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J. H. Peng · T. Fahima · M. S. Röder
Y. C. Li · A. Dahan · A. Grama
Y. I. Ronin · A. B. Korol · E. Nevo

Microsatellite tagging of the stripe-rust resistance gene *YrH52* derived from wild emmer wheat, *Triticum dicoccoides*, and suggestive negative crossover interference on chromosome 1B

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Abstract Stripe rust caused by *Puccinia striifomis* West. is one of the most devastating diseases relating to wheat production. Wild emmer wheat, *Triticum dicoccoides*, the tetraploid progenitor of cultivated wheat, has proven to be a valuable source of novel stripe-rust resistance genes for wheat breeding. For example, *T. dicoccoides* accessions from Mt. Hermon, Israel, are uniformly and highly resistant to stripe-rust. The main objective of the present study is to map a stripe-rust resistance gene, derived from the unique Mt. Hermon population of wild emmer, using microsatellite markers. An F_2 mapping population was established by crossing stripe-rust resistant *T. dicoccoides* accession H52 from Mt. Hermon with the *Triticum durum* cultivar Langdon. The stripe-rust resistance derived from accession H52 was found to be controlled by a single dominant gene which was temporarily designated as *YrH52*. Out of 120 microsatellite markers tested, 109 (91%) showed polymorphism between the parental lines. Among 79 segregating microsatellite loci generated from 56 microsatellite primer pairs, nine were linked to *YrH52* with recombination frequencies of 0.02–0.35, and LOD scores of 3.56–54.22. A genetic map of chromosome 1B, consisting of ten microsatellite loci and the stripe-rust resistance gene *YrH52*, was constructed with a total map length of 101.5 cM. *YrH52* is also closely linked to RFLP marker *Nor1* with a map distance of 1.4 cM and a LOD value of 29.62. Apparent negative crossover interference was observed in chromosome 1B, especially in the region spanning the centromere. Negative crossover interference may be a common characteristic of gene-rich regions or gene clusters in specific chromosomes.

Key words Genetic mapping · Microsatellite marker · Stripe-rust resistance · *Puccinia striiformis* · Negative crossover interference · *Triticum dicoccoides* · Wild emmer wheat

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J. H. Peng · T. Fahima · Y. C. Li · A. Dahan · Y. I. Ronin
A. B. Korol · E. Nevo (✉)
Institute of Evolution, University of Haifa, Mount Carmel,
Haifa 31905, Israel
Fax: +972-4-8246554
E-mail: nevo@research.haifa.ac.il

M. S. Röder
Institute for Plant Genetics and Crop Plant Research,
Corrensstrasse 3, D-06466 Gatersleben, Germany

A. Grama
Agricultural Research Organization, The Volcani Center,
P. O. Box 6, Bet Dagan 50250, Israel

Introduction

Wheat is the foremost food crop in the world, and becomes even more important as the global population increases. However, modern plant breeding practices have reduced the genetic diversity in cultivated plants, including wheat, making them increasingly susceptible to diseases, pests, and environmental stresses (Nevo 1995). Consequently, maintenance of the world food supply is threatened. Stripe rust (also called yellow rust), caused by *Puccinia striiformis* f. sp. *tritici* West., is generally considered one of the most devastating diseases of wheat throughout the world. In an infected field, the reduction in yield can reach 50% due to the shriveled grains and reduced spikes caused by this disease. In extreme situations, it can result in yield losses of up to 100% (Roelfes et al. 1992). In Israel, and especially in northern Israel, stripe rust has become a major threat to wheat production due to the appearance of new virulent races (Manistersky and Eyal 1994).

Growing resistant cultivars is the most economical method of controlling the stripe-rust disease. The breeding of resistant varieties offers an effective

approach to reduce or eliminate fungicide application and to minimize yield losses due to this disease (Roelfes et al. 1992). Previously, a series of resistance genes *Yr1–Yr28* has been identified, as well as other provisionally designated genes (McIntosh et al. 1998). However, with the breakdown of *Yr5* resistance in India and Australia, all of the described major resistance genes have become susceptible to one or more of the new races of the pathogen (Stubbs 1985; Roelfes et al. 1992). The greatest hope for future crop genetic improvement lies in exploiting the gene pools of the wild relatives of crop plants. The wild gene pools of wheat contain many economically important alleles for resistance to diseases and pests, high grain-protein content, tolerance to many kinds of ecological stresses, and earliness, that might be transferred to cultivated wheat and utilized in its improvement (Feldman and Sears 1981; Nevo 1983, 1988, 1995; Nevo et al. 1985, 1986, 1991). Gerechter-Amitai and Stubbs (1970) and Nevo et al. (1986) found that wild emmer wheat, *Triticum dicoccoides*, populations indigenous to Israel are valuable sources of stripe rust resistance. Several novel resistance genes to stripe rust in wild emmer wheat have been identified (Gerechter-Amitai et al. 1989 a, b; Van Silfhout et al. 1989 a, b). Only one of them, designated *Yr15*, harbored in accession G-25 collected from Rosh Pinna, Israel, has been characterized in detail and mapped (Gerechter-Amitai et al. 1989 a; McIntosh et al. 1996; Sun et al. 1997; Chagué et al. 1999).

An interesting fact is that all the tested accessions from Mt. Hermon, Israel, are highly resistant to stripe rust at both the seedling and adult stages, and a large number of the highly resistant accessions to stripe rust in Israeli wild emmer wheat are from the Mt. Hermon population. Nevo et al. (1986) tested 113 wild emmer accessions from 11 populations using rust race 2EO, and found that all the accessions from the Mt. Hermon population were entirely immune to stripe rust at both seedling and adult stages, and accounted for 55% and 67% of the total highly resistant adult and seedling accessions, respectively. The et al. (1993) tested 541 accessions from 23 Israeli wild emmer populations using rust race 110 E143 A+, and also found that all accessions from the Mt. Hermon population were highly resistant to stripe rust, and accounted for 74% of the total highly resistant accessions. Van Silfhout et al. (1989 b) tested about 850 wild emmer accessions collected from 31 locations using 28 stripe rust isolates from 19 countries, and found that 19 (28%) of the 68 promising resistant accessions again derived from Mt. Hermon. However, no further studies have been conducted regarding the genetics of stripe-rust resistance from this unique Mt. Hermon population.

