Chromosome engineering to enhance utility of alien-derived stem rust resistance

Xu SS[1], Dundas IS[2], Pumphrey MO[3], Jin Y[4], Faris JD[1], Cai X[5], Qi LL[6], Friebe BR[6], Gill BS[6]

[1]USDA-ARS Northern Crop Science Laboratory, Fargo, ND 58105, USA, [2]School of Agriculture, Food and Wine, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia, [3]USDA-ARS Plant Science and Entomology Research Unit, Manhattan, KS 66506, USA, [4]USDA-ARS Cereal Disease Laboratory, St. Paul, MN 55108, USA, [5]Departments of Plant Sciences, North Dakota State University, Fargo, ND 58105, [6]Wheat Genetic and Genomic Resources Center, Kansas State University, Manhattan, KS 66506, USA

ABSTRACT

In the past 50 years, a number of stem rust (*Sr*) resistance genes identified from wild relatives of wheat have been incorporated into wheat genomes through chromosome engineering. Some of these genes, including *Sr25, Sr26*, *Sr32*, *Sr37*, *Sr39*, *Sr40*, *Sr43*, *Sr44*, *SrR*, and three unnamed novel *Sr* genes from *Aegilops speltoides* and *Haynaldia villosa*, are effective against Ug99. However, the alien chromosomal segments possess deleterious genes in addition to the *Sr* genes. To enhance the utility of these *Sr* genes in wheat breeding, we have been eliminating deleterious linkage drag associated with these *Sr* genes through homoeologous recombination. The *ph1b* mutant was used to induce recombination between the alien chromosomal segments and their homoeologs in wheat. Recombinants with reduced alien chromatin were identified and characterized through stem rust testing, molecular marker analysis, and fluorescent genomic *in situ* hybridization (FGISH). To date, several resistant lines with modified or shortened alien chromosomal segments have been developed. Lines containing modified alien chromosome segments with genes *SrR*, *Sr26*, *Sr32*, and *Sr39* are undergoing backcrossing and field evaluation for yield and quality characteristics in wheat breeding programs.

INTRODUCTION

Stem rust (caused by *Puccinia graminis* f.sp. *tritici* Eriks. & Henn.) is a serious disease of cultivated wheats, including durum (*Triticum turgidum* L. ssp. *durum*) and common wheat (*T. aestivum* L.). Deployment of resistance genes in commercial cultivars has provided effective control for the disease worldwide for decades. However, emergence of a new stem rust race, Ug99 (or *TTKS*) in Africa (8) and its spread to other areas has posed a serious threat to world wheat production because the race is virulent to most currently deployed *Sr* resistance genes (10). Thus, there is a need to identify and deploy *Sr* resistance genes that are effective against Ug99 into commercial wheat cultivars.

Cultivated wheats have a large number of relatives, which are useful sources of genes for wheat improvement. In the past 50 years, a number of *Sr* resistance genes identified in wheat relatives, such as *Ae. speltoides, Thinopyrum ponticum,* and rye (*Secale cereale* L.), have been incorporated into the wheat genomes through chromosome translocations. Some of these genes have been found to be highly effective against Ug99 (4, 10). However, most of the alien-derived *Sr* resistance genes are associated with deleterious linkage drag, which usually results in yield reduction and inferior quality (6). Thus, they are virtually unusable in their current form.

To enhance the utility of *Sr* genes in wheat breeding, we have initiated work to eliminate the deleterious linkage drag associated with these *Sr* genes through homoeologous recombination. In this paper, we review sources of alien-derived stem rust resistance genes and describe our strategy and progress in improving several translocations.

STEM RUST RESISTANCE DERIVED FROM WILD RELATIVES OF WHEAT

Alien Stem Rust Resistance Genes Introgressed into Wheat Genomes

Among 50 genes for stem rust resistance in wheat (7), 15 are derived from alien chromosomes of wheat relatives. Except for *Sr31* (*S. cereale*), *Sr34* (*Ae. comosa*), and *Sr38* (*Ae. ventricosa*), 12 alien resistance genes were found to be effective against Ug99 (Table 1).

