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Cytogenetic studies in wheat XVII. Monosomic analysis and linkage relationships of gene *Yr15* for resistance to stripe rust

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Summary

The highly effective stripe rust resistance gene, *Yr15*, derived from *Triticum dicoccoides*, was located in chromosome 1BS. *Yr15* showed linkage of 0.30 (34 cM) with *Yr10* and 0.07 with the centromere. *Yr15* was preferentially transmitted relative to its alternate allele.

Introduction

Genetic and cytogenetic studies on resistance to stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* were initiated in Australia soon after the introduction of the pathogen in 1979 (O'Brien et al. 1980). Although greater emphasis in breeding for resistance was given to sources of adult plant resistance, some cytogenetic studies were directed at understanding the genetics of seedling resistance. In this paper we describe the chromosome location of, and linkage studies involving, *Yr15*, a gene originally transferred from wild emmer, *Triticum turgidum* L. var. *dicoccoides* (*T. dicoccoides*), to common wheat (Gerechter-Amitai et al. 1989).

Materials and methods

Parental material and monosomic analysis

Several hexaploid wheat lines with high levels of seedling resistance to stripe rust were obtained from Dr. A. Grama, The Volcani Centre, Bet Dagen, Israel. These were derivatives of *T. dicoccoides* G-25. One or other of two lines, V763-2312 or V763-254, with pedigree Bluebird/Tordo/3/G-25/2/Nursit 163 (durum)//2**T. aestivum*, was crossed as male parent to a partially generated monosomic series of the common wheat line CSP44 (Condor Single Plant Selection Plant 44). Because the resistance gene was derived from a tetraploid wheat, crossing was restricted to monosomic lines representing the A and B genomes which are shared by the two species. Monosomic hybrids were not successfully generated from monosomics 3A and 5A.

F2 seedlings from F1 plants in the various crosses were inoculated with *P. striiformis* pathotype (pt.) 108 E141 A+ (PBIC culture 831917) chosen for virulence on seedlings of CSP44 which carries *Yr6* and *YrA* (Wellings et al. 1988), and avirulence for *Yr15*.

Telocentric mapping

For telocentric mapping, a line possessing a telosome carrying *Yr15* was crossed with Kalyansona and F1 hybrids with 41 normal chromosomes and the telosome were pollinated with Kalyansona. The chromosome constitutions of the resulting testcross plants were determined and seedlings were tested with *P. striiformis* pt. 108 E141 A+ which is virulent on seedlings of Kalyansona. The breeding history is summarised in Fig. 1.

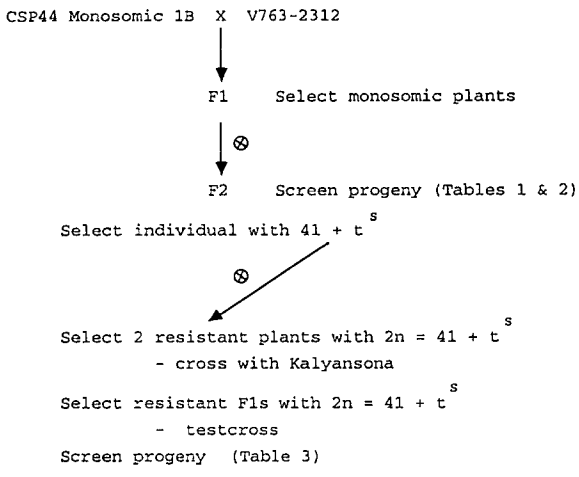

Figure 1. Flow diagram for steps involved in the cytogenetic analysis of *Yr15*. ⊗ indicates self-pollination.

Table 1. F2 segregation for stripe rust reaction in crosses of CSP44 monosomics and either V763-254 or V763-2312

Chromosome[a]	Resistant	Susceptible
1A	138	23
2A	101	28
4A	57	14
6A	112	27
7A	115	19
1B	85	6
2B	102	16
3B	96	25
4B	106	21
5B	108	25
6B	108	26
7B	89	30
Total (Excluding 1B)[b]	1132	254

[a] Chromosomes 3A and 5A were not tested.
[b] $\chi^2_{3:1} = 32.9$; $P<0.01$.

Linkage studies

Line V763-2312 was crossed with two wheats known to possess marker genes on chromosome 1B, namely a Warigal backcross derivative with *Yr10* and SUN89D which carries genes *Yr9* as well as *Sr31* for resistance to stem rust and *Lr26* for resistance to leaf rust in the rye component of a translocation T1BL·1RS chromosome.

