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Chromosomal locations in common wheat of three new leaf rust resistance genes from *Triticum monococcum*

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Summary

Monosomic analysis was conducted to determine chromosomal locations of three new leaf rust resistance genes recently transferred to common wheat (*Triticum aestivum*) from *T. monococcum*. The resistance gene in wheat germplasm line KS92WGRC23 was transferred from *T. monococcum* ssp. *monococcum*. The resistance genes found in KS93U3 and KS96WGRC34 were transferred from *T. monococcum* ssp. *aegilopoides*. Allelism tests showed that the three resistance genes were unlinked. The three lines were crossed with each of the seven A-genome Wichita monosomic lines. The leaf rust resistance genes in KS92WGRC23, KS93U3, and KS96WGRC34 were located on chromosomes 6A, 1A, and 5A, respectively, by monosomic analysis. These results demonstrate that the three new genes derived from *T. monococcum* are each different. They also differ from previously reported *Lr* genes. This information on chromosome location and the development of mapping populations will facilitate molecular tagging of the new genes.

Introduction

Common wheat (*Triticum aestivum* L.) frequently suffers significant losses due to leaf rust in Kansas and other states of the southcentral Great Plains region of the United States (Marshall, 1988; Roelfs, 1988, Watson, 1996). The use of cultivars resistant to the wheat leaf rust pathogen, *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* (Eriks. & E. Henn.) D.M. Henderson, is potentially the most efficient and economical means of controlling this disease (Knott, 1989; Line, 1993; McVey & Long, 1993). Unfortunately, some major cultivars like TAM 107 contain no detectable leaf rust resistance genes (McVey and Long, 1993). Other widely used cultivars like 2163, Karl, and TAM 200 contain leaf rust resistance genes that have been defeated by one or more leaf rust races. New sources of leaf rust resistance could be useful for improving the diversity of resistance genes in Great Plains wheat cultivars.

The Wheat Genetics Resources Center (WGRC) at Kansas State University has recently transferred three new leaf rust resistance genes from diploid *T. monococcum* L. (2n=2×=14, AA) to hexaploid common wheat (2n=6×=42, AABBDD). The gene in the germplasm line KS92WGRC23 was transferred from cultivated einkorn wheat, *T. monococcum* ssp. *monococcum*. The genes found in the lines KS93U3 and KS96WGRC34 were transferred from wild *T. monococcum* ssp. *aegilopoides* (Link) Thell. [synonym *T. monococcum* var. *boeoticum*].

Although these three new resistance genes are from *T. monococcum*, their low infection types (ITs) and field performances are different (Hussien et al., 1997). Information on the chromosomal locations of these genes will help clarify their relationships to other leaf rust resistance genes and will aid in development of molecular markers. This study was undertaken to determine the chromosomal locations of the three leaf rust resistance genes in the wheat lines KS92WGRC23, KS93U3, and KS96WGRC34 using monosomic analysis.

Materials and methods

KS92WGRC23 (Reg. no. GP-393, PI566672) is a hard red winter wheat germplasm line with the pedigree 'Karl*3//PI266844/PI355520'. It was developed cooperatively by the WGRC of Kansas State University, the USDA-ARS, and the Kansas Agricultural Experiment Station (Cox et al., 1994). The seedling IT ranged from immune (0) to a few hypersensitive flecks (0;) when inoculated with avirulent leaf rust races. Jacobs et al. (1996) described the mechanisms associated with this resistance reaction. Cox et al. (1994) reported that the resistance in this line is controlled by a single dominant gene. Hussien et al. (1997) noted that KS92WGRC23 contains an additional gene for intermediate resistance that can be detected with isolate PRTUS6 of *P. recondita* f.sp. *tritici* but not with isolate PRTUS25. Isolate PRTUS6 belongs to race PBJ-10 according to the nomenclature of Long and Kolmer (1989) and is virulent to leaf rust resistance genes *Lr1*, *Lr2c*, *Lr3a*, *Lr10*, *Lr11*, and *Lr17*. Isolate PRTUS25 belongs to race MFB-10 and is virulent to leaf rust resistance genes *Lr1*, *Lr3a*, *Lr10*, *Lr24*, and *Lr26*.

