Lr46: A Gene Conferring Slow-Rusting Resistance to Leaf Rust in Wheat

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ABSTRACT

Singh, R. P., Mujeeb-Kazi, A., and Huerta-Espino, J. 1998. *Lr46*: A gene conferring slow-rusting resistance to leaf rust in wheat. *Phytopathology* 88:890-894.

Wheat (*Triticum aestivum*) cultivar Pavon 76 carries slow-rusting resistance to leaf rust that has remained effective in Mexico since its release in 1976. 'Pavon 76' was crossed with two leaf rust-susceptible wheat cultivars, Jupateco 73S and Avocet S, and between 118 and 148 individual F_2 plant-derived F_3 and F_5 lines were evaluated for adult-plant leaf rust resistance at two field sites in Mexico during different seasons. Evaluation of F_1 plants and parents indicated that the slow-rusting resistance was partially dominant. Segregation in the F_3 and F_5 generations indi-

cated that the resistance was based on two genes with additive effects. Monosomic analysis was carried out to determine the chromosomal locations of the resistance genes. For this purpose, two or three backcross-derived cytogenetic populations were developed by crossing 'Pavon 76' with a monosomic series of adult-plant leaf rust-susceptible cultivar Lalbahadur. Evaluation of such BC_2F_3 and BC_3F_3 lines from 16 confirmed 'Lalbahadur' monosomics indicated that one slow-rusting gene was located in chromosome 1B of 'Pavon 76'. This gene, designated as *Lr46*, is the second named gene involved in slow-rusting resistance to leaf rust in wheat.

Additional keywords: *Puccinia recondita*.

Leaf rust, caused by *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* (Eriks. & E. Henn.) D.M. Henderson, is an important disease of wheat (*Triticum aestivum* L.) worldwide. Genetic resistance is the most economical and environmentally safe measure to reduce crop losses. Sayre et al. (13) estimated the genetic progress in reducing grain yield losses through breeding for leaf rust resistance in International Maize and Wheat Improvement Center (CIMMYT) germ plasm-derived Mexican wheat cultivars. Average grain yield losses due to leaf rust of these cultivars, released between 1966 and 1988, ranged between 6.6 and 62.7% for immune 'Oasis 86' and most susceptible 'Jupateco 73', respectively. 'Pavon 76', released in 1976, displayed slow-rusting or partial resistance (3,10) that has remained effective in Mexico and other parts of the world where it has been released and grown. Relative area under the disease progress curve of 'Pavon 76' was only 9% of 'Jupateco 73', with a 17% loss of the grain yield compared with the yield in fungicide-protected plots. Singh and Rajaram (21) postulated the presence of genes *Lr1*, *Lr10*, and *Lr13* in 'Pavon 76' and indicated that the slow-rusting resistance to Mexican races of *P. recondita* f. sp. *tritici* must be conferred by additional unknown genes, because the races used in their studies carried virulences for all of the above *Lr* genes. Gene *Lr34* is known to confer slow-rusting resistance to Mexican races of *P. recondita* f. sp. *tritici* (19); however, Singh (18) indicated its absence in 'Pavon 76'.

The objectives of our study were (i) to determine the number and type of genes that confer adult-plant resistance to *P. recondita* f. sp. *tritici* in Mexican spring wheat cultivar Pavon 76, and (ii) to identify the chromosomes carrying genes involved in the adult-plant resistance of 'Pavon 76'.

MATERIALS AND METHODS

Genetic analysis. 'Pavon 76' was crossed with two susceptible wheat cultivars, Jupateco 73S and Avocet S. The pedigrees of these

