Theor Appl Genet (1995) 90: 982–990

G. M. Schachermayr · M. M. Messmer · C. Feuillet
H. Winzeler · M. Winzeler · B. Keller

Identification of molecular markers linked to the *Agropyron elongatum*-derived leaf rust resistance gene *Lr24* in wheat

Received: 31 August 1994 / Accepted: 30 September 1994

Abstract The objective of this study was to identify molecular markers linked to the wheat leaf rust resistance gene *Lr24* derived from *Agropyron elongatum* (3DL/3Ag translocation). Two near isogenic lines (NILs), 'Arina' and *Lr24/7** "Arina", were screened for polymorphism at the DNA level with 115 RFLP probes. Twenty-one of these probes map to the homoeologous group 3. In addition, 360 RAPD primers were tested on the NILs. Six RFLP probes showed polymorphism between the NILs, and 11 RAPD primers detected one additional band in the resistant NIL. The genetic linkage of the polymorphic markers with *Lr24* was tested on a segregating F_2 population (150 plants) derived from a cross between the leaf rust resistant *Lr24/7** "Arina" and the susceptible spelt (*Triticum spelta*) variety 'Oberkulmer'. All 6 RFLP markers were completely linked to *Lr24*: one was inherited as a codominant marker (PSR1205), one was in coupling phase (PSR1203) and 4 were in repulsion phase (PSR388, PSR904, PSR931, PSR1067) with *Lr24*. The localization of these probes on chromosome 3D was confirmed by nulli-tetrasomic analysis. Distorted genotypic segregation was found for the codominant RFLP marker PSR1205. This distortion can be explained by the occurrence of hemizygous plants. One of the 11 RAPD markers (OPJ-09) also showed complete linkage to the *Lr24* resistance gene. The polymorphic RAPD fragment was cloned and sequenced. Specific primers were synthesized, and they produced an amplification product only in the resistant plants. This specific marker allows a reliable and rapid screening of a large number of genotypes in practical breeding. Analysis of 6 additional lines containing *Lr24* revealed that 3 lines have a smaller chromosomal segment of *A. elongatum* than lines derived from 'Agent', a commonly used gene donor for the *Lr24* resistance gene.

Key words Leaf rust · RFLP · RAPD · Wheat · *Agropyron elongatum*

Introduction

Many resistance genes against wheat leaf rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks. & Henn.) (*Lr* genes) have been introgressed into wheat from wild relatives (Knott 1989; Baum et al. 1992). The wild species *Agropyron elongatum* has been used as a donor for several *Lr* genes, among them *Lr24* (Knott 1989). The leaf rust resistance of the cultivar 'Agent' was derived from *A. elongatum* as a result of a natural translocation involving wheat chromosome 3D (Smith et al. 1968). The resistance was caused by a dominant gene, first called *LrAg* (Browder 1973) and later renamed *Lr24* (McIntosh et al. 1976). The cultivar 'Agent' was subsequently used as a *Lr24* donor line to produce a number of leaf rust resistant lines and cultivars in different countries.

Another source for the *Lr24* resistance gene are the translocation lines developed by Sears (1973). He generated several transfers of the 3Ag *A. elongatum* chromosome to wheat chromosome 3D by induced homoeologous pairing. All these 3Ag/3D translocation lines carried the *Lr24* resistance gene. From chromosome pairing studies, there is evidence that the transferred Ag chromosome segments of some of these lines are smaller than the one in 'Agent' (Sears 1973; McIntosh et al. 1976).

In several European countries, including Switzerland, no virulence has yet been found on the *Lr24* gene (von Kröcher et al. 1992; Siharulidze et al. 1993, our unpublished data). In the United States and Canada matching virulence increased with the use of cultivars containing *Lr24* (Kolmer 1993; Long et al. 1993; Martens and Dyck 1988). This shows that the *Lr24* gene is not a durable source of resistance and should be used only in combinations with other *Lr* genes. Worldwide, no virulence has been reported on the combination of *Lr9* and *Lr24* (Roelfs et al. 1992). Therefore, this combination is of particular interest for resistance breeding.

Communicated by G. Wenzel

G. M. Schachermayr · M. M. Messmer · C. Feuillet · H. Winzeler
M. Winzeler · B. Keller (✉)
Department of Plant Breeding, Swiss Federal Research Station for Agronomy (FAP), Reckenholzstrasse 191, CH-8046 Zürich, Switzerland

The selection of genotypes carrying two or more leaf rust resistance genes using traditional host-parasite interactions is very time-consuming and often not possible due to a lack of isolates with specific virulence genes. The development of markers that are closely linked with the respective resistance genes, therefore, is essential for the selection of such gene combinations. We have recently found molecular markers for the *Lr9* resistance gene in wheat (Schachermayr et al. 1994). There, near-isogenic lines (NILs) were successfully used to find molecular differences between the resistant and susceptible lines. Specific primers enabled the development of a rapid, polymerase chain reaction (PCR)-based assay for the presence of the *Lr9* gene. Similar strategies using NILs also resulted in closely linked markers for other genes in cereals, such as the powdery mildew resistance gene *Pm3* in wheat (Hartl et al. 1993), the powdery mildew resistance gene *Ml-a* in barley (Schüller et al. 1992), the oat stem rust resistance gene *Pg3* (Penner et al. 1993) and the rice bacterial blight disease resistance locus *Xa21* (Ronald et al. 1992). Strategies using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers (for reviews see Tingey and del Tufo 1993; Waugh and Powell 1992) have both been successfully used and are often complementary (Schachermayr et al. 1994).

In the present article we describe the identification of molecular markers linked to the *Lr24* resistance gene of wheat. One RAPD and 6 RFLP markers were completely linked to the *Lr24* gene. The conversion of the RAPD marker into a sequence-tagged site (STS, Olson et al. 1989) will allow a rapid screening for *Lr24* in breeding programs.