The hard red winter wheat line KS93U3 has the pedigree 'Wrangler//Mustang*2/TA213'. In seedling tests, the IT of KS93U3 was a heterogeneous mixture of mostly smaller pustules (X-). Resistance is controlled by a single dominant gene (T. S. Cox, unpublished).

The hard red winter wheat germplasm line KS96WGRC34 has the pedigree 'TAM107/TA749// Wrangler'. In seedling tests, the IT of KS96WGRC34 was a hypersensitive fleck with more chlorosis than usual (;C). KS96WGRC34 carries a single dominant gene conditioning resistance (T.S. Cox, unpublished).

For allelism tests, the three resistant germplasm lines were intercrossed. F_1 plants were allowed to self pollinate. Seeds of F_2 progenies were planted in 7-cm-plastic pots (eight seeds per pot) filled with vermiculite. Pots were refrigerated at 4 °C for 5 days to break dormancy and initiate uniform germination of seeds. Subsequently, pots were transferred to a greenhouse maintained at 21±5 °C with a 12 hr photoperiod. After 10 days, seedlings were inoculated using the urediniospore oil suspension inoculation technique (Browder, 1971). Ampoules containing urediniospores of race MFB-10 were retrieved from storage in liquid nitrogen and heat shocked in a 40 °C water bath for 5 minutes. Inoculum was applied with an atomizer, then seedlings were placed in a moist chamber for 16 hours at 16 °C without light. Plants were then returned to the greenhouse.

Seedlings were scored for disease 10-12 days after inoculation using the scale developed by Stakman et al. (1962) and modified by Roelfs et al. (1992): 0 = no uredinia or other macroscopic signs of infection, ; = no uredinia, but hypersensitive necrotic or chlorotic flecks present, 1 = small uredinia surrounded by necrosis, 2 = small to medium-sized uredinia often surrounded by chlorosis or necrosis (green island may be surrounded by chlorotic or necrotic border), X = random distribution of variable-sized uredinia on single leaf, 3 = medium-sized uredinia that may be associated with chlorosis, and 4 = large uredinia without chlorosis. A range of variation in ITs is recorded by indicating the range, with the most prevalent infection type listed first (e.g., 12 or ;1). ITs of 3 or greater were considered susceptible.

For monosomic analysis, the seven A-genome monosomic stocks (1A to 7A) in a 'Wichita' (CI 11952) background were provided by the WGRC. Only the A-genome monosomics were used because the resistance genes were obtained from *T. monococcum*, which contains only the A-genome. Monosomic plants (2n=41 chromosomes) were identified by chromosome counts of root-tip cells (Endo and Gill, 1984). The 41-chromosome Wichita monosomic stocks were crossed as females with the resistant lines KS92WGRC23, KS96WGRC34, and KS93U3. The 41-chromosome F_1 plants were self pollinated to produce F_2 populations. F_2 seedlings were screened for resistance as described above. *P. recondita* f. sp. *tritici* race PBJ-10 was used for segregating populations derived from crosses with KS96WGRC34 and KS93U3. Race MFB-10 was used for those derived from KS92WGRC23.

The critical cross identifying the chromosomal location of the resistance gene is expected to exhibit an excess of resistant plants in the F_2 (McIntosh, 1987). In the noncritical crosses, however, a normal ratio of 3 resistant (R) to 1 susceptible (S) plants will be obtained. The critical and noncritical crosses were identified through a χ^2 goodness-of-fit test for a 3R:1S ratio.

Results

In the allelism tests, the R:S ratio did not differ from the expected 15R:1S ratio in any of the crosses (Table 1). This indicated that the three genes were genetically independent.

