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Identification of molecular markers linked to the *Yr15* stripe rust resistance gene of wheat originated in wild emmer wheat, *Triticum dicoccoides*

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Abstract The *Yr15* gene of wheat confers resistance to the stripe rust pathogen *Puccinia striiformis* West., which is one of the most devastating diseases of wheat throughout the world. In the present study, molecular markers flanking the *Yr15* gene of wheat have been identified using the near-isogenic-lines approach. RFLP screening of 76 probe-enzyme combinations revealed one polymorphic marker (*Nor*/*Taq*I) between the susceptible and the resistant lines. In addition, out of 340 RAPD primers tested, six produced polymorphic RAPD bands between the susceptible and the resistant lines. The genetic linkage of the polymorphic markers was tested on segregating F_2 population (123 plants) derived from crosses between stripe rust-susceptible *Triticum durum* wheat, cv D447, and a BC_3F_9 resistant line carrying *Yr15* in a D447 background. A 2.8-kb fragment produced by the *Nor* RFLP probe and a 1420-bp PCR product generated by the RAPD primer OPB13 showed linkage, in coupling, with the *Yr15* gene. Employing the standard maximum-likelihood technique it was found that the order $OPB13_{1420}$–*Yr15*–*Nor1* on chromosome 1B appeared to be no less than 1000-times more probable than the closest alternative. The map distances between $OPB13_{1420}$–*Yr15*–*Nor1* are 27.1 cM and 11.0 cM for the first and second intervals, respectively. The application of marker-assisted selection for the breeding of new wheat cultivars with the stripe rust resistance gene is discussed.

Key words Gene mapping · RAPD · RFLP · Stripe rust · *Triticum dicoccoides* · *Triticum durum*

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Introduction

Stripe rust (yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is one of the most devastating diseases of wheat throughout the world. Breeding of resistant varieties is an effective approach to eliminate the use of fungicides and minimize crop losses due to this disease. However, most of the described major genes for resistance to stripe rust in cultivated wheat have become ineffective to one or more of the known pathogenic races when acting singly (Stubbs 1985). In all wheat-growing areas of the world, the virulence of the races has been shown to increase step by step (Stubbs 1985), often breaking down even combinations of resistance genes.

Depletion of effective genes for resistance to stripe rust in cultivated wheat has led to a search for new resistance genes among wild wheats. A particularly promising source for yellow rust resistance is wild emmer wheat, *Triticum dicoccoides* Korn, the progenitor of all cultivated wheat (Gerechter-Amitai and Stubbs 1970). This wild species, discovered in northern Israel by Aaronsohn (1910), was found to be a valuable genetic resource for wheat improvement, including resistance to several diseases (Nevo 1983, 1989, 1995). One accession, *T. dicoccoides* G-25, was shown to be highly resistant to more than 20 stripe rust races from six countries (Gerechter-Amitai and Stubbs 1970).

Further studies showed that this resistance was conferred by one dominant gene, designated as *Yr15* (Gerechter-Amitai and Grama 1974; Gerechter-Amitai et al. 1989).

Both restriction fragment length polymorphism (RFLP) (Botstein et al. 1980; Dweikat et al. 1994; Schachermayer et al. 1994; Williams et al. 1994) and random amplified polymorphic DNA (RAPD) (Williams et al. 1990) have been used as molecular markers in wheat. Near-isogenic lines (NILs) differing in a specific trait have been successfully used to isolate genetic markers for the gene determining that trait. This approach has given tightly linked RFLP and RAPD markers for several important disease resistance genes, e.g. the *Tm-2a* viral resistance gene in tomato (Young and Tanksley 1989), the black root rot resistance gene in tobacco (Bai et al. 1995), the *ml-0* resistance locus (Hinze et al. 1991), the *Ml-a* (Schuller et al. 1992) and *Rh* loci (Barua et al. 1993) in barley, the *Ht1* gene in maize (Bentolila et al. 1993), and the *Pm3* (Hartl et al. 1993), *Lr9* (Schachermayr et al. 1994) and *Cre* (Williams et al. 1994) genes in wheat.