Chromosome counts

Somatic chromosome counts were performed on root tips pre-treated in ice-water, fixed in acetic alcohol (1:3) and prepared by the Feulgen procedure. Meiotic chromosome preparations were made on pollen mother cells. Entire spikes were fixed in Carnoy Solution (6 absolute ethyl alcohol : 3 chloroform : 1 glacial acetic acid) for 48 hours and stored in 90% alcohol until used. Individual anthers were removed from spikes, squashed and stained in aceto-carmine before examination at metaphase I.

Rust infection and scoring

Stripe rust

After inoculation, seedlings were held in a high humidity chamber at 11 °C for 24 hours before transference to a naturally-lit greenhouse, temperature-controlled to approximately 17 °C. Reactions were recorded after 14–16 days. Infection types (IT) were scored on a 0 (no visible symptoms), ; (fleck), 1 (necrotic lesion with slight sporulation) to 4 (full sporulation with necrosis or chlorosis) scale. For analysis, plants with IT 2 to 3 or higher were included in the susceptible class.

Stem rust and leaf rust

Following inoculation, seedlings were held in a high humidity chamber at normal greenhouse temperatures for 24 hours (18–25 °C before transference to benches. Reactions were scored 12–14 days after infection. Seedlings with *Sr31* produced IT ;1 to 2- (infection types according to Stakman et al. 1962) and were readily distinguished from the alternative susceptible segregates with IT 3+. Seedlings with *Lr26* produced IT ;1 to X- and were easily distinguished from susceptible segregates.

Results

Monosomic analysis

Seedlings with *Yr15* produced IT 0 to ;, whereas those of CSP44 and other susceptible controls gave IT 23 or higher. The F2 results for monosomic analysis are given in Table 1. The ratios of seedlings scored resistant and susceptible generally exceeded 3:1, the ratio expected for normal segregation of alleles at a single locus. After excluding the results for chromosome

Table 2. Association between chromosome number and stripe rust response in pooled F2 populations from CSP44 monosomic 1B/V763-2312

Chromosome number	Resistant	Susceptible
42	41	1
41	88	2
40	3	2
41 + t^L (3 sats)[a]	1	–
41 + t^S (4 sats)	1	–
40 + t^S (3 sats)	1	–
	135	5

[a] Satellites.

Table 3. Chromosome number and stripe rust reactions of testcross progeny of monotelodisomic plants heterozygous for *Yr15*

Chromosome number	Resistant	Susceptible
42	2	44
41 + t^S [a]	35 [b]	4
Subtotal	37	48
41 + t^L	–	2
42 + t^S	16	1
42 + i^S	–	1
41	1	42
40	–	2
Total	54	96

[a] t^S = short telosome with satellite, t^L = long telosome, no satellite, i^S = iso chromosome with terminal satellites.
[b] Includes one plant with 40 + t and meiotic pairing of $19'' + 1t' + 1'$.

1B on the basis of its extreme deviance, the ratio for the pooled results was 4.46 resistant : 1 susceptible. In stripe rust infection experiments there is often a proportion of seedlings that escape infection. In this instance, because resistant seedlings with IT 0 could not be distinguished from escapes, there could have been a consistent bias increasing the numbers of seedlings scored resistant. The data given in Table 1 are homogeneous when the results for chromosome 1B are excluded (contingency table $\chi^2 = 10.9$; P 10d.f. > 0.25).

To investigate the likelihood that chromosome 1B was the carrier of *Yr15*, somatic chromosome counts and rust responses were determined for a further 140 F2 seedlings from two monosomic plants in the original cross. Because chromosome 1B is satellited, as far as possible the number of satellited chromosomes was also recorded. The results (Table 2) provided further evidence for the location of *Yr15* in chromosome 1B, however occasional plants with 2n = 41 or 42 were susceptible and three of five plants with 2n = 40 were resistant. If *Yr15* were located in chromosome 1B, then resistance of the plant with 40 + t^S indicated that the gene must be located in the shorter satellited arm. When grown to maturity this plant was almost sterile.