wheats are given in Table 1. A total of 118 individual F_2 plant-derived F_3 and F_5 lines from the cross 'Jupateco 73S' $\times$ 'Pavon 76' and 148 F_3 and 146 F_5 lines from the cross 'Avocet S' $\times$ 'Pavon 76' were used in the adult-plant inheritance studies. The F_5 lines were obtained by harvesting a random spike from each F_3 line and a random plant from each F_4 line. The F_3 lines from 'Jupateco 73S' $\times$ 'Pavon 76' were grown at CIMMYT's highland research station at El Batán (Mexico State) near Mexico City during the 1991 crop season, which begins in mid-May. The F_1 seeds from the above cross were included in the planting. Other populations were grown at CIMMYT's research station at Ciudad Obregon (Sonora State) in northwestern Mexico during different crop seasons (Table 2). At this site, sowing was done during the last week of November. Approximately 60 seeds of each F_3 or F_5 line were sown in 75-cm-wide paired-row plots, 1 m in length, with 20-cm row spacing and a 50-cm pathway between plots. Rows of the two parents were planted at the beginning of each population. The leaf rust epidemic was initiated approximately 6 weeks after sowing by spraying spreader rows of highly susceptible cultivar Morocco, planted on one side of the plots in the pathway, with a suspension of urediniospore in a light-weight mineral oil (Soltrol 170; Phillips 66 Co., Bartlesville, OK). Both field sites (El Batán and Ciudad Obregon) have a favorable environment for leaf rust development. Mexican *P. recondita* f. sp. *tritici* race TBD/TM was used for studies during the 1991 and 1992 to 1993 seasons, and race MCJ/SP was used for studies during the 1995 to 1996 and 1996 to 1997 seasons (Table 2). Nomenclature of these races is based on Singh (16) with the following avirulence/virulence formula: TBD/TM, *Lr3ka*, 9,11,16,19,21,23,24,25,26,29,30,32,33/1,2a,2b,2c,3,3bg,10,12,13,14a,14b,15,17,18,20,22b,27+31,28; and MCJ/SP, *Lr2a*,2b,2c,3ka,9,16,19,21,24,25,28,29,30,32,33/1,3,3bg,10,11,12,13,14a,14b,15,17,18,20,22b,23,26,27+31. Race TBD/TM was one of the predominant races in Mexico before 1994. Race MCJ/SP and a few variants related to it appeared in Mexico during the 1994 to 1995 season and have replaced the earlier races (6). Vacuum-dried (12) urediniospores of these races are stored at CIMMYT. Fresh inoculum obtained by multiplying urediniospores on the susceptible cultivar Morocco in the greenhouse was used in various studies.

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Publication no. P-1998-0716-01R
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The visual estimations of adult-plant leaf rust severities were based on the modified Cobb scale (11). The host response to infection (reaction) followed the scale described by Roelfs et al. (12). The first leaf rust severity and reaction were recorded on flag leaves at about the milk stage of grain development, when the susceptible parent displayed a response between 80S and 100S. The severity range (the most resistant and most susceptible plant) was recorded for F_3 and F_5 lines; whereas, for F_5 lines, average leaf rust severity was also visually estimated. The F_3 and F_5 lines were evaluated a second time approximately 12 to 15 days after the first evaluation, when leaf rust had killed leaves of the susceptible parent used in the cross. Our previous studies (20,22) have shown that the timing of the first rating is critical to identify plants or lines that are similar in susceptibility level to the susceptible parent, whereas the second rating is critical to identify plants or lines similar in resistance levels to the resistant parent. On the basis of the two data sets, the F_3 and F_5 lines were classified into four categories: homozygous parental-type resistance (HPTR); homozygous parental-type susceptibility (HPTS); either segregating or homozygous for disease levels higher than that of the resistant parent, but less than that of the susceptible parent (SegI); and segregating with disease

levels reaching the susceptible parent's response (SegS). Chi-square analyses were carried out to compare the distribution of F_3 and F_5 phenotypic frequencies with those expected for segregation of two additive genes (Table 2).

Chromosomal location of resistance genes. Since 'Chinese Spring' carries adult-plant leaf rust resistance genes *Lr12* and *Lr34* (5), its aneuploids (14) could not be used for chromosomal location work. Therefore, we used a monosomic series of adult-plant leaf rust-susceptible Indian cultivar Lalbahadur, which was developed and provided by R. A. McIntosh (Plant Breeding Institute, University of Sydney, Australia).

