Chromosomal Locations of Genes for Stem Rust Resistance in Monogenic Lines Derived from Tetraploid Wheat Accession ST464

Daryl L. Klindworth,* James D. Miller, Yue Jin, and Steven S. Xu

ABSTRACT

The genetics of resistance to stem rust (caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. and Henn.) in durum (*Triticum turgidum* L. ssp. *durum*) is not as well understood as for bread wheat *(T. aestivum* L.). Our objective was to determine the chromosomal location of genes for stem rust resistance in four monogenic lines derived from the Ethiopian tetraploid landrace ST464. The four monogenic lines were crossed to a set of stem rust susceptible aneuploids based on the tetraploid line 47-1. We observed chromosome pairing in the hybrids and made testcrosses to 'Rusty' durum. Monogenic lines ST464-A1 and ST464-A2 were observed to carry a 2A/4B translocation, and subsequent crosses proved that the translocation was derived from ST464. Testcross F_2 seedlings were inoculated with one of three stem rust pathotypes and classified for segregation for resistance to identify the critical chromosome for each monogenic line. The stem rust resistance genes in monogenic lines ST464-A1, ST464-A2, and ST464-C1 were located to chromosomes 6A, 2B, and 6A, respectively. The gene in ST464-B1 may be located to chromosome 4A, because it appeared it was not located on any of the other 13 chromosomes. The four ST464 monogenic lines and hexaploid lines carrying *Sr9e* and *Sr13* were then tested with eight stem rust pathotypes with the objective of postulating the genes present in the monogenic lines. The genes in ST464-A2 and ST464-C1 were postulated to be *Sr9e* and *Sr13*, respectively.

D.L. Klindworth, J.D. Miller (retired), and S.S. Xu, USDA-ARS Northern Crop Science Lab., P.O. Box 5677, State Univ. Stn., Fargo, ND 58105; Y. Jin, USDA-ARS Cereal Disease Lab., Univ. of Minnesota, St. Paul, 55108. Received 25 May 2006. *Corresponding author (Daryl.Klindworth@ars.usda.gov).

Abbreviations: IT, infection type; MI, metaphase I.

The genetics, including chromosomal location and allelic identity, of stem rust (caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. and Henn.) resistance genes is fairly well understood for bread wheat (*Triticum aestivum* L.). However, compared to bread wheat, the genetics of stem rust resistance in durum wheat (*T. turgidum* L. ssp. *durum*) is not as well understood. The major reason for this has been the lack of a stem rust susceptible set of aneuploids in tetraploid wheat that would be suitable for either traditional aneuploid analysis or for developing chromosome substitution lines that could be used in molecular marker studies. Use of the 'Langdon' disomic substitutions for stem rust studies is limited because Langdon, and hence the disomic substitutions, has at least three genes (N.D. Williams, unpublished data, 1998) conferring resistance to stem rust. We have developed a set of stem rust susceptible aneuploids in durum wheat which could be used directly in aneuploid analysis or to develop additional genetic stocks that could be used for molecular marker studies. These aneuploids are based on a stem rust susceptible genetic stock, designated Line 47-1, and obtained from L.R. Joppa, USDA-ARS, retired.

In response to the stem rust epidemics of North America in the 1950s, researchers incorporated resistance genes in bread wheat and durum wheat from a number of sources. In durum,

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677 S. Segoe Rd., Madison, WI 53711 USA

two important sources of resistance were Khapli emmer (*T. turgidum* L. ssp. *dicoccum*) (CItr 4013) and ST464 (PI 191365), a durum landrace from Ethiopia. In North Dakota, Khapli was the source of stem rust resistance in Langdon (CItr 13165) (Heermann and Stoa, 1956) and 'Wells' (CItr 13333) (Heyne, 1962); and, ST464 was the source of genes in 'Leeds' (CItr 13768) (Lebsock et al., 1967). Two or more of these cultivars, or lines derived from them, appear in the parentage of all North Dakota durums released since 1978. Therefore, understanding the genetics of stem rust resistance in Khapli and ST464 provides an insight into the genetics of stem rust resistance of current durum cultivars.

The genetics of stem rust resistance in Khapli has been more thoroughly studied than that of ST464. The resistance in Khapli was transferred to Khapstein (PI 245108), which was found to carry *Sr7a*, *Sr13*, and *Sr14* (Knott, 1962), although McIntosh et al. (1995, p. 104–105) stated that Khapli was not the source of *Sr7a* in Khapstein. All three of these genes have been mapped to specific chromosomes. Depending on the pathotypes used, the number of genes reported in ST464 varies from two (Kenaschuk et al., 1959) to three (Ataullah, 1963). The International Virulence Gene Survey (Luig, 1983), in which the stem rust resistance genes were postulated by reaction of the genotypes to a broad range of pathotypes, indicated that Leeds carries *Sr9e* and *Sr13*, and hence ST464 should carry these two genes. In contrast to the conclusions of Luig (1983), Knott (1996) conducted a monosomic analysis of hexaploid derivatives of ST464 and concluded that ST464 had *Sr7a* in chromosome 4A and a second gene whose location was undetermined. While the studies cited above indicate a specific number of genes in ST464 and Khapli, each carries additional stem rust resistance genes. Watson and Stewart (1956) found that Khapstein does not possess all of the resistance genes from Khapli. Williams and Gough (1965) found evidence for a fourth gene in Khapli and cited evidence for a fifth.