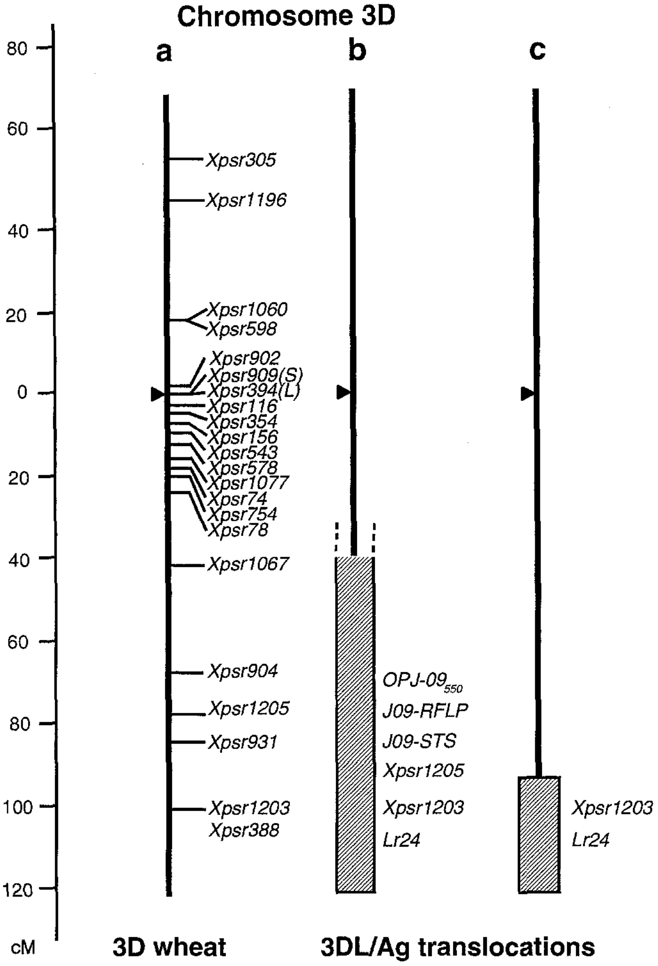


Fig. 1a Linkage map of RFLP probes of the wheat chromosome 3D used for this study according to Devos and Gale (1993). The approximate position of the centromere is indicated by the *arrowhead.* **b** Schematic illustration of the 3DL/Ag translocation of *Lr24* lines related to 'Agent' (*Lr24/7** "Arina", 'Agent', RL6064, RNS173). **c** Schematic illustration of the 3DL/Ag translocation of *Lr24* lines related to 3Ag3 (RNS184, RNS189, RNS219). The *Agropyron elongatum* chromosome segments are indicated by *hatched bars.* The precise size of the translocation is not known

Materials and methods

Plant material

The *Lr24* donor line RL6064 ('Agent'/6* "Thatcher") was backcrossed seven times to the susceptible Swiss winter wheat cultivar 'Arina' and afterwards selfed to produce a leaf rust resistant F_8 (NIL) of 'Arina' designated as *Lr24/7** "Arina". The resistant *Lr24/7** "Arina", the susceptible 'Arina' and the susceptible Swiss spelt (*Triticum spelta*) variety 'Oberkulmer' were used for the RAPD and RFLP screening to find markers putatively linked to the *Lr24* resistance gene.

A segregating F_2 population of 150 plants from a cross between the resistant NIL *Lr24/7** "Arina" and 'Oberkulmer' was used for the linkage analysis. *Triticum spelta* was used as susceptible parent to increase the chances of detecting polymorphic RFLP loci on chromosome 3D (Liu et al. 1990, Schachermayr et al. 1994). Each F_2 plant was screened for leaf rust resistance at the seedling stage and was assayed with the putatively linked RAPD and RFLP markers.

The aneuploid line nullisomic 3D – tetrasomic 3A (N3DT3A) of 'Chinese Spring' (CS) (Sears 1966) and CS were used to verify the location of the linked RFLP fragments on the wheat chromosome 3D.

In addition, the resistant cultivar 'Agent' (Smith et al. 1968), the *Lr24* donor line RL6064 used for our resistant NIL, and 4 'Kalyansona' backcross lines (RNS173, RNS184, RNS189, RNS219) were tested for the presence of the RAPD and RFLP fragments linked to the *Lr24* gene. RNS173 is derived from a cross 'Agent'/5* "Kalyansona", whereas RNS184, RNS189, RNS219 are derived from a different *Lr24* source, TR380.27/4* "3Ag3", four times backcrossed to 'Kalyansona'. Seeds were kindly provided by Dr. P. Dyck (Winnipeg, Agriculture Canada) and Dr. R. Basant (ICAR, India).

Fifty-one winter wheat and 9 spring wheat varieties (Siedler et al. 1994) lacking the *Lr24* resistance gene, as deduced from pedigree data, were also tested for the absence of the RAPD and RFLP fragments linked to the *Lr24* gene.

RFLP analysis

Isolation of genomic DNA, Southern analysis and probe labelling with [^{32}P] were performed as described by Graner et al. (1990). The genomic DNA of *Lr24/7** "Arina", 'Arina' and 'Oberkulmer' was digested with seven restriction endonucleases (*Eco*RI, *Hin*dIII, *Xba*I, *Eco*RV, *Dra*I, *Bam*HI and *Bgl*II) and hybridized with 115 DNA probes of a set of wheat clones kindly provided by Dr. M.D. Gale (Cambridge Laboratory, Norwich) and Dr. P. Gay (Ciba-Geigy, Basel). Of these probes 21 have been mapped on homoeologous chromosomes of group 3 by Devos and Gale (1993) (Fig. 1a), 10 belong to the homoeologous group 3 (Devos et al. 1992; Devos and Gale

1993) and the remaining 84 probes map to the other 6 homoeologous groups of wheat. The genomic DNA of the 150 F_2 plants, CS, N3DT3A, the 6 *Lr24* containing cultivars and the 51 wheat cultivars was digested with the restriction enzyme that showed a clear polymorphism in the parental screening, and was hybridized with the putatively linked RFLP probes.

RAPD analysis

RAPD reactions similar to those described by Williams et al. (1990) were performed with some minor modifications. Amplification reactions contained 10 m*M* TRIS-HCl, pH 8.3, 50 m*M* KCl, 2 m*M* $MgCl_2$, 0.001% gelatine, 100 μ*M* of each dNTP, 15 ng 10-mer primer (Operon Technologies, Calif.), 5 ng genomic DNA, and 0.5 units *Taq* DNA polymerase (Perkin Elmer Cetus, Switzerland) in a volume of 25 μl. Amplifications were performed in a PTC-100 thermocycler (MJ-Research, BioConcept, Switzerland) programmed at 94°C for 6 min, followed by 45 cycles at 92°C for 1 min, at 35°C for 1 min and at 72°C for 2 min. The extension of the amplified fragment was achieved at 72°C for 5 min. The amplification products were separated in 1.2% agarose gels and visualized under UV light after ethidium bromide staining. A total of 360 primers were screened to identify polymorphism between the resistant NIL *Lr24/7** "Arina" and the susceptible 'Arina'. The primers that showed polymorphism between the NILs were tested for polymorphism between the parental lines of the F_2 population, *Lr24/7** "Arina" and 'Oberkulmer'.