Based on cytogenetic analysis, McIntosh et al. (1996) revealed that the stripe rust resistance gene, *Yr15*, resides in the short arm of chromosome 1B of wheat at a distance of about 7 cM from the centromere. This analysis was conducted employing telocentric mapping, and the authors mentioned that with full chromosome pairing and normal disjunction this recombination level may have been different (McIntosh et al. 1996). Furthermore, the results obtained did not allow a decision on whether *Yr15* is proximal or distal to *Nor-B1*. Thus, the question of the map location of *Yr15*, and especially the position of this gene relative to the interval *centromere–NorB1*, remains unclear. Therefore, the objective of our study was to reveal molecular markers flanking this important resistance gene, and to map *Yr15* relative to already mapped loci.

Materials and methods

Plant materials and isolation of DNA

The plant materials used for this study consisted of the resistant *Yr15* donor line, *T. dicoccoides*, G-25, a *T. durum* Desf. line susceptible to stripe rust (cv D447 = LD393/2 Langdon ND58-322), and resistant BC_3F_8 (B1, B2) and BC_3F_9 lines (B9 and B10) which are based on the introgression of *Yr15* into *T. durum* (D447), with selection for resistance and morphological similarity to the cultivar in each generation. In this study, we refer to the lines B9, B10 and D447 as near-isogenic lines. The resistant lines, B9 and B10, were crossed to the susceptible parent D447 and the F_1 hybrids were self-crossed to produce segregating populations for linkage analysis.

Leaf tissue samples were collected from the F_2, G-25, D447 and NILs, frozen in liquid nitrogen, and kept in $-80°C$. DNA was extracted according to the method described by Junghans and Metzlaff (1990).

Resistance tests

Resistance to stripe rust was tested at the seedling stage as described by Gerechter-Amitai et al. (1989), using the stripe rust isolate WYR-004 (race 2E18).

RFLP analysis

Wheat DNA for Southern analysis was digested with the restriction enzymes *Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I and *Taq*I following the manufacturer's instructions. Electrophoresis was conducted in an 0.8% agarose gel for *Eco*RI-, *Eco*RV-, *Hin*dIII- and *Dra*I-digested products (10 µg DNA / lane), and a 1% agarose gel for *Taq*I-digested products (3 µg DNA / lane), with 1 × TAE buffer, at 50 V overnight. The DNA was then transferred onto nylon membranes (Hybond-N +, Amersham).

Membranes were pre-hybridized at 65°C for 2–4 h in a 6 × SSC, 5 × Denhard's solution (0.02% BSA, PVP360 and Ficol 1400, w/v), with 50 µg/ml of salmon-sperm DNA and 0.5% SDS. The hybridization was performed overnight at 65°C, with ^{32}P-labelled probe. The hybridized membranes were washed for 20 min with 2 × SSC and 0.1% SDS at 65°C, then for 15 min with 1 × SSC and 0.1% SDS at 65°C. Occasionally, it was necessary to carry out an additional wash with 0.5 × SSC and 0.1% SDS for 10 min at 65°C. The membranes were exposed to X-ray film (XAR-5, Kodak) at $-70°C$.

RFLP probes

Probes for RFLP were prepared by PCR amplification of plasmid DNA using the M13R and 21M13 universal primers. The *Nor*-wheat rDNA intergenic spacer (IGS) 3.0-kb insert was digested with *Taq*I from the recombinant plasmid *pTa71* (described in Gerlach and Bedbrook 1979), kindly provided by M. O'Dell (Plant Breeding Institute, Cambridge). Other RFLP probes (located on chromosome 1B) were kindly provided by M. Sorrells (Cornell University, Ithaca) and M. D. Gale (Cambridge Laboratory, Norwich).

RAPD analysis

Amplification reactions were performed based on the standard protocol of Williams et al. (1990). PCR reaction mixtures of 20 µl contained 20 ng of template DNA, incubation buffer (IV) and 0.5 U of Thermostable DNA polymerase from Advanced Biotechnologies (Leatherhead, Surrey, UK), 2 mM of $MgCl_2$, 0.1 mM of each: dCTP, dGTP, dTTP and dATP, and 12 ng of a single 10-mer primer obtained from Operon Technologies or the University of British Columbia. Amplifications were performed in DNA Thermo-cycler (Perkin Elmer) programmed for 45 consecutive cycles each consisting of 1 min at 94°C, 2 min at 37°C and 2 min at 72°C. Following amplification, the samples were subjected to electrophoresis in 1.4% agarose gels, stained with 0.5 µg/ml ethidium bromide, and viewed under UV light.