Use of monogenic lines for analysis of stem rust resistance was proposed by Knott (1966), who outlined five advantages for their use in studies of genetics or host–parasite interactions. Williams and Miller (1982) used monogenic lines developed from four tetraploid wheats to determine allelic relationships of their stem rust resistance genes. Among the lines developed by N.D. Williams are four monogenic stem rust resistant lines derived by crossing Marruecos 9623 (PI 192334) with ST464 (Williams and Gough, 1968). Marruecos 9623 is known to carry only a single thermosensitive gene, temporarily designated *SrM* (USDA-ARS Cereal Disease Laboratory, 2004; Klindworth et al., 2006). When tested with a broad set of stem rust pathotypes, the virulence–avirulence pattern and the infection types of the monogenic lines do not match those of *SrM*; therefore, the single genes in these lines must be derived from ST464 rather than Marruecos 9623. Use of these lines in studies of host–pathogen interactions would be enhanced if the gene present in each line were identified. Also, identification of the genes in the lines would provide additional information on the genes present in ST464. The objective of this study was to determine the chromosomal location of the stem rust resistance genes in the four ST464 monogenic lines using the aneuploid durum stocks of Line 47-1. Based on those results, we conducted virulence tests using eight stem rust pathotypes to partially postulate the identity of the genes in the ST464 monogenic lines. We also found that two of the ST464 lines had reciprocal translocations, and additional crosses were made with the objective of determining whether ST464 was the source of the translocations.

MATERIALS AND METHODS

Selection of Stem Rust Susceptible Aneuploids

We produced a set of stem rust susceptible aneuploids of durum wheat by backcrossing the Langdon D-genome disomic substitutions to Line 47-1. However, we had selected disomic substitutions for only six of the possible 14 aneuploid stocks. Those stocks were 1D(1A), 7D(7A), 1D(1B), 2D(2B), 3D(3B), and 5D(5B). These stocks had $13^{II} + 1^{II}_{D}$ at metaphase I (MI) of meiosis, except that 3D(3B) and 5D(5B) were maintained as $13^{II} + 1^{II}_{D} + 1^{I}_{B}$, which is identical to how the Langdon D-genome disomic substitutions are maintained for those two chromosomes (Joppa and Williams, 1988). The remaining stocks were maintained as double-monosomics ($13^{II} + 1^{I}_{A\text{ or }B} + 1^{I}_{D}$). For the six stocks that had been converted to disomic substitutions, the identity of the missing chromosomes was confirmed by crossing to the appropriate double ditelocentric line and observing pairing in the hybrids. For the eight stocks maintained as double monosomics, the identity of the monosomes was assumed to be correct based on the parentage of each stock. Each aneuploid stock was tested for reaction to stem rust pathotypes (isolate) *Pgt-JCMN* (gb121), *Pgt-TPMK* (TNMK-sp1), and *Pgt-LBBL* (r111) using the procedures described below. For all aneuploid stocks, susceptible infection types of 3, 33^{+}, or 34 were observed for all three pathotypes.

Inoculation Procedures

Seedlings were inoculated using the techniques of Williams et al. (1992). Briefly, urediniospores were suspended in nonphytotoxic, parafinic oil and sprayed on 6- to 8-d-old seedlings. The plants remained in a subdued light mist chamber for 24 h following inoculation. Seedlings were then moved to a greenhouse at 20 to 23°C with supplemental fluorescent light to maintain a 14/10-h (day/night) photoperiod. Seedlings were classified for stem rust infection type (IT) 12 to 14 d postinoculation by scoring the infected primary leaf from each plant (Stakman et al., 1962; Roelfs and Martens, 1988).

Cytogenetic Techniques and Aneuploid Analysis

To prepare for crossing, those aneuploid stocks that were double-monosomic ($2n - 1 + 1 = 28$) were screened for chromosome

pairing configurations. An immature spike from each plant was fixed in Carnoy's solution (95% ethanol/chloroform/glacial acetic acid in a 6:3:1 ratio) for 24 to 48 h, and anthers were excised and squashed in an acetocarmine solution to microscopically observe chromosome pairing at MI of meiosis (Smith, 1947). Only aneuploid plants having $13^{II} + 2^{I}$ at MI were used for crossing. The four monogenic lines derived from ST464 that were used in this study were identified as ST464-A1, ST464-A2, ST464-B1, and ST464-C1. To initiate aneuploid analyses of the genes, each monogenic line of ST464 was crossed to the susceptible aneuploid stocks of Line 47-1. For those crosses to double-monosomics, the procedure was identical to using the disomic substitutions with the exception that, as suggested by Joppa and Williams (1977, 1988), the F_1 plants were screened for chromosome pairing at MI of meiosis. The F_1 plants had either 14^{II} (euploid), $13^{II} + 1^{I}$ (monosomic), $13^{II} + 2^{I}$ (double-monosomic), or $14^{II} + 1^{I}$ (monosomic-addition), and all plants that were not double-monosomic or monosomic were discarded.

After selecting double-monosomic or monosomic F_1 plants, testcrosses of the selected plants were made to the stem rust susceptible genotype, 'Rusty' (PI 639869) (Klindworth et al., 2006). Because there is preferential transmission of the A- or B-genome chromosome through the pollen of double-monosomic plants (Joppa and Williams, 1988), our objective was to make all crosses using Rusty as the female, as this would result in a higher frequency of euploid plants. However, poor pollen shedding in some double-monosomic plants forced us to use Rusty as the male for some testcrosses, and these testcrosses are identified in the results.

The F_1 seedlings from testcrosses were inoculated with an appropriate pathotype (*Pgt-TPMK* on ST464-A1 and ST464-C1, *Pgt-LBBL* on ST464-B1, and *Pgt-HKHJ* on ST464-A2) of stem rust using the procedures described previously. The testcross F_1 plants were grown in the greenhouse and chromosome pairing at MI was determined for each plant. A population of 25 to 30 F_2 seedlings (a testcross F_1 family) was tested with the appropriate stem rust pathotype for each testcross F_1 plant that had been grown. Segregation among the families and within a cross was tested for goodness of fit to monogenic ratios using the chi-square test.