The resistant plant with 2n = 41 + t^S was grown to maturity (Fig. 1). Two progeny with 2n = 41 + t^S and stripe rust resistance were crossed with Kalyansona. Meiotic examination of these plants showed that the telosome was paired with its normal homologue in 33% and 47% of metaphase I cells. Several F1 plants with 2n = 41 + t^S were selected and testcrossed as female parents with Kalyansona. The pooled results for these testcrosses are given in Table 3. Only 56% of plants inherited a normal chromosome complement of 42 chromosomes reflecting the poor chromosome pairing of the telosome and its entire homologue. Both nondisjunction of the 1B chromosomes and misdivision to generate the long telosome and short isochromosome were apparent. Some of the testcross plants were grown to maturity and examined at meiosis. Resistant plants with 2n = 41 + t^S chromosomes continued to show poor chromosome pairing (6 plants). On the other hand, two plants examined of the four susceptible plants with 41 + t^S showed almost complete pairing. These results indicated that the *T. dicoccoides* chromosome segment in which *Yr15* was located, was somewhat divergent from the homologous chromosome segment in hexaploid wheat. If only the individuals that inherited a telosome and a normal 1B chromosome are considered, the recombination value can be estimated at 0.071 ± 0.003. However, with full chromosome pairing and normal disjunction this recombination level may have been different.

Genetic linkage of Yr15 *and* Yr9/Sr31/Lr26

When tested for reaction to stripe rust, F2 families and F3 lines derived from cross V763-2312/SUN89D with one exception were uniformly resistant. The exceptional F3 line segregated 15 resistant : 2 susceptible. Because *Yr9* is known to be completely associated with *Lr26* (McIntosh et al. 1995), tests for leaf rust response using the *Lr26*-avirulent *P. recondita* pathotype 104-2,3,6,11 (culture 84045) and *Sr31*-avirulent pathotype 34-1,2,3,4,5,6,7 (culture 74-L1) enabled classification for the *Lr26* and *Sr31* loci, respectively. The stripe rust resistance of seedlings not possessing *Lr26* and

Sr31, and hence *Yr9*, was presumed to be conferred by *Yr15*. F3 lines were classified 9 *Lr26Lr26 Sr31Sr31* (*Yr9Yr9*), –: 51 *Lr26lr26 Sr31sr31* (*Yr9yr9*), *Yr15* –: 26 *lr26lr26 sr31sr31* (*yr9yr9*), *Yr15Yr15*. These results were consistent with a location of *Yr15* in chromosome 1B. However, if *Yr15* was located in 1BS, the single F3 line which segregated for response to stripe rust and leaf rust would have to be attributed to outcrossing or aneuploidy for chromosome 1B.

Genetic linkage of Yr15 *and* Yr10

Progenies (20-25 seedlings) from five F1 plants were tested on three separate occasions and the pooled segregation data for the progeny of each F2 plant were used to decide the phenotypic class to which it belonged. Because no Australian *P. striiformis* pathotype is virulent for *Yr15* or *Yr10*, the two genes which could not be distinguished on the basis of infection type were treated as duplicates.

A total of 743 F2 plants and 133 F3 lines was tested (Table 4). Among these, 17 F2 plants and 5 F3 lines were scored susceptible and homozygous susceptible, respectively, whereas assuming segregation at independent loci, 46 susceptible F2 plants and 8 homozygous susceptible F3 lines would be expected. It was concluded that the genes were linked in repulsion (854 : 22; $\chi^2_{15:1} = 20.9$; $P < 0.01$). These results were used to make a preliminary estimate of linkage (p) based on the maximum likelihood expression:

$$854[\frac{-2p}{4-p^2}] + 22[\frac{2}{p}] = 0$$

$p (= p_1) = 0.32$ with a standard error of 0.03

This value of linkage enabled production of expectation classes for individual F3 lines depending on genetic constitution (Table 4).

As observed in F2, the segregation in repulsion heterozygotes would be 38.8 : 1 (854:22). In populations with less than 120 seedlings it was not possible to distinguish all repulsion heterozygotes from the non-segregating resistant genotypes. However, when heterozygotes were in coupling, segregation of 7.6 resistant : 1 susceptible would be expected and with the population sizes available, this group was usually distinguishable from both the repulsion heterozygotes and the 3 : 1 segregation class. In cases where insufficient numbers were available allocation to one or other class was made on the basis of the lower χ^2 value when tested against the respective expected ratios.

