Linkage mapping of powdery mildew and greenbug resistance genes on recombinant 1RS from ‘Amigo’ and ‘Kavkaz’ wheat–rye translocations of chromosome 1RS.1AL

Yehia Mater, Stephen Baenziger, Kulvinder Gill, Robert Graybosch, Lynda Whitcher, Cheryl Baker, James Specht, and Ismail Dweikat

Abstract: Cultivated rye (Secale cereale L., 2n = 2x = 14, RR) is an important source of genes for insect and disease resistance in wheat (Triticum aestivum L., 2n = 6x = 42). Rye chromosome arm 1RS of S. cereale ‘Kavkaz’ originally found as a 1BL.1RS translocation, carries genes for disease resistance (e.g., Lr26, Sr31, Yr9, and Pm8), while 1RS of the S. cereale ‘Amigo’ translocation (1RS) carries a single resistance gene for greenbug (Schizaphis graminum Rondani) biotypes B and C and also carries additional disease-resistance genes. The purpose of this research was to identify individual plants that were recombinant in the homologous region of 1AL.1RSV and 1AL.1RSA using both molecular and phenotypic markers.

Secale cereale ‘Nekota’ (1AL.1RSA) and S. cereale ‘Pavon 76’ (1AL.1RSV) were mated and the F1 was backcrossed to ‘Nekota’ (1AL.1AS) to generate eighty BC1F2:3 families (i.e., (‘Nekota’ 1AL.1RSA × ‘Pavon 76’ 1AL.1RSV) × Nekota’ 1AL.1AS). These families were genotyped using the secalin–gliadin grain storage protein banding pattern generated with polyacrylamide gel electrophoresis to discriminate 1AL.1AS/1AL.1RS heterozygotes from the 1AL.1RSA+V and 1AL.1AS homozygotes. Segregation of the secalin locus and PCR markers based on the R173 family of rye specific repeated DNA sequences demonstrated the presence of recombinant 1AL.1RSA+V families. Powdery mildew (Blumeria graminis) and greenbug resistance genes on the recombinant 1RSA+V arm were mapped in relation to the Sec-1 locus, 2 additional protein bands, 3 SSRs, and 13 RFLP markers. The resultant linkage map of 1RS spanned 82.4 cM with marker order and spacing showing reasonable agreement with previous maps of 1RS. Fifteen markers lie within a region of 29.7 cM next to the centromere, yet corresponded to just 36% of the overall map length. The map position of the RFLP marker probe mwg68 was 10.9 cM distal to the Sec-1 locus and 7.8 cM proximal to the powdery mildew resistance locus. The greenbug resistance gene was located 2.7 cM proximal to the Sec-1 locus.

Key words: microsatellites, SSRs, RFLP, secalin-gliadin, alien genes introgression.

Résumé : Le seigle cultivé (Secale cereale L., 2n = 2x = 14, RR) constitue une importante source de gènes de résistance aux insectes et aux maladies du blé (Triticum aestivum L., 2n = 6x = 42). Le bras chromosomique 1RS de S. cereale ‘Kavkaz’, lequel était initialement impliqué dans une translocation 1BL.1RS, porte plusieurs gènes de résistance (par exemple, Lr26, Sr31, Yr9 et Pm8), tandis que le bras 1RS de la translocation S. cereale ‘Amigo’ (1RS) porte un gène de résistance aux biotypes B et C du puceron vert des graminées (Schizaphis graminum Rondani) ainsi que d’autres gènes de résistance à des maladies. Le but de ces travaux était d’identifier des individus recombinants au sein des régions homologues 1AL.1RSV et 1AL.1RSA à l’aide de marqueurs phénotypiques et moléculaires. Secale cereale ‘Nekota’ (1AL.1RSA) et S. cereale ‘Pavon 76’ (1AL.1RSV) ont été croisés et la F1 a été rétrocroisée avec ‘Nekota’ (1AL.1AS) pour produire 80 familles BC1F2:3 (i.e., (‘Nekota’ 1AL.1RSA × ‘Pavon 76’ 1AL.1RSV) × ‘Nekota’ 1AL.1AS). Ces familles ont été génotypées en examinant les motifs des protéines de réserve (sécalines–gliadines) sur gels de polyacrylamide afin de distinguer les hétérozygotes 1AL.1AS/1AL.1RS des homozygotes 1AL.1RSA+V ou 1AL.1AS. La ségrégation du locus de la sécaline et des marqueurs PCR basés sur la famille de séquences répétitives R173 (spécifiques du seigle) a


Corresponding Editor: J.P. Gustafson.

