1996).

Cenci et al. (2004) have recently mapped three enzymes of the carotenoid biosynthesis pathway (phytoene synthase, phytoene desaturase, and ζ -carotene desaturase), but none of them map to chromosomes of the homoeologous group-7. The same is true for the three lipoxygenase genes (*LoxA*, *LoxB*, and *LoxC*), which have been mapped in homoeologous groups 4 and 5 (Graner and van Mechelen 1993; Hessler et al. 2002). These results indicate that the differences in color observed in the 7EL translocation lines carrying *Y* is not the direct effect of a polymorphism in the *cis*-regulation of these enzymes.

The HPLC analysis of the seed pigments demonstrated that the presence of the 7EL distal chromosome segment carrying the *Y* gene did not result in the presence of new peaks. The main difference was an increase in the abundance of the preexisting pigments in the lines with the 7E *Y* allele relative to the lines with the wheat allele. This increase was observed in both hexaploid lines with low basal levels of pigments and tetraploid lines with relatively higher levels of yellow pigments (Fig. 2). The large number of peaks affected by the introduction of the 7EL segment suggests that the *Y* gene is either a more efficient enzyme in the early steps of the carotenoid biosynthetic pathway or a regulatory factor that affects several steps of this biosynthetic pathway.

Orthologues to the *L. ponticum* *Y* gene seem to be also present in tetraploid and hexaploid wheat. The QTLs for yellow pigment have been mapped to the distal end of the long arms of homoeologous group 7 in both bread- and pasta-wheat mapping populations that are not segregating for the 7EL translocation. In hexaploid wheat, a QTL for flour color was identified linked to *Xcdo347* (Parker et al. 1998), one of the most distal markers on chromosome arm 7AL (Nelson et al. 1995). This QTL explained almost 60% of the genetic variation in flour color in a single seed descent population from the cross 'Schomburgk' $\times$ 'Yarralinka' (Parker et al. 1998). Additional QTLs for flour yellow color were

identified in the distal region of chromosome arm 7AL in the double haploid populations 'Cranbrook' × 'Halberd' and 'Sunco' × 'Tasman' (Mares and Campbell 2001). The peak of the 7AL QTL on the first population included the locus *Xpsr680* also mapped closely linked to *Y* on the 7EL segment in our recombinant lines (Table 1). A QTL for flour yellow color was also identified in the colinear region of chromosome 7BL in the double haploid population 'CD87' × 'Ketpwa'. This QTL explained 10% of the variation of flour yellow color and was mapped between RFLP loci *Xpsr680* and *Xpsr121* (Mares and Campbell 2001).

In tetraploid wheat, QTLs for yellow pigment were identified at the distal ends of the long arms of chromosomes 7A and 7B (Elouafi et al. 2001). These QTLs explained 13% and 53% of the variation in yellow pigment in the tetraploid backcross mapping population Omrabi5/Omrabi5 × *T. dicoccoides* 600545 (Elouafi et al. 2001). The presence of allelic variation for the *Y* gene in tetraploid wheat suggests that different effects on yellow pigment levels might be obtained by the introgression of the *Y-7E* allele in different genetic backgrounds. These will depend on the effects of the *Y-7E* allele relative to the *Y-7A* allele that is being replaced by the homoeologous alien segment.

The comparative mapping results discussed above suggest that *Y* is an important gene for the accumulation of seed yellow pigments not only in *L. ponticum* but also in tetraploid and hexaploid wheat.

Segregation distortion

The original T4 translocation and Sears recombinant chromosome 7DS·7DL–7EL#1 (used as starting point for our recombinant lines) showed preferential transmission associated with the presence of the segregation distortion locus *Sd1*. This locus was mapped proximal to *Lr19* (Zhang and Dvorak 1990), between the centromere and *Xpsr165* (Prins et al. 1996). We observed a similar preferential transmission in the recombinant lines carrying the proximal part of the 7DS·7DL–7EL#1, confirming its proximal location.

An additional segregation distortion locus, designated *Sd2*, was proposed to explain the self-elimination in pollen of the 7EL/7BL recombinant line *Lr19*-149 (Marais et al. 2001; Prins and Marais 1998; Prins et al. 1997). *Lr19*-149 (first designated 88M22-149) was produced by *ph1b*-induced homoeologous recombination and a 7E segment was apparently relocated to chromosome 7BL, perhaps in a double crossover event (Prins and Marais 1998). The 7EL segment present in *Lr19*-149 includes *Lr19* and RFLP loci *Xpsr129* and *Wsp-D1c* but not *Y*. Four secondary recombinants from *Lr19*-149, including shorter 7EL segments, showed very comparable self-elimination rates to the original *Lr19*-149 line (Marais et al. 2001).

An alternative explanation for the self-elimination of *Lr19*-149 in pollen is the presence of an altered chro-

mosome structure resulting from this particular translocation of 7EL to 7BL (Prins et al. 1997). This alternative hypothesis was partially supported by the presence of a duplicated region including the *Wsp-B1* locus. Both the *Wsp-B1* and the *Wsp-E1* were detected in this line (Prins et al. 1997). The segregation rates of the recombinant chromosomes produced in this study support the second hypothesis. None of the 18 terminal segments of 7EL in wheat chromosomes 7DL or 7AL (Table 1) showed self-elimination in pollen. These lines included six recombinants (Table 1) that have retained *Xpsr129*, the most proximal marker present in the 7EL/7BL *Lr19*-149 line. The unfavorable selection of the 7EL segment in *Lr19*-149 is most likely the result of some deletion or duplication originated in this particular interstitial translocation rather than the effect of a second segregation distortion locus within the 7EL segment.

Concluding remarks

The normal transmission of the distal 7EL recombinant lines 1-22 and 1-23 will facilitate the incorporation of the *Lr19* and *Y* genes into durum breeding programs. However, the effect of the incorporation of the *Y-7EL* allele on yellow pigment in different durum germplasm is difficult to predict a priori. Pigment content comparison between 7EL and non-7EL lines in early generations of segregating populations would be necessary to determine the relative value of the *Y-7EL* and *Y-7AL* allele present in a particular genetic background.

In common wheat breeding programs, the introgression of the recombinant 1-96 7EL segment will be favored by the presence of *Sd1*, which increases the transmission of *Lr19*. This particular segment is expected to increase leaf rust resistance without affecting the white color of the flour and should provide an additional tool to wheat breeders. The available STS marker (Parker and Langridge 2000) can be used to accelerate the introgression of this segment by marker-assisted selection.

The development of isogenic lines for the distal 7EL segment in durum wheat lines UC1112 and UC1113 and for the proximal 7EL segment in hexaploid line 1-96, may provide valuable information for the location of the 7EL chromosome region associated with yield increases under irrigated conditions (Monneveux et al. 2003; Singh et al. 1998).

Acknowledgements J. Dubcovsky acknowledges financial support from USDA–CSREES competitive grant IFAFS 2001-04462 and NSF–Americas Grant INT-0404590. The authors express their gratitude to Mike Gale, A. Graner, and M. Sorrells for supplying the RFLP clones.

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