14. Cytogenetic manipulation to enhance the utility of alien resistance genes

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Abstract

Although many wild relatives in the Triticeae tribe have been exploited to transfer stem rust resistance genes to wheat, the derived germplasms have often not been immediately useful in wheat breeding programs. Too frequently, large chromosome segments surrounding desirable genes also harbor deleterious genes that result in unacceptable yield or quality. Recombination between chromosomes of wheat and chromosomes of distant relatives is very rare due to genetic restrictions on chromosome pairing in polyploid wheat. However, chromosome pairing can be manipulated by utilizing mutant stocks that relax this tight genetic control. The *ph1b* mutant produced by E.R. Sears over 30 years ago is an invaluable chromosome engineering tool, readily employed in the age of high-throughput molecular genetics. Shortened translocations have already been produced for stem rust resistance genes *Sr26* and *SrR* using *ph1b*-induced homoeologous recombination. We are currently using induced-homoeologous recombination to reduce the sizes of alien chromosome segments surrounding TTKSK-effective genes *Sr32*, *Sr37*, *Sr39*, *Sr40*, *Sr43*, *Sr47*, *SrTt3, Sr2S#1* and *SrAeg5* to eliminate linkage drag putatively associated with these genes. Additional TTKSK-effective genes *Sr44, SrHv6, SrAsp5,* and *SrAse3* were first targeted for development of compensating translocation stocks and then for shortening the size of each alien segment. Population development is also underway to characterize several potentially new sources of resistance.

Keywords

Stem rust, wheat, Ug99, translocations

Introduction

The Triticeae relatives of cultivated wheat are valuable sources of genes for wheat improvement. Genes from close relatives, or homologous genomes, are readily deployed in elite germplasm due to more "normal" recombination rates, which provide a mechanism to recombine chromosomes and select superior germplasm. However, a delicate balance must be achieved between desirable target traits and undesirable "wild" genes or chromosome regions when transferring genes from more diverged, or homoeologous genomes. Only a handful of large translocation chromosomes have been successfully used in global wheat production, such as T1BL·1RS or T1AL·1RS (Villareal et al. 1995). Despite persistent breeding attempts, the majority of large translocations have not been exploited in agriculture.

Chromosome pairing in wheat is restricted to strict homologs by genetic control, preventing recombination among homoeologous chromosomes in the polyploid nucleus. This control maintains the stability of the polyploid genome, resulting in diploid-like pairing of 21 bivalents in metaphase I of meiosis for hexaploid wheat, and 14 bivalents in tetraploid wheat. Pairing homoeologous loci *Ph1* and *Ph2* (Riley and Chapman 1958; Mello-Sampayo 1971; Sears 1977), located on the long arm of chromosome 5B and the short arm of chromosome 3D, respectively, are primarily responsible for suppressing homoeologous recombination. While crucial to maintaining stability of the wheat genome, the *Ph* genes present an obstacle when transferring agronomically important traits from diverged genomes. Sears (1977) produced a deletion mutant of the *Ph1* locus, *ph1b*, in a Chinese Spring (CS) background that enables straightforward transfer of desirable genes from homoeologous genomes. In the absence of *Ph1*, chromosome pairing is enhanced between wheat chromosomes and alien homoeologs and recombinants may be recovered.

Even with *ph1b* stocks, the limiting steps in alien gene transfer have long been the ability to screen and detect desired genotypes for population development and to select recombinant progeny, constrained by the laborious and highly-technical nature of the required cytogenetic techniques. Fortunately, advances in DNA marker development and application have allowed a shift from cytogenetic observations to molecular genetic screening. Homozygous *ph1b* genotypes can be selected by DNA markers (Roberts et al. 1999), simplifying population development. Although many alien donor species have poor to non-existent molecular marker resources, and wheat microsatellite (SSR) markers are rarely transferable, PCR-based markers sufficient for detecting target alien translocations can be routinely developed from mapped expressed sequence tags (ESTs) (Qi et al. 2007).

