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ORIGINAL PAPER

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# Locating the broad-spectrum wheat leaf rust resistance gene *Lr52* (*LrW*) to chromosome 5B by a new cytogenetic method

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**Abstract** This study was conducted to genetically map a potentially new wheat leaf rust resistance gene (*LrW*) using a novel genetic method and to test its effectiveness against current races of leaf rust (*Puccinia triticina* Eriks.) in Canada. Undoubled haploids of a near-isogenic line of Thatcher carrying the resistance gene (RL6107) were pollinated with a contrasting susceptible cultivar to generate an array of hybrids with random deficiencies arising from irregular meiosis of the haploid. Genetic analysis of the deficiencies in such populations can be used to locate qualitative traits by which the two parents differ through a process that we have called haploid deficiency mapping. In the present case, 5/417 hybrids were both susceptible to leaf rust (i.e. lacked the resistance gene) and also lacked several polymorphic microsatellite alleles from RL6107 that are specific to chromosome 5B. This correlated failed transmission of the resistance gene and deficiency for chromosome 5B. Analysis of an $F_2$ population showed that the factor conditioning resistance was located on the short arm of 5B, 16.5 cM distal to the locus of the microsatellite *Xgwm443*. Since no other leaf rust resistance genes have been mapped to this region, *LrW* was re-designated *Lr52*. RL6107 was tested with 29 isolates of *P. triticina*, encompassing a diversity of virulence found in North America, with none showing virulence. The effectiveness and novelty of *Lr52* make it a promising source of resistance for North American wheat cultivars.

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## Introduction

Leaf rust, caused by *Puccinia triticina* Eriks., is a globally distributed disease of wheat (*Triticum aestivum* L.) which causes significant reductions in grain yield and quality (Samborski 1985). While host genetic resistance is a desirable method of disease control, as the pathogen evolves new virulence to overcome resistance, new effective genes need to be identified (Dyck and Kerber 1985). As potentially new leaf rust resistance (*Lr*) genes are discovered, genetic mapping is required to ensure their novelty and distinction from previously identified genes. Many *Lr* genes have been assigned to chromosomes using monosomic analysis (e.g. Sears 1961; Dyck and Kerber 1981; Dyck et al. 1987; Hussien et al. 1997; Singh et al. 2001). Dyck and Jedel (1989) identified an *Lr* gene with wide-spectrum resistance, temporarily designated *LrW*, that has not been genetically mapped.

The purpose of this study was to assess the novelty and usefulness of *LrW* by assigning it to a chromosome using a newly proposed technique that utilizes haploid-derived aneuploids, mapping it to identify linked microsatellite markers and testing its effectiveness against a set of diverse isolates of *P. triticina*. Based on the evidence presented here, the coordinator of the wheat genetic map, R.A. McIntosh, has renamed *LrW* as *Lr52*, and this name is used in the remainder of the paper.

## Materials and methods

### Generating and pollinating haploids

Haploids ($n=3$, $x=21$, ABD) carrying *Lr52* were generated from the wheat line RL6107 (Thatcher* 6/V336; Dyck and Jedel 1989) following the method of Thomas et al. (1997), except that dicamba (100 ppm) was used in place of 2,4-dichlorophenoxyacetic acid (2,4-D). Unemasculated florets of haploid plants were pollinated four or five heads at a time by the twirl method using a

wheat variety (AC Foremost) that is susceptible to prevalent Canadian leaf rust isolates. Haploids do not need to be emasculated because they are male-sterile.

### Testing for leaf rust-susceptible hybrids

Hybrids derived from pollinated haploids were inoculated at the seedling stage with *Puccinia triticina* virulence phenotype MBDS (Long and Kolmer 1989; McCallum and Seto-Goh 2003) following the procedure of McCallum and Seto-Goh (2003). Thatcher and RL6107 were co-inoculated as checks, and all infection types were recorded (McIntosh et al. 1995; Stakman et al. 1962).

### Determining chromosome deficiencies in hybrids derived from haploids

DNA was extracted from lyophilized leaf tissue of both the parents and leaf rust-susceptible hybrid plants using a modified CTAB extraction technique (Kleinhoffs et al. 1993) (i.e. no phenol). To identify informative DNA markers, we screened RL6107 and AC Foremost for polymorphism for 58 microsatellites distributed on all 21 chromosomes of the wheat genome.