The use of DNA based-markers for the genetic analysis of plant species has become a major research strategy for a broad spectrum of scientists, ranging from those interested in identifying and cloning new genes to plant breeders seeking more efficient methods for scoring segregating populations and identifying desirable genotypes (Brown et al. 1996). The development of genetic markers presents a useful tool for plant breeding, as the presence of a gene can be detected without waiting for phenotypic expression in the plants (Feuillet et al. 1995). In wheat, many mapping projects have been conducted regarding agronomically important resistance genes using both RFLP and RAPD markers. Linked RFLP or RAPD markers have revealed genes resistant to leaf rust (Feuillet et al. 1995), stem rust (Paull et al. 1994), stripe rust (Sun et al. 1997; Chagué et al. 1999), powdery mildew (Hartl et al. 1995), Hessian fly (Ma et al. 1993), and nematodes (Williams et al. 1994).

Recently, a new type of genetic marker, the microsatellite, also called an SSR (simple sequence repeat), has been developed in terms of repeated DNA sequence variation. These markers are based on a 1–6 nucleotide core element, such as $(GT)_n$ or $(CT)_n$, that is tandemly repeated from two to tens of times. A different "allele" occurs at a microsatellite locus as a result of changes in the number of times the core element is repeated, altering the length of the repeated region. Microsatellites are ubiquitously interspersed in eukaryotic genomes, and can find applications as highly variable and multiallelic PCR-based genetic markers (Brown et al. 1996). Microsatellite loci have been added to maps of a number of cereal crops, including maize (Senior and Heun 1993), rice (Wu and Tanksley 1993), and barley (Liu et al. 1996). In wheat, Röder et al. (1995, 1998) have developed the Gatersleben wheat microsatellite (GWM) markers and published a molecular map consisting of 279 microsatellite loci amplified by 230 primer sets. GWM markers can work in different species of the genus *Triticum*, including *T. aestivum* (AABBDD), *T. durum* (AABB), *T. monococcum* (AA) or *T. tauschii* (DD) (Röder et al. 1998), *T. aethiopicum* (Plaschke et al. 1995), and *T. dicoccoides* (Fahima et al. 1998). Microsatellites present a much higher polymorphism and informativeness in hexaploid bread wheat than any other marker system (Plaschke et al. 1995; Röder et al. 1995). GWM markers are mainly genome-specific, provide genome-wide coverage, and thus are suitable for mapping agronomically important genes in wheat (Röder et al. 1998).

The major objective of the present study is to reveal microsatellites linked with the provisionally named stripe-rust resistance gene *YrH52*, derived from the unique Mt. Hermon population of *T. dicoccoides*, and to locate this gene to a specific chromosome by the aid of mapped microsatellite markers.

Materials and methods

Plant materials

T. dicoccoides accession H52 from the Mt. Hermon population was shown to be highly resistant to the Australian race 110 E143 A+ (The et al. 1993), to Chinese epidemic races Tiaozhong 31 and Tiaozhong 32 (WY Yang, Crop Institute, Sichuan Academy of

Agricultual Science, Chengdu, China, 1996, personal communication), and to the Israeli race 134E132 (Grama et al., unpublished data) of stripe rust. By contrast, *T. durum* cultivar Langdon was highly susceptible to the Chinese and Israeli races. A hybrid cross was made between H52 and Langdon. The true F_1 plants were planted and artificially selfed under controlled greenhouse conditions in Haifa, Israel, to obtain F_2 seeds. The 238 F_1-derived F_2 plants were also grown in the greenhouse. At around the elongation stage of wheat plants, about 3 g of young and healthy leaves were sampled from each individual F_2 plant separately, frozen in liquid nitrogen, and stored at $-80°C$.

Genomic DNA extraction

Genomic DNA was extracted using the plant genomic DNA isolation reagent DNAzol^{TM}ES (Molecular Research Center, Inc., Cincinnati, Ohio, USA), with some modification. The frozen leaves (0.3–0.4 g) of F_2 individual plants were pulverized in liquid nitrogen using a mortar containing a small amount of glass powder and a pestle. The frozen, fine leaf powder was transferred to a 2-ml centrifuge tube containing 0.7 ml of DNAzol^{TM}ES solution and 70 ng of RNase A. Genomic DNA was extracted from the pulverized sample by mixing the tube by inversion and incubating the sample for 5 min with occassional shaking. Chloroform/isoamyl alcohol (24 : 1, 0.75 ml) was added to the extract, and the resulting mixture was vigorously shaken for 15 s. The mixture was stored for 5 min and then centrifuged at 12 000 g for 10 min. The aqueous phase was transferred to a fresh 1.5-ml tube. The DNA was precipitated by adding 650 µl of 100% ethanol and centrifuging at 5000 g for 4 min. The DNA pellet was washed with DNAzol^{TM}ES-ethanol solution (0.35 ml) and 95% ethanol (0.75 ml), respectively. After the ethanol was removed, the DNA pellet was dissolved in 100 µl of 8 mM NaOH by overnight incubation at room temperature, and the pH was adjusted to 8.0 by adding 11.5 µl of 0.1 M HEPES. The insoluble material, if any, in the DNA solution was removed by centrifugation at 12 000 g.

Stripe-rust test

One hundred and fifty F_3 families were chosen from the available F_2 mapping population. Stripe-rust challenge tests were conducted during the 1997–98 cropping season at Bet Dagan Experimental Farm of the Volcani Center in central Israel and at the Neve Yaar Agricultural Research Center in northern Israel. Each F_3 family was represented by three replications for the Bet Dagan experiment and one replication for the Neve Yaar experiment, with 12 individual plants in each replication. The parents, H52 and Langdon, were planted once each in 30 rows as controls, and Langdon was also used as a spreader of stripe-rust spores. Plants of the Bet Dagan experiment were artificially inoculated when the second leaf appeared using the new Israeli stripe-rust culture 5016 (race 134E132), while the Neve Yaar experiment was naturally infected. The plants were scored using 0–9 scales of infection type (IT) (Line and Qayoum 1992) when the rust fully developed in March, 1998. Nine plants (three randomly taken from each replication) were scored for the Bet Dagan experiment, and all the plants (9–12) were scored for the Neve Yaar experiment. Altogether, about 3000 F_3 plants were scored. The plants with an IT of 0–3 (no sporulation) were considered as resistant and those with an IT of 5–9 (rich sporulation) as susceptible. Based on the reaction of F_3 families to the stripe rust, the corresponding F_2 individual plants were classified into three types (see Table 1), i.e. homozygous resistant (RR, all plants in an F_3 family had a 0–3 IT value), heterozygous resistant (Rr, most of the plants in an F_3 family were resistant and the minority were susceptible) and homozygous susceptible (rr, all plants in an F_3 family had a 5–9 IT value).