Y.M. Mater,1 P.S. Baenziger, J.E. Specht, and I. Dweikat.2 Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583, U.S.A.
K.S. Gill. Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164, U.S.A.
R.A. Graybosch. USDA–ARS, University of Nebraska, Lincoln, NE 68583-0915, U.S.A.
L. Whitcher. USDA–ARS, North Carolina State University, Raleigh, NC 27695-7616, U.S.A.
C.A. Baker. USDA–ARS, Stillwater, OK 74075, U.S.A.

1Deceased.
2Corresponding author (e-mail: idweikat2@unl.edu).
Introduction

Rye (Secale cereale L.) is an important source of alien genes because of its considerable tolerance to biotic and abiotic stresses. It has thus been used extensively for the improvement of wheat by alien introgression methods. Rye chromosome arm 1RS, transferred to wheat via 1AL.1RS, 1BL.1RS, or 1DL.1RS wheat–rye chromosomal translocation, has been the most successfully deployed segment (Zeller and Hsam 1984; Porter et al. 1993). To date, wheat cultivars carrying rye translocations occupy more than 5 000 000 hectares of cultivated wheat (Rabinovich 1998; Villareal et al. 1998). Chromosome arm 1RS contains many genes that confer resistance to numerous insects and diseases, and may confer some yield advantages, especially under dry conditions (Zeller and Fuchs 1983; Carver and Rayburn 1994; Moreno-Sevilla et al. 1995; Villareal et al. 1998). The disease resistance and agronomic advantages associated with these translocations have led to their widespread use in breeding programs throughout the world. Indeed, several wheat cultivars with these translocations have been released (Zeller 1973; Rabinovich 1998; Mujeeb-Kazi et al. 2000). The wheat–rye translocations originally found in the wheat cultivars S. cereale ‘Amigo’ and S. cereale ‘Kavkaz’ have been used independently for many years to achieve resistance to several pathogens and insects, to broaden adaptation, and to increase yield.

The source of the ‘Kavkaz’ translocation 1BL.1RS was S. cereale ‘Petkus’ (Zeller 1973), while the source of the ‘Amigo’ translocation 1AL.1RS was S. cereale ‘Insave’ (Sebesta and Wood 1978). The ‘Kavkaz’ translocated rye arm, now designated 1RSV, carries genes for resistance to leaf rust, Puccinia recondita Rob. ex Desm. (Lr26); stem rust, Puccinia graminis Pers., (Sr31); yellow rust, Puccinia striiformis Westend. (Yr9); and powdery mildew, Blumeria graminis (DC) E.O. Speer (Pm8). The ‘Amigo’ translocated arm, now designated 1RSA, carries a single resistance gene for greenbug (Schizaphis graminus) biotypes B and C. It also carries genes conferring resistance to powdery mildew (Pm17) and stem rust (SrR). To date, no rust strains have been reported that distinguish between Sr31 and SrR resistance. Therefore, the allelic relationship of the stem rust resistance genes SrR and Sr31 is still unknown. However, previous studies showed that Pm8 and Pm17 are allelic (Hsam and Zeller 1997). The replacement of Pm8 by Pm17 in wheat–rye 1BL.1RS translocation lines is now becoming essential in most European countries, because powdery mildew resistance conferred by Pm8 has been overcome (Lutz et al. 1992).

The short arm of rye (1RS) on chromosome 1BL.1RS of ‘Kavkaz’ was translocated to the homoeologous chromosome 1A of ‘Pavon 76’ by Lukaszewski (1997). Moving the 1RS translocation from 1BL to 1AL should decrease the detrimental effects on end-use quality of having 1RS in substituting for a group one short chromosome arm (Kumlay et al. 2003).