Leaf rust-susceptible hybrids were tested with polymorphic microsatellite markers to identify chromosome deficiencies. Chromosomes with apparent deficiencies (monomorphic markers) were tested with additional microsatellites. All PCR analyses were performed following the procedure of Röder et al. (1998), except that the extension time per cycle was 50 s and the final extension time was 5 min. The PCR products were separated on 5% denaturing polyacrylamide gels in TBE buffer (89 mM Tris, 89 mM boric acid, 20 mM EDTA) at 85 W for 2 h and subsequently stained with silver (Promega, Madison, Wis.).

### Screening for markers linked to *Lr52*

$F_2$ populations were produced by selfing two resistant hybrids from the cross between haploid RL6106 and AC Foremost. The DNA was extracted from 169 $F_2$ seedlings. $F_{2:3}$ families were inoculated with *P. triticina* (MBDS) in 3-foot rows at Glenlea, Manitoba, Canada in the summer of 2003 and were scored as either 'resistant', 'segregating' or 'susceptible'. Polymorphic microsatellite markers were used with homozygous resistant and homozygous susceptible $F_2$ plants to test for linkage between the microsatellites and *Lr52*. The linkage map was constructed with MAPMAKER VER. 3.0B (Lander et al. 1987) using the Kosambi (1944) mapping function.

### Assessing gene effectiveness

Seedlings of RL6107 were inoculated at the two- to three-leaf stage with 29 different *P. triticina* isolates (McCallum and Set-Goh 2003). These isolates represented 23 different virulence phenotypes. Three different isolates of MBDS and TJBJ, and two different isolates of SBDG were tested, plus a mixed inoculum that included a representative and proportional sample of most *P. triticina* virulence phenotypes found in western Canada in 2000 (McCallum and Seto-Goh 2003). Seedling infection types were scored 12 days post-inoculation.

## Results

### Generating hybrids and testing for leaf rust-susceptible hybrids

About 7,000 spikelets, representing 455 heads and 20 haploid plants of RL6107, were pollinated with AC Foremost to obtain 540 seeds. Seed set was therefore about 1.2 seeds per head pollinated and 0.1 seeds per spikelet pollinated. From 440 seeds, five plants (1-109, 1-178, 1-280, 1-369 and 1-438) were susceptible to leaf rust (virulence phenotype MBDS), and 412 plants were resistant.

### Determining chromosome deficiencies in hybrids derived from haploids

Three susceptible haploid-derived hybrids were analysed with 21 microsatellite markers (i.e. one marker per chromosome; Table 1). One plant had a chromosome 4A RL6107 microsatellite allele deficiency, while

**Table 1** Transmission of RL6107 microsatellite alleles to three leaf rust-susceptible hybrids

| Marker[a] | Chromosome | Transmission of RL6107 allele[b] | | |
|---|---|---|---|---|
| | | 1-109 | 1-178 | 1-280 |
| GWM136 | 1A | Y | Y | Y |
| GWM413 | 1B | Y | Y | Y |
| GWM642 | 1D | Y | Y | Y |
| GWM372 | 2A | Y | Y | Y |
| GWM148 | 2B | Y | Y | Y |
| GWM30 | 2D | Y | Y | Y |
| GWM674 | 3A | Y | Y | Y |
| GWM493 | 3B | Y | Y | Y |
| GWM383 | 3D | Y | Y | Y |
| GWM397 | 4A | N | Y | Y |
| GWM368 | 4B | Y | Y | Y |
| GDM125 | 4D | Y | Y | Y |
| GWM156 | 5A | Y | Y | Y |
| GWM67 | 5B | N | N | N |
| GWM190 | 5D | Y | Y | Y |
| GWM570 | 6A | Y | Y | Y |
| GWM219 | 6B | Y | Y | Y |
| GWM325 | 6D | Y | Y | Y |
| GWM332 | 7A | Y | Y | Y |
| GWM537 | 7B | Y | Y | Y |
| GWM295 | 7D | Y | Y | Y |

[a]All GWM markers are from Röder et al. (1998); GDM125 is from Pestova et al. (2000)
[b]Y, the RL6107 allele was present in the hybrid; N, the RL6107 allele was not present in the hybrid