Table 1. Segregation for reaction to leaf rust race MFB-10 in crosses among three resistant germplasm lines derived from *Triticum monococcum*

Cross	Number of F_2 plants		χ^2(15:1)	P-value
	Resistant	Susceptible		
KS92WGRC23 / KS93U3	49	4	0.15	>0.10
KS96WGRC34 / KS93U3	130	11	0.58	>0.10
KS96WGRC34 / KS92WGRC23	266	18	0.00	>0.10

Table 2. F_2 segregation in progenies of F_1 monosomic plants from crosses of the A-genome Wichita monosomics and three leaf rust resistant lines

Monosome	KS92WGRC23			KS93U3			KS96WGRC34		
	R	S	χ^2	R	S	χ^2	R	S	χ^2
1A	21	1	4.91*	96	10	13.60**	103	55	8.12
2A	60	16	0.63	67	19	0.35	104	35	0.002
3A	44	10	1.21	64	25	0.45	94	36	0.50
4A	31	7	0.88	77	21	0.67	74	24	0.01
5A	52	15	0.24	71	30	1.19	107	13	12.84**
6A	54	3	11.84**	60	22	0.15	121	38	0.10
7A	53	1	15.43**	78	27	0.03	97	35	0.16

*Significantly greater than 3:1 ratio at P= 0.05
**Significantly greater than 3:1 ratio at P=0.01

In the monosomic analysis of KS92WGRC23, F_2 plants of the 2A, 3A, 4A, and 5A families produced the expected 3R:1S ratios (Table 2). Thus, the resistance gene could not be located on these chromosomes. Unexpectedly, three families (1A, 6A, and 7A) had a significant excess of resistant plants and could not be eliminated as the critical cross for the resistance gene. Presumably, disease escapes accounted for the excess in two of the families. Since the IT of KS92WGRC23 is 0 to 0; (nearly immune), escapes may be confused with resistant plants.

In order to resolve the issue, twenty putatively resistant F_2 plants from each of the three crosses, 1A, 6A, and 7A were progeny tested. Between 14 and 48 F_3 seedlings from each F_2 plant were inoculated. In the critical cross, some F_3 families should produce a few susceptible nullisomic plants which may vary from 3 to 10% in different monosomic plant progenies. However, none of the F_3 families from the critical cross should segregate 3R:1S. Twelve of twenty F_3 families for chromosome 1A segregated in a 3R:1S ratio, thus eliminating chromosome 1A. Fourteen of twenty F_3 families for chromosome 7A segregated 3R:1S, thus eliminating chromosome 7A. In contrast, none of the F_3 families for chromosome 6A segregated in a 3R:1S ratio (P=0.05). Fifteen F_3 families produced only resistant progeny. Five F_3 families each included a single susceptible seedling and the rest were resistant. Therefore, the resistance gene in KS92WGRC23 must be located on chromosome 6A.

The monosomic analysis for the resistance gene in line KS93U3 identified six of the monosomics (2A, 3A, 4A, 5A, 6A, and 7A) as noncritical families (Table 2). The monosomic 1A family, however, had significantly more resistant plants than expected. Therefore, the gene in the resistant line KS93U3 must be located on chromosome 1A. A progeny test of nineteen resistant F_2 plants confirmed this result. Fifteen F_3 families produced only resistant progeny. Four F_3 families each produced a few susceptible progeny and the rest were resistant. The ratios of resistant to susceptible seedlings in each of the four families were significantly different from a 3:1 ratio (P=0.10).

Monosomic analysis for the gene in KS96WGRC34 identified a significant excess (P<0.001) of resistant plants only in the cross involving chromosome 5A (Table 2). A progeny test of twenty resistant F_2 plants confirmed the location of the gene on chromosome 5A. Fourteen F_3 families produced only resistant progeny. Six F_3 families each produced a few susceptible plants and the rest were resistant. The R:S ratio in each of the six families was significantly different from 3:1.

Discussion

The new leaf rust resistance genes in wheat lines KS92WGRC23, KS93U3, and KS96WGRC34 were previously shown to have different ITs and field performances (Hussien et al., 1997). The race specificity of the gene in KS92WGRC23 was unique, but the race specificities of the genes in KS93U3 and KS96WGRC34 were similar when tested against nine isolates of *P. recondita* f. sp. *tritici* (Hussien et al., 1997). In the present study, allelism tests showed that the leaf rust resistance genes in the three wheat lines were unlinked. Using monosomic analysis, the leaf rust resistance genes in KS92WGRC23, KS93U3, and KS96WGRC34 were located on chromosomes 6A, 1A, and 5A, respectively. These results confirm that the three new genes derived from *T. monococcum* are different.