Cloning and sequencing of a RAPD product

The polymorphic PCR product ($OPJ\text{-}09_{550}$) was cloned in a pGEM-T vector (Promega, Switzerland) as recommended by the manufacturer. Transformation of Epicurian Coli® competent cells (Stratagene, Switzerland) resulted in recombinant clones pJ09/3 and pJ09/27 containing the fragment in both orientations. Double-strand sequencing (Sequenase® Version 2.0 DNA Sequencing Kit, USB™, Switzerland) was done by the dideoxy-chain termination method using the M13 universal primer (Sanger et al. 1977). The cloned PCR fragment was used as an RFLP probe, called J09-RFLP.

STS design and analysis

Based on the sequence of the polymorphic amplification product $OPJ\text{-}09_{550}$ two specific primers, J09/1 and J09/2, 20 bases in length, were designed and synthesized by Microsynth (Windisch, Switzerland). The sequence of the specific primers is: J09/1, 5′ TCTAGTCTGTACATGGGGGC 3′; J09/2, 5′TGGCACATGAACTCCATACG 3′. Amplification of the specific marker J09-STS was done in a 25 μl volume containing 10 m*M* TRIS-HCl, pH 8.3, 50 m*M* KCl, 2 m*M* $MgCl_2$, 0.001% gelatine, 100 μ*M* of each dNTP, 40 n*M* of each primer, 5 ng genomic DNA, and 0.5 units DNA *Taq* polymerase (Perkin Elmer Cetus, Switzerland). Amplifications were performed in a PTC-100 thermocycler (MJ-Research, BioConcept, Switzerland) programmed at 94°C for 4 min, followed by 40 cycles at 92°C for 1 min, at 60°C for 1 min and at 72°C for 2 min. The extension of the amplified fragment was achieved at 72°C for 5 min. The amplification products were loaded onto a 1.4% agarose gel and visualized under UV light after ethidium bromide staining.

Screening for leaf rust resistance

For the screening of the 150 F_2 plants for leaf rust resistance, seedlings were grown in a growth chamber (Conviron IG, Switzerland) at a 20°/16°C day/night temperature, 85/99% day/night relative humidity and a photoperiod of 16 h (360 $\mu Em^{-2}s^{-1}$ photosynthetic photon flux density). When the first leaf was fully extended, segregation of the *Lr24* gene was evaluated by artificial inoculation with a mixture of two leaf rust isolates avirulent on *Lr24*. The pots were placed on a rotating plate in a hood and sprayed with the urediospores as a suspension with mineral oil Soltrol 170 (Phillips Petroleum, Paris). Plants were visually scored for rust reaction 10 days after inoculation: susceptible plants showed large uredia, while resistant plants showed a clearly hypersensitive reaction. Seedlings of 'Arina' were used as susceptible standards.

Results

Screening of the near-isogenic lines with RFLPs and RAPDs

Polymorphism between the NILs, *Lr24/7** "Arina" and 'Arina', was detected with 6 RFLP markers (PSR1067, PSR904, PSR1205, PSR931, PSR1203, PSR388) in combination with four to seven restriction enzyme digests. All of these probes have been previously mapped to the long arm of chromosome 3DL (Fig. 1a), except for PSR388, which has been mapped to homoeologous group 2 (Devos et al. 1993). The probes PSR1067, PSR904, PSR931 (Fig. 2) and PSR388 showed null alleles for the band on chromosome 3D, i.e. the fragment was present in the susceptible 'Arina' but was absent in the resistant NIL *Lr24/7** "Arina". Probes PSR1203 (Fig. 3) and PSR1205 (Fig. 4) revealed RFLP bands of different sizes for the two NILs. All 6 probes were polymorphic between *Lr24/7** "Arina" and 'Oberkulmer' and were, therefore, useful for the segregation analysis. In addition, 14 probes belonging to the homoeologous chromosome group 3 showed polymor-

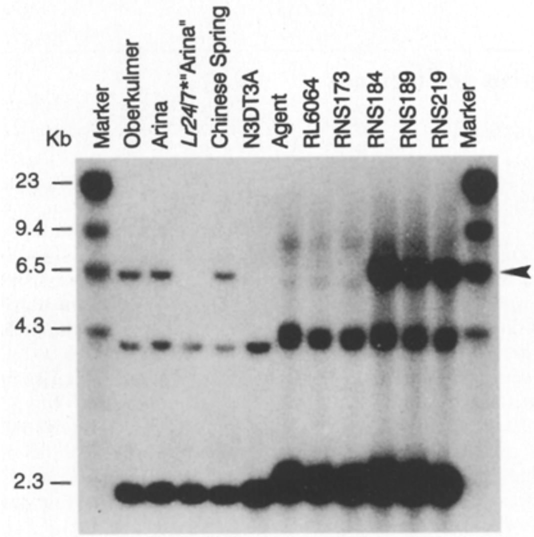


Fig. 2 Southern hybridization pattern of *Eco*RV-digested genomic DNA with probe PSR931. The *arrowhead* indicates an RFLP fragment of about 6.5 kb absent in the resistant *Lr24* lines related to 'Agent' (*Lr24/7** "Arina", 'Agent', RL6064, RNS173) and present in the susceptible lines 'Oberkulmer', 'Arina', 'Chinese Spring' and the resistant *Lr24* lines related to 3Ag3 (RNS184, RNS189, RNS219). The location of this fragment on chromosome 3D was determined by the aneuploid line nullisomic 3D – tetrasomic 3A (N3DT3A) of 'Chinese Spring'. The molecular weight marker is λDNA digested by *Hin*dIII