Data analysis

Chi-square (χ^2) tests were used in analyses of the inheritance of stripe rust resistance, and the polymorphic RFLP and RAPD markers. The RAPD marker nomenclature used by Michelmore et al. (1991) was employed in describing RAPD loci. Linkage analysis was performed employing the maximum-likelihood (ML) approach (Bailey 1961; Korol et al. 1994).

Results

Analysis of near-isogenic lines with RAPDs and RFLPs

DNA samples from the stripe rust-resistant line, B9 and the susceptible line, D447, were used as templates for screening polymorphism at the molecular level. Since the genome of the susceptible recurrent parent cv D447 and the resistant lines B9 and B10 are supposed to be identical, except for the regions flanking the gene of interest, we referred to them as NILs. Therefore, any probe showing polymorphism between the recurrent parent cv D447 and its resistant NILs should be located near the gene of interest. Out of 340 random 10-mer oligonucleotide primers, 245 produced scorable amplification products. A total of 3218 discernible DNA fragments ranging from 200 to 2600 bp were produced, corresponding to an average of 9.46 fragments per primer. Forty primers showed polymorphisms between D447 and B9 in the first run. These primers were then used to test for polymorphisms between the susceptible line, D447, the resistant NILs, B9 and B10, and the *Yr15* donor parent, G25. Six primers showed polymorphism between the susceptible line and all resistant lines, in which an additional band was found in the resistant lines (Table 1, Fig. 1).

Because cytogenetic analysis has demonstrated that the stripe rust resistance gene is located on chromosome 1B (R. A. McIntosh, personal communication; McIntosh et al. 1996), a set of RFLP markers that have been assigned to chromosome 1B was used to screen for polymorphism between the recurrent parent *T. durum* cv D447, the resistant lines B1, B2, B9 and B10, and *T. dicoccoides* G-25. A total of 76 probe-enzyme combinations were surveyed. When DNA was digested with the restriction enzyme *Taq*I and hybridized with the *Nor* RFLP probe, polymorphism was found between the susceptible recurrent parent D447 and the resistant NILs, and the *Yr15* donor parent G-25 (Fig. 2). All the resistant lines (B1, B2, B9 and B10) show a 2.8-kb band which is also present in the resistant parent *T. dicoccoides* (G-25) but absent in the susceptible recurrent parent *T. durum* cv D447 (Fig. 2). Furthermore,

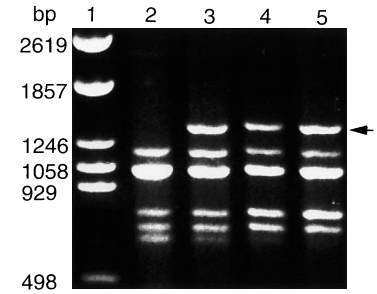


Fig. 1 RAPD products generated from the susceptible *T. durum* parent cv D447 (*lane 2*), the resistant near-isogenic lines B9 (*lane 3*) and B10 (*lane 4*), and the *Yr15* gene-donor parent, *T. dicoccoides* G25, (*lane 5*) with the primer OPB13. The *arrowhead* indicates a polymorphic band (1420 bp) differentiating between the susceptible and the resistant lines. The molecular-weight marker is pBR322 digested with *Alw*-441/*Mval* (*lane 1*)

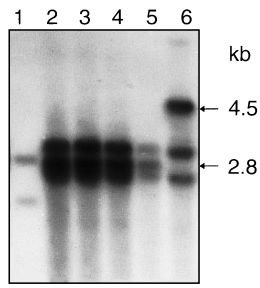


Fig. 2 Southern-hybridization pattern (*Nor* probe, *Taq*I digest) of the *Yr15* gene-donor parent, *T. dicoccoides* G25, (*lane 1*), the resistant near-isogenic lines B1, B2, B9 and B10 (*lanes 2–5*, respectively), and the susceptible *T. durum* parent cv D447 (*lane 6*). Genomic DNA was digested with *Taq*I, and hybridized with the *Nor* RFLP probe (*pTa71*). The *arrowheads* indicate the presence of a RFLP fragment of about 4.5 kb in the susceptible line D447 and a 2.8-kb fragment present in the resistant lines, G25 and B1, B2, B9 and B10