The chromosomal locations indicate that the new resistance genes are also different from previously described wheat leaf rust resistance genes. No other leaf rust resistance genes have been reported on chromosomes 5A or 6A (McIntosh et al., 1995; Roelfs et al., 1992). Therefore, the genes in KS92WGRC23 and KS96WGRC34 are unique. The gene in KS93U3 was located on chromosome 1A, which is also the location of *Lr10* (McIntosh et al., 1995). However, the IT of KS93U3 is heterogeneous (X-) whereas the IT of *Lr10* is typically a fleck (;) with some isolates giving small or medium-sized uredinia (12) (McIntosh et al., 1995). The race specificities are also clearly different. Most common races in the Great Plains are virulent on plants with *Lr10*, but most were avirulent for the gene in KS93U3 (Hussien et al.,1997). Although the gene in KS93U3 and *Lr10* reside on the same chromosome, their linkage relationships are yet to be determined.

Confirmation of the results of the monosomic analyses is desirable before new gene symbols are allocated to these genes. Populations have been developed that will allow mapping of the new *Lr* genes with respect to known molecular markers. Identification of molecular markers flanking the genes will be useful in screening for the presence of these new genes in breeding materials. This will be particularly helpful for the development of resistance gene combinations.

T. monococcum has been a good source of resistance genes to both stem rust and leaf rust of wheat. Stem rust resistance genes *Sr21*, *Sr22*, and *Sr35* were transferred from *T. monococcum* to hexaploid wheat (McIntosh et al., 1984). *Sr21* and *Sr22* are reportedly common in accessions of *T. monococcum* (The, 1976). The (1976) found that all accessions in a collection of 121 *T. monococcum* lines were resistant to leaf rust. The majority were highly resistant, producing infection types ranging from hypersensitive flecks (;) to nearly immune with a few flecks (0;). In Australia, no leaf rust isolate with virulence on the *T. monococcum* accession W10 was found despite extensive tests over several years (The, 1976). Vallega (1979) tested 102 accessions of *T. monococcum* and found all accessions to possess resistance to five races of the leaf rust fungus. Gill et al. (1983) also reported the existence of leaf rust resistance in this species.

There is not yet an officially named gene for leaf rust resistance transferred to hexaploid wheat from *T. monococcum*. However, Valkoun et al. (1986) reported that a dominant leaf rust resistance gene, tentatively designated Lr Tm_1, was transferred to hexaploid wheat from *T. monococcum*. The IT was a hypersensitive fleck with a few tiny pustules (;1). The gene was located on chromosome 3A by monosomic analysis (Valkoun et al., 1988). This result has not yet been confirmed. The IT and chromosomal location of Lr Tm_1 do not match those of the three genes from *T. monococcum* reported in the present study. Dyck and Bartos (1994) recently attempted to transfer another leaf rust resistance gene from *T. monococcum* to hexaploid wheat. However, this gene was found to be the same as *Lr33* (RL6057).

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References

Browder, L.E., 1971. Pathogenic specialization in cereal rust fungi, especially *Puccinia recondita* f.sp. *tritici*: concepts, methods of study, and application, US Dept. Agr Tech Bull 1432.

Cox, T.S., R.G. Sears, B.S. Gill & E.N. Jellen, 1994. Registration of KS91WGRC11, KS92WGRC15, and KS92WGRC23 leaf rust-resistant hard red winter wheat germplasms. Crop Sci 34: 546–547.

Dyck, P.L. & P. Bartos, 1994. Attempted transfer of leaf rust resistance from *Triticum monococcum* and durum wheat to hexaploid wheat. Can J Plant Sci 74: 733–736.

Endo, T.R. & B.S. Gill, 1984. Somatic karyotype, heterochromatin distribution, and nature of chromosome differentiation in common wheat, *Triticum aestivum* L. em Thell. Chromosoma 89: 361–369.