Homologous recombination between the ‘Insave’ rye arm and the ‘Petkus’ rye arm of chromosome 1AL.1RS would be expected if ‘Pavon 76’ (1AL.1RSA) were to be mated to S. cereale ‘Nekota’ (1AL.1RSA), because both 1AL and 1RS arms should pair. The purpose of this research was to develop recombinant wheat–rye lines for 1RS between 1AL.1RSA and 1AL.1RSV to identify recombinant chromosomes using molecular and phenotypic markers, and to determine linkage relationships between genes on 1RS when present in a wheat genetic background.

Materials and methods

Plant material

‘Nekota’ 1AL.1RSA, a selection from the heterogeneous cultivar ‘Nekota’ and that carries 1RS from ‘Amigo’ translocation 1AL.1RSA was mated to ‘Pavon 76’ 1AL.1RSV, which carries 1RS from ‘Kavkaz’ translocation. This mating generated an F1 heterozygote (designated 1AL.1RSA+V), in which homologous recombination could occur between the ‘Amigo’- and ‘Kavkaz’-derived rye arms. The F1 was backcrossed to a different ‘Nekota’ 1AL.1RSA (i.e., ‘Nekota’ 1AL.1RSA × ‘Pavon 76’ 1AL.1RSV) × ‘Nekota’ 1AL.1RSV). These crosses were possible because ‘Nekota’ is a heterogeneous cultivar for 1AL.1RSA and 1AL.1RSV (Haley et al. 1996), hence ‘Nekota’ 1AL.1RSA and ‘Nekota’ 1AL.1RSV selections were isolated. ‘Nekota’ 1AL.1RSV and ‘Nekota’ 1AL.1RSV selections were agronomically similar (Espitia-Rangel et al. 1999). This backcross was designed to ensure that no further recombination occurred after the initial recombinants were generated in the 1RS chromosome arm of 1AL.1RSA+V. The BC1F1 thus had only two genotypes: those with two copies of 1AL.1RSA and those with one copy of 1AL.1RSA and one copy of 1AL.1RSA+V (Fig. 1). Eighty BC1F1 1AL.1RSA/1AL.1RSA+V genotypes were identified and selected for selfing. From the selfed progeny, homozygous genotypes of

© 2004 NRC Canada
the recombinant 1AL.1RS_{A+V} were obtained and harvested individually.

**Protein analyses**

The BC_{1}F_{2} progeny of each of the 80 selfed BC_{1}F_{1} plants was genotyped based on the secalin–gliadin banding pattern using SDS–PAGE (sodium dodecyl sulphate – polyacrylamide gel electrophoresis) according to Graybosch et al. (1999). The use of Gli-A1 and Sec-I loci as markers for the two chromosome arms 1AS and 1RS, respectively, facilitated the discrimination between the 1AL.1RS_{A+V}/1AL.1AS heterozygotes and the 1AL.1RS_{A+V} or the 1AL.1AS homozygotes.

At least nine seeds from each of the 80 selfed BC_{1}F_{1} plants were identified and planted to obtain BC_{1}F_{2} tissues for DNA extraction and to increase seeds for resistance screening. Secalin locus bands and PCR markers based on R173 family of rye specific repeated DNA sequences (Rogowsky et al. 1992) were scored to distinguish between recombinants.
(e.g., 1RS_{A+V}) and the non-recombinant 1AL. 1RS_{A+V} families.