Table 1 RAPD polymorphisms between the susceptible parent (D447) and the resistant NILs (B9 and B10). +, presence of the RAPD marker; −, absence of the RAPD marker

RAPD primers	Primer sequence (5′ to 3′)	Size of polymorphic RAPD band (bp)	Parents		NILs	
			D447	G-25	B9	B10
UBC475	CCAGCGTATT	550	−	+	+	+
		720	+	+	+	−
UBC532	TTGAGACAGG	490	−	+	+	+
UBC541	GCCCCTTTAC	640	+	+	+	−
UBC561	CATAACGACC	1050	−	+	+	−
UBC321	ATCTAGGGAC	1700	−	+	+	+
OPA20	GTTGCGATCC	1060	−	−	+	−
OPB8	GTCCACACGG	660	−	+	+	+
OPB13	TTCCCCCGCT	1420	−	+	+	+
OPF15	CCAGTACTCC	510	−	+	+	+
		430	+	+	+	−
OPK4	CCGCCCAAAC	940	+	−	+	−
OPT16	GGTGAACGCT	830	−	+	+	−

a 4.5-kb fragment that is present in line D447 is absent in G-25 and is also missing in all four resistant lines (B1, B2, B9 and B10) (Fig. 2).

Inheritance of the *Yr15* gene and the polymorphic markers, and their linkage test in the F_2 population

To test for an association between the revealed DNA polymorphisms and the stripe rust resistance gene (*Yr15*), the six polymorphic RAPD primers were used to amplify genomic DNA of the F_2 mapping population. Only one of the RAPD markers, OPB13 (1420 bp), produced polymorphism in 123 F_2 individuals (Fig. 3). The RAPD band $OPB13_{1420}$ segregated as: 95:28 in the F_2 population (test for deviation from the expected 3:1 ratio gives $\chi^2 = 0.328$, $df = 1$, $P = 0.5$–0.7) (Table 2). Linkage between $OPB13_{1420}$ and the *Yr15* gene was tested using 123 F_2 individuals of the mapping population. The results obtained indicated that $OPB13_{1420}$ was linked, in coupling, to *Yr15* (see below).

To establish an association between the rDNA gene *Nor1* (*pTa71*) and the resistance gene *Yr15*, genomic DNAs of 114 F_2 individuals were hybridized with this probe (Fig. 4). The 2.8-kb band of the *T. dicoccoides* (G-25) parent segregated 86:28 in the F_2 mapping population, which fits a 3:1 distribution ($\chi^2 = 0.012$, $df = 1$, $P > 0.90$). The co-segregation of the scored stripe rust resistance and the 2.8-kb restriction fragment in 114 offspring indicated the presence of close linkage between the RFLP marker and the resistance gene *Yr15*.

Let us denote the resistance locus as R/S, and the DNA markers as A/B (*Nor*1) and M/m ($OPB13_{1420}$). The segregation data presented in Table 2 served to estimate linkage between the three loci and the most probable order using three-point analysis. A standard maximum-likelihood (ML) technique was employed (Bailey 1961; Korol et al. 1994).

Table 2 Segregation of the F_2 mapping population for the resistance gene *Yr15* and the DNA markers *Nor1* and *OPB13*[a]

RFLP marker	AA		AB		BB		F	
RADP marker	M	m	M	m	M	m	M	m
Resistant	34	7	37	6	8	1	7	1
Susceptible	0	1	1	0	7	12	1	0

[a] A: a 2.8-kb fragment detected by the *Nor* RFLP probe; B: a 4.5-kb fragment detected by the *Nor* RFLP probe; F: F_2 plants for which RFLP data is missing; M: a 1420-bp PCR product generated by RAPD primer OPB13; m: F_2 plants without the RAPD product $OPB13_{1420}$