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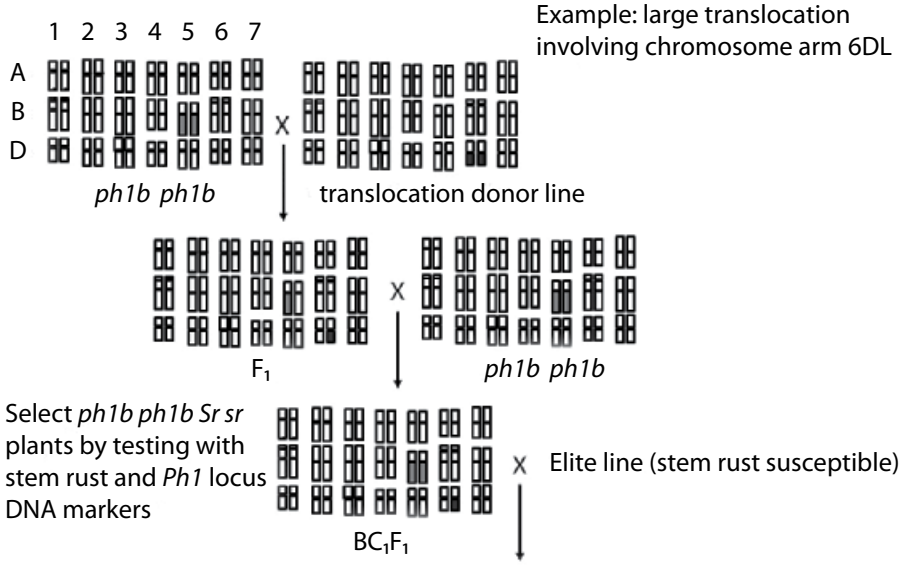
Fig. 1 Population development and screening strategy to reduce the size of an alien translocation with a desirable stem rust resistance gene. A hypothetical whole-chromosome arm translocation replacing 6DL is presented (shaded by dark gray). After crossing and backcrossing, a large translocation donor line with the *ph1b* mutant stocks (*ph1b* mutation indicated by light gray shading), progeny are selected that are homozygous *ph1b ph1b* and hemizygous for the target translocation and homoeologous wheat chromosome. Recombination between wheat chromosomes and homoeologous translocations is enhanced in these selected plants and they are crossed to an elite wheat line. The resulting progeny are then screened for the resistance gene by rust phenotyping and for shortened alien segments using one or more DNA markers that tag the translocation chromosome. Plants with shorter alien segments should lack specific alien markers while retaining rust resistance.



Screen 100s-1000s of progeny with stem rust and translocation-specific DNA markers to identify recombinants

Our goal is to make useful to variety development programs, effective stem rust resistance genes derived from wild species. Numerous stem rust resistance genes (*Sr)* from wheat relatives, such as *Aegilops speltoides* Tausch*, Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey*, Triticum timopheevii* (Zhuk.) Zhuk., and *Secale cereale* L., have been incorporated into wheat genomes in the form of chromosome translocations. More than a dozen of these genes are effective against race TTKSK (Ug99) and related derivatives (Singh et al. 2006; Jin et al. 2007). Unfortunately, most of them are associated with deleterious linkage drag. Reducing the amount of alien chromatin increases the likelihood of a translocation having commercial value. Their manipulation and use is important given the overarching goal of long-lasting rust protection in wheat crops worldwide, particularly when two or more broadly effective genes are pyramided and/or combined with minor-gene resistance in a single cultivar.

The specific gene targets of this project are *Sr32*, *Sr37*, *Sr39*, *Sr40*, *Sr43*, *Sr44, Sr47*, *SrAeg5, SrAsp5, SrAse3, SrHv6, SrTt3,* and *Sr2S#1*. Traditional hybridization and chromosome manipulation methods are coupled with DNA marker development, stem rust phenotyping, and genotyping of large populations to identify recombinant progeny with smaller translocations. After reducing the amount of alien chromatin, the *Sr* genes are transferred to elite wheat germplasm adapted to Africa and/or Asia. The following summaries document progress in our ongoing chromosome engineering efforts on each alien-derived stem rust resistance gene.