Seeds of the 21 'Lalbahadur' monosomics (Table 1) were germinated in petri plates in the dark at room temperature. Root tips were collected from each germinating seedling, which had been pretreated with colchicine plus dimethylsulfoxide for 3.5 h and fixed in 2% aceto-orcein (9). Plants with 41 chromosomes were retained and transplanted to the field. Crosses of 'Pavon 76' (used as the male parent) were made with each of the 21 monosomics. F_1 seeds from the 21 crosses were germinated, and plants with $2n = 41$ were identified by mitotic root tip chromosomal counts. These monosomic plants were used as male parents in backcrosses with the corresponding $2n = 41$ 'Lalbahadur' monosomics to develop a chromosome substitution series (15). Two to three backcrosses were made as described above. Two BC_2 (or, in three cases, BC_1) monosomic plants from each of the 21 crosses were selfed to obtain F_2 populations from these backcrosses. About 20 space-sown (15 cm between plants) plants of each of the above F_2 populations were grown to develop backcross F_2 -derived F_3 lines. About 20 BC_1F_3 or BC_2F_3 substitution (both disomic and monosomic) lines from each cross (two families within each cross, each carrying 10 lines) were grown during the 1994 and 1995 crop seasons at El Batan research station and tested for resistance with races TBD/TM and MCJ/SP, respectively (Table 3). The plot sizes, planting, and inoculation procedures were the same as described for genetic analysis. The 'Lalbahadur' monosomics and 'Pavon 76' were included as parental checks. The lines were classified for leaf rust response. It is expected that in a cross involving the critical chromosome (chromosome carrying the resistance gene), the disomic substitution F_3 lines (those derived from $2n = 42$ plants) will be homozygous resistant, because the substituted chromosome pair carries the resistance gene in homozygous state from the resistant parent. The monosomic substitution lines (those derived from $2n = 41$ plants) in a critical cross will segregate, because a few susceptible plants with $2n = 40$ will lack the resistance allele, and the plants with $2n = 41$ could be partially resistant if resistance is not completely dominant (as the case is in our study). Homozygous-susceptible F_3 lines, derived mostly from $2n = 40$ plants, are rarely expected in a critical cross, because the nullisomic plants are usually eliminated from the F_2 populations because of the lack of vigor or male sterility (14). In noncritical crosses, the substitution F_3 lines from the backcrosses could be distributed in all three categories in a Mendelian segregation ratio, or all could be homozygous susceptible, depending on the number of backcrosses and the probability of the carry-

TABLE 1. Wheat cultivars, 'Lalbahadur' monosomics (Lalb Mono), their pedigrees, and reaction range to leaf rust tested in the field over four seasons

Cultivar/ monosomic	Pedigree	Leaf rust reaction range ^a
'Pavon 76'	Vcm//Cno/7C/3/Kal/Bb	5-10MSS
'Jupateco 73S'	II12300//Lr64/8156/3/Nor	100S
'Avocet S'	WW119/WW15//Egret	80-100S
'Lalbahadur'	S54723/R531	100S
Lalb Mono 1A	Mx86.98.1/Kal//5*Lalb	100S
Lalb Mono 2A	Oxley Mono 2A/Kal//5*Lalb	Seg ^b (5-10MSS, 100S)
Lalb Mono 3A	Mx86.96.6/Kal//5*Lalb	100S
Lalb Mono 4A	Oxley Mono 4A/Kal//5*Lalb	100S
Lalb Mono 5A	Oxley Mono 5A/Kal//5*Lalb	100S
Lalb Mono 6A	Oxley Mono 6A/Kal//5*Lalb	100S
Lalb Mono 7A	Oxley Mono 7A/Kal//5*Lalb	100S
Lalb Mono 1B	Oxley Mono 1B/Kal//5*Lalb	100S
Lalb Mono 2B	Mx86.97.2/Kal//4*Lalb	100S
Lalb Mono 3B	Oxley Mono 3B/Kal//5*Lalb	100S
Lalb Mono 4B	Oxley Mono 4B/Kal//5*Lalb	100S
Lalb Mono 5B	Oxley Mono 5B/Kal//5*Lalb	100S
Lalb Mono 6B	Oxley Mono 6B/Kal//5*Lalb	100S
Lalb Mono 7B	Oxley Mono 7B/Kal//5*Lalb	100S
Lalb Mono 1D	Mx86.100.2/Kal//5*Lalb	100S
Lalb Mono 2D	Egret Mono 2D/CSP44/Kal/3/5*Lalb	10-20MSS
Lalb Mono 3D	Oxley Mono 3D/Kal//5*Lalb	100S
Lalb Mono 4D	Egret Mono 4D/Kal//2*Lalb	5-10MSS
Lalb Mono 5D	Mx86.101.6/Kal//5*Lalb	Seg (5-20MSS, 100S)
Lalb Mono 6D	Oxley Mono 6D/Kal//5*Lalb	100S
Lalb Mono 7D	Mx86.106.4/Kal//4*Lalb	100S

^a Leaf rust reaction has two components: percent severity based on the modified Cobb scale (11) and response to infection based on Roelfs et al. (12), where MSS = moderately susceptible to susceptible and S = susceptible response to infection.