Gill, B.S., L.E. Browder, J.H. Hatchett, T.L. Harvey, T.J. Martin, W.J. Raupp, H.C. Sharma & J.G. Waines, 1983. Disease and insect resistance in wild wheats. In: S. Sakamoto (ed), Proc 6th Int Wheat Genet Symp. Plant Germplasm Institute, pp. 785–792. Kyoto Japan.

Hussien, T., R.L. Bowden, B.S. Gill, T.S Cox & D.S. Marshall, 1997. Performance of four new leaf rust resistance genes transferred to common wheat from *Aegilops tauschii* and *Triticum monococcum*. Plant Dis 81: 582–586.

Jacobs, A.S., Z.A. Pretorius, F.J. Kloppers & T.S. Cox, 1996. Mechanisms associated with wheat leaf rust resistance derived from *Triticum monococcum*. Phytopathology 86: 588–595.

Knott, D.R., 1989. The Wheat Rusts: Breeding for Resistance. Springer-Verlag Publ, Berlin Germany.

Line, R.F., 1993. Integrated pest management for wheat: IPM in a wide ranging system. Plant Dis 77: 303–307.

Long, D.L. & J.A. Kolmer, 1989. A North American system of nomenclature for *Puccinia recondita* f.sp. *tritici*. Phytopathology 79: 525–529.

Marshall, D., 1988. Characteristics of the 1984–1985 wheat leaf rust epidemic in central Texas. Plant Dis 72: 239–241.

McIntosh, R.A., 1987. Gene location and gene mapping in hexaploid wheat. In: E.G. Heyne (Ed), Wheat and Wheat Improvement, pp. 269–287. ASA Madison WI.

McIntosh, R.A., P.L. Dyck, T.T. The, J.E. Cusick & D.L. Milne, 1984. Cytogenetical studies in wheat XIII. *Sr35* – a third gene from *Triticum monococcum* for resistance to *Puccinia graminis tritici*. Zeitschrift für Pflanzenzüchtung 92: 1–14.

McIntosh, R.A., C.R. Wellings & R.F. Park, 1995. Wheat Rusts: An Atlas of Resistance Genes. CSIRO, East Melbourne, Victoria 3002, Australia.

McVey, D.V. & D.L. Long, 1993. Genes for leaf rust resistance in hard red winter wheat cultivars and parental lines. Crop Sci 33: 1373–1381.

Roelfs, A.P., 1988. Resistance to leaf and stem rusts in wheat. In: N.W. Simmonds & S. Rajaram (Eds), Breeding Strategies for Resistance to the Rusts of Wheat pp. 10–22. CIMMYT, Mexico D.F.

Roelfs, A.P., R.P. Singh & E.E. Saari, 1992. Rust Diseases of Wheat: Concepts and Methods of Disease Management. CIMMYT, Mexico, D.F.

Stakman, E.C., D.M. Stewart & W.Q. Loegering, 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici* US Dept Agr, Agr Res Service E617.

The, T.T., 1976. Variability and inheritance studies in *Triticum monococcum* for reaction to *Puccinia graminis* f. sp. *tritici* and *Puccinia recondita*. Zeitschrift für Pflanzenzüchtung 76: 287–298.

Valkoun, J., D. Kucerova & P. Bartos, 1986. Transfer of leaf rust resistance from *Triticum monococcum* L. to hexaploid wheat. Zeitschrift für Pflanzenzüchtung 96: 271–278.

Valkoun, J., L. Kucera, D. Kucerova & J. Kosner, 1988. Chromosomal location in wheat of a gene from *Triticum monococcum* L. for low reaction to leaf rust. Scientia Agriculturae Bohemoslavaca, Tomus 20 (XXXVll): 21–24.

Vallega, V., 1979. Search for useful characters in diploid *Triticum* spp. In: S. Ramanujan (Ed), Proc Fifth Int Wheat Genetics Symp, pp. 156–162. Indian Soc Genetics and Plant Breeding. New Delhi India.

Watson, S., 1996. Wheat Varieties for Kansas 1997. Lone Tree Publishing Co., Topeka Kansas.