With one co-dominant and two dominant loci, the total number of distinguishable segregation classes in F_2 is $3 \times 2 \times 2 = 12$. Clearly, for three linked loci one can have three potential orders, and the estimation procedure should allow us to choose the true one. The observed numbers of these classes (n_i) enter into the logarithm of the ML-function as:

$$\lg L(r_1, r_2) = c + \sum_{i=1}^{12} n_i \lg [p_i(r_1, r_2)],$$

where r_1 and r_2 are the recombination rates between the loci in two neighbouring intervals, n_i is the observed number of plants in the ith ($i = 1, \ldots, 12$) class, while $p_i(r_1, r_2)$ is its expected frequency which depends on the chosen configuration (one of the three possibilities) and the level of interference. Our subsequent analysis was conducted for two different interference models, 'Kosambi interference' and 'no interference', resulting in similiar results. A small paucity of susceptible plants was observed in the data. Thus, we have to allow for a mis-classification of some proportion (α) of susceptible genotypes as 'resistant'. Such a possibility is modelled by correction of the expected frequencies p_i in the following way: for each susceptible class, p_i was replaced by the term (1-α) p_i and the quantity α p_i was

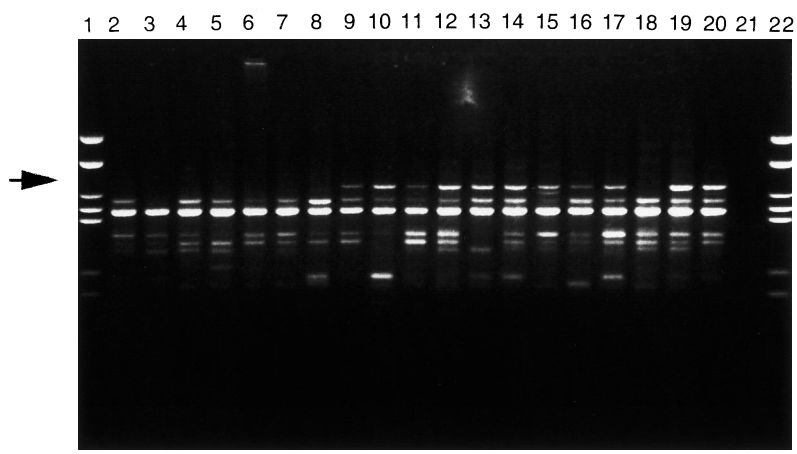


Fig. 3 PCR amplification of genomic DNA with the primer OPB13 in segregating F_2 individuals from the cross D447/B9. *Lanes 2–8* are the susceptible F_2 individuals. *Lanes 9–17* are the resistant F_2 individuals. *Lane 19* is the resistant parent B9, *lane 18* is the susceptible parent, *T. durum* cv D447, *lane 20* is the *Yr15* gene-donor line, *T. dicoccoides* G25. *Lanes 1 and 22* are the molecular-weight marker, pBR322 digested with *Alw*441/*Mval*. *Lane 21* is a negative control (no DNA). The *arrowhead* indicates a dominantly inherited DNA fragment of about 1420 bp linked to the *Yr15* resistance gene

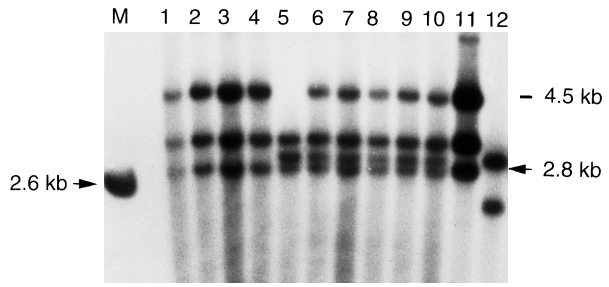
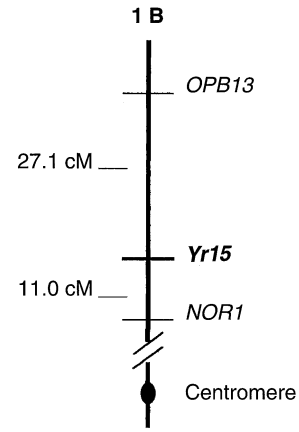