**RFLP analyses**

Genomic DNA of the ‘Nekota’ (1AL.1RS_{A}) and ‘Pavon 76’ (1AL.1RS_{V}) parents and the 80 BC_{1}F_{2} plants was extracted from leaves of 2- to 4-week-old greenhouse-grown plants by the cetyltrimethylammonium bromide (CTAB) method (Shah et al. 1999). Total genomic DNA (10 µg) of the parents and 80 BC_{1}F_{2} plants was digested with EcoRI, HindIII, and BamHI restriction endonucleases (Promega, Madison, Wis.), then subjected to electrophoresis on 0.8% agarose gels for 18–20 h at 2.4 V/cm, before being blotted to Hybond N membrane (Amersham) following the manufacturer’s recommendations. Fifty probes specific for wheat homoeologous group 1 chromosomes were used for the gel blot DNA analysis of the two parental lines (Sandhu et al. 2001). The cDNA and gDNA clones used as probes were kindly provided by the curator of the USDA–ARS central probes repository, Albany, Calif., and by Dr. A. Graner, Federal Center for Breeding Research on Cultivated Plants, Grünbach, Germany. Genomic DNA digestion, Southern blot analyses, probe labeling and purification, hybridization, and autoradiography were performed as described by Gill et al. (1991).

**PCRs and gel electrophoresis**

A total of 15 SSR markers that are known to map to either the 1AS of wheat or 1RS of rye were screened in this study. Primers for SSR markers were synthesized based on their published sequences (Roder et al. 1998; Saal and Wricke 1999). The PCR mixtures (25 µL total volume) consisted of 10 mM Tris–HCl (pH 8.8, 25 °C), 50 mM KCl, 2.0 mM MgCl_{2}, nucleotides dATP, dTTP, dCTP, and dGTP (200 µM each), 0.2 µM primers, 30 ng template DNA, and 1.0 U Taq DNA polymerase (Promega). Amplifications were carried out in an MJ Research (Waltham, Mass.) PTC-100 thermocycler programmed for 32 cycles of 1 min at 94 °C, 1 min at 53 °C, 1 min at 72 °C, and ending with 5 min at 72 °C. The PCR products (25 µL) were gel fractionated on 12% w/v polyacrylamide using a Hoefer vertical-gel apparatus (Promega). The PCR products (25 µL) were gel fractionated on 0.8% agarose gels for 18–20 h at 2.4 V/cm, before being blotted to Hybond N membrane (Amersham) following the manufacturer’s recommendations. Fifty probes specific for wheat homoeologous group 1 chromosomes were used for the gel blot DNA analysis of the two parental lines (Sandhu et al. 2001). The cDNA and gDNA clones used as probes were kindly provided by the curator of the USDA–ARS central probes repository, Albany, Calif., and by Dr. A. Graner, Federal Center for Breeding Research on Cultivated Plants, Grünbach, Germany. Genomic DNA digestion, Southern blot analyses, probe labeling and purification, hybridization, and autoradiography were performed as described by Gill et al. (1991).

**Screening procedure**

In the BC_{1}F_{2} families from each BC_{1}F_{1} parent, at least two BC_{1}F_{2} homozygous 1AL.1RS_{A+V} plants were grown to produce BC_{1}F_{3} families. BC_{1}F_{2} seedlings of 1AL.1RS_{A+V} families and their 1AL.1AS counterparts were screened for resistance to greenbug biotype C and to powdery mildew. Plants screened for resistance to greenbug biotype C were grown in Scott’s Redi-earth Plug and Seedling Mix in black polystyrene 73-hole growing trays, where the holes represent pots of size 3.7 cm x 4.5 cm. Three seeds of each entry were planted (2.5 cm deep) per hole. The 3-seed test was repeated six times to insure unambiguous result totaling 18 per entry. Seedlings were infested on emergence (usually 5 d after planting) by laying leaves containing infested greenbug colonies between the rows of plants. Standard greenbug culture and resistance evaluation protocols were used (Starks and Burton 1977). The test was conducted in a greenhouse under ambient light conditions. Plants were evaluated immediately after the susceptible checks were given a greenbug injury score of 9.0 (i.e., dead plant), which was usually 2–3 weeks after planting. The greenbug injury scoring range was 1–3 (resistant), 4–6 (moderately resistant to moderately susceptible), and 7–9 (susceptible). An entry was classified as resistant if the 18 seedlings had a score of less than 4, or susceptible if greater than 6. Greenbug resistance was thus mappable as a quantitative trait.