Table 1. Alien species-derived stem rust (*Sr*) resistance genes that are effective against Ug99 (race *TTKS*).

Gene	Donor species	Chro.[1]	IT[2]	Line
Sr24	*Th. ponticum*	3DL	L	Agent/9*LMPG
Sr25	*Th. ponticum*	7DL	L	Agatha/9*LMPG
Sr26	*Th. ponticum*	6AL	L	Eagle
Sr27	*S. cereale*	3A	L	73,214,3-1/9*LMPG
Sr32	*Ae. speltoides*	2A,2B	L	ER5155
Sr36	*T. timopheevi*	2BS	L	W2691SrTt-1
Sr37	*T. timopheevi*	4BL	L	W3563
Sr39	*Ae. speltoides*	2B	L	RL6082
Sr40	*T. timopheevi*	2BS	L	RL 6088
Sr43	*Th. intermedium*	7D	L	KS10-2
Sr44	*Th. intermedium*	7DS	L	Taf-2
SrR	*S. cereale*	1D	L	DRA-1

[1] Wheat chromosomes/arms involved in the translocations (7), [2] IT, infection type to race *TTKS* at seedling stage was based on Jin et al. (4) except for *Sr43* and *SrR* (unpublished data). Low infection types (L) are indicative of host resistance.

Potential Novel Sources of Stem Rust Resistance

In addition to the resistance genes listed in Table 1, we have identified a number of wheat-alien species derivatives with resistance to multiple stem rust races including Ug99 (Table 2). Among these derivatives, the Chinese Spring (CS)-*H. villosa* chromosome 6V disomic addition line, CS-*Ae. speltoides* 5S disomic addition line and Alcedo-*Ae. caudata* disomic addition line AIII should carry novel genes for stem rust resistance because none of the *Sr* genes currently available are derived from *Ae. speltoides* chromosome 5S, *H. villosa*, and *Ae. caudata*.

Table 2. Potential novel sources of stem rust resistance from wild relative species of wheat.

Line and Pedigree	2n	IT to Ug99[1]
T. durum-*Ae. speltoides* translocation	28	L
CS-*H. villosa* 6V addition	44	L
CS-*Ae. speltoides* 5S addition	44	L
T. *aestivum* cv. Alcedo	42	H
AIII (Alcedo-*Ae. caudata* addition)	44	L
Zhong 4 (*T. aestivum*/*Th. intermedium*)	56	L
Zhong 5 (*T. aestivum*/*Th. intermedium*)	56	L
Zhong 6 (*T. aestivum*/*Th. intermedium*)	56	L
Zhong 7 (*T. aestivum*/*Th. intermedium*)	56	L
Zhong 8 (*T. aestivum*/*Th. intermedium*)	56	L
78829 (*T. aestivum*/*Th. intermedium*)	56	L
SS5 (*T. aestivum*/*Th. ponticum*)	56	L
LDN/*Ae. speltoides* PI369581	41	L
LDN/*Ae. speltoides* PI369600	42	L
LDN/*Ae. speltoides* PI369609	42	L
LDN 5D(5B)/*Ae. speltoides* PI393494	42	L
LDN 5D(5B)/*Ae. speltoides* PI442448	42	L

[1] IT, infection type at seedling stage was based on the unpublished data. Low infection types (L) are indicative of host resistance and high infection types (H) are indicative of host susceptibility.

The durum wheat-*Ae. speltoides* chromosome translocation line was originally developed by transferring an *Sr* gene from *Ae. speltoides* PI 369590 to stem rust-susceptible durum line 47-1 (L. R. Joppa, unpublished). The *Sr* gene has recently been determined to be located on a translocated 2B/2S chromosome using FGISH and molecular markers. The majority of the translocated chromosome is derived from *Ae. speltoides* 2S (J. D. Faris, S. S. Xu, and X. Cai, unpublished). Based on its reactions to multiple stem rust races, this resistance gene is different from *Sr32* and *Sr39*, two *Sr* genes located on chromosome 2S of *Ae. speltoides*. This *Ae. speltoides* chromosomal fragment, therefore, may contain a novel stem rust resistance gene locus or allele.