^b Seg = segregating.

TABLE 2. Distribution of F_3 and F_5 lines in the crosses of adult leaf rust-resistant wheat cultivar Pavon 76 with leaf rust susceptible 'Jupateco 73S' and 'Avocet S'

Cross	Generation	Season tested	Pathotype	Number of lines with reaction ^a				χ^2 value ^b	<i>P</i> ^c	Number of genes
				HPTR	HPTS	SegI	SegS			
'Jupateco 73S' × 'Pavon 76'	F_3	1991	TBD/TM	4	4	43	67	4.21	>0.10	2
'Jupateco 73S' × 'Pavon 76'	F_5	1992-1993	TBD/TM	17	23	58	20	3.25	>0.25	2
'Avocet S' × 'Pavon 76'	F_3	1995-1996	MCJ/SP	7	9	61	71	1.22	>0.75	2
'Avocet S' × 'Pavon 76'	F_5	1996-1997	MCJ/SP	29	26	72	19	0.20	>0.95	2

^a Reaction categories: HPTR = homozygous for the parental-type resistance (homozygous for both resistance alleles); HPTS = homozygous for the parental-type susceptibility (homozygous, lacking both resistance alleles); SegI = either segregating or homozygous for disease levels higher than that of the resistant parent, but less than that of the susceptible parent (homozygous for one resistance allele, the second resistance allele either absent or present in heterozygous condition); SegS = segregating with disease levels reaching the susceptible parent response (heterozygous for one resistance allele and the second resistance allele absent; or heterozygous for both resistance alleles).

^b χ^2 values for ratios HPTR/HPTS/SegI/SegS (1:1:6:8) in F_3 and HPTR/HPTS/SegI/SegS (0.1914:0.1914:0.4922:0.1251) in F_5 .

^c *P* values higher than 0.05 indicate nonsignificant values of χ^2 .

over of resistance allele from one backcross generation to the next. Testing of these substitution F₃ lines from one or two backcrosses can help identify several noncritical chromosomes, which can be eliminated from further backcrossing. Based on results obtained during the 1994 and 1995 seasons, we made one additional backcross and grew BC₂- or BC₃-derived substitution F₃ lines from 10 crosses during the 1996 season at El Batan and tested them using race MCJ/SP (Table 4) to verify earlier results on the chromosomal location of resistance.

The 'Lalbahadur' monosomics and one apparently critical chromosome in the substitution work were diagnosed for their correct identification using the modified giemsa C-banding technique (7).

We have excluded the results for chromosomes 1A, 3A, 4A, 1D, and 3D, because they were identified as being incorrect.

The disomic 'Lalbahadur' ('Pavon 1B') chromosome substitution lines and the parents were grown in the field during the 1997 season at El Batan, inoculated with race MCJ/SP, and evaluated for leaf rust progress four times at weekly intervals (Fig. 1).

RESULTS AND DISCUSSION

Genetic analysis. 'Pavon 76' displayed between 5 and 10% leaf rust severity when the susceptible parents 'Jupateco 73S' and 'Avocet S' displayed approximately 80 to 100% leaf rust (Table 1),

TABLE 3. Distribution of BC₁F₃ or BC₂F₃ lines for leaf rust reaction when various 'Lalbahadur' monosomic (Lalb Mono) chromosomes were substituted with respective chromosomes from 'Pavon 76', tested during 1994 and 1995 using *Puccinia recondita* f. sp. *tritici* pathotypes TBD/TM and MCJ/SP, respectively