Reaction of Monogenic Lines to Eight Stem Rust Pathotypes

After determining the chromosomal location of the genes in the monogenic ST464 lines, the ST464 lines were tested for reaction to a group of eight diverse pathotypes of the stem rust pathogen. Pathotypes (isolates) used were *Pgt-TPMK* (TNMK-sp1), -*TMLK* (72-41 sp2), -*JCMN* (gb121), -*RTQQ* (72.00), -*HKHJ* (R29M), -*MCCF* (A-5), -*QCCJ* (QCC-2), and -*TTTT* (01MN81A). Reactions of the monogenic lines to these pathotypes were compared to checks which included ST464, Rusty, and hexaploid monogenic lines Vernstein (PI 442914), which carries *Sr9e*, and Line S (PI 442913), which carries *Sr13*.

Identification of the Source of the Translocation

After observing chromosome-pairing configurations in the hybrids and observing that a reciprocal translocation was present, we made additional crosses with Langdon, ST464, Line 47-1, Leeds, and selected Langdon D-genome disomic substitutions. Unfortunately, the line of ST464 from which ST464-A1 and ST464-A2 were derived was not maintained at Fargo. ST464 was obtained from the National Small Grains collection at Aberdeen, ID. When grown, we observed that the ST464 population segregated for plants having white or black chaff. We made initial crosses with plants of both types of chaff color. Based on the chromosome pairing observed in the F_1 plants, we made additional crosses only with the progeny of a plant that had black chaff and observed chromosome pairing in F_1 plants.

RESULTS

Although our main objective was to determine the chromosomal location of the stem rust resistance genes in the monogenic lines of ST464, a translocation was determined to be present in two of the ST464 monogenic lines, and this resulted in different pairing configurations being

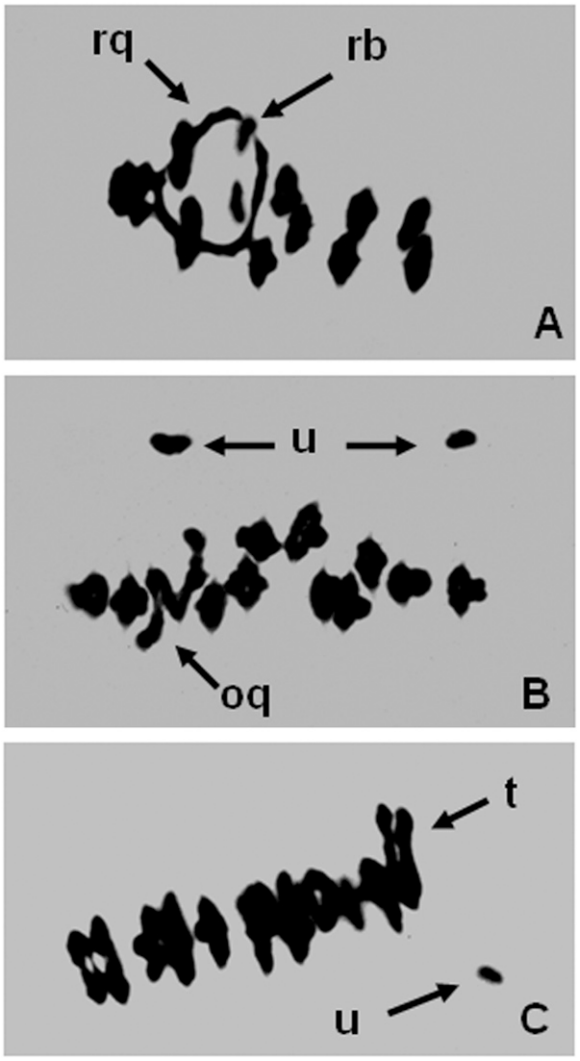

Figure 1. Chromosome pairing in three hybrids having ST464-A1 or ST464-A2 in their parentage. Pairing configurations and pedigrees are: A = $1^{IV} + 12^{II}$ in a Rusty//1D(1A)/ST464-A2 hybrid; B = $1^{IV} + 11^{II} + 2^{I}$ in a Langdon 4D(4A)/ST464-A1 hybrid; and C = $1^{III} + 12^{II} + 1^{I}$ in a Line 47-1 4D(4B)/ST464-A2 hybrid. Legend: rq, ring quadrivalent; rb, rod bivalent; u, univalent; oq, open quadrivalent; t, trivalent.

observed in the critical crosses for the translocation. In explaining the stem rust segregation data, it is helpful to clearly show the reason for our choice of F_1 plants which were used as parents in making testcrosses. In addition, in determining the chromosomes involved in the translocation, the chromosome pairing data indicated that the 4D(4A) double-monosomic was incorrectly identified, and it is helpful to present these data before the stem rust segregation data to explain our treatment of the data.

Identification of the Translocation

Our objective had been to produce double-monosomic F_1 plants to use for making testcrosses, and we achieved this objective in all but three crosses involving the double-monosomic 6D(6B) aneuploid with ST464-A1, ST464-A2, and ST464-C1. In the fifty-three crosses in which we observed chromosome pairing configurations in either double-monosomic or monosomic F_1 plants, all F_1 hybrids of crosses to ST464-B1 and ST464-C1 had $13^{II} + 2^{I}$ at MI. This indicated that these two lines had a standard chromosome arrangement relative to Line 47-1. However, quadrivalents or trivalents were observed in crosses of ST464-A1 and ST464-A2 with the Line 47-1 aneuploids (Fig. 1A). This indicated that a reciprocal translocation was present in these two lines. Maximum chromosome pairing configurations of $I^{IV} + 11^{II} + 2^{I}$ (Fig. 1B) were observed in double-monosomic plants for 10 of the 14 possible cross combinations (Table 1). In crosses involving aneuploids of 2D(2A), 4D(4A), and 4D(4B), double-monosomic plants did not occur; but, instead plants having $1^{III} + 12^{II} + 1^{I}$ were observed (Fig. 1C). Since trivalents should only have been observed in the two critical crosses; one of the three aneuploids stocks, 2D(2A), 4D(4A), or 4D(4B), must have been incorrectly identified.