Among the newly identified resistance lines, six wheat-*Th. intermedium* partial amphiploids (Zhong 4, Zhong 5, Zhong 6, Zhong 7, Zhong 8, and 78829), one wheat-*Th. ponticum* partial amphiploid (SS5), three durum Langdon (LDN)-*Ae. speltoides* amphiploids, and two LDN 5D(5B)-*Ae. speltoides* amphiploids showed near-immunity or high levels of resistance to North American stem rust races and Ug99 (Table 2). These lines may provide additional novel genes for stem rust resistance.

ENHANCEMENT OF UTILITY OF ALIEN-DERIVED STEM RUST RESISTANCE VIA CHROMOSOME MANIPULATIONS

The common procedure for enhancing utility of alien-derived genes for desirable characters in wheat is to reduce the size of the alien segments by inducing new recombination between alien segments and wheat chromosomes. Generally, the smaller the alien chromatin, the more likely the translocation will have commercial value. In our research, we are using the *ph1b* mutant and durum 5D(5B) substitutions to induce new translocations in hexaploid and tetraploid wheats, respectively.

Inducing Homoeologous Recombination in Hexaploid Wheat

For reducing the sizes of the alien chromatin carrying *Sr32 Sr37*, *Sr39, Sr40,* and *Sr43* in hexaploid wheat, we used the previously described procedure (3, 9) that involves producing F_2 populations from crosses between the translocation lines and CS *ph1bph1b,* followed by the use of molecular markers and FGISH to identify new translocation lines carrying the *Sr* genes. We have also used a modified procedure to improve efficiency for induction and identification of new translocations. The F_1 plants from crosses between the translocation lines and CS *ph1bph1b* are backcrossed to CS *ph1bph1b* plants and the remaining spikes are self-pollinated to produce F_2's. The BC_1F_1 plants are evaluated for reaction to stem rust and genotyped at the *Ph1* locus. The BC_1F_1 plants that are homozygous for *ph1b* and hemizygous for the alien segment are backcrossed to CS or elite stem rust-susceptible lines. Then we evaluate the BC_2 population for stem rust resistance. The resistant individuals selected from the population are characterized for the size of alien segments using FGISH and molecular markers.

Efforts to reduce the size of alien chromatins containing *Sr32, Sr37*, *Sr39*, *Sr40,* and *Sr43* are currently underway in the two USDA-ARS laboratories in Manhattan, KS and Fargo, ND. To date, the F_2 or BC_1 populations derived from the original translocation stocks crossed with CS *ph1bph1b* have been developed. The University of Adelaide has developed several new lines with shortened alien chromatins carrying *Sr* genes *SrR* and *Sr26* (1, 2). Dundas et al. (2) also produced a number of lines with shortened or modified alien chromosome segments carrying *Sr32*, *Sr37*, *Sr39*, and *Sr40* but used the *ph1b* mutant in a cv. Angas background. Lines containing modified alien chromosome segments with genes *SrR*, *Sr26*, *Sr32*, and

Sr39 are undergoing backcrossing and field evaluation for yield and quality characteristics in wheat breeding programs in Australia. In the new line with *SrR*, the deleterious linkage drag affecting dough-quality appears to have been removed.

The gene *Sr44* is currently available on a non-compensating translocation chromosome (T7DS-7Ai#1L·7Ai#1S). The original 7Ai#1 disomic addition line (Vilmorin 27-DA 7Ai#1) has been crossed to CS monosomic for chromosome 7D (CS M7D) at USDA-ARS in Manhattan, KS. Double monosomic progeny of 'CS M7D'/'Vilmorin 27-DA 7Ai#1' population are expected to produce compensating centromeric translocation lines among the F_2 progeny. A combination of molecular marker screening, cytology, and *Sr* disease screening will enable us to identify the compensating T7DL.7Ai#1S Robertsonian translocations with *Sr44*. In addition, the resistant CS-*Ae. speltoides* 5S disomic addition line (CS-AESP DA 5S) was crossed with CS monosomic for chromosome 5D (CS M5D), and similarly the resistant CS-*H. villosa* 6V disomic addition line (CS-HVIL DA 6V) has been hybridized with CS monosomic for chromosome 6D (CS M6D). The 'CS M5D'/' CS-AESP DA 5S' and 'CS M6D'/'CS-HVIL DA 6V' populations have been characterized as outlined above in order to identify stem rust-resistant compensating translocations. A second round of chromosome engineering using *ph1b*-induced homoeologous recombination is underway to further reduce the size of the alien chromosome segments and reduce linkage drag.