Substitution	Year tested	Number of lines with reaction ^a					
		Family 1 ^b			Family 2 ^b		
		HR	Seg	HS	HR	Seg	HS
Lalb Mono 2A*3/'Pavon 76'	1994	4	2	3	3	4	2
	1995	3	3	3	2	4	1
Lalb Mono 5A*3/'Pavon76'	1994	0	0	10	0	0	10
	1995	0	0	10	0	0	10
Lalb Mono 6A*3/'Pavon76'	1994	0	0	10	0	0	10
	1995	0	0	10	0	1	9
Lalb Mono 7A*3/'Pavon76'	1994	0	0	10	0	0	10
	1995	0	0	10	0	0	10
Lalb Mono 1B*3/'Pavon76'	1994	3	7	0	5	5	0
	1995	3	7	0	5	5	0
Lalb Mono 2B*3/'Pavon76'	1994	0	0	9	2	4	4
	1995	0	0	9	2	3	5
Lalb Mono 3B*3/'Pavon76'	1994	0	0	10	2	7	1
	1995	0	0	10	3	7	0
Lalb Mono 4B*3/'Pavon76'	1994	0	0	10	1	6	3
	1995	0	0	10	2	7	1
Lalb Mono 5B*2/'Pavon76'	1994	2	7	1	1	8	1
	1995	4	5	1	2	7	1
Lalb Mono 6B*3/'Pavon76'	1994	0	0	10	0	0	10
	1995	0	0	10	0	0	10
Lalb Mono 7B*3/'Pavon76'	1994	0	8	2	0	1	8
	1995	0	8	2	0	2	8
Lalb Mono 2D*2/'Pavon76'	1994	5	2	3	2	2	6
	1995	4	3	3	2	3	5
Lalb Mono 4D*3/'Pavon76'	1994	2	5	3	10	0	0
	1995	2	5	3	10	0	0
Lalb Mono 5D*2/'Pavon76'	1994	4	5	1	0	0	10
	1995	3	6	1	0	0	10
Lalb Mono 6D*3/'Pavon76'	1994	1	3	6	1	4	5
	1995	0	4	6	1	5	4
Lalb Mono 7D*3/'Pavon 76'	1994	0	0	10	0	4	6
	1995	0	0	10	0	6	4

^a Reaction categories: HR = homozygous resistant, Seg = segregating, and HS = homozygous susceptible.

^b Families 1 and 2 were derived from two different monosomic (2n = 41) BC₁ or BC₂ plants.

TABLE 4. Distribution of BC₂F₃ or BC₃F₃ lines for leaf rust reaction when various 'Lalbahadur' monosomic (Lalb Mono) chromosomes were substituted with respective chromosomes from 'Pavon 76', tested during 1996 using *Puccinia recondita* f. sp. *tritici* pathotype MCJ/SP

Substitution	Number of lines with reaction ^a								
	Family 1 ^b			Family 2 ^b			Family 3 ^b		
	HR	Seg	HS	HR	Seg	HS	HR	Seg	HS
Lalb Mono 2A*4/'Pavon 76'	0	0	14	3	5	5	...	...	...
Lalb Mono 5A*4/'Pavon 76'	0	0	14	0	0	14	0	0	14
Lalb Mono 6A*4/'Pavon 76'	0	0	14	0	0	14	...	...	...
Lalb Mono 1B*4/'Pavon 76'	1	13	0	3	11	0	2	12	0
Lalb Mono 5B*3/'Pavon 76'	2	8	4	0	0	14	...	...	...
Lalb Mono 7B*4/'Pavon 76'	0	0	14	0	0	14	0	0	14
Lalb Mono 2D*3/'Pavon 76'	6	8	0	4	9	1	4	12	0
Lalb Mono 5D*3/'Pavon 76'	6	6	2	3	5	6	0	0	14
Lalb Mono 6D*4/'Pavon 76'	0	0	14	0	0	14	0	0	14
Lalb Mono 7D*4/'Pavon 76'	0	0	14	0	0	14	0	0	14

^a Reaction categories: HR = homozygous resistant, Seg = segregating, and HS = homozygous susceptible.

^b Families 1, 2, and 3 were derived from three different monosomic (2n = 41) BC₂ or BC₃ plants.