Approaches to induce homoeologous recombination

The preferred method for reducing the size of alien segments is to employ the *ph1b* mutant of hexaploid wheat. In this approach, either F_2 or BC_1F_1 populations are firstly produced from crosses between the translocation lines and *ph1b* mutant stocks. DNA markers that detect the *ph1b* mutation, and stem rust phenotypic screening, are applied to progeny to identify individual plants homozygous *ph1b ph1b* and hemizygous for the target translocation and homoeologous wheat chromosomes (Fig. 1). Populations developed from the selected plants are then screened for stem rust resistance and genotyped for translocation-specific marker alleles to identify putative recombinant progeny. Fluorescent genomic *in situ* hybridization (GISH) is then applied on putative recombinants to confirm shorter translocation lines carrying each *Sr* gene.

The crossing and screening procedure for tetraploid wheat is similar to that for hexaploid wheat, but homoeologous pairing is induced in durum 5D(5B) substitution lines lacking *Ph1*, rather than using *ph1b* or *ph1c* mutants. Durum lines 'Rusty' and '47-1' are widely susceptible to stem rust races and provide good backgrounds to investigate genetics of stem rust resistance (Klindworth et al. 2006).

Chromosome engineering of cataloged *Sr* genes

Sr26. The University of Adelaide has developed several lines with shortened alien segments carrying *Sr26* (Dundas et al. 2007). Dundas and Shepherd (1994; 1996; 1998) described the isolation of nine plants identified with 6Ae#1 chromosome segments of reduced size compared to that in Australian cv. Eagle. Seven carried *Sr26* (viz. WA1, WA2, WA5, WA6, WA8, WA9 and WA12) and two (WA7 and WA11) did not (Dundas et al. 2007). *Sr26* was localized to the extreme distal portion of chromosome 6Ae#1 and was closely linked to loci *Xmwg573-6Ae#1*, *Xmwg798-6Ae#1*, and *Xmwg2053-6Ae#1*. Lines WA1, WA5, WA6 and WA9 were provided to wheat breeding programs in Australia and the USA, and are currently in advanced stages of backcrossing to Australian cultivars. A simple PCR-based marker is available for selection of *Sr26* on shortened segments (Mago et al. 2005).

Sr32. Fifteen lines carrying modified segments of the *Ae. speltoides* 2S#1 chromosome were selected from 97 putative recombinants showing dissociation of chromosome 2S#1-specific markers in a *ph1b ph1b* genotype (Dundas et al. 2007). These were derived from the original T2DL-2S#1L·2S#1S translocation produced by E.R. Sears. Eleven were stem rust resistant; two lines carry *Sr32* on the short arm of the 2S#1 chromosome (lines 2S#1/ 102 and 2S#1/ 122a). In situ hybridization studies on these lines confirmed that the original 2S#1 segment was altered in structure. Lines carrying *Sr32* were resistant to the east African pathotypes TTKSK (Ug99), TTKST, and TTTSK and Yemani pathotypes TRTT at the USDA-ARS Cereal Disease Laboratory (Table 1). Both lines have a largely cv. Angas background. All resistant lines were initially backcrossed to Angas, and are currently undergoing backcrossing with Australian cv. Westonia and Indian cv. HUW234. Crosses with Westonia are at the BC_2 stage.

Sr37. The translocation line W3563 carrying *Sr37* on chromosome 4G from *T. timopheevi* (2n=4x=28, A^tA^tGG) was originally developed by McIntosh and Gyarfas (1971). W3563 has a 4B/4G chromosome translocation (Friebe et al. 1996) and is resistant to TTKSK (Jin et al. 2007) and certain North American races. A total of 17 representative lines with confirmed shortened *T. timopheevii* chromosome 4G#1 segments were selected from about 50 initial lines after screening for dissociation of *Sr37* and 4G#1 markers (Dundas et al. 2007) in a *ph1b ph1b* genotype. Three lines showing modified *T. timopheevii* 4G#1 chromosome segments derived from the 4B-4G#1 chromosome were backcrossed to cv. Angas and are currently undergoing backcrossing to Westonia and HUW234. Lines 4G#1/ 327, 4G#1/ 361 and 4G#1/ 376 were selected from others on the basis of fertility and growth habit.