Inducing Homoeologous Recombination in Tetraploid Wheat

Homoeologous pairing can be induced in aneuploids nullisomic for chromosome 5B. Durum lines Rusty and 47-1 are near-universally susceptible to stem rust races (5) and their 5D(5B) substitution lines are currently available for inducing crossing-over of homoeologous chromosomes in tetraploid wheat. Thus, shortening the size of the alien segment in the durum-*Ae. speltoides* translocation line is being performed using the Rusty and 47-1 5D(5B) substitution lines.

The crossing and testing procedure is similar to that for hexaploid wheat. Currently, the durum-*speltoides* line has been crossed to Rusty 5D(5B) double monosomic and to the 47-1 5D(5B) disomic substitution at USDA-ARS in Fargo, ND. The double-monosomic (13'' + 2') hybrid plants have been backcrossed to their respective parent (*ie.* Rusty 5D(5B) and 47-1 5D(5B) disomic substitutions). In the coming season, the BC_1 plants will be tested for resistance to stem rust. The resistant BC_1 plants will then be screened using the molecular markers for the *Ph1* locus. The resistant plants nullisomic for chromosome 5B will be backcrossed to Rusty. Similar to transfers in hexaploid wheat, the hybrids would initially be tested for stem rust resistance followed by molecular marker analysis. Any plants identified as having a reduced alien segment would be examined by FGISH to verify a reduced alien segment size.

The development and selection of translocation stocks is usually a slow process. However, the availability of various cytogenetic stocks and modern molecular techniques make the implementation of the research more feasible and efficient than ever before. As a part of a global cooperative effort in confining the Ug99 threat, the new lines with eliminated or reduced deleterious drag, once they become available, will be used to develop superior wheat cultivars/germplasm adapted to various environments.

REFERENCES

1. Anugrahwati DR, Shepherd WK, Verlin CD, Zhang P, Mirzaghaderi G, Walker E, Francki GM, and Dundas SI (2008) Isolation of wheat-rye 1RS recombinants that break the linkage between the stem rust resistance gene *SrR* and secalin. Genome 51:341-349.
2. Dundas IS, Anugrahwati DR, Verlin DC, Park RF, Bariana HS, Mago R, and Islam AKMR (2007) New sources of rust resistance from alien species: meliorating linked defects and discovery. Aust J Agric Res 58:545–549.
3. Friebe B, Jiang J, Raupp WJ, McIntosh R, and Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91:59-87.
4. Jin Y, Singh RP, Ward RW, Wanyera R, Kinyua M, Njau P, Fetch T, Pretorius ZA, and Yahyaoui A (2007) Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race *TTKS* of *Puccinia graminis* f. sp. *tritici*. Plant Dis 91:1096-1099.
5. Klindworth DL, Miller JD, and Xu SS (2006) Registration of Rusty durum wheat. Crop Sci 46:1012-1013.
6. Lukaszewski AJ (2000) Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. Crop Sci 40:216-225.
7. McIntosh, RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers J, Appels R (2005) Catalogue of gene symbols for wheat. MacGene 2005 (http://www.grs.nig.ac.jp/wheat/komugi/genes/download.jsp, verified June 24, 2008).
8. Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis*. f. sp. *tritici* in Uganda. Plant Dis 84:203.
9. Qi L, Friebe B, Zhang P, and Gill BS (2007) Homoeologous recombination, chromosome engineering and crop improvement. Chrom Res 15:3-19.
10. Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P, and Ward RW (2006) Current status, likely migrations and strategies to mitigate the threat to wheat production from race Ug99 (*TTKS*) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 2006, 1, No. 054, 13 pp.