To resolve the chromosomes involved in the translocation and to identify which aneuploid stock was incorrectly identified, we crossed ST464-A1 and ST464-A2 to the five Langdon disomic substitutions and observed chromosome pairing in the hybrids (Table 2). A quadrivalent was observed in crosses to 4D(4A), 2D(2B), and 6D(6B), indicating that these chromosomes were not involved in the translocation. A trivalent and a univalent were again observed in the 2D(2A) and 4D(4B) crosses. Therefore, the chromosomes involved in the translocation were 2A and 4B, and the aneuploid stock of Line 47-1 that was incorrectly identified was 4D(4A).

To confirm that ST464 was the source of the translocation, we crossed ST464 to Langdon, Langdon aneuploids, and other genotypes of interest. We grew 15 plants of ST464 and observed that ST464 was heterogeneous for chaff color and plant height. Crosses were made with six white- and two black-chaffed plants of ST464. When we grew the F_1 plants, we observed quadrivalents in both crosses involving a black-chaffed ST464 parental plant. In the crosses involving the white-chaffed parental plants, no quadrivalents were observed in crosses involving five of the white-chaffed ST464 parental plants, and heterogeneity for the presence of the quadrivalent was observed in the case of the final white-chaffed ST464 parental plant. We concluded that ST464 was heterogeneous for a translocated chromosome as well as chaff color and plant height. While both black-chaffed plants of ST464 that were tested were determined to carry a translocation, the number of plants tested was too small to conclude that all black-chaffed plants of ST464 carry the translocation. The progeny of one of the black-chaffed plants shown to carry a translocation was used to make all of the additional crosses for tests of chromosome pairing.

Results from the crosses involving a black-chaffed ST464 parental plant are shown in Tables 2 and 3. The crosses of black-chaffed ST464 with the Langdon aneuploids indicated that the chromosomes involved in the translocation were 2A and 4B. The crosses ST464-A2/ST464 and ST464-A2/ST464-A1 did not exhibit a quadrivalent, and this indicated

Table 1. Classification of hybrids of Line 47-1 aneuploids to ST464-A1 and ST464-A2 for maximum chromosome pairing at metaphase I (MI) of meiosis.

Line 47-1 aneuploid	No. of F_1 plants		F_1 pairing configurations†
	ST464-A1	ST464-A2	
1D(1A)	4	4	Quad.
2D(2A)	2	7	Triv.
3D(3A)	3	1	Quad.
4D(4A)	9	5	Triv.
5D(5A)	4	3	Quad.
6D(6A)	9	6	Quad.
7D(7A)	4	2	Quad.
1D(1B)	4	3	Quad.
2D(2B)	3	4	Quad.
3D(3B)	2	5	Quad.
4D(4B)	3	4	Triv.
5D(5B)	5	5	Quad.
6D(6B)	0	0	missing
7D(7B)	3	3	Quad.

†Quad. indicates that the double-monosomic plants had $I^{IV} + 11^{II} + 2^{I}$ at MI of meiosis or that monosomic plants had $I^{IV} + 11^{II} + 1^{I}$ at MI. Triv. indicates that double-monosomic plants did not occur, but were replaced by a class of plants having $1^{III} + 12^{II} + 1^{I}$ or plants having $1^{III} + 12^{II}$ at MI of meiosis.

Table 2. Classification of hybrids of Langdon aneuploids to ST464-A1, ST464-A2, and ST464 for maximum chromosome pairing at metaphase I of meiosis.†

Langdon aneuploid	Pairing
2D(2A)	$1^{III} + 12^{II} + 1^{I}$
2D(2B)	$I^{IV} + 11^{II} + 2^{I}$
4D(4A)	$I^{IV} + 11^{II} + 2^{I}$
4D(4B)	$1^{III} + 12^{II} + 1^{I}$
6D(6B)	$I^{IV} + 11^{II} + 2^{I}$

†The three ST464 lines were crossed to each of the five aneuploids except that the LDN 4D(4B)/ST464-A2 cross was not made. For ST464, a black-chaffed plant was used as the parent for all crosses. Three hybrid plants were analyzed for each cross.

that these three durums carried the same 2A/4B translocation. Therefore, the results confirmed that ST464 was the source of the translocation that was carried in ST464-A1 and ST464-A2. Two crosses involving Leeds, a cultivar deriving its resistance to stem rust from ST464, are also shown in Table 3. The results of these crosses indicate that Leeds did not carry the translocation from ST464.

Table 3. Maximum chromosome pairing observed in F_1 hybrids having parents used in the study of rust resistance in ST464 durum wheat.

Cross	Chromosome pairing
Langdon/ST464†	$I^{IV} + 12^{II}$
Leeds/ST464†	$I^{IV} + 12^{II}$
Langdon/Leeds	14^{II}
Langdon/Line 47-1	14^{II}
ST464-A2/ST464†	14^{II}
ST464-A2/ST464-A1	14^{II}

†A black-chaffed plant of ST464 that was known to carry the 2A/4B translocation was used as the parent for crosses.

Location of Resistance Genes

In making testcrosses, our objective was to use double monosomic or monosomic F_1 plants for crossing. But, because of the 2A/4B translocation located in ST464-A1 and ST464-A2, the six populations of F_1 plants that tested chromosome 2A, 4A, and 4B did not have double monosomic plants, but instead had plants having either $1^{III} + 12^{II} + 1^{I}_{D}$ ($2n = 28$) or $1^{III} + 12^{II}$ ($2n = 27$), and these plants were used for crossing. For both of these pairing configurations, the trivalent was composed of a single nontranslocated chromosome derived from the Line 47-1 aneuploid, and two translocated chromosomes derived from ST464-A1 or ST464-A2.