Powdery mildew evaluations were carried out using the standard detached leaf method (Bennett 1981), where two 2.5-cm sections of the primary leaf of test plants are placed abaxial side down on 0.5% water agar in 9-cm diameter petri plates containing 50 ppm benimidazole to delay senescence. A simple settling tower placed over each plate was used to inoculate the leaf sections with individual isolates of Blumaria graminis (DC.) E.O. Speer f.sp. tritici Em. Marchal. The plates were then placed in a growth chamber set at 17 °C with 12 h light : 12 h dark. Each leaf section was rated 9–11 d after inoculation using a scale from 0 to 9 where 0–3 represented a resistant reaction, 4–6 was intermediate, and 7–9 was susceptible (Leath and Heun 1990).

In a preliminary powdery mildew evaluation, the lines ‘Pavon’ 1AL/1RS_{V}, ‘Pavon’ 1AL/1RS_{A}, ‘Pavon’ 1AL/1AS, ‘Nekota’ 1A/1RSV line #20, ‘Nekota’ 1AL/1AS line #21 (two different sources of ‘Nekota’), ‘Nekota’ 1AL/1RS_{A}, and the check cultivars ‘Amigo’ (PI578213), ‘Kavkaz’ (PI367723), and S. cereale ‘Chancellor’ (CI12333) (susceptible check) were evaluated (2 replications/line or cultivar) for powdery mildew reaction to 7 different isolates of B. graminis f.sp. tritici (data not shown). Isolate No. 156b1 produced consistent resistant reactions on ‘Amigo’ and consistent susceptible reactions on ‘Kavkaz’. To estimate the level of heterogeneity in the 80 paired progeny (160 populations total) of the 1AL.1RS and 1AL.1AS populations, 20 plants from each of five 1AL.1RS populations and 20 plants from each of three 1AL.1AS populations were evaluated. These results found greater heterogeneity in the 1AL.1RS populations than in the 1AL.1AS populations. As a result, 6 plants (each plant replicated twice) from each of the 80 1AL.1RS and 2 plants (each replicated twice) from each of the 80 1AL.1AS populations were evaluated for mildew reaction along with the checks ‘Amigo’, ‘Kavkaz’, and ‘Chancellor’. A progeny was classified as resistant if its mean plant rating was less than 4, or as susceptible if greater than 6.

**Data analysis**

The linkages among the protein, SSR, and RFLP markers were analyzed using data obtained from BC_{1}F_{2} populations, and with the computer software program “MapManager” (Manly 1993). A logarithm of odds (LOD) score of 4.0 was used as a linkage threshold to construct the linkage map, with maximum recombination threshold set to fraction as
3.0. The Kosambi transformation (Kosambi 1944) was used to translate recombination frequencies into centimorgans (cM).

Results and discussion

A total of 783 seeds from the 80 BC1F1:2 families were evaluated with respect to their SDS–PAGE phenotypes (Table 1). The non-significant \( \chi^2 \) indicated a reasonable fit of the observed ratio to the expected 1:2:1. Several techniques are now used to genotype the 1RS.1AL/1A.1AS heterozygotes, such as C-banding (Ter-Kuile et al. 1990), A–PAGE (acid polyacrylamide gel electrophoresis) (William et al. 1992) and SDS–PAGE (Koebner and Shepherd 1986). We found the SDS–PAGE procedure to be a rapid and convenient method for genotyping the 1RS\(_A\)/1AL.1AL.1AS heterozygotes.

The rye-specific PCR marker R173-3, previously mapped by Rogowsky et al. (1992) on 1RS, produced an amplicon polymorphism between 'Nekota' 1AL.1RSA and 'Pavon 76' 1AL.1RSV. The two bands of different mobility associated with 1RSV and 1RSA were identified and scored in BC1F2 families. The same primer pair (PawS5 and PawS6 targeting the border of R173-3) was used by Rogowsky et al. (1992) to amplify two polymorphic bands between 1RS of *S. cereale* 'King II' and 1RS of 'Petkus'. These two markers were mapped as Paw 173-3I and Paw 173-3P 2.3 m.u. from xcd 580 (Fig. 2).