^c Not tested.

indicating that the adult-plant resistance of 'Pavon 76' was easily distinguished in each season. The F_1 plants from the cross 'Jupateco 73S' $\times$ 'Pavon 76' displayed about 30% leaf rust severity as compared with 5% for 'Pavon 76' and 100% for 'Jupateco 73S' during the 1991 season. This indicated that resistance was partially dominant. In all seasons, 'Pavon 76' matured with disease ratings of 30 to 40% (Fig. 1, the 1997 crop season). The uredinia on 'Pavon 76' were scored as moderately susceptible to susceptible (moderate to large uredinia without chlorosis or necrosis), compared with susceptible (large uredinia without chlorosis or necrosis) for 'Jupateco 73S' and 'Avocet S' (Table 1), which indicates the compatible nature of response to infection, a known feature of slow-rusting or partial resistance (3,10).

Frequency distributions of leaf rust severity in F_5 lines at first evaluation are shown in Figures 2 and 3 for 'Jupateco 73S' $\times$ 'Pavon 76' and 'Avocet S' $\times$ 'Pavon 76' crosses, respectively. The lines were distributed continuously. Distributions of F_3 and F_5 lines in both crosses were in accordance to ratios expected for segregation at two independent loci, assuming that the two resistance alleles acted in additive manner (Table 2). These assumptions were made because $1/16$ of the F_3 lines and about 19% of the F_5 lines were phenotypically similar to the resistant or susceptible parent's response. Race selection in studies conducted in different years did not influence the estimation of the number of resistance genes. From these results, we conclude that the slow-rusting resistance of 'Pavon 76' is based on the additive effects of two genes. Our conclusion that the slow-rusting resistance to leaf rust involves a few additive genes is congruent with those published earlier (1,2,8,22).

Because both 'Pavon 76' and 'Avocet S' carry race-specific genes *Lr10* and *Lr13* (21; R. P. Singh, unpublished data), the F_3 and F_5 lines derived from their crosses are expected to be homozygous. Therefore, the two additive genes involved in the slow-rusting resistance of 'Pavon 76' are different from *Lr10* and *Lr13*. Genes *Lr10* and *Lr13* are expected to be ineffective because of the presence of virulence in the *P. recondita* f. sp. *tritici* races we used.

Chromosomal location of resistance. Evaluations of the 'Lalbahadur' monosomics for leaf rust resistance indicated that monosomics for chromosomes 2D and 4D were resistant to leaf rust, and monosomics for 2A and 5D segregated. This implies that results for crosses involving these monosomics require careful interpretation. The remaining monosomics were similar in their susceptibility to cultivars Lalbahadur, Jupateco 73S, and Avocet S.

Distribution of leaf rust reactions of BC_1F_3 or BC_2F_3 lines in 16 substitutions (both monosomic and disomic) is given in Table 3. Results for substitutions involving chromosomes 1A, 3A, 4A, 1D, and 3D are not presented, because 'Lalbahadur' monosomics for

these chromosomes were identified as being incorrect by the C-banding diagnostic. Chromosome 1B of 'Pavon 76' appeared to be critical, because the F_3 lines were either homozygous resistant or segregating. In all other substitutions, the F_3 lines were either homozygous susceptible or segregation occurred and homozygous-susceptible lines were observed frequently. One family in the substitution involving 'Lalbahadur' monosomic 4D had all homozygous-resistant lines. This was to be expected, because the monosome itself was resistant (Table 1); therefore, backcrosses resulted in recovering the parental resistance phenotype of the monosome parent. Based on these results, we infer that several chromosomes either did not carry the resistance gene or may have lost the targeted chromosome from 'Pavon 76' through the transmission of male gametes with $n = 20$ chromosomes. In either case, further backcrossing may not be desirable, because similar results will be obtained. Results for F_3 lines of 10 substitutions derived from one additional backcross to the 'Lalbahadur' monosomic are summarized in Table 4. Because BC_3F_3 lines in each of the three families for the substitution involving chromosome 1B were either homozygous resistant or segregating, it was again evident that chromosome 1B of 'Pavon 76' carried the resistance allele. Segregation for resistance in substitutions involving chromosomes 2A, 2D, and 5D is due to the fact that these 'Lalbahadur' monosomics were not susceptible (Table 1). Most segregating lines in the 'Lalbahadur' 1B substitution lacked completely susceptible plants. This is to be

Fig. 2. Frequency distribution of adult-plant leaf rust severities of 118 F_5 lines from the cross 'Jupateco 73S' $\times$ 'Pavon 76'.