Microsatellite genotyping

From the mapped microsatellite markers of wheat (Röder et al. 1998), 137 markers covering the A and B genomes were first chosen for polymorphism tests using the two parents of the mapping population. Then, based on the map distribution of the polymorphic markers, 56 markers (2–7 per chromosome) were chosen to genotype DNA samples of the mapping population, consisting of 150 F_2 individuals, two parentals and one control (Chinese Spring wheat). The microsatellite markers were characterized by Röder et al. (1998). The PCR reaction was performed in a 25-µl volume using a Perkin-Elmer 9600 thermocycler. The reaction mixture contained: 250 nM of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1 U of *Taq*-polymerase and 100 ng of template DNA. After 3 min of denaturation at 94°C, 45 cycles were performed with 1 min at 94°C, 1 min at either 50, 55 or 60°C (depending on the individual microsatellite marker), 2 min at 72°C, and a final extension step of 10 min at 72°C. Fragment analysis was carried out on an automated laser fluorescence (ALF) sequencer (Pharmacia) using a short gel cassette. Denaturing gels (0.35-mm thick) with 6% polyacrylamide were prepared using SequaGel XR (Biozym). The gels were run in 1 × TBE buffer [0.09 M Tris-borate (pH 8.3) and 2 mM EDTA] at 600 V, 40 mA, and 50 W with 2 mW laser power and a sampling interval of 0.84 s. The gels were re-used four to five times. In each lane, fragments with known sizes were included as standards. Fragment sizes were calculated using the computer program Fragment Manager Version 1.2 (Pharmacia) by comparison with the internal size standards.

For data collection of the microsatellite genotypes, individual fragments were tentatively scored as present/absent markers if they were extremely different in size and if there were more than two fragments from the same primer pair. A given pair of fragments may represent two alternative alleles at a single locus, two redundant components of a single allele, or alleles at two distinct (linked or unlinked) loci. Doubts were resolved by linkage analysis (Van den Berg et al. 1997). Therefore, two completely, or very closely, linked fragments in repulsion were considered as co-dominant alleles at a single locus, those in coupling phase were considered as redundant fragments representing a single allele, while loosely linked or unlinked fragments were considered as different dominant/recessive alleles at two distinct loci.

RFLP genotyping

In order to compare the chromosomal location of the previously mapped *T. dicoccoides*-derived stripe resistance gene *Yr15* and the new Mt. Hermon population resistance gene *YrH52*, the *Yr15*-linked RFLP marker *Nor1* (Sun et al. 1997) was used to genotype our mapping population. Genomic DNA was digested with the restriction enzyme *Taq*I at 65°C for approximately 20 h. The digested products (3 µg/lane) were separated overnight in a 1% agarose gel with 1 × TAE buffer at 30 V. The DNA then was transferred onto nylon membranes (Hybond^{TM}-N+, Amersham), using the alkaline blotting procedure according to Amersham's protocol.

The *Nor*-wheat rDNA intergenic spacer (IGS) 3.0-kb insert was digested with *Taq*I from the recombinant plasmid *pTa*71 (described by Gerlach and Bedbrook 1979), kindly provided by M. O'Dell (Plant Breeding Institute, Cambridge). The *Nor1* spacer was labelled with [α-^{32}P]dCTP using a Megaprime DNA labelling system (Amersham Life Science, England). Membranes were pre-hybridized at 65°C for 30 min with Rapid-hyb buffer (Amersham Life Science). The hybridization was performed overnight at 65°C with the ^{32}P-labelled *Nor1* probe. The hybridized membranes were washed twice for 20 min at 65°C with each of the following solutions: 2 × SSPE + 0.1% SDS, 1 × SSPE + 0.1% SDS, and 0.5 × SSPE + 0.1% SDS. The membranes were exposed to X-ray film (Kodak, BioMax MR) at $-80°C$ for 30–60 min.

Genetic mapping

The mapping analysis was conducted using MAPMAKER 3.0b (Lincoln et al. 1992). The resulting tentative order of loci was evaluated using the "ripple" procedure to obtain the most-likely sequence of loci. The "error detection" command was used to check for unexpected mistakes in scoring and data input. Multiple loci detected by a single marker had a suffix a, b, c, … added following the regular marker name. This linkage group was assigned to a specific chromosome by using the well-mapped locus Xgwm131a (Röder et al. 1998) in this group as an "anchor".

The Kosambi (1944) mapping function was used in MAPMAKER to estimate map distances. This means that the expected frequency of a double-crossover, r_{12}, in two adjacent intervals 1 and 2 obeys the condition $r_{12} = c(r)r_1r_2 = 2rr_1r_2$, where $c(r) = 2r$ is the coefficient of coincidence under Kosambi interference, and r_1, r_2 and r are the rates of recombination in intervals 1, 2, and 1 + 2, respectively. To test whether this assumption is valid in our case, the real crossover interference level was estimated directly from consequent adjacent interval pairs using the maximum-likelihood (ML) procedure (Bailey 1961). The LOD score was applied to test the significance of deviation of the observed level of crossover interference from Kosambi expectation or from the "no interference" model.