Endosperm storage protein analysis showed that, of the 80 BC1F2 homozygous 1RSA+V.1AL families, 47 had the secalin bands associated with the 'Kavkaz' translocation (three extra faster-moving secalin bands), whereas 33 had the secalin band (single band) associated with the 'Amigo' translocation (Fig. 3). A possible explanation for the failure of additional secalins to be expressed by the 'Amigo' translocation is that a different source of rye ('Insave' vs. 'Petkus') contributed the 1RS segments (Berzonsky et al. 1991). Two bands proposed to be related to the Sec-1 locus were scored and mapped as two different protein markers. One band was associated with 1RS\(_A\) and was designated Sec-1I, while the other was associated with 1RS\(_V\) and was designated as Sec-1P (Fig. 2). Genotyping our population with the R173–3 and Sec-1 markers resulted in the first evidence that homologous recombination had occurred between the two rye arms, and apparently with ample frequency to screen for disease resistance loci and investigate for the allelic relationship of genes located on 1RS of 'Insave' and 1RS of 'Petkus'.

Of the 50 probes used to screen for RFLPs between parental varieties, 19 detected polymorphism with at least one of the five restriction enzymes employed. We were able to position 13 RFLPs on the map using a LOD 5.0 criterion (Fig. 2). The RFLP marker *psr381* remained unlinked possibly because of the high segregation distortion. Of 15 PCR-based markers, 6 were linked and grouped to the other markers at the same LOD criterion. Only one SSR marker, Barc176, remained unlinked at the minimum LOD score 3.0 used to construct our map.

Of the total of 26 phenotypic, protein, and DNA markers, 23 were grouped into one linkage group with certainty (LOD score 5.0). Ordering the 23 markers was accomplished by MapManager. The map span of the recombinable 1RS segment was 82.4 cM. Clustering of loci in a small centromeric region of the recombinant 1RS was very obvious. The order of the markers generated from MapManager followed the same order on the physical map of the corresponding chromosome arm in wheat except for the RFLP markers *xcd 580*, *xmwg 2056*, *xcd 442*, and *xcd 1188*, which were placed in the predicted order derived from a collection of previous linkage and physical maps (Sandhu and Gill 2002).

The RFLP marker *xmwg 68*, recently mapped proximal to the stem rust region on 1RS (Mago et al. 2002), and previously mapped along with *xmwg 60* to flank the complex *Mla* powdery mildew resistance locus in the corresponding re-
region on 1H (Wei et al. 1999), was mapped as being 10.9 cM distal to the Sec-1 locus and 7.8 cM proximal to the Pm locus. Hsam et al. (2000) mapped Pm17 6.1 cM proximal to the rust resistance gene Lr26. Hence, if the rust genes (Sr33, Lr26, and Yr8) of ‘Petkus’ rye were mapped ~5 cM distal to the Sec-1 locus (Singh et al. 1990). However, in a recent study by Hsam et al. (2000), Lr26 was mapped 9.1 cM distal to the Sec-1 locus, which would be in agreement with our map.

The population was screened for the greenbug resistance gene located on 1RS from ‘Amigo’. The designation Gb2 was assigned by Tyler et al. (1987) for this gene. Biotype C was used to genotype families into 36 resistant and 44 susceptible. In the present study, Gb2 was mapped proximal to the Sec-1 locus (2.7 cM). These molecular markers should serve as a diagnostic tool to speed up the selection process for resistance and improving quality characteristics.

Despite the numerous advantageous effects of having the rye chromosome arm 1RS present in the wheat genome, deleterious influence on wheat end-use quality have been reported in hard wheat (Zeller and Hsam 1984; Dhaliwal et al. 1987; Graybosch et al. 1993; Fenn et al. 1994; and Seo et al. 1995) and to a lesser extent on soft wheat (McKendry et al. 1996). These deleterious effects are mostly associated with 1BL.1RS, where as such effects are attenuated in 1AL.1RS (Graybosch et al. 1993; Kumlay et al. 2003). The agronomic evaluations of 1AL.1RSV+A have been initiated to determine if the creation of new recombinant 1AL.1RS lines may allow recombinatory improvement of both agronomic and the end-use quality effect of IRS in wheat.

References


© 2004 NRC Canada