Fig. 4 Southern hybridization pattern of F_2 individuals from the cross D447/B9 segregating for *Nor 1*. Genomic DNA was digested with *Taq*I and hybridized with the *Nor* RFLP probe (*pTa71*). *Lane 1–4* are the susceptible F_2 individuals, *lanes 5–10* are the resistant F_2 individuals. *Lane 11* is the susceptible parent *T. durum* cv D447, *lane 12* is the *Yr15* gene-donor line, *T. dicoccoides* G25. The *arrowhead* indicates an RFLP fragment of about 2.8 kb present in the resistant lines. *M* is pGEM-3 DNA digested with *Hin*fI, *Rsa*I and *Sin*I

Fig. 5 Linkage map showing the region near the *Yr15* gene. All distances are given in centiMorgans using Kosambi's mapping function and were derived from the multipoint analysis



added to the expected frequency of the corresponding resistant class. Thus, our expected frequencies are now functions of three parameters, $p_i = p_i(r_1, r_2, \alpha)$. The numerical values of r_1, r_2, and α providing the maximum $\lg L(r_1, r_2, \alpha)$ are considered as ML-estimates $(\hat{r}_1, \hat{r}_2, \hat{\alpha})$ of the unknown parameters.

The standard errors of the parameter estimates can be obtained as described in Bailey (1961). Namely, we can calculate the matrix of second derivatives of the function $\ln L(r_1, r_2, \alpha)$ at the values $\hat{r}_1, \hat{r}_2, \hat{\alpha}$ and then obtain an inversion matrix with the opposite sign. The diagonal of the resulting matrix is composed of squared standard errors of the parameter estimates. The correlation for mis-classification and the standard errors were calculated only for the chosen (putatively true) order of the three involved loci.

The results obtained for the three possible gene orders are given in Table 3. As can be easily seen from this table, the third order, M/m–R/S–A/B, gives the highest value of the log-likelihood function (e.g. -22.30 versus -25.35 for the closest result related to the order M/m–A/B–R/S, in the case of Kosambi metrics at $\alpha = 0$). Correspondingly, the chosen order is about 1000-times more probable than M/m–A/B–R/S. Noteworthy, the chosen order also fits another criterion usually used in revealing the true order in multipoint mapping: the minimum sum of recombination rates. Here it gives $r_1 + r_2 = 25.2 + 12.8 = 38\%$ (compared to $37.4 + 11.3 = 48.7$ in the closest alternative).

The last question to be addressed is the significance of linkage between *Yr15* and the flanking markers. According to the ML-method, one could consequently put $r_1 = 50\%$ and $r_2 = 50\%$ into $\lg L$ and repeat the maximization in order to obtain the estimates of the remainder parameters, provided there is free recombination in one of the intervals. The resulting values of $\lg L(r_1 = 50\%)$ and $\lg L(r_2 = 50\%)$ are compared to the initial $\lg L(r_1, r_2)$ (LOD score test), giving a chi-square for linkage. In our case the resulting χ^2 was 15.75 ($df = 1$, $P < 0.02\%$) for the first interval, and 50.28 ($df = 1$, $P < 0.0001\%$) for the second interval (for the Kosambi metric). The derived map in the vicinity of the *Yr15* gene is shown in Fig. 5.

Discussion

In most of the studies in which strong linkage has been reported between RFLP or RAPD markers and disease

Table 3 Parameter estimation for three possible positions of *Yr15* relative to the revealed two DNA markers

Order	Metric	$\hat{r}_1$(%)	$\hat{r}_2$(%)	$\hat{\alpha}$(%)	$\lg L$
r_1 r_2 ↓ ↓					
A/B–M/m–R/S	H[a]	33.8	22.4		− 31.45
	K[a]	38.3	24.1		− 32.45
M/m–A/B–R/S	H	35.5	11.1		− 24.95
	K	37.4	11.3		− 25.35
M/m–R/S–A/B	H	25.9	12.9		− 22.48
	H[b]	25.7 ± 5.3	11.1 ± 3.9	8.5 ± 11.1	− 21.83
	K	25.2	12.8		− 22.30
	K[b]	24.7 ± 5.2	10.8 ± 3.6	9.3 ± 10.8	− 21.61