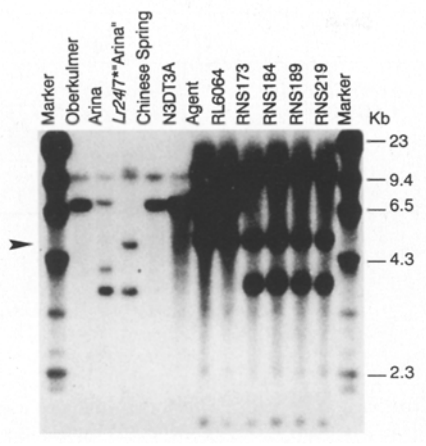


Fig. 3 Southern hybridization pattern of *EcoRV*-digested genomic DNA with probe PSR1203. The *arrowhead* indicates an RFLP fragment of about 4.5 kb present in the resistant *Lr24* containing lines *Lr24/7** “Arina”, ‘Agent’, RL6064, RNS173, RNS184, RNS189 and RNS219, and absent in the susceptible lines ‘Oberkulmer’, ‘Arina’, ‘Chinese Spring’, N3DT3A. This fragment is completely linked in coupling phase with the *Lr24* resistance gene. The molecular weight marker is λDNA digested by *Hin*dIII

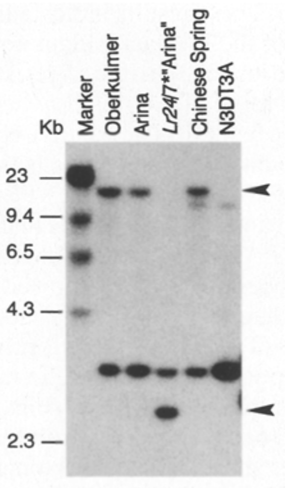
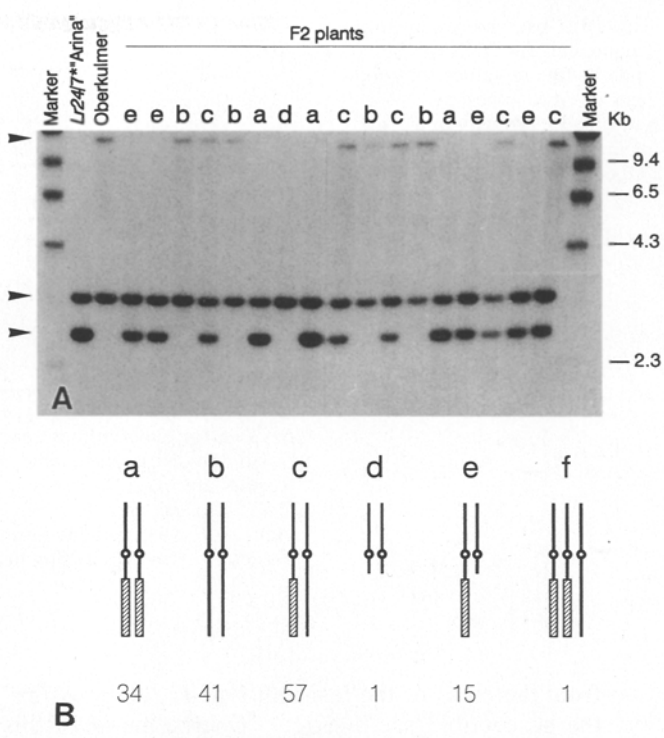


Fig. 4 Southern hybridization pattern of *Hin*dIII-digested genomic DNA with probe PSR1205. The *arrowheads* indicate the allelic RFLP fragments of about 3 kb in the resistant parent *Lr24/7** “Arina” and of about 20 kb in the susceptible parent ‘Oberkulmer’. The location of this fragment on chromosome 3D was determined by the aneuploid line nullisomic 3D-tetrasomic 3A (N3DT3A) of ‘Chinese Spring’. The molecular weight marker is λDNA digested by *Hin*dIII



ig. 5A Southern hybridization pattern of *Hin*dIII digested genom- DNA of segregating F_2 plants, their resistant parent *Lr24/7** “Ari- ” and their susceptible parent ‘Oberkulmer’ with probe PSR1205. e *arrowheads* indicate the codominant RFLP fragments of about and 20 kb and the monomorphic RFLP fragment of about 3.5-kb ed as reference for the scoring of the band intensity of the 3-kb *24/7** “Arina” fragment. The 3-kb fragment was completely linked ith the *Lr24* gene. The *small letters* correspond to the putative genopes shown in Fig. 5b. The molecular weight marker is λDNA disted by *Hin*dIII. **B** Putative composition of 3DL chromosomes of e segregating F_2 population of a cross *Lr24/7** “Arina” and ‘Oberkulmer’ according to the Southern hybridization pattern of *Hin*dIII-digested genomic DNA with probe PSR1205. Presence and absence of the allelic 3-kb fragment from *Lr24/7** “Arina” and the 20-kb fragment from ‘Oberkulmer’, as well as the band intensity of the 3-kb fragment were taken into consideration. The observed numbers of F_2 plants with the different chromosome compositions are also given. **a** indicates the chromosome composition of resistant F_2 plants homozygous for the 3DL/3Ag translocation derived from *Lr24/7** “Arina”, **b** indicates the chromosome composition of susceptible F_2 plants homozygous for the wheat chromosome 3DL derived from ‘Oberkulmer’, **c** indicates the chromosome composition of resistant F_2 plants heterozygous for the 3DL/3Ag translocation, **d** indicates the chromosome composition of the susceptible F_2 plant no. 7 nullisomic for 3DL, **e** indicates the chromosome composition of the resistant F_2 plants monosomic for 3DL, **f** indicates the chromosome composition of the resistant F_2 plant no. 86 trisomic for 3DL

phism between the parents of the mapping population, but they were monomorphic for the NILs.

Of the 360 RAPD primers tested 30 gave no amplification products at all for ‘Arina’ and *Lr24/7** “Arina”, while the others amplified 3–12 major fragments ranging from 400 to 2000 bp. Eleven (3%) primers resulted in 1 additional band in the resistant line *Lr24/7**“Arina”, whereas several other bands generated by the same primers were identical in both the resistant and susceptible NILs. The 11 primers also showed polymorphism between *Lr24/7** “Arina” and ‘Oberkulmer’, the parents of the segregating F_2 population.