**Table 2** Microsatellite marker transmission for chromosomes 4A and 5B in leaf rust-susceptible hybrids

| Marker[a] | Chromosome | Distance from centromere (cM)[b] | Transmission of RL6107 allele[c] | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1-109 | 1-178 | 1-280 | 1-369 | 1-438 |
| GWM397 | 4AL | 18 | N | Y | Y | – | – |
| WMC161 | 4AL | 40 | N | Y | Y | – | – |
| WMC262 | 4AL | 49 | N | Y | Y | – | – |
| WMC313 | 4AL | 71 | N | Y | Y | – | – |
| GWM443 | 5BS | 66 | N | N | N | N | N |
| WMC149 | 5BS | 26 | N | N | N | N | N |
| GWM133 | 5BS | 9 | N | N | N | N | N |
| GWM67 | 5BL | 6 | N | N | N | N | N |
| WMC75 | 5BL | 55 | N | N | N | N | N |
| WMC235 | 5BL | 80 | N | N | N | N | N |

[a]All GWM markers are from Röder et al. (1998); WMC markers are from Somers et al. (2004)
[b]Distance estimates are based on consensus maps by Somers et al. (2004)
[c]N, the RL6107 allele was not present in the hybrid; Y, the RL6107 allele was present in the hybrid; –, the microsatellite marker was not tested with the hybrid

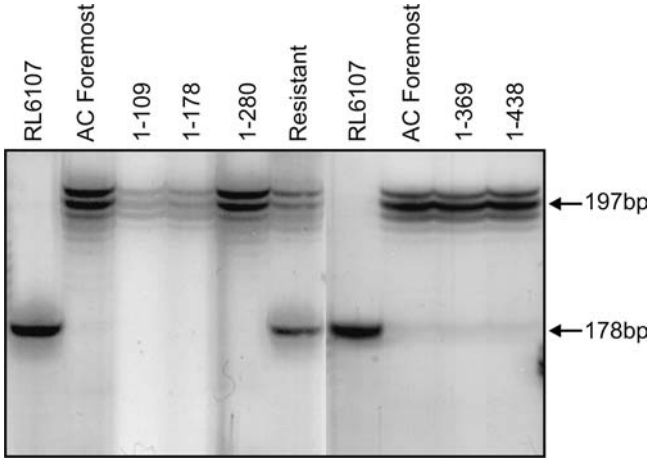


**Fig. 1** Example of a deficient microsatellite (WMC75) allele in susceptible hybrids missing chromosome 5B from wheat line RL6107. Plants 1-109, 1-178, 1-280, 1-369 and 1-438 are susceptible hybrids and have failed to receive the RL6107 allele of wmc75 (178 bp), but they do have the allele (197 bp) from the susceptible parent AC Foremost. An example of a resistant hybrid, in the lane labeled *Resistant*, received both alleles

all three had deficiencies on chromosome 5B. Further testing of three and six microsatellites, on chromosomes 4A and 5B, respectively, confirmed that all three susceptible hybrids were deficient for chromosome 5B but that only one of the three had the additional deficiency for chromosome 4A (Table 2, Fig. 1). Two additional susceptible hybrids were analysed, and chromosome 5B was deficient for the RL6107 alleles in both of these hybrids (Table 2). In summary, all five susceptible hybrids were deficient for RL6107 microsatellite alleles on chromosome 5B (Fig. 2).