For aneuploid analysis of tetraploid wheat, a testcross has advantages over analysis of an F_2 population. In an F_2 population, some of the plants will be disomic substitutions, and in some instances, depending on transmission frequencies and the morphological similarity of euploid and disomic substitution plants, this will interfere with identification of the critical chromosome. Also, the D-genome chromosome may carry an inhibitor of the trait being studied. By making a testcross to a euploid genotype and examining chromosome pairing, all testcross plants having 14^{II} at MI will be known to be euploid, thereby eliminating any effects of D-genome chromosomes in the analysis. Also, by making a testcross, the total number of plants analyzed can be reduced.

The segregation observed in the testcrosses should fit a 1:1 ratio in all noncritical crosses. In the F_1 plants of the critical crosses, the resistance gene will be located on the A- or B-genome monosome. Therefore, in the critical cross, all euploid testcross plants will carry the gene and all F_1 families derived from euploid testcross plants will segregate for resistance. At the same time, in the critical cross, none of the double monosomic plants will carry the resistance gene, and all F_1 families of double-monosomic testcross plants will be homozygous susceptible.

ST464-A1

For ST464-A1, with the exception of the crosses to 6D(6B), all testcrosses were made using Rusty as the female parent. There were six F_1 plants established in the case of the 6D(6B) cross, however, none of them were double monosomics and no testcross F_1 plants were produced to test chromosome 6B. Double-monosomic F_1 plants were used to produce testcross F_1 plants for all crosses except 2A and 6A, where monosomic plants had to be used. The consequence of using monosomic plants for crosses was that double-monosomic testcross F_1 plants did not occur in either the 2A or 6A populations (Table 4).

When the testcross F_1 plants and F_1 families were tested with *Pgt-TPMK*, the observed segregation ratios and ITs indicated that the gene in ST464-A1 was incompletely dominant with ITs in heterozygous testcross F_1 plants ranging from $11^{-}0$;3c (resistant) to 34 (susceptible). Data from all populations except 2A, 4A, 6A, and 4B were pooled, and the chi-square test indicated that the pooled segregation of 38:54:40:49 fit a 1:1:1:1 segregation ratio ($P = 0.287$), confirming that the translocation breakpoint was not linked to the stem rust resistance gene (Table 4). Because there was no linkage of the stem rust resistance gene and the translocation breakpoint, we pooled the 14^{II} and $1^{IV} + 12^{II}$ classes for chi-square tests to a 1:1 ratio. For example, chromosome 1A was tested to a pooled segregation of 13 homozygous susceptible to 7 segregating families. All populations fit a 1:1 segregation ratio except the 6A population, where all 16 families segregated for stem rust resistance, indicating that the stem rust resistance gene in ST464-A1 was located in chromosome 6A.

ST464-A2

In the case of ST464-A2, all testcrosses were made using Rusty as the female parent except for crosses to 5D(5A) and 6D(6B). For the 6B testcross, two F_1 plants were established, but both had $1^{IV} + 12^{II}$ and testcrosses for chromosome 6B could not be made. For chromosome 5A, the F_1 plants did not produce enough pollen for crossing, so late tillers were crossed as female to Rusty to produce seed. Double-monosomic F_1 plants were used for making all testcrosses.

We compared testcross F_1 plants and F_1 families and observed that heterozygous testcross F_1 plants had ITs to *Pgt-HPHJ* ranging from 1 to 12, indicating that the stem rust resistance gene in ST464-A2 was dominant. To determine the chromosome carrying the gene, the 14^{II} and $1^{iv} + 12^{II}$ classes were pooled for chi-square tests as had been done previously. All populations fit a 1:1 segregation ratio except the 2B population, where all 28 families segregated for stem rust resistance, indicating that the stem

Table 4. Segregation for chromosome pairing configuration at metaphase I (MI) of meiosis in BC_1F_1 plants having the generalized pedigree Rusty//Line 47-1 aneuploid/ST464-A1, and stem rust reaction of the BC_1F_1 families to *Pgt-TPMK*.

Chromosome Tested	BC_1F_1 pairing configuration and classification of the BC_1F_1 families for stem rust reaction†										
	14^{II}		$1^{IV} + 12^{II}$		Prob. (>χ^2)‡	$13^{II} + 2^{I}$		$1^{IV} + 11^{II} + 2^{I}$		Other§	
	HS	Seg	HS	Seg	Test ratio 1:1	HS	Seg	HS	Seg	HS	Seg
1A	4	4	9	3	0.180	3	1	2	2	1	1
2A	0	0	11	12	0.835	0	0	0	0	0	0
3A	5	7	1	7	0.074	4	1	2	0	3	0
5A	5	8	3	5	0.275	1	1	0	0	2	1
6A	0	8	0	8	<0.001	0	0	0	0	0	1
7A	1	6	4	1	0.564	3	2	5	2	0	0
1B	4	3	5	9	0.513	1	2	0	3	0	0
2B	8	4	6	6	0.414	0	0	0	0	0	0
3B	4	4	3	4	0.796	3	4	3	2	0	2
4B	0	0	13	10	0.532	9	2	0	0	0	1
4A¶	0	0	16	16	1.000	4	6	0	0	1	1
5B	4	8	4	9	0.072	0	0	1	0	1	0
6B	0	0	0	0	–	0	0	0	0	0	0
7B	3	10	5	5	0.144	0	1	2	0	0	1
Total#	38	54	40	49	0.063	15	12	15	9	–	–

†HS, homozygous susceptible; Seg, segregating 3 resistant/1 susceptible.

‡Probabilities for chi-square (χ^2) goodness of fit tests to a 1:1 ratio were determined after pooling the 14^{II} and the $1^{IV} + 12^{II}$ classes. Therefore, for chromosome 1A, the pooled segregation of 13 homozygous susceptible/7 segregating was tested to a 1:1 ratio.