Sr39. Stem rust resistance gene *Sr39* was transferred to hexaploid wheat cv. Thatcher (Tc) from *Ae. speltoides* by Kerber and Dyck (1990). Seven lines with shortened *Ae. speltoides* chromosome segments were produced after screening for dissociation of 2S#2 RFLP markers in chromosome T2BL-2S#2L·2S#2S-2BS (Kerber and Dyck 1990) in a *ph1b ph1b* genotype. Stem rust resistant lines (+*Sr39*) 2S#2/ 163, 2S#2/ 220 and 2S#2/ 247 showed obvious structural alterations relative to the *Ae. speltoides* chromosome after in situ hybridization. All of these lines and 2S#2/ 151 (+*Sr39*) were backcrossed to cv. Angas and are currently being backcrossed to Westonia and HUW234. Lines 2S#2/ 151 and 2S#2/ 247 are at the BC_2 stage with Westonia.

Sr40. Eleven plants were confirmed by progeny testing to carry shortened segments of the *T. timopheevii* 2G#2 chromosome derived from the T2BL-2G#2S translocation chromosome (Dyck 1992). Six of these are resistant to stem rust, but only lines 2G#2/ 286, 2G#2/ 300, 2G#2/ 301 and 2G#2/ 305 showed adequate fertility and vigor in field plots. These four lines are undergoing backcrossing with Westonia and HUW234.

Sr43. *Sr43* was transferred from *Th. elongatum* (Host) D.R. Dewey to wheat chromosome 7D using *ph1b*-induced homoeologous recombination (Knott et al.1977; Kibirige-Sebunya and Knott 1983). Three stocks with *Sr43*, viz. KS10-2, KS24-1, and LMq6-28-1a, were obtained from D.R. Knott, Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Two lines, KS10-2 and KS24-1, were chosen for this study. The translocation chromosomes in KS10-2 and KS24-1 were identified as T7DL-7Ae#2L·7Ae#2S and T7DS·7Ae#2L, respectively (Kim et al. 1993; McIntosh et al. 2008). The results from FGISH analysis showed that the long arm and about 50% of short arm of the interchanged chromosome in KS10-2 came from *Th. elongatum*, and only the short arm of the interchanged chromosome in KS24-1 was from *Th. elongatum*. The TTKSK infection type for KS10-2 was fleck, but for KS24-1 was ;1 in our testing (Xu et al. 2009). These two lines were crossed and backcrossed to the CS *ph1b* mutant. Over 2,500 hybrid seeds for each translocation line were produced from crosses of resistant BC_1F_1 plants with

CS. Eighteen specific SSR markers associated with alien chromatin were developed. Hybrid seeds consisting of *ph1b*-induced homoeologous recombinants are ready to be screened with molecular markers and stem rust inoculations with TTKSK.

Sr44. *Sr44* is currently available on a non-compensating translocation chromosome (T7DS-7Ai#1L·7Ai#1S) that is not useful for wheat breeding or amendable to directed chromosome manipulations (Friebe et al. 1996). We are first producing new compensating translocation stocks. The original 7Ai#1 disomic addition line (Vilmorin 27-DA 7Ai#1; Cauderon et al. 1973) was crossed to a CS plant monosomic for chromosome 7D (CS M7D). Double monosomic progeny of a 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 stem rust response screening was applied to approximately 300 F_2 plants to identify compensating T7DL·7Ai#1S Robertsonian translocations with *Sr44*. Four progeny are potential candidates at this time, based on the presence of *Sr44* and 7Ai#1S DNA marker alleles, and the absence of 7Ai#1L DNA marker alleles. These progeny will be characterized by GISH to rule out the possibility of telosomic chromosomes. Once produced, the compensating translocation stock(s) will be crossed and backcrossed to *ph1b ph1b* genotypes to initiate reduction in the size of this alien segment.