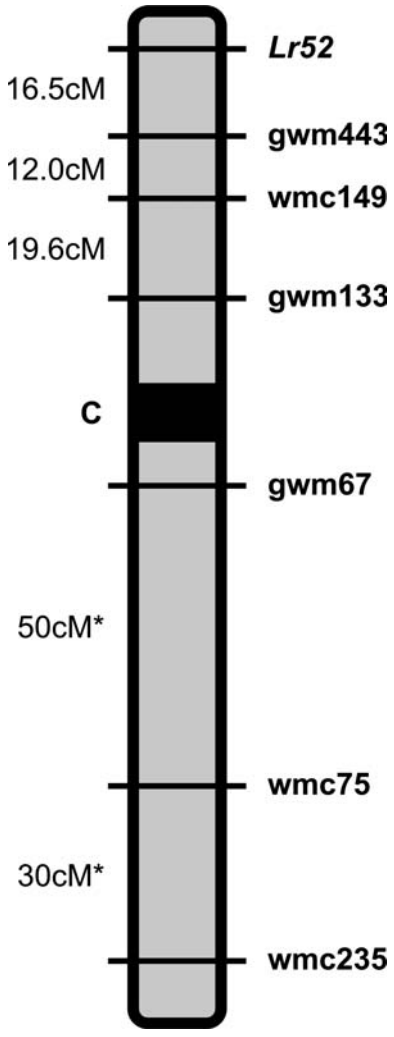


**Fig. 2** Microsatellites *Xgwm443*, *Xwmc149*, *Xgwm133*, *Xgwm67*, *Xwmc75* and *Xwmc235* demonstrated the failed transmission of chromosome 5B from RL6107 to all leaf rust-susceptible hybrids. Mapping with the $F_2$ population revealed linkage between *Lr52* and microsatellites shown on 5BS. Distances are in centiMorgans as calculated with the Kosambi mapping function (Kosambi 1944). *C* denotes the centromere. *Genetic distances are estimates based on maps by Somers et al. (2004)

## Screening for markers linked to *Lr52*

Based on progeny tests of $F_2$-derived $F_3$s, $F_2$s between RL6107 and AC Foremost fit the expected genotypic ratio for a single gene (out of 169 families, 42 were resistant, 83 segregated and 44 were susceptible; $\chi^2_{1:2:1} = 0.101$, $P = 0.95$). Ten leaf rust-susceptible $F_2$ plants (representing 20 gametes) were screened with polymorphic microsatellite markers on chromosome 5B. Microsatellite *Xgwm443* and the *Lr52* locus appeared to be linked, thus 39 homozygous resistant and 41 homozygous susceptible individuals were tested with GWM443. The genetic distance between *Xgwm443* and *Lr52* was 16.5 cM with a LOD score of 17. Microsatellites GWM133 and WMC149 were also tested with the $F_2$ population, revealing that *Lr52* is 16.5 cM distal of *Xgwm443* (Fig. 2).

**Table 3** Virulence phenotypes and infection types of the *Puccinia triticina* isolates tested with RL6107 and Thatcher

| Isolate | Virulence phenotype[a] | RL6107[b] | Thatcher[b] |
|---|---|---|---|
| –[c] | BBB | ;1 | 3+4 |
| – | CBDJ | ;1– | 34 |
| 2001 | Epidemic[d] | ;11– | 3+4 |
| 12–3 | MBDS | ;1 | 34 |
| 00-13-1 | MBDS | ;1– | 3+ |
| 00-7-2 | MBDS | ;1– | 4 |
| – | MBRJ | ;1 | 4 |
| 00-52-2 | MCPS | ;1– | 4 |
| 99-46-2 | MDRJ | ;11– | 3+ |
| 99-127-1 | MFMJ | ;11– | 3+4 |
| – | MGBJ | ;11+ | 3+4 |
| – | NBBR | ;11– | 34 |
| – | PBDG | ;1 | 3+4 |
| 99-231-2 | PBLR | ;11– | 3+4 |
| 99-228-1 | PBMR | ;1 | 3+4 |
| – | PCLR | ;1– | 34 |
| – | SBDG | ;1 | 4 |
| 00-148-2 | SBDG | ;11+ | 3+ |
| 00-74-1 | SGBJ | ;1+– | 3+ |
| 00-24-1 | TBPS | ;11– | 3+4 |
| 00-44-2 | TCMJ | ;1 | 4 |
| 99-8-1 | TFMJ | ;1 | 3+4 |
| 99-93-1 | TFRJ | ;11+ | 34 |
| 00-53b-1 | TGLJ | ;11– | 3+ |
| 00-179-1 | THBJ | ;1– | 4 |
| 00-30-1 | THMJ | ;11+ | 4 |
| – | TJBJ | ;1 | 3+4 |
| 00-32-1 | TJBJ | ;1 | 3+4 |
| 00-30-2 | TJBJ | ;1 | 3+4 |

[a]Nomenclature as described by Long and Kolmer (1989)
[c]No isolate number was assigned
[b]Infection types as described McIntosh et al. (1995)
[d]Epidemic mixture is representative of virulence phenotypes found in western Canada in 2000 (McCallum and Seto-Goh 2003)

### Effectiveness of *Lr52*

Seedlings of RL6107 were resistant to 29 rust isolates representing 23 virulence phenotypes (Table 3). The virulence phenotype codes used in Table 3 are explained in Long and Kolmer (1989). All infection types of RL6107 were resistant (';' to ';11+') including several cases involving different isolates of common virulence phenotypes (TJBJ and MBDS; Table 3).