§Chromosome pairing configurations at MI of meiosis included six plants having $14^{II} + 1^{I}$, four plants with $1^{IV} + 12^{II} + 1^{I}$, and six plants having heteromorphic bivalents or unpaired telosomes.

¶The 4A population was shown to be incorrect and is a 4B population.

#Excludes plants from crosses testing chromosomes 2A, 4A, 6A, and 4B.

Table 5. Segregation for chromosome pairing configuration at metaphase I (MI) of meiosis in BC_1F_1 plants having the generalized pedigree Rusty//Line 47-1 aneuploid/ST464-A2, and stem rust reaction of the BC_1F_1 families to *Pgt-HPHJ*.

Chromosome Tested	BC_1F_1 pairing configuration and classification of the BC_1F_1 families for stem rust reaction†										
	14^{II}		$1^{IV} + 12^{II}$		Prob. (>χ^2)‡	$13^{II} + 2^{I}$		$1^{IV} + 11^{II} + 2^{I}$		Other§	
	HS	Seg	HS	Seg	Test ratio 1:1	HS	Seg	HS	Seg	HS	Seg
1A	4	10	3	6	0.061	0	1	2	0	0	1
2A	0	0	10	12	0.670	0	0	0	0	1	3
3A	2	1	2	0	0.157¶	1	0	1	0	0	0
5A	2	2	2	0	0.361¶	0	0	0	0	0	1
6A	2	5	6	3	0.796	1	0	0	0	0	0
7A	4	3	5	3	0.439	0	2	0	4	0	0
1B	3	5	2	3	0.405	0	0	0	0	0	0
2B	0	17	0	11	<0.001	0	0	1	0	0	0
3B	3	5	1	1	0.527	1	0	0	0	0	0
4B	0	0	5	13	0.059	5	3	0	0	0	0
4A#	0	0	4	13	0.029	3	5	0	0	0	0
5B	4	8	4	13	0.016	0	0	0	0	1	0
6B	0	0	0	0	–	0	0	0	0	0	0
7B	3	2	3	3	0.763	0	0	0	0	1	0
Total††	27	41	28	32		3	3	3	4		

†HS, homozygous susceptible; Seg, segregating 3 resistant/1 susceptible.

‡Probabilities for chi-square (χ^2) goodness of fit tests to a 1:1 ratio were determined after pooling the 14^{II} and the $1^{IV} + 12^{II}$ classes. Therefore, for chromosome 1A, the pooled segregation of 7 homozygous susceptible/16 segregating was tested to a 1:1 ratio.

§All eight plants in this class had chromosome pairing configurations of $1^{IV} + 12^{II} + 1^{I}$ at MI of meiosis.

¶Chi-square values were corrected for continuity.

#The 4A population was shown to be incorrect and is a 4B population.

††Excludes plants from crosses testing chromosomes 2A, 4A, 2B, and 4B.

Table 6. Segregation for chromosome pairing configuration at metaphase I of meiosis in BC_1F_1 plants having the generalized pedigree Rusty//Line 47-1 aneuploid/ST464-B1, and stem rust reaction of the BC_1F_1 families to *Pgt-LBBL*.

Chromosome Tested	BC_1F_1 pairing configuration and classification of the BC_1F_1 families for stem rust reaction†						
	14^{II}		Prob. (> χ^2)‡	$13^{II} + 2^{I}$		Other§	
	HS	Seg	Test ratio 1:1	HS	Seg	HS	Seg
1A	4	4	0.724§	1	0	0	0
2A	2	7	0.089§	2	3	0	0
3A	2	4	0.361§	1	0	0	1
5A	11	11	1.000	0	0	0	0
6A	9	8	0.808	1	1	0	1
7A	11	8	0.491	2	6	0	0
1B	7	10	0.467	3	2	0	0
2B	9	11	0.655	0	1	3	1
3B	6	8	0.593	3	0	0	0
4B	11	7	0.346	3	11	0	0
4A¶	5	6	0.763	5	8	0	2
5B	10	6	0.317	0	0	0	0
6B#	13	9	0.394	–	–	1	2
7B	11	12	0.835	0	4	1	0
Total	111	111	1.000	21	36		

†HS, homozygous susceptible; Seg, segregating 1 resistant/3 susceptible.

‡Includes 11 plants having $14^{II} + 1^{I}$ and one plant having $13^{II} + 1^{I}$.

§Chi-square values were corrected for continuity.

¶The 4A population was shown to be incorrect and is a 4B population.

#To avoid sterility problems, the pedigree of the 6B population was Line 47-16D(6B)/ST464-B1//Rusty. All four double-monosomic plants in the 6B population were sterile.

rust resistance gene in ST464-A2 was located in chromosome 2B (Table 5). After pooling data from all populations except 2A, 4A, 2B, and 4B, the observed segregation of 27:41:28:32 fit a 1:1:1:1 segregation ratio ($P = 0.282$), confirming that the translocation breakpoint was not linked to the stem rust resistance gene.

ST464-B1

For ST464-B1, all crosses were made using double-monosomic plants as the male and Rusty as the female parent, with the exception of the 6D(6B) cross. Only two of the seven F_1 plants in the 6D(6B) cross were double-monosomics, and they were crossed using Rusty as the male parent to produce testcross F_1 seeds. The testcross F_1 plants were inoculated with *Pgt-LBBL*. Reactions among testcross F_1 plants and F_1 families indicated that the stem rust resistance gene in ST464-B1 was incompletely dominant with ITs in heterozygous plants ranging from 1^- to 1^-13cn^-. Segregation within testcross F_1 families indicated that plants homozygous for the stem rust resistance gene had an IT of 0;1 which was identical to the IT of ST464-B1.