An estimate of linkage was made from the pooled F3 and F2 data.

$$98[\tfrac{-2}{2-p}] + 14[\tfrac{2}{p}] + 16[\tfrac{1-2p}{p(1-p)}] + 5[\tfrac{2}{p}] +$$

$$726[\tfrac{-2p}{4-p^2}] + 17[\tfrac{2}{p}] = 0$$

$p (= p_2) = 0.33$

A standard error of 0.03 for p_2 was estimated from $s = \sqrt{\frac{1}{I}}$

where

$$I = 743\left[1 + \frac{p^2}{4-p^2}\right] + 133\left[4 + \frac{1-2p}{p(1-p)}\right]$$

The F3 line distribution was tested for goodness of fit to the expected values based on the estimated linkage value of $p_2 = 0.33$. There was a significant deviation from this distribution. The allocation of F3 lines to one or other of the 7.6 : 1 and 3 : 1 segregation classes was based on the assumption that both the *Yr15* and *Yr10* would segregate 3 : 1 when present alone. However, both the monosomic analysis and other data (Khan et al., Arts and McIntosh, unpublished) indicate that the *Yr15* allele is often preferentially transmitted relative to the alternate allele. In the analysis this would result in the likelihood that some lines allocated to the 7.6 : 1 class could be lines segregating only for *Yr15*. When the 7.6 : 1 and 3 : 1 segregation classes were pooled there was a satisfactory fit to a distribution of 98 : 30 : 5 ($\chi^2 = 1.76$; $P > 0.25$).

A further estimate of linkage based on these values combined with the F2 results was made

$$98[\tfrac{-2}{2-p}] + 30[\tfrac{2(1-p)}{p(2-p)}] + 5[\tfrac{2}{p}] +$$

$$726[\tfrac{-2p}{4-p^2}] + 17[\tfrac{2}{p}] = 0$$

$p (= p_3) = 0.33 \pm 0.03$

Discussion

The monosomic analysis and linkage studies described above show that *Yr15* is located in chromosome 1B. Although *Yr15* appeared to be stable in common wheat backcross derivatives, a telocentric 1BS chromosome that arose by misdivision during chromosome location paired with a normal wheat homologue in less than

50% of meiocytes. This indicated that at least a component of the 1B chromosome in *T. dicoccoides* was considerably divergent from that of common wheat. Linkage of *Yr15* with the centromere was estimated at 0.07 when only gametes receiving either the short telosome or the entire 1B chromosome were considered. The location of *Yr15* in chromosome 1BS was confirmed by its failure to recombine with disease resistance genes located in a T1BL·1RS chromosome and its linkage of 0.30 with *Yr10*. This latter gene is located within a few centimorgans of *Rg1* (brown glumes) (Metzger & Silbaugh 1970) and *Gli-B1* (gliadin locus) (Payne et al. 1986) in the satellite region. It is not clear whether *Yr15* is proximal or distal to *Nor-B1*, located at the non-staining constriction separating the satellite from the remainder of the chromosome.

Linkage between *Yr15* and *Yr10* was indicated from tests of F2 populations and of F3 families. However, estimation of linkage was complicated by limited F3 population sizes and the two classes of digenic heterozygotes, viz. repulsion (parental) and coupling (recombinant), as well as lines segregating monogenically. Segregation ratios in progenies of digenic heterozygotes are dependent on recombination value and rapidly diverge in opposite directions as ratios move from 15 : 1 (for independence) to no apparent segregation (for complete linkage). In the present study, an estimate of linkage (p_1) made from the F2 segregation was used to estimate frequencies of genotypes in each segregation class. With an estimate of 39 resistant : 1 susceptible for digenic repulsion heterozygotes it was clear that this group should be pooled with homozygotes leaving digenic coupling heterozygotes (7.6 : 1), monogenic groups and homozygous susceptibles as potentially distinguishable classes. However, when the estimated linkage value (p_2) was used to predict class frequencies, there was a significant deviation from the observed values. The deviation was attributable to apparent failure to accurately distinguish digenic coupling and monogenic heterozygotes possibly due to preferential segregation in favour of the *Yr15* allele. A further linkage estimate (p_3) based on pooled coupling heterozygote and monogenic classes provided a value of 0.30 ± 0.03 (or 34 ± 2cM) which gave an excellent fit between predicted and observed frequencies.

Although G-25, the *T. dicoccoides* source of *Yr15*, was highly resistant to *P. striiformis* collections from a wide geographical area, it proved susceptible to one collection from Afghanistan (van Silfhout et al. 1989) and another from Saudi Arabia (G.H.J. Kema, personal communication). While this highly effective gene could have a role in wheat breeding, the disease resistance that it confers is unlikely to be durable. Therefore *Yr15* should be used in combinations with other genes for seedling resistance or in genetic backgrounds with at least moderate levels of adult plant resistance. Such genotypes will be difficult to construct in the absence of a *Yr15*-virulent culture of the pathogen or of molecular markers to select those genes less effective than *Yr15*. A molecular probe for *Yr15* would be less desirable even though it may be easier to find.

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