Linkage analysis at the *Lr24* region

Linkage studies were performed for the *Lr24* resistance gene and the DNA markers using a segregating F_2 popula-

Table 1 Observed segregation frequencies for DNA markers and chi-square values for goodness-of-fit to expected ratio for F2 progeny of a cross of _Lr24/7_* “Arina” and ‘Oberkulmer’

Genotype[a]	DNA marker						
	Dominant: PSR1203, OPJ-09, J09-RFLP, J09-STS		Codominant: PSR1205[b]			Recessive: PSR388, PSR904, PSR931, PSR1067	
	A_	BB	AA	AB	BB	AA	B_
Expected ratio	3	1	1	2	1	1	3
Observed frequency[c]	108	40	49	58	41	49	99
Chi-square	0.324			7.784		5.189	
P[d]	0.569			0.020		0.023	
Resistant phenotype	108	0	49	58	0	49	58
Susceptible phenotype	0	40	0	0	41	0	41

[a] AA corresponds to a genotype homozygous for _Lr24/7_* “Arina”, BB corresponds to a genotype homozygous for ‘Oberkulmer’, and AB corresponds to a heterozygous genotype; A_ indicates that AA and AB can’t be distinguished and B_ indicates that AB and BB can’t be distinguished

[b] Results are based on the scoring for presence or absence of the allelic 3 kb RFLP fragment from _Lr24/7_* “Arina” and the 20 kb fragment from ‘Oberkulmer’

[c] Plant no. 7 was excluded because of irregularities detected with probe PSR1205 in the *Hin*dIII digest

[d] Values smaller than 0.05 indicate significant deviations from goodness-of-fit

tion from the cross of the resistant NIL _Lr24/7_* “Arina” and the susceptible spelt variety ‘Oberkulmer’. In this mapping population the resistance gene followed a monogenic dominant 3:1 segregation: 108 plants were resistant and 42 susceptible (chi-square=0.720, P=0.396). The DNA markers tested followed a dominant, codominant or recessive segregation and were completely linked with the *Lr24* gene (Table 1).

The probe PSR1205 was scored as a codominant marker in the F$_2$ population digested with restriction enzyme *Hin*dIII. A fragment of 3 kb was present in the parental line _Lr24/7_* “Arina”, whereas ‘Oberkulmer’ had a band of about 20 kb (Fig. 5A). When scoring for the presence or absence of the bands, we found a significant deviation from the genotypic 1:2:1 segregation in favor of both homozygous classes (Table 1). The allele frequency was 0.53 for the 3 kb fragment derived from _Lr24/7_* “Arina” and 0.47 for the 20 kb fragment derived from ‘Oberkulmer’, and showed no deviation from the expected 1:1 ratio (chi-square=0.865, P=0.352). All plants homozygous or heterozygous for the 3 kb fragment were leaf rust resistant, while the 41 plants homozygous for the 20 kb ‘Oberkulmer’ fragment were susceptible. The susceptible plant no. 7 showed irregular inheritance: the fragments of both parents were missing (d in Fig. 5A). Therefore, plant no. 7 was excluded from the linkage analysis shown in Table 1. In addition to scoring for the presence or the absence of the respective bands in the F$_2$ plants, the intensity of the 3 kb fragment was taken into consideration. Plants homozygous for the 3 kb fragment cosegregating with the resistance gene were expected to show the same band intensity as the resistant parent _Lr24/7_* “Arina”, whereas the band of heterozygous plants should be of half-intensity. Out of 107 resistant plants 15 were heterozygous for the _Lr24/7_* “Arina” allele based on band intensity but lacked the corresponding allele of the susceptible parent ‘Oberkulmer’ (Fig. 5A). Another type of irregularity was observed in the resistant plant no. 86: the probe revealed full band intensity for the _Lr24/7_* “Arina” allele, and hybridized also to the allele of ‘Oberkulmer’ (data not shown). These results indicate irregularities during the meiosis of the F$_1$ plants. Plant no. 7 would be homozygous for the loss of 3DL, the 15 resistant plants would be heterozygous for the loss of 3DL whereas plant no. 86 would be trisomic for 3DL. The putative chromosome composition of these different genotypes is shown in Fig. 5B.

The probes PSR1067 (*Dra*I), PSR931 (*Eco*RV), PSR904 (*Eco*RI) and PSR388 (*Dra*I) that showed the null alleles in _Lr24/7_* “Arina” were inherited as recessive markers. The 4 recessive markers showed complete cosegregation and deviated significantly (P< 0.05) from the expected 1:3 ratio (Table 1). The respective bands, which were scored for presence or absence in the F$_2$ population, were in repulsion phase with *Lr24* (Fig. 6). No recombination was found between these 4 probes and *Lr24* (i.e. all susceptible plants showed the ‘Oberkulmer’ bands) except for plant no. 7. However, this could be explained by the hypothesis that this plant is homozygous for the loss of chromosome 3DL, as discussed above. All other F$_2$ plants lacking the ‘Oberkulmer’ bands were homozygous for the _Lr24/7_* “Arina” fragment of the codominant marker PSR1205.

Probe PSR1203 hybridized to an *Eco*RV fragment of about 4.5 kb in the resistant _Lr24/7_* “Arina” that was missing in ‘Oberkulmer’ (Fig. 3) and was inherited as a dominant marker. This fragment was in coupling phase with the *Lr24* gene and cosegregated completely with the resistance gene (Table 1).

One of the 11 RAPD primers distinguishing the NILs (primer OPJ-09) amplified the indicative fragment of 550 bp in all resistant F$_2$ plants (Fig. 7a). In addition, for 2 of the 42 susceptible F$_2$ plants a band of the same size was amplified (data not shown). The cloned PCR fragment (J09-RFLP) hybridized to a 2.3 kb *Eco*RV fragment linked

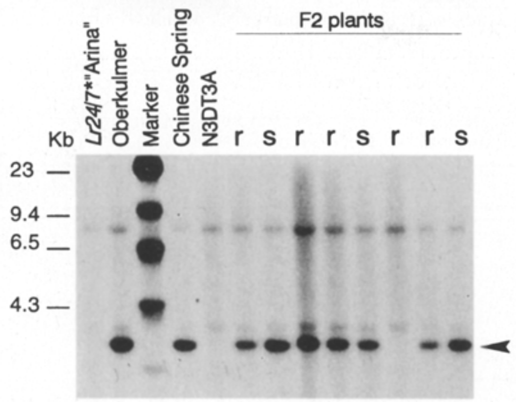


Fig. 6 Southern hybridization pattern of *Dra*I digested genomic DNA isolated from segregating F_2 plants (*r*=resistant, *s*=susceptible), their resistant parent *Lr24/7** "Arina" and their susceptible parent 'Oberkulmer' with probe PSR1067. The *arrowhead* indicates an RFLP fragment of about 3 kb that is completely linked in repulsion phase with the *Lr24* gene. The location of this fragment on chromosome 3D was determined by the aneuploid line nullisomic 3D – tetrasomic 3A (N3DT3A) of 'Chinese Spring'. The molecular weight marker is λDNA digested by *Hin*dIII

with the resistance gene and was inherited as a dominant marker (Fig. 7b). The 2 susceptible plants showing the RAPD band did not have this *Eco*RV fragment. Thus, no recombination was found between the *Lr24* resistance gene and the OPJ-09 product when the latter was used as an RFLP probe.