Results

Inheritance of the stripe-rust resistance derived from *T. dicoccoides* accession H52

The stripe rust developed well in the two experimental locations, Bet Dagan and Neve Yaar, because of the cold and humid weather during January–March, 1998, in Israel. About 3000 F_3 plants in total were scored using the scale introduced by Line and Qayoum (1992). They were easily divided into two classes based on the infection type (IT), i.e. non-sporulating (0–3) and sporulating (5–9) classes. The 150 F_2 individuals (scored as F_3 families) were classified into three groups, RR, Rr and rr (Table 1). Among the 43 RR families at Bet Dagan, six were scored as Rr at Neve Yaar because of an infection escape at the former location. Only one of the 37 plants scored as rr at Neve Yaar was scored as Rr at Bet Dagan, perhaps because of random sampling error. Chi-square values were calculated as 1.080 and 0.0267 for Bet Dagan and Neve Yaar, respectively, based on the observed values and a theoretical ratio of 1 : 2 : 1. These χ^2 values were significantly lower than the threshold value $\chi^2_{0.05,2} = 5.99$. Therefore, the segregation ratio for the stripe-rust reaction of the mapping population at the two different locations fitted the theoretical ratio, and expression of the resistance gene was similar at the two locations. After pooling data from the two locations, a new χ^2 value was calculated as 0.1200, which again indicated that the segregation of F_2 plants for reaction to stripe-rust in our mapping population fitted the theoretical ratio of 1 : 2 : 1 ($df = 2$, $P > 0.90$). From this result, it can be inferred that the stripe-rust resistance of *T. dicoccoides* accession H52 from Mt. Hermon, Israel, is controlled by a single, dominant gene. According to the rules of gene symbolization in wheat (McIntosh et al. 1998), we temporarily designate this resistance gene as *YrH52*.

Microsatellite polymorphism between the parental genotypes

Based on the distribution of microsatellite markers (MM) on the A and B genomes of wheat (Röder et al. 1998), 137 MM were chosen to identify polymorphism between *T. dicoccoides* accession, H52 and the *T. durum* cultivar Langdon, and 120 of these successfully supported PCR amplification. Among the 120 markers, 109 (91%) generated polymorphisms between the parents. This means that the mapping population should be polymorphic for these microsatellite markers.

Microsatellite loci linked to *YrH52*

Of the polymorphic microsatellite markers, 56 were chosen to genotype the F_2 mapping population, and 79 segregating microsatellite loci were identified. As an example, Fig. 1 shows the segregation pattern of the

Table 1 F_2 segregation pattern of reaction to stripe rust in the cross Langdon (*T. durum*) × H52 (*T. dicoccoides*). RR = homozygous resistant F_3 families, Rr = heterozygous resistant F_3 families, rr = homozygous susceptible F_3 families. O = observed value, E = expected value at a theoretical ratio of 1 : 2 : 1. χ^2_T = the χ^2 value for the pooled data of the two locations. $\chi^2_{0.05,2} = 5.99$

Location	Neve Yaar				Statistical test			
	Category	RR	Rr	rr	O	E	$(O-E)^2/E$	χ^2
Bet Dagan	RR	37	6	0	43	37.5	0.8067	
	Rr	0	70	1	71	75	0.2133	1.0800
	rr	0	0	36	36	37.5	0.0600	
Statistical test	O	37	76	37	$N = 150$			
	E	37.5	75	37.5		$N = 150$		
	$(O-E)^2/E$	0.0067	0.0133	0.0067				
	χ^2		0.0267					$\chi^2_T = 0.1200$

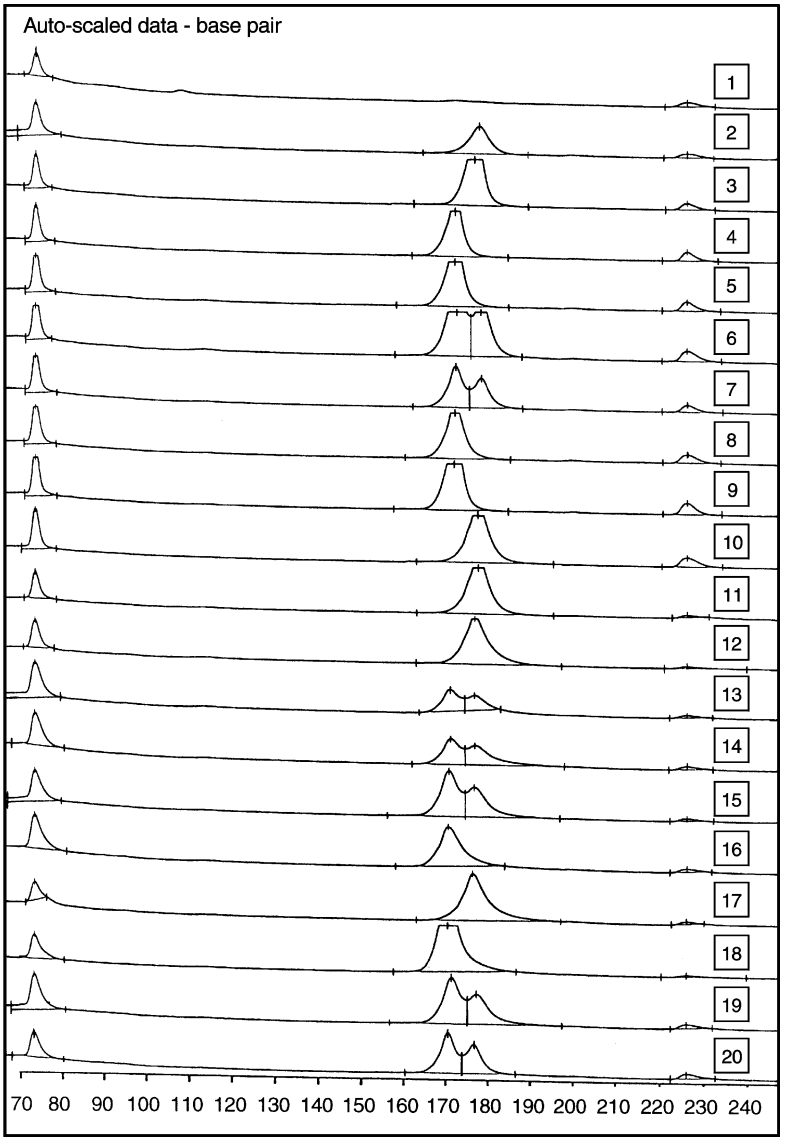

Fig. 1 F_2 segregation pattern of microsatellite GWM18 shown as a printout of the computer program Fragment Manager V1.2. The horizontal scale represents size in bp. The vertical scale represents intensity of fluorescences. The peaks represent DNA fragments. Sizes in bp were calculated by comparison with the added internal standards in each lane (73 bp and 226 bp). *Lanes 1*, *2*, *3 and 4* represent the results for a negative control without DNA, Chinese Spring wheat showing a 178-bp band, *T. dicoccoides* accession H52 showing a 178-bp band, and *T. durum* cultivar Langdon showing a 172-bp band, respectively. *Lanes 5–20* represent the results for 16 F_2 individual plants: *lanes 5*, *8*, *9*, *16 and 18* are *T. durum*-type homozygotes; *lanes 10*, *11*, *12 and 17* are *T. dicoccoides*-type homozygotes; *lanes 6*, *7*, *13*, *14*, *15*, *19*, *and 20* are heterozygotes between H52 and Langdon