## Discussion

A deficiency for chromosome 5B correlated with the loss of *Lr52* in five cases out of five. Therefore, it can be concluded that *Lr52* is on chromosome 5B. The only other *Lr* gene to be identified on chromosome 5B is *Lr18*, which is on the long arm (McIntosh 1983). Linkage to microsatellite markers on the short arm of chromosome 5B (Fig. 2) confirmed the location of *Lr52*. Since this excludes *Lr52* as an allele of *Lr18*, *Lr52* is distinct from all other previously identified leaf rust resistance genes.

Dyck and Jedel (1989) showed that *Lr52* in RL6107 is effective against nine *P. triticina* isolates. In this study, *Lr52* was effective against all 23 virulence phenotypes of *P. triticina* against which it was tested. Resistance to the epidemic mixture indicated seedling resistance to the current virulence phenotypes found in western Canada, making this a promising source of leaf rust resistance for Canadian wheat cultivars.

One premise for the reliable application of haploid deficiency mapping is that the small group of critical individuals (those showing loss of the gene) are identified without error. In prior studies, linked SCAR (sequence characterized amplified region) markers were used to demonstrate gene loss (Thomas et al. 2001; J. Thomas unpublished). In the present case, phenotyping (i.e., rust infection) was reliable because the contrast between resistant and susceptible hybrids was comparable to that between the near-isogenic line RL6107 and its susceptible recurrent parent Thatcher (i.e., the level of dominance of *Lr52* was high).

Sears (1939) suggested two principle routes for the origin of monosomes in the progeny of pollinated haploids. One likely route is the random meiotic elimination of one or more univalents from a partially restituted egg sac. If such an ovule is pollinated, the normal result is a single or multiple monosomic of the standard (untranslocated) karyotype. However, monosomy can also arise from disjunction of the mostly open bivalents that are observed in haploid meiosis (Gaines and Aase 1926; Person 1955; Riley and Chapman 1957; Kimber and Riley 1963; Jauhar et al. 1991; Thomas et al. 1997). Where subsequent metaphase I disjunction of all or most of the univalents is followed by restitution of the second division, the recovery of such ovules by pollination should give rise to monosomic-trisomic duplications and deficiencies. Since bivalents present in the original haploid meiocyte are usually intergenomic (Jauhar et al. 1991, 1999) and usually involve homœologues, the resulting duplication-deficiencies are expected to show homœologous compensation. As expected, plants that combine trisomes (indicated by tri-radial trivalents such as 'Y's and frying pans) with monosomes are encountered among the progeny of haploids. One of these trisomes is a probable translocation. Isolated terminal markers from otherwise deficient chromosomes have been detected in the progeny of haploids, presumably translocated to a homœologue by non-homologous crossover (Thomas et al. 2001; J. Thomas unpublished). No such cases were detected in the present study. Presumably, the five instances of gene loss of *Lr52* were all occasioned by standard 5B monosomes arising from the random elimination of 5B univalents (Sears 1939).

The rate at which particular chromosome deficiencies are observed appears to be variable. In this study, transmission failure of *Lr52*, caused by the deficiency of chromosome 5B, occurred at a rate of one in 83.4 plants, which is tenfold less frequent than the failed transmission of two different SCAR markers on chromosomes 2B and 3B (Thomas et al. 2001). Variable rates of specific defi-

ciencies have also been observed in studies with other uncharacterized leaf rust resistance genes (C. Hiebert unpublished). The rate of deficiency of the critical chromosome cannot yet be predicted. Since the recovery of leaf rust-susceptible hybrids in this study was approximately 1%, it is recommended that at least 300–400 hybrid seed should be generated for phenotypic screening. This should ensure that several hybrids with the critical deficiency can be identified for further analysis. It is imperative to find agreement in chromosome deficiencies between several hybrids because some individuals may have more than one deficiency, as was the case with the susceptible hybrid that was monosomic for chromosomes 4A and 5B.

Haploid deficiency mapping appears to be an efficient method for assigning genes to chromosomes in wheat. Standard monosomic analysis requires the ongoing maintenance and checking of aneuploid stocks and the screening of 21 $F_2$ populations (100–200 individuals per cross) to test for distorted segregation. By contrast, haploid deficiency mapping requires the testing of a smaller number of individuals (400) for phenotypic screening and a low number of plants for genetic analysis (in our case, five). Most of the labour for haploid deficiency mapping is the production of a few haploids and the generation of sufficient hybrid seed. In this respect, some haploids are more sterile than others. Particular haploids may be sufficiently sterile to preclude the use of this method. In this case it is recommended that haploids should be made from hybrids, and resistant haploids selected for haploid deficiency mapping. This is now our usual procedure.