In order to get a reliable PCR assay, specific 20 mer primers (J09/1 and J09/2) were used to amplify the linked $J09_{550}$ fragment of *Lr24/7** "Arina" in the F_2 population. All resistant plants amplified a DNA fragment of 350 bp, while none of the susceptible plants showed an amplification product (Fig. 7c). Thus, these primers identify a sequence-tagged site (Olson et al. 1989) in wheat (J09-STS) that is completely linked with the *Lr24* gene.

Chromosomal localization of the markers

All 6 PSR probes and J09-RFLP were hybridized with genomic DNA of N3DT3A and 'Chinese Spring' digested by the same enzyme that was used for linkage analysis. For the markers PSR388, PSR904, PSR931, PSR1067 (Fig. 6) and PSR1205 the relevant fragment linked to the *Lr24* resistance gene was present in 'Chinese Spring' but absent in the N3DT3A line of 'Chinese Spring'. For the dominant markers PSR1203 and J09-RFLP the linked fragment was absent in both 'Chinese Spring' and the N3DT3A line.

Use of the markers in plant breeding material

To test whether the markers found in this study are useful for detecting the *Lr24* gene in different genetic backgrounds, 6 additional lines containing the *Lr24* gene were assayed. The results are summarized in Table 2. Lines

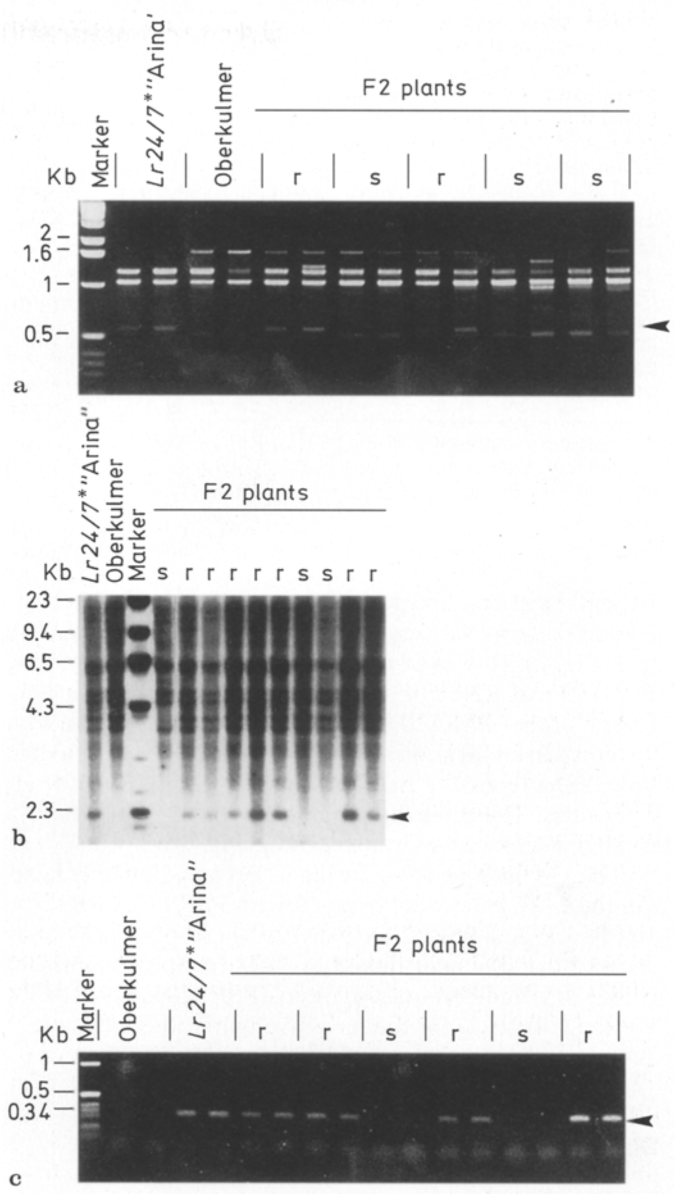


Fig. 7a PCR amplification of genomic DNA by primer OPJ-09 in segregating F_2 plants (*r*=resistant, *s*=susceptible), their resistant parent *Lr24/7** "Arina" and susceptible parent 'Oberkulmer'. The *arrowhead* indicates a dominantly inherited DNA fragment of about 550 bp completely linked in coupling phase with the *Lr24* resistance gene. The molecular weight marker is the 1 kb ladder (Gibco BRL, Switzerland). **b** Southern hybridization pattern of *Eco*RV-digested genomic DNA of segregating F_2 plants (*r*=resistant, *s*=susceptible), their resistant parent *Lr24/7** "Arina" and their susceptible parent 'Oberkulmer' with the cloned PCR fragment of 550 bp amplified by primer OPJ-09 (J09-RFLP). The *arrowhead* indicates a fragment of about 2.3 kb completely linked in coupling phase with the *Lr24* resistance gene. The molecular weight marker is λDNA digested by *Hin*dIII. **c** PCR amplification of genomic DNA by the specific primers J09/1 and J09/2 in segregating F_2 plants (*r*=resistant, *s*=susceptible), their resistant parent *Lr24/7** "Arina" and susceptible parent 'Oberkulmer'. The *arrowhead* indicates a dominantly inherited DNA fragment of about 350 bp completely linked in coupling phase with the *Lr24* resistance gene. The molecular weight marker is the 1 kb ladder (Gibco BRL, Switzerland)

Table 2 Identification of the *Lr24* resistance gene in different genetic backgrounds with the DNA markers completely linked to *Lr24* in the segregating F_2 population of a cross *Lr24/7** "Arina" and 'Oberkulmer'

Wheat lines containing *Lr24*	DNA markers								
	Dominant[a]				Codominant[a]	Recessive[b]			
	PSR1203	OPJ-09	J09-RFLP	J09-STS	PSR1205	PSR1067	PSR904	PSR931	PSR388
*Lr24/7** "Arina"	+	+	+	+	+	–	–	–	–
Agent	+	+	+	+	+	–	–	–	–
RL6064	+	+	+	+	+	–	–	–	–
RNS173	+	+	+	+	+	–	–	–	–
RNS184	+	–	–	–	–	+	+	+	–
RNS189	+	–	–	–	–	+	+	+	–
RNS219	+	–	–	–	–	+	+	+	–