microsatellite locus Xgwm18 detected by the ALF sequencer. In the two-point test of MAPMAKER 3.0b (Lincoln et al. 1992), using the "near" command with a LOD score ≥ 3.0 and a maximum Kosambi distance of 50 cM, nine microsatellite loci detected by eight microsatellite primer pairs (Table 2) were found to be linked to *YrH52*, with recombination frequencies ranging from 0.02 to 0.35, and LOD scores ranging from 3.56 to 54.22 (Table 3). Except for Xgwm124, which was relatively far from *YrH52* (43.37 cM), the linkages between the other eight loci and *YrH52* were stronger, with linkage distances less than 30.00 cM. Xgwm264a and Xgwm18 were very closely linked to *YrH52*, with genetic distances of 2.00 and 3.00 cM, respectively. Xgwm131a and Xgwm636b were also relatively close to *YrH52*, with genetic distances of 10.1 and 16.6 cM, respectively. Other *YrH52*-linked microsatellite loci were Xgwm264c, Xgwm403a, Xgwm153 and Xgwm550a, though with larger genetic distances. Among the nine microsatellite loci, four exhibited co-dominant expression, with the remainder being present/absent. All the microsatellite loci exhibited perfect Mendelian inheritance (Table 3).

Genetic mapping of *YrH52*

By the aid of the multiple-point analysis of MAPMAKER 3.0b (Lincoln et al. 1992), a highly reliable linkage group consisting of ten microsatellite loci and the stripe-rust resistance gene *YrH52* was established from the 79 microsatellite loci through the "group" command, with a LOD score of 8 and a maximum recombination frequency of 0.5. By using the well-mapped locus Xgwm131a as an anchor (Röder et al. 1998), all of the ten microsatellite loci and the resistance

Table 2 Characteristics of the screened microsatellite markers

Marker	Repeat type	Annealing temperature (°C)	Fragment size (bp) in Chinese Spring	Chromosomal location[a]
GWM18	$(CA)_{17}GA(TA)_4$	50	186	1B
GWM124	$(CT)_{27}(GT)_{18}$imp	60	213	1B
GWM131	$(CT)_{22}$	60	131	1B
GWM153	$(GA)_{18}$	60	188	1B
GWM264	$(CA)_{33}$	60	159	1B
GWM403	$(CA)_{13}$	55	133	1B
GWM550	$(CT)_8(GT)_{18}$	55	150	1B
GWM636[b]	$(GA)_{28}$imp	50	110	2A

[a] According to Röder et al. (1998)
[b] GWM636 generated two loci. The main locus, a, was mapped onto chromosome 2A by Röder et al. (1998), the additional locus, b, was allocated to chromosome 1B in the present study

Table 3 Microsatellite loci linked to stripe-rust resistance gene *YrH52*

Locus	Type	Segregation[a]		Linkage to *YrH52*		
		Ratio	χ^2	RF[b]	LOD[c]	Distance (cM)
Xgwm264a	Co-dominant	A : H : B = 1 : 2 : 1	0.120	0.02	54.22	2.0
Xgwm18	Co-dominant	A : H : B = 1 : 2 : 1	0.013	0.03	50.23	3.0
Xgwm131a	Dominant	A : C = 1 : 3	0.009	0.10	18.30	10.1
Xgwm636b	Dominant	A : C = 1 : 3	0.436	0.16	12.40	16.6
Xgwm264c	Dominant	A : C = 1 : 3	0.436	0.21	8.16	22.4
Xgwm403a	Dominant	D : B = 3 : 1	0.009	0.21	8.76	22.4
Xgwm153	Dominant	A : C = 1 : 3	0.222	0.26	5.05	28.8
Xgwm550a	Co-dominant	A : H : B = 1 : 2 : 1	0.120	0.26	9.02	28.8
Xgwm124	Co-dominant	A : H : B = 1 : 2 : 1	1.080	0.35	3.56	42.4
Nor1[d]	Dominant	A : C = 1 : 3	0.080	0.03	29.62	3.0

[a] A = homozygote for the allele from *T. dicoccoides* accession H52 at this locus; B = homozygote for the allele from *T. durum* cultivar Langdon at this locus; H = heterozygote carrying both alleles from H52 and Langdon at this locus; C = not a homozygote for the allele from H52; D = not a homozygote for the allele from Langdon; $\chi^2_{0.05,1} = 3.84$, $\chi^2_{0.05,2} = 5.99$
[b] RF = recombination frequency
[c] LOD = $\log_{10}$[L(r)/L(0.5)], the decimal logarithm of likelihood ratio. Usually, the linkage is reliable if LOD $\geq$ 3.0 (Van den Berg et al. 1997)
[d] *Nor1* is an RFLP marker

gene *YrH52* were assigned to chromosome 1B. Through using the "order", "map" and "ripple" commands of MAPMAKER 3.0b (Lincoln et al. 1992), a microsatellite genetic map involving *YrH52* was constructed with a total map length of 101.5 cM (Fig. 2). *YrH52* was flanked on either side by Xgwm264a and Xgwm264c, with map distances of 2.2 and 24.5 cM, respectively. From the distal side of Xgwm264a, four other microsatellite loci, Xgwm18, Xgwm131a, Xgwm636b and Xgwm403a, followed in an interval of < 23.0 cM.