Because we were able to make the cross for 6D(6B), we had data for all chromosomes except 4A (Table 6). A 1:1 segregation ratio was observed for all 13 crosses. However, there were three populations (1A, 2A, and 3A) which were small in size. When making the testcrosses using Rusty as the female, only one or two selfed seeds in the critical cross may result in misidentification of the critical cross when population sizes are small. However, 2A cannot be the critical cross because double-monosomic plants whose progenies segregate for stem rust resistance cannot occur in the critical cross, and three such families were observed for the 2A testcross. With four homozygous susceptible, euploid families in the 1A testcross, chromosome 1A is not a good candidate for the location of the gene. There were only two homozygous susceptible, euploid families in the 3A testcross, and if both of these were selfs, then the gene would be located on chromosome 3A. However, because there is no data for chromosome 4A, the gene in ST464-B1 may be located in chromosome 4A. An additional trial of chromosomes 3A and 4A will be necessary when the 4D(4A) disomic substitution becomes available.

ST464-C1

For ST464-C1, all crosses were made using Rusty as the female and all male plants had $13^{II} + 2^{I}$ at MI of meiosis. In the case of the cross involving Line 47-1 6D(6B), eight F_1 plants were grown, but none were double-monosomic, and testcross F_1 seeds could not be produced.

When tested with *Pgt-TPMK*, the gene in ST464-C1 had a dominant gene action, producing ITs ranging from 1^-1 to 21 in heterozygous testcross F_1 plants while those testcross F_1 plants that produced homozygous susceptible families had ITs ranging from 32 to 34. Tests of the observed segregation ratios indicated good fits to

a 1:1 ratio for 10 of the 12 chromosomes tested (Table 7). The population testing chromosome 7B did not fit a 1:1 segregation ratio, but the gene could not be on this chromosome because one double-monosomic plant was observed in which the progeny segregated for stem rust resistance. The population testing chromosome 6A also failed to fit a single gene segregation ratio. The genetic expectation was that in the critical cross there should be no homozygous susceptible families derived from a euploid testcross plant, and one homozygous susceptible family was observed in the population testing chromosome 6A. However, because Rusty was used as the female parent in making testcrosses, the homozygous susceptible family in the 6A population could be derived from a selfed seed. In the 6A population, all double-monosomic testcross F_1 plants produced homozygous susceptible families as would be expected in the critical cross. Therefore, the gene in ST464-C1 was located in chromosome 6A.

Seedling Reactions to Seven Stem Rust Pathotypes

Because we identified genes in the monogenic lines that are located on chromosome 6A, and because of prior reports that ST464 has *Sr13*, we should observe that one of the two ST464 monogenic lines carrying a gene on 6A produces a virulence–avirulence pattern similar to the hexaploid *Sr13* line. The two ST464 monogenic lines having genes located on chromosome 6A were compared to Line S. ST464-C1 and Line S had similar ITs to the eight pathotypes. At the same time ST464-A1 differed from Line S by having a susceptible IT to *Pgt-JCMN*, *-HKHJ*, *-MCCF*, *-QCCJ*, and *-TTTT*. The gene in ST464-C1 was postulated to be *Sr13*.

The gene in ST464-A2 was located on chromosome 2B, and since Luig (1983) reported that ST464 carries *Sr9e*, which is also located on chromosome 2B, we compared reactions of ST464-A2 to the hexaploid-monogenic line Vernstein which carries *Sr9e*. For the seven pathotypes, ST464-A2 and Vernstein had similar reactions to all pathotypes except *Pgt-TPMK* and *Pgt-TMLK*. However, in previous trials (unpublished), ST464-A2 had produced a susceptible (IT $33^{+}2$) reaction to both *Pgt-TPMK* and *Pgt-TMLK*. Therefore, while the cause of the differential reactions for ST464-A2 between the two trials was unknown, the virulence–avirulence patterns and the genetic data supports the conclusion of Luig (1983) that ST464 carries *Sr9e*.

Progeny of one of the selected black-chaffed plants of ST464 was included in our virulence tests (Table 8). The black-chaffed selection was included in the trials because it carried the 2A/4B translocation, and since two of the ST464 monogenic lines carried this translocation, it was thought to be representative of the parental ST464 plants used in selecting the monogenic lines. However, the test of *Pgt-TTTT* on the black-chaffed ST464 selection indicated that the black-chaffed selection did not carry *Sr13*. Progeny of a white-chaffed selection of ST464 has been

Table 7. Segregation for chromosome pairing configuration at metaphase I (MI) of meiosis in BC_1F_1 plants having the generalized pedigree Rusty//Line 47-1 aneuploid/ST464-C1, and stem rust reaction of the BC_1F_1 families to *Pgt-TPMK*.

Chromosome Tested	BC_1F_1 pairing configuration and classification of the BC_1F_1 families for stem rust reaction†						
	14˝		Prob. (> χ^2)	13˝ + 2′		Other‡	
	HS	Seg	Test ratio 1:1	HS	Seg	HS	Seg
1A	10	12	0.670	6	4	0	0
2A	14	13	0.847	2	1	2	1
3A	9	13	0.394	0	4	1	1
5A	11	5	0.134	0	0	0	1
6A	1	17	<0.001	9	0	1	1
7A	12	9	0.513	3	2	0	1
1B	9	8	0.808	5	5	0	0
2B	14	8	0.201	2	2	0	1
3B	6	4	0.527	12	4	1	1
4B	14	9	0.297	2	4	2	2
4A§	9	8	0.808	5	4	1	1
5B	16	8	0.102	0	0	0	2
6B	0	0		0	0	0	0
7B	6	16	0.033	1	1	1	0
Total¶	130	130	1.000	42	31		

†HS, homozygous susceptible; Seg, segregating 3 resistant/1 susceptible.

‡Includes 14 plants having $14^{II} + 1^{I}$, three plants having $13^{II} + 1^{I}$, two plants having $14^{II} + t^{I}$, and two plants with abnormal chromosome configurations.

§The 4A population was shown to be incorrect and is a 4B population.