[a] + indicates the presence of the PCR or RFLP fragments linked in coupling phase with the *Lr24* resistance gene

[b] – indicates the absence of the RFLP fragments linked in repulsion phase with the *Lr24* resistance gene, i.e., the respective fragments are present (+) in the susceptible parent but absent (–) in the resistant parent

'Agent', RL6064 and RNS173 showed the same hybridization patterns as *Lr24/7** "Arina" for all DNA markers (e.g. Fig. 2). However, the 3 'Kalyansona' lines RNS184, RNS189 and RNS219 showed the expected pattern only for the probes PSR1203 (Fig. 3), a dominant marker with the most distal location on chromosome 3DL (Fig. 1a) and PSR388, a recessive marker showing a null allele for all *Lr24* lines. These results suggest that those 3 lines have a smaller *A. elongatum* translocation (Fig. 1c).

The 51 winter and nine spring wheat breeding lines lacking the *Lr24* gene were assayed with PSR931, PSR1205, J09-RFLP and PSR1203. None of these 60 wheat lines showed the fragment linked to *Lr24* for the codominant (PSR1205) and dominant markers (PSR1203, J09-RFLP), nor the null allele for the recessive marker PSR931.

Discussion

Six RFLP markers and one RAPD marker converted to a STS were found to be completely linked to the *Lr24* resistance gene in wheat. According to the map of Devos and Gale (1993) the 5 RFLP markers PSR1067, PSR904, PSR1205, PSR931 and PSR1203 span a genetic distance of 60 cM, whereas in our F_2 population all DNA markers are completely linked (i.e. 0 cM), indicating suppressed recombination. Thus, the genetic distance between the linked DNA markers does not correspond to the physical distance. The resistant parent of our F_2 population, *Lr24/7** "Arina", was derived from RL6064 seven times backcrossed with 'Arina', and the line RL6064 was itself derived from 'Agent' six times backcrossed with 'Thatcher'. This implies that the translocated *A. elongatum* segment of 'Agent' is inherited as a whole complex of genes, among them the *Lr24* resistance gene, and that linkage drag could not be reduced by recurrent backcross breeding. The lack of recombination could be explained by incomplete chromosome pairing of the translocated segment of the *A. elongatum* chromosome with the wheat 3DL chromosome during meiosis (Sears 1973). The high frequency of non-allelic RFLP fragments (five out of six) linked in coupling or repulsion with the *Lr24* gene suggests that a considerable part of the translocated *A. elongatum* chromosome is not homoeologous to the chromosome 3DL of 'Oberkulmer' (*T. spelta*).

The analysis of the F_2 population from the cross *Lr24/7** "Arina"×'Oberkulmer' with the codominant probe PSR1205 based on band intensity led to the discovery of plants hemizygous for the *Xpsr1205* locus on chromosome 3DL. Loss of this locus occurred in 14% of all resistant plants and explains the distorted segregation found for PSR1205. Unfortunately, scoring based on band intensity was only reliable for this probe and the 3 kb fragment. The F_2 plant no. 7 was null allelic for all tested RFLP probes and susceptible to leaf rust. This indicates that this line was not only homozygous for the loss of the *Xpsr1205* locus but has lost part or even the whole of chromosome 3D. Cytogenetic analysis should allow to determine the physical size of the lost fragment. Although the mechanism of chromosome loss is unclear, it could be due to the lack of pairing between the spelt and *A. elongatum* chromosome arm during meiosis in the F_1 plants.

In additional lines carrying the *Lr24* resistance gene, two groups could be distinguished based on the RFLP pattern with probes PSR1205, PSR1067, PSR931, PSR904 and J09-RFLP. The first group of lines is related to 'Agent' (Lr24/7* "Arina", 'Agent', RL6064, RNS173) and shows the presence of the respective bands for the dominant and codominant markers and absence of the band for the recessive markers. The second group of lines is related to 3Ag3 (RNS184, RNS189, RNS219) and showed the presence of the relevant band only for the dominant marker PSR1203 and the absence of the band for the recessive marker PSR388. *Xpsr*1203 is the most distal locus mapped on wheat chromosome 3DL. Thus, it is likely that the 3Ag3 *Lr24* translocation (Fig. 1c) is considerably smaller than the one in 'Agent' (Fig. 1b) and is located at the end of the chromosome. Possibly, the 3Ag3 *Lr24* translocation corresponds to translocation no. 3 described by Sears (1973)

(R. Basant, personal communication). These 'Kalyansona' backcross lines might, therefore, be better gene donors for the *Lr24* resistance gene in breeding programs.

Probe PSR388 has been mapped close to the centromere of chromosome 2A and 2B (Devos et al. 1993). In our present investigation we found linkage of *Xpsr388* to markers on chromosome 3DL and the *Lr24* resistance gene. The location of the respective band on chromosome 3D was confirmed by nullisomic analysis. The trivial explanation of our finding is a possible error in probe handling. However, *Xpsr388* maps to 3DL and shows a hybridization pattern that is clearly distinct from the other linked RFLP probes. In addition, there is no other *Xpsr* clone that maps to this chromosomal region of 3DL. Thus, PSR388 maps not only to the homoeologous group 2 but also to 3DL.

The number of RAPD polymorphisms between NILs that were linked to the *Lr24* resistance gene was surprisingly low (1 RAPD marker out of 360 primers tested). Given the fact that the *A. elongatum* translocation comprises more than half of a chromosome arm on the genetic map, a larger number of polymorphisms was expected. In an earlier study, a short telomeric translocation (B. Friebe and B. S. Gill, personal communication) derived from *Aegilops umbellulata* resulted in 3 RAPD markers (out of 395 primers tested) (Schachermayr et al. 1994).

The RAPD marker OPJ-09 amplified the 550 bp fragment of *Lr24*/7* "Arina" in all of the resistant F_2 plants. However, 2 of the 42 susceptible lines also showed the characteristic band. The 2 false positive F_2 plants were not recombinants between the OPJ-09_{550} fragment and *Lr24* which we could show by using the amplified fragment as an RFLP probe. There, complete linkage of the J09-RFLP probe with the *Lr24* gene was demonstrated. The possibility of being able to use the amplified fragment as a RFLP probe is quite rare in wheat: only one in ten RAPD bands does not contain highly repetitive sequences (Devos and Gale 1992).