Linkage between *YrH52* and *Nor1*

The two parental lines and all 150 F_2 individuals of our mapping population were also genotyped using the RFLP probe *Nor1*. The *Nor1* probe detected three fragments in the *T. durum* cultivar Langdon, i.e., 2.7-, 3.0-, and 4.5-kb bands, but only one band (2.8 kb) in the *T. dicoccoides* accession H52 (Fig. 3). The 2.7- and 3.0 kb-bands in Langdon co-migrated. The segregation of Langdon fragments fitted the theoretical ratio of 3 : 1 ($\chi^2 = 0.080$, $P = 0.88$ for 4.5-kb band; and $\chi^2 = 0.222$, $P = 0.84$ for the 2.7- and 3.0-kb bands). The segregation of the single 2.8-kb band in H52 deviated significantly from the theoretical ratio of 3 : 1, and approximately fitted a 15 : 1 ratio, due to the co-segregation of two fragments, i.e. a 1B fragment and a 6B fragment (Flavell et al. 1986). Therefore, we ignored this band and scored only the 4.5-kb band as a dominant marker. By the aid of MAPMAKER 3.0b (Lincoln et al. 1992), the 4.5-kb *Nor1* marker was found to be closely linked to *YrH52* with a recombination frequency of 0.03 and a LOD-score of 29.62 (Table 2). Using the "try" command of MAPMAKER 3.0b, the *Nor1* marker was placed between *YrH52* and Xgwm264a with a 1.4-cM map distance from *YrH52* (Fig. 2). Sun et al. (1997) showed that another *T. dicoccoides*-derived stripe-rust resistance gene, *Yr15,* was 11.0 cM apart from *Nor1*. Based on this result, the map distance between *YrH52*

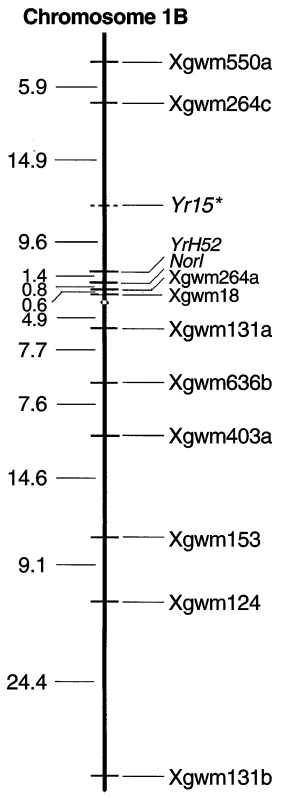

Fig. 2 Microsatellite maping of stripe-rust resistance gene *YrH52* on chromosome 1B. Locus names and corresponding locations on the genetic map are indicated on the right side. Map distances (Kosambi) are in centi-Morgans, shown on the left side of the map. The relative locations of the centromere and *Yr15* are cited from Sun et al. (1997) and Röder et al. (1998)

and *Yr15* can be calculated as 9.6 cM (Fig. 2). However, genotypic differences between the two hybrids in the rate of recombination cannot be excluded thus reducing the reliability of this estimation.

Crossover interference on chromosome 1B

The obtained ML-estimates of the coefficient of coincidence (c) indicate that negative crossover interference (i.e. $c > 1$) may be a characteristic of some regions of chromosome 1B (Fig. 4). In particular, high values of c were obtained for the pairs of small segments 4–5, 5–6 ($c = 9.9$), 6–7,7–8 ($c = 9.0$), and 7–8, 8–9 ($c = 20.0$), with significant deviations from the 'no interference' Haldane recombination scheme. Clearly, the deviations from Kosambi interference in such cases should be even more significant. For example, the LOD-score test for comparing the ML-estimate $c = 9.0$ versus $c = 1$ gave a $P_H = 0.07$, while comparison of $c = 9.0$ versus Kosambi interference gave a $P_K = 0.007$. Correspondingly, Monte-Carlo testing of significance resulted in a $P_H = 0.05$ and a $P_K = 0.02$. The trend revealed was confirmed for the foregoing regions by using larger intervals from one or both sides of the central point of the interval pair (as shown in Fig. 4), hence with a lower dependence of the estimates on a unique double recombinant. The same result, $c > 1$, was found for the majority of such cases, although the deviations from 'no interference' ($c = 1$) were less extreme. We would like to stress that the highest level of negative crossover interference was found in the region spanning the centromere. Lukaszevski and Curtis (1993) also found indications of negative crossover interference on chromosome 1B and some other chromosomes in a similar mapping population (F_2 of the *T. dicoccoides* × *T. durum* cultivar Langdon), although they were rather skeptical about the reality of this phenomenon.

Discussion

Chromosomal location of *YrH52* derived from the unique Mt. Hermon population of *T. dicoccoides*

Israeli wild emmer wheat, *T. dicoccoides*, is potentially an important genetic resource for the improvement of wheat stripe-rust resistance (Gerechter-Amitai and Stubbs 1970; Nevo et al. 1986; The et al. 1993). The *T. dicoccoides* population from Mt. Hermon in northern Israel represents a unique group of accessions highly resistant to stripe rust. All the tested Mt. Hermon accessions have proven to be highly resistant (Nevo et al. 1986; The et al. 1993). Through a relatively large-scale field experiment testing about 3000 F_3 progenies, we found that the resistance of Mt. Hermon accession H52 to the new Israeli stripe-rust race 134E132 was conferred by a single dominant gene (Table 1). This resistance gene from the unique Mt. Hermon population is temporarily designated as *YrH52*.

Among the 28 catalogued stripe-rust resistance genes, 24 were allocated to specific chromosomes (McIntosh et al. 1998). However, only *Yr15* has been mapped using DNA markers (Sun et al. 1997; Chagué et al. 1999). F_2 progenies are probably the most widely used populations for the creation of linkage maps in inbreeding species, because a given population size represents twice as many potentially informative meioses as would be the case for a backcross with the same homozygous parents (Van den Berg et al. 1997). In the present study, therefore, we used an F_2 mapping population and microsatellite markers (Röder et al. 1998) to molecularly tag the temporarily designated stripe-rust resistance gene *YrH52*. This resistance gene was mapped onto chromosome 1B (Table 3 and Fig. 2). According to the wheat microsatellite map (Röder et al. 1998), Xgwm264a and Xgwm18 are located on the short arm, while Xgwm131a is on the long arm, of chromosome 1B. The linkages between *YrH52* and Xgwm264a and Xgwm18 are significantly stronger than that between *YrH52* and Xgwm131a, and *YrH52* lies outside of the interval Xgwm264a – Xgwm18 – Xgwm131a on the Xgwm264a side (Fig. 2). It may be concluded, therefore, that *YrH52* is located on the short arm of chromosome 1B.