¶ Excludes plants from the cross testing chromosome 6A.

tested with only two of the pathotypes shown in Table 8, *Pgt-QCCJ* and *Pgt-TTTT*. Low ITs of 0; and 2− were obtained for the tests with *Pgt-QCCJ* and *Pgt-TTTT*, respectively. This result indicated that the white-chaffed selection of ST464 carried both *Sr9e* and *Sr13*. Therefore, there was heterogeneity in the original ST464 population for the presence of *Sr13*.

DISCUSSION

Univalent shift is the most likely explanation for the 4D(4A) double-monosomic being misidentified. In hexaploid wheat, univalent shift can convert a monosomic stock to any of the other 20 monosomics. In tetraploid wheat, compensation by the D-genome chromosomes limits univalent shift in double monosomic plants to homoeologous chromosomes (Joppa and Williams, 1977). Therefore, a shift from double monosomic 4D(4A) to 4D(5A) would produce a plant of such poor vigor or fertility that it would be unlikely that such a stock could be maintained. But, a univalent shift of 4D(4A) to 4D(4B) is possible. Reselection of the 4D(4A) line is underway.

We have not attempted to map the translocation breakpoint in ST464, and it appears that mapping of this translocation is not warranted. It is likely that the 2A/4B translocation in ST464 is the same as the 2A/4B translocation found in all 15 tetraploid landraces of Ethiopian origin studied by Kawahara and Taketa (2000). These translocations were mapped by C-banding. The study of Kawahara and Taketa (2000) suggests a monophyletic origin of Ethiopian landraces; however, our finding that five of the six white-chaffed plants of ST464 did not carry the translocation suggests an area for further study of the origin of Ethiopian tetraploid wheat. Additional studies of the translocation would also be warranted if it could be shown that the translocation was associated with a gene for stem rust resistance or any other desirable trait. Knott (1996), conducted a monosomic analysis of stem rust resistance genes in two hexaploid derivatives of ST464 and was unable to conclusively locate one of the genes. However, that study did not include studies of chromosome pairing; therefore no association of resistance with a translocation was established. The translocation was not transmitted to Leeds; and, although ST464 appears in the parentage of other cultivars such as Crosby (CItr 17282) and Rolette (CItr 15326) from North Dakota and Stewart 63 (CItr 13771) from Canada (Knott, 1963), their complex parentage makes it unlikely that this translocation occurs in any cultivars.

Knott (1996) reported that ST464 carries *Sr7a* on chromosome 4A. In the present study, three of the ST464 monogenic lines had stem rust resistance genes located on chromosomes other than 4A. The stem rust resistance gene in the fourth line, ST464-B1, may be located to chromosome 4A, but since this gene conferred resistance only to *Pgt-LBBL*, it could not be *Sr7a*. ST464 is a landrace in which we observed variation for heading date, plant height, chaff color, and presence of a 2A/4B translocation. Our test of black-chaffed and white-chaffed selections of ST464 with *Pgt-TTTT* indicated that ST464 is also heterogeneous for the presence of *Sr13*. If there is also heterogeneity in ST464 for other *Sr* genes; and, if the original ST464 parental plants which Williams and Gough (1968) used for crossing did not carry *Sr7a*, then none of the selected ST464 monogenic lines would have carried *Sr7a* even though the gene was present in the original population.

When comparing seedling reactions of monogenic lines, we observed instances where the virulence–avirulence patterns did not match even when it was postulated that the lines carried the same gene. This may be caused by differences in minor genes on the A- or B-genome, or it may be due to the D-genome in the hexaploid-monogenic lines which may carry either inhibitors or modifiers of resistance. (Kerber and Green, 1980; Williams et al., 1992). Minor genes may be present either in the hexaploid genetic stocks or in the ST464 monogenic lines. The ST464 monogenic lines were produced by crossing to Marruecos 9623 which carries a minor gene identified as *SrM* (USDA-ARS Cereal Disease Laboratory, 2004).

Table 8. Seedling infection types of four ST464 monogenic lines to eight pathotypes of *Puccinia graminis* f. sp. *tritici*.†

Entry	Comments	Pathotype							
		TPMK	*TMLK*	*JCMN*	*RTQQ*	*HKHJ*	*MCCF*	*QCCJ*	*TTTT*
ST464-B1‡		34	34	3	34	34	34	34	4
ST464-A1	on 6A	t1⁻,3	0;	34	0;1	34	34	34	33+
ST464-C1	on 6A	1	1	1	10;	1	1	12	2–
Line S§	*Sr13*	12	1	1	1	23⁻	1	32	2++
ST464-A2	on 2B	1	12	10;	10;	1	1⁻10;	0;1	33+
Vernstein§	*Sr9e*	34	34	1	1	1	11⁻0;	10:	4
ST464 (black chaff)		23	23⁻	10;	0;1	1	1⁻0;	0;1	33+
Rusty		34	34	34	34	34	34	34	4

†Infection types are described by Stakman et al. (1962).

‡*Pgt-LBBL* was not included in this trial, however ST464-B1 had an IT of 0;1 to *Pgt-LBBL* in prior trials.

§Hexaploid monogenic line.

By itself, *SrM* confers resistance only to *Pgt-LBBL* at low temperatures, but there may also be epistatic interactions of *SrM* with other *Sr* genes.

The results of the virulence–avirulence tests indicated that the gene in ST464-C1 was *Sr13*; but the gene in ST464-A1, which was also located to chromosome 6A, was not identified. In addition to *Sr13, Sr8* and *Sr26* are also located on chromosome 6A. However, *Sr26* was derived from *Agropyron elongatum* (Host) P. Beauv. (Knott, 1961); therefore, the genes in ST464-A1 cannot be *Sr26*. Considering the failure to observe exact matches in the avirulence–virulence tests, it will be necessary to conduct allelism tests to complete the identification of the genes in the monogenic lines of ST464.

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