## Conclusion

This study used haploid deficiency mapping to locate *Lr52* on chromosome 5B. Analysis of an $F_2$ population revealed that *Lr52* is 16.5 cM distal of the microsatellite marker *Xgwm443* on the short arm of chromosome 5B. As no other *Lr* genes have been located on chromosome 5BS, *Lr52* is a novel gene. This gene is effective against *P. triticina* populations in North America and is a promising resistance source for North American wheat breeding programs.

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## References

Dyck PL, Jedel PE (1989) Genetics of resistance to leaf rust in two accessions of common wheat. Can J Plant Sci 69:531–534

Dyck PL, Kerber ER (1981) Aneuploid analysis of a gene for leaf rust resistance derived from the common wheat cultivar Terenzio. Can J Genet Cytol 23:405–409

Dyck PL, Kerber ER (1985) Resistance of the race-specific type. In: Roelfs AP, Bushnell WR (eds) The cereal rusts, vol 2. Academic Press, Orlando, pp 469–500

Dyck PL, Kerber ER, Lukow OM (1987) Chromosome location and linkage of a new gene (*Lr33*) for reaction to *Puccinia recondita* in common wheat. Genome 29:463–466

Gaines EF, Aase HC (1926) A haploid wheat plant. Am J Bot 13:373–385

Hussien T, Bowden RL, Gill BS, Cox TS (1997) Chromosome location of leaf rust resistance gene *Lr43* from *Aegilops tauschii* in common wheat. Crop Sci 37:1764–1766

Jauhar PP, Riera-Lizarazu O, Dewey WG, Gill BS, Crane CF, Bennett JH (1991) Chromosome pairing relationships among the A, B, and D genomes of bread wheat. Theor Appl Genet 82:441–449

Jauhar PP, Almouslem AB, Peterson TS, Joppa LR (1999) Inter- and intragenomic chromosome pairing in haploids of durum wheat. J Hered 90:437–445

Kimber G, Riley R (1963) The relationships of the diploid progenitors of hexaploid wheat. Can J Genet Cytol 5:83–88

Kleinhoffs A, Kilian A, Saghai MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Long DL, Kolmer JA (1989) A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology 79:525–529

McCallum BD, Seto-Goh P (2003) Physiologic specialization of wheat leaf rust (*Puccinia triticina*) in Canada in 2000. Can J Plant Pathol 25:91–97

McIntosh RA (1983) Genetic and cytogenetic studies involving *Lr18* for resistance to *Puccinia recondita*. In: Sakamota S (ed) Proc 6th Int Wheat Genet Symp. Faculty of Agriculture, Kyoto University, pp 777–783

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO Publ, East Melbourne, Australia

Person C (1955) An analytical study of chromosome behaviour in a wheat haploid. Can J Bot 33:11–30

Pestova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Riley R, Chapman V (1957) Haploids and polyhaploids in *Aegilops* and *Triticum*. Heredity 11:195–207

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Samborski DJ (1985) Wheat leaf rust. In: Roelfs AP, Bushnell WR (eds) The cereal rusts. vol 2. Academic Press, Orlando, pp 39–59

Sears ER (1939) Cytogenetic studies with polyploid species of wheat. I. Chromosomal aberrations in the progeny of a haploid of *Triticum vulgare*. Genetics 24:509–523

Sears ER (1961) Identification of the wheat chromosome carrying leaf-rust resistance from *Aegilops umbellulata*. Wheat Inf Serv 12:12–13

Singh D, Park RF, Bariana HS, McIntosh RA (2001) Cytogenetic studies in wheat. XIX. Chromosome location and linkage studies of a gene for leaf rust resistance in the Australian cultivar 'Harrier'. Plant Breed 120:7–12

Somers DJ, Isaac P, Edwards K (2004) A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici*. Agric Res Serv E617

Thomas J, Chen Q, Howes N (1997) Chromosome doubling of haploids of common wheat with caffeine. Genome 40:552–558

Thomas J, Riedel E, Penner G (2001) An efficient method for assigning traits to chromosomes. Euphytica 119:217–221