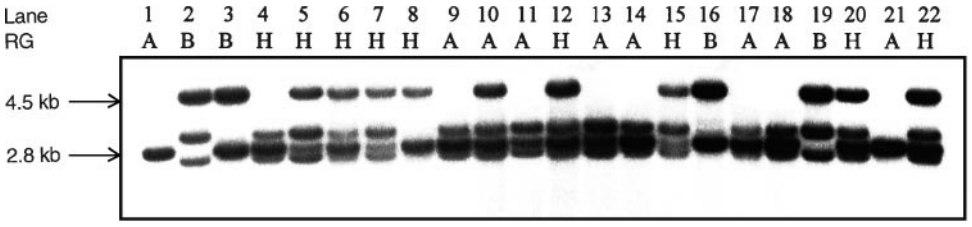

Fig. 3 Southern-blot hybridization pattern of stripe-rust-resistant *T. dicoccoides* accession H52 (*lane 1*), susceptible *T. durum* cultivar Langdon (*lane 2*), and 20 F_2 individuals (*lanes 3–22*). Genomic DNA was digested with *Taq*I, and then hybridized with the RFLP probe *Nor1*. The arrows indicate the 4.5-kb band from Langdon and the 2.8-kb band from H52. *RG* = stripe-rust-reaction genotype of individuals: *A* = RR, i.e., homozygous resistance; *B* = rr, i.e., homozygous susceptible; *H* = Rr, heterozygous resistance

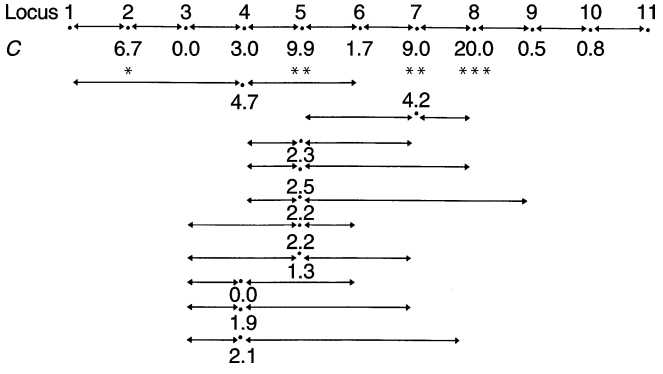

Fig. 4 Crossover interference on chromosome 1B: maximum likelihood estimates of the coefficients of coincidence for different pairs of adjacent intervals. *c* is the ML-estimate of the coefficient of coincidence. The significance of deviations from $c = 1$ are marked by $*P < 0.1$, $**P < 0.05$, and $***P < 0.01$. The code 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 represents Xgwm131b, Xgwm124, Xgwm153, Xgwm403a, Xgwm636b, Xgwm131a, Xgwm18, Xgwm264a, *YrH52*, Xgwm264c and Xgwm550a, respectively, in Fig. 2

In the present study, the stripe-rust resistance gene *YrH52* was flanked on either side by the microsatellite loci Xgwm264a and Xgwm264c, with genetic distances of 2.2 and 24.4 cM, respectively (Fig. 2). Moreover, *YrH52* was linked to nine microsatellite loci with recombination frequencies of 0.02–0.35 and LOD scores of 3.56–54.22 (Table 3), and also closely linked to the RFLP marker *Nor1* with a genetic distance of 1.4 cM (Fig. 2). Another catalogued locus, and the only stripe-rust resistance gene tagged till now by molecular markers, is *Yr15*. It was flanked by both a RAPD ($OPB13_{1420}$) and a RFLP (*Nor1*) marker with genetic distances of 27.1 and 11.0 cM, respectively (Sun et al. 1997), and by a RAPD ($UBC199_{700}$) and a microsatellite (Xgwm33) marker with genetic distances of 4.7 and 4.5 cM, respectively (Chagué et al. 1998).

Relationship between stripe-rust resistance genes *YrH52* and *Yr15*

The stripe-rust resistance gene *Yr15* is also derived from *T. dicoccoides*, and has been well characterized (Gerechter-Amitai et al. 1989 a, b). It was mapped onto chromosome 1B by cytogenetic analysis (McIntosh et al. 1996) and molecular markers (Sun et al. 1997; Chagué et al. 1999). Although, both *YrH52* and *Yr15* originate from *T. dicoccoides*, they derive from different geographical populations: *YrH52* is from the Mt. Hermon population (altitude 1300 m), while *Yr15* is from the Rosh Pinna population (altitude 700 m) north of the Sea of Galilee. Ecogeographical factors, e.g. altitude, mean annual temperature, mean day-night temperature difference and mean annual rainfall, are different between these two locations (Nevo et al. 1986). The reactions (infection type) of these two populations to stripe-rust races in both Australia and Israel are also extremely different (Nevo et al. 1986; The et al. 1993). In a reaction test using 28 isolates of stripe rust from 19 countries, the *Yr15*-carrier G25 was resistant to most of the races but not to race 7E134 from Afghanistan, while eight Mt. Hermon accessions were resistant to all the races including the one from Afghanistan (Van Silfhout et al. 1989 b). Among the 19 *T. dicoccoides* accessions with different reaction patterns to 15 isolates of stripe rust, four Mt. Hermon accessions were different from each other and also different from the *Yr15*-carrier G25 (Van Silfhout et al. 1989 b). Of the 11 *T. dicoccoides* accessions with one more pair of resistance genes in addition to *Yr15*, four derive from Mt. Hermon (Gerechter-Amitai et al. 1989 b). An obvious genetic differentiation between the Mt. Hermon and Rosh Pinna populations has also been detected by using allozyme markers (Nevo and Beiles 1989) and microsatellite markers (Fahima et al. 1998). In total, it is estimated that at least 8–11 major stripe-rust resistance genes exist in the Israeli wild emmer populations (Van Silfhout et al. 1989 a, b). Furthermore, many *T. dicoccoides* accessions (including those from Mt. Hermon) carry one dominant gene different from *Yr15* (Gerechter-Amitai et al. 1989 b). The RFLP analysis using the *Yr15*-linked marker *Nor1* suggests that these two resistance genes may be located approximately 9.6 cM apart on the short arm of chromosome 1B, *YrH52* being closer to the centromere than *Yr15* (Fig. 2). Therefore, we assume that *YrH52* and *Yr15* may be different genes present in a complex region on chromosome 1BS.