[a] H and K denote no interference (Haldane's metric) and Kosambi's interference respectively
[b] The mapping model takes into account a possible classification error with a probability $\alpha(S \rightarrow R)$

resistance loci, the latter traits were derived from introgression via interspecific crosses (Martin et al. 1991; Paran and Michelmore, 1991; Ronald et al. 1992; Ishii et al. 1994; Schachermayr et al. 1994; Williamson et al. 1994; Bai et al. 1995). The present study has used the NILs approach to identify RAPD and RFLP markers linked to the stripe rust resistance gene *Yr15*. To further support the putative linkage between the polymorphic markers and the *Yr15* gene, a segregation analysis was carried out on an F_2 population. One RFLP marker and one RAPD marker were found to be linked to the stripe rust resistance gene *Yr15*, which was introgressed into *T. durum* from wild emmer wheat, *T. dicoccoides*. A standard maximum-likelihood technique was employed to analyze the linkage between *Yr15* and two linked marker loci. The order $OPB13_{1420}$–*Yr15–Nor1* gives the highest value of the log-likelihood function. The chosen order is no less than 1000-times more probable than the closest alternative. The map distances between $OPB13_{1420}$ and the *Yr15* gene, and the RFLP marker *Nor1* and the *Yr15* gene, are 27.1 cM and 11.0 cM respectively. Thus, our mapping efforts allowed us to find two molecular markers flanking the *Yr15* gene.

According to the published genetic maps, the distance between *Nor1* and the centromere is in a range of 1.7–8.6 cM (Hart et al. 1993; Gale et al. 1995; Van Deynze et al. 1995). Probably, the variation depends on the genetic background of the mapping populations used in the different studies. Based on telocentric mapping, McIntosh et al. (1996) found the distance between *Yr15* and *Nor1* to be 7 cM. However, McIntosh et al. (1996) note that the specific material used in their mapping (mono-telodisomic plants heterozygous for *Yr15*) might affect the rate of recombination. Thus, our estimate of the distance between *Yr15* and *Nor1* corroborates other mapping results. Altogether, the available data allow us to conclude that the segment $OPB13_{1420}$–*Yr15–Nor1* is oriented from the telomere towards the centromere.

Martin et al. (1991) used RAPDs and near-isogenic lines to identify markers linked to the *pto* locus (conferring resistance to *Pseudomonas syringae*) in tomato. Less than 150 primers were required to isolate seven markers, three of which were confirmed by segregation analysis to be linked to the target locus. In our study, out of 340 primers screened, only one RAPD marker was found to be linked in coupling to the *T. dicoccoides* transfer containing the gene for resistance to stripe rust. The number of RAPD polymorphisms between NILs that were linked to the *Yr15* resistance gene was surprisingly low. Similiar results were reported for screening RAPD markers for linkage with the *Lr24* gene, introgressed from *Agropyron elongatum* into wheat. Only one RAPD marker, out of 360 primers tested, was found to be linked to the *Lr24* gene (Schachermayr et al. 1995). These problems may be related to the complex genome of wheat, which contains a large fraction of repetitive DNA. The development of an RFLP map has been much more difficult in wheat than in most other crops due to the polyploid nature of the crop, a high proportion of repetitive DNA, and unusually low levels of DNA polymorphism within the genome (Chao et al. 1989). Levels of polymorphism in wheat detected by RAPD analysis using agarose-gel electrophoresis are similiar to those detected by RFLP analysis (Devos and Gale 1992).

Conventional gene-pyramiding techniques rely on field and green-house screening with differential rust races. They may also require the use of controlled environmental facilities if the resistance genes are temperature-sensitive. Consequently, the process is complex and time consuming. Thus, the availability of linked molecular markers should accelerate the introgression of the resistance gene into current wheat cultivars. It will also facilitate the accumulation of several resistance genes in a target cultivar. Clearly, the accuracy of marker-assisted selection can be improved if selectable markers are available on both sides of the gene of interest. This was achieved in the present study. Further efforts are needed to narrow down the marked interval spanning the target gene.

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