Fig. 1. Progress of leaf rust on 'Pavon 76' (Pvn), 'Lalbahadur' (Lalb), 'Lalbahadur' monosomic 1B (Lalb Mono 1B), and 'Lalbahadur' ('Pavon 1B') disomic chromosome substitution lines observed during the 1997 season.

Fig. 3. Frequency distribution of adult-plant leaf rust severities of 146 F_5 lines from the cross 'Avocet S' $\times$ 'Pavon 76'.

expected if resistance is partially effective in hemizygous chromosomal state (i.e., only one copy of the resistance gene is present in the plant). The rare susceptible plants in segregating families are, therefore, either nullisomic ($2n = 40$) or secondary aneuploids as a consequence of chromosomal misdivision.

The chromosomal status of all 6 homozygous resistant and 10 randomly chosen segregating BC_3F_3 lines from the chromosome 1B substitution (in the cross 'Lalbahadur' monosomic 1B*4 $\times$ 'Pavon 76'; Table 4) was determined by doing mitotic root tip counts on five seedlings per line. If all five seedlings were disomic ($2n = 42$), we interpreted that the line was derived from a disomic plant. But if at least two of the five seedlings had chromosome counts of $2n = 41$ or 40, we inferred that the line was derived from a monosomic plant. As expected, all six homozygous-resistant lines were disomic, and all segregating lines were derived from monosomic plants. Monosomic plants from segregating families were C-banded to validate the status of chromosome 1B and ratify our results. The observations for chromosome 1B were correct. Therefore, we conclude that one of the two slow-rusting genes present in 'Pavon 76' is located on chromosome 1B and designated as *Lr46*. Chromosomal arm location is currently not possible because of the unavailability of a susceptible monotelosomic series. However, we are developing populations suitable for identifying the linked molecular marker to establish chromosomal arm location. The disomic BC_3F_3 lines (Table 4) could be called 'Lalbahadur' ('Pavon 1B') chromosome substitution lines and used as tester lines for gene *Lr46*. These lines have about 94% recovery of the 'Lalbahadur' genotype for 20 other chromosomes and are expected to possess gene *Lr1*, because both the 'Lalbahadur' and 'Pavon 76' parents carry it. This should not pose any major problem in testing the usefulness of *Lr46* at other sites, because virulence for *Lr1* is common in most areas of the world.

Because the substitution of chromosome 7D of 'Pavon 76' in 'Lalbahadur' did not confer any resistance (Tables 3 and 4), we conclude that it lacked the slow-rusting gene *Lr34* located on chromosome 7DS (4). This result is in agreement with Singh (18), who inferred the absence of *Lr34* in 'Pavon 76' from the absence of leaf tip necrosis, which is tightly linked to it (17). Therefore, gene *Lr46* is the second named gene conferring slow rusting to leaf rust in wheat.

Our genetic analysis results have shown that 'Pavon 76' contains two slow-rusting genes. However, in the current study, we were unable to determine the chromosomal location of the second gene. There are three possible reasons for this failure: (i) the second gene may be located on one of the five chromosomes for which information is lacking; (ii) the targeted chromosome of 'Pavon 76' may have been missed because of transmission of a 20 chromosome male gamete, since we used monosomic plants as male parents in backcrossing onto 'Lalbahadur' monosomics; and (iii) the second gene may also be located on chromosome 1B, on the opposite arm, or further away on the same arm. The third possibility can be ruled out, because disease progressed much faster on 'Lalbahadur' ('Pavon 1B') chromosome substitution lines than on 'Pavon 76', which carried both slow-rusting genes (Fig. 1). This result further indicates that the presence of the single slow-rusting gene *Lr46* does not give enough protection under high leaf rust pressure, as the last rating of 60% (Fig. 1) was too high for acceptance in breeding programs. The gene must be present in combination with other slow-rusting genes of additive effects to impart adequate resistance, as seen earlier with gene *Lr34* (22).

ACKNOWLEDGMENTS

We thank R. A. McIntosh for providing the 'Lalbahadur' monosomic series and A. McNab for editorial review of the manuscript.

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