However, in order to confirm the genic relationship between *YrH52* and *Yr15*, further analysis is needed using additional DNA markers common to both mapping populations.

Classical genetic and molecular data show that genes for disease resistance are not randomly distributed over the genome of a species; rather, they frequently occur in clusters on particular chromosomes (Islam et al. 1989) and encode a leucine-rich repeat (LRR) region (Michelmore and Meyers 1998). It has been observed repeatedly that disease resistance genes are often located on a chromosome in a complex region containing different pathogen race specificities (Hartl et al. 1995; Michelmore and Meyers 1998). Among the 24 catalogued and allocated stripe-rust resistance genes, *Yr9*, *Yr10*, *Yr15* and *Yr24* were located on chromosome 1BS, and *Yr3* and *Yr21* on 1B (McIntosh et al. 1998). In our study, the provisionally named stripe-rust resistance gene *YrH52* derived from the unique Mt. Hermon population of *T. dicoccoides* was also mapped onto chromosome 1BS by the aid of microsatellite markers. Therefore, stripe-rust resistance genes in wheat are not distributed randomly over the genome. Furthermore, leaf-rust resistance gene *Lr26* (completely linked with *Yr9*), stem rust resistance gene *Sr31*, and powdery mildew resistance gene *Pm8* are also located on chromosome 1BS (McIntosh et al. 1996, 1998). Chromosome 1BS in wheat may thus be an important carrier of a cluster of disease resistance genes, including the two *T. dicoccoides*-derived stripe-rust resistance genes.

It now appears that many DNA markers and genes in wheat are clustered in a few regions of corresponding chromosomes. Most of chromosome group–1 RFLP markers derived from cDNA clones (86%) and 14 agronomically important genes are present in five clusters that encompass only 10% of the physical length of the chromosomes (Gill et al. 1996). The clustering of resistance genes may be the result of the co-evolution of plant species and their pathogens, and adaptation to abiotic stress. Michelmore and Meyers (1998) emphasize divergent selection acting on arrays of solvent-exposed residues in the LRR, resulting in the evolution of individual resistance genes within a haplotype. These clusters are the reservoirs of variation for resistance specificities rather than rapidly evolving dynamic groups of genes. The presence of multiple complex clusters of resistance genes, each with arrays of potential ligand binding sites, suggests how plants can generate and maintain large numbers of resistance specificities against ever-changing pathogen populations (Michelmore and Meyers 1998). The fact that the *T. dicoccoides*-derived *YrH52*, *Yr15*, and other resistance genes, may be located in the same complex region in 1BS indicates that a higher efficiency for resistance gene introgression from wild relatives into cultivated crops, as well as a pyramiding strategy of resistance genes, would be expected.

Apparent negative crossover interference on chromosome 1B

Positive crossover interference, i.e. a reduced frequency of adjacent double-crossovers compared to that expected from the assumption of independence, is with only very few exceptions, a characteristic of meiotic organisms (Egel-Mitani et al. 1982). Consequently, it is generally assumed that negative crossover interference is mainly associated with intragenic recombination. Nevertheless, cases are known of a higher than expected frequency of double-crossovers in adjacent chromosome segments with a small genetic, but large physical, length. In *Drosophila melanogaster*, within a segment 4-cM long accounting for about 25% of the physical length of chromosome 3 and spanning the centromere, a significant excess of multiple exchanges has been found (Sinclair 1975). Similar results have been obtained in other *Drosophila* studies with autosomes (Green 1975; Dennell and Keppy 1979; Korol et al. 1994), but not with the X-chromosome (Lake 1986). Significant negative crossover interference was also found in barley (Søgaard 1977). Dennell and Keppy (1979) suggested that negative chromosome interference may be a characteristic of all regions exhibiting a very low average density of recombination per unit physical length. This generalization fits the results of the present study. We found a significant excess of double exchanges in genetically very short segments spanning the centromere of chromosome 1B that comprise about 50–70% of the physical length of the chromosome (Lukaszewski and Curtis 1993; Gill et al. 1996). Interest in the problem of coincident crossovers is due to the current large-scale genome mapping efforts and the growing evidence that the length of the genetic maps of some plants tend to increase with the number of molecular markers employed. The simplest explanation is to assume that double-crossovers can occur, at least in some organisms, at much smaller distances than is usually accepted. Our data support this view, corroborating other results (Gill et al. 1996; Takahashi et al. 1997) and an apparent non-correspondence between the total chiasma frequency and genome length of some species, including cereals (Nilsson et al. 1993).

One difficulty with our data is in the alternation of full positive and strong negative crossover interference. The simplest explanation for this may be that the size of the mapping population ($n = 150$) is too small to allow reliable conclusions based on such kinds of patterns. Although this may indeed be the case, we cannot renounce speculating that the observed pattern of alternating interference meets the recent findings of Gill et al. (1996). They demonstrated the existence of gene-rich islands in wheat chromosomes with higher frequencies of recombination in these regions than in gene-poor segments. Thus, we can assume that the 'positive-negative interference' doubted by Lukaszewski and

Curtis (1993) may be a real phenomenon in wheat, if double-crossovers indeed occur within the foregoing islands and recombination in one island reduces the chance of crossovers in adjacent segments. One important aspect of our results is that the foregoing recombination pattern was revealed using microsatellite markers, which may not necessarily follow the island-like distribution of structural genes [half of the RFLP markers used by Gill et al. (1996) were cDNA probes]. If negative crossover interference is indeed a real phenomenon in wheat, then gene introgression via homologous recombination from relatively close wild relatives of cultivated wheat can be considered much more probable than previously thought.

The assumption of Kosambi interference is usually included in mapping models and software. Such a critical assumption, even if valid in many situations, is often accepted without a test. This may lead to serious biases in recombination estimates in current genetic maps without considering such contradictory aspects such as negative crossover interference. Other factors, like correlated variation between meioses in recombination rates for different chromosome segments, may generate an apparent negative crossover interference (Säll and Bengtsson 1989). Therefore, more attention should be paid to planning multi-point mapping experiments and data analysis. In no case should assumptions about the mode of interference (or "no interference") be automatically accepted.

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