The conversion of the linked RAPD marker to a STS for *Lr24* using 2 specific primers overcomes the problems of unspecific amplification that occurred with the shorter primer OPJ-09. Interestingly, the fragment amplified by the specific primers was shorter than the RAPD fragment. Possibly, there is an internal binding site for one primer in the same fragment.

The J09-STS marker allows a reliable and rapid screening of a large number of genotypes for the presence of the *Lr24* gene derived from 'Agent'. In addition, the codominant and recessive inherited RFLP markers, especially PSR1205 and PSR388, can be useful in practical breeding as they allow the selection of plants homozygous for the *Lr24* resistance gene. However, the scoring for the presence of a band linked to the *Lr24* resistance gene should be preferred, since it avoids misclassification due to loss of chromosome segments (e.g. plant no. 7). The combined use of the STS marker found for *Lr24* and the STS available for *Lr9* (Schachermayr et al. 1994) greatly facilitates pyramiding the two leaf-rust resistance genes by marker-assisted selection and should allow a more efficient breeding for durable resistance against this disease.

Acknowledgments We would like to thank Christine Friedrich-Baumgartner for her excellent technical assistance. We thank Padruot Fried and Philipp Streckeisen for NILs with *Lr24* and other wheat breeding lines as well as for helpful discussions. We would like to thank Dr. P. Dyck and Dr. R. Basant for providing the *Lr24* lines. We are grateful to Mike Gale, Katrien Devos and Philippe Gay for providing us the PSR RFLP probes. This work was supported by the Swiss Priority Program Biotechnology (no. 5002-34559).

References

Baum M, Lagudah ES, Appels R (1992) Wide crosses in cereals. Annu Rev Plant Physiol Plant Mol Biol 43:117–143

Browder LE (1973) Probable genotype of some *Triticum aestivum* 'Agent' derivatives for reaction to *Puccinia recondita* f. sp. *tritici*. Crop Sci 13:203–206

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84:567–572

Devos KM, Gale MD (1993) Extended genetic maps of the homoeologous group 3 chromosomes of wheat rye and barley. Theor Appl Genet 85:649–652

Devos KM, Atkinson MD, Chinoy CN, Liu CJ, Gale MD (1992) RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. Theor Appl Genet 83:931–939

Devos KM, Millan T, Gale MD (1993) Comparative RFLP maps of the homoeologous group-2 chromosomes of wheat, rye and barley. Theor Appl Genet 85:784–792

Graner A, Siedler H, Jahoor A, Herrmann RG, Wenzel G (1990) Assessment of the degree and type of restriction fragment length polymorphism in barley (*Hordeum vulgare*). Theor Appl Genet 80:826–832

Hartl L, Weiss H, Zeller FJ, Jahoor A (1993) Use of RFLP markers for the identification of alleles of the *Pm3* locus conferring powdery mildew resistance in wheat (*Triticum aestivum* L.). Theor Appl Genet 86:959–963

Knott DR (1989) The wheat rusts - breeding for resistance. Monographs on theoretical and applied genetics 12. Springer, Berlin Heidelberg New York

Kolmer JA (1993) Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1991. Can J Plant Pathol 15:34–36

Liu YG, Mori N, Tsunewaki K (1990) Restriction fragment length polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat. Jpn J Genet 65:367–380

Long DL, Roelfs AP, Leonard KJ, Roberts JJ (1993) Virulence and diversity of *Puccinia recondita* f. sp. *tritici* in the United States in 1991. Plant Dis 77:786–791

Martens JW, Dyck PL (1988) Occurrence and virulence of *Puccinia recondita* in Canada in 1986. Can J Plant Pathol 10:268–272

McIntosh RA, Dyck PL, Green GJ (1976) Inheritance of leaf rust and stem rust resistances in wheat cultivars 'Agent' and 'Agatha'. Aust J Agric Res 28:37–45

Olson M, Hood L, Cantor CH, Botstein D (1989) A common language for physical mapping of the human genome. Science 24:1434–1435

Penner GA, Chong J, Levesque-Lemay M, Molnar SJ, Fedak G (1993) Identification of a RAPD marker linked to the oat stem rust gene *Pg3*. Theor Appl Genet 85:702–705

Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico, D.F.

Ronald PC, Albano B, Tabien R, Abenes L, Wu KS, McCouch S, Tanksley SD (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Mol Gen Genet 236:113–120

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Schachermayr G, Siedler H, Gale MD, Winzeler H, Winzeler M, Keller B (1994) Identification and localization of molecular markers linked to the *Lr9* leaf rust resistance gene of wheat. Theor Appl Genet 88:110–115

Schüller C, Backes G, Fischbeck G, Jahoor A (1992) RFLP markers to identify the alleles on the *Mla* locus conferring powdery mildew resistance in barley. Theor Appl Genet 84:330–338

Sears ER (1966) Chromosome mapping with the aid of telocentrics. In: MacKey J (ed) Proc 2nd Int Wheat Genet Symp. Hereditas Suppl 2, Lund, pp 370–381

Sears ER (1973) *Agropyron*-wheat transfers induced by homoeologous pairing. In: Sears ER, Sears LMS (eds) Proc 4th Wheat Genet Symp. University of Missouri, Columbia, Mo., pp 191–199

Siedler H, Messmer MM, Schachermayr GM, Winzeler H, Winzeler M, Keller B (1994) Genetic diversity in European wheat and spelt breeding material based on RFLP data. Theor Appl Genet 88:994–1003

Siharulidze Z, Naskidashville G, Smirnova LA (1993) Virulence structure of the wheat leaf rust population in Georgia during 1987–1991. Cereal Rusts Powdery Mildew Bull 21:35–40

Smith EL, Schlehuber AM, Young HC, Edwards, LH (1968) Registration of 'Agent' wheat. Crop Sci 8:511–512

Tingey S, del Tufo JP (1993) Genetic analysis with random amplified polymorphic DNA markers. Plant Physiol 101:349–352

von Kröcher C, Bartels G, Fehrmann H (1992) Studies on the physiological specialization of leaf rust in wheat (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks. & Henn.). J Plant Dis Prot 99:137–144

Waugh R, Powell W (1992) Using RAPD markers for crop improvement. Trends Biotechnol 10:186–191

Williams GK, Kubelik AR, Kenneth JL, Rafalski A, Scott VT (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535