Sr47. *Sr47* was described by Faris et al. (2008). The gene is carried in the tetraploid stock DAS15, which has a background of line 47-1, a stem rust susceptible durum. The gene originated from *Ae. speltoides* and is carried in a T2BL-2SL·2SS translocation chromosome. The translocation in DAS15 was characterized using FGISH by Faris et al. (2008). Line DAS15 was reported as having IT 2= to Ug99, and in subsequent tests was shown to be resistant to Ug99 variants. To reduce linkage drag associated with *Sr47*, DAS15 was crossed to Rusty 5D(5B) and the F_1 crossed to 47-1 5D(5B). These F_1 plants will be crossed to Rusty in 2009. DAS15 was crossed to CS *ph1b*, and two backcrosses to CS *ph1b* have been completed in an attempt to transfer *Sr47* to hexaploid wheat. SSR markers additional to those identified by Faris et al. (2008) are presently being sought for use in shortening the alien segment in this line.

Chromosome engineering of tentatively designated Sr genes

2S#1. Of 11 stem rust resistant recombinants derived from the original T2DL-2S#1L·2S#1S stock with *Sr32*, nine had a second gene (temporarily named *Sr2S#1*) on the long arm of the 2S#1 chromosome (lines 2S#1/ 44, 2S#1/ 45, 2S#1/ 52, 2S#1/ 70, 2S#1/ 122b, 2S#1/ 122c, 2S#1/ 142, 2S#1/ 145b and 2S#1/ 287). Line 2S#1/ 45 is stem rust resistant but carries a telocentric chromosome of the long arm of the original T2DL-2S#1L·2S#1S chromosome. This evidence confirms that the *Sr2S#1* gene is located on the long arm of the original translocation chromosome. Because it has a telocentric chromosome, line 2S#1/ 45 will not be suitable for agricultural use.

SrR. The University of Adelaide has developed several new lines with shortened alien segments carrying *SrR* derived from Imperial rye. Koebner and Shepherd (1986a, b) induced homoeologous recombination between rye chromosome arm 1RS of the translocation line 1DL·1RS and the short arm of homoeologous wheat chromosome 1D in an attempt to break the linkage between *SrR* and the secalin gene. Intercrossing two of these primary recombinants resulted in the secondary recombinant DRA-1 with an interstitial rye segment carrying *SrR* and *Sec-1* (Koebner and Shepherd 1988; Rogowsky et al. 1991, 1993). Anugrahwati et al. (2008) produced the tertiary recombinant T6-1 derived from DRA-1 with *SrR* and lacking *Sec-1*. T6-1 is currently undergoing backcrossing in Australian wheat breeding programs.

SrTt3. This *T. timopheevii*-derived *Sr* gene, linked to *Sr36*, is located on chromosome 2G#3, (McIntosh et al. 1995). The translocation line AH (McIntosh et al. 2005) was crossed with Angas *ph1b ph1b*. A population of 100 F_3 plants derived from F_2 *ph1b ph1b* genotypes heterozygous for chromosomes 2B and 2B-2G#3 was screened for dissociation of 2G#3 markers generated with probes ABC252 and ABG58. Putative recombinants are now undergoing progeny-testing to confirm the marker patterns.

SrAge5. Screening a set of *Ae. geniculata* Roth addition lines in a CS background (*Ae. geniculata* donor accession TA2899) revealed that TA7659, a disomic addition line with $5M^g$ (21"+1" $5M^g$#1), was resistant to a composite stem rust infection; TA7670, a ditelosomic addition line with the short arm of $5M^g$ (21"+t"$5M^g$#1S), was susceptible; a long arm ditelosomic addition line was not available. These results prompted us to test existing $5M^g$ translocation stocks for stem rust resistance (Kuraparthy et al. 2007). Although the *Ae. geniculata* donor accession, TA10437, used to develop the *Lr57/ Yr40* transfers (Kuraparthy et al. 2007) was a different accession, the line TA5599 (T$5M^g$S·$5M^g$L-5DL) in a WL711 background was resistant, whereas TA5602 [T5DL·5DS-$5M^g$S (0.95)] and WL711 were susceptible. The infection types were similar between the TA7659 and TA5599 and both showed low infection types against TTKSK. To further characterize these materials, a population

Table 1 Infection types of wheat lines carrying SrR (rye), Sr32 or Sr2S#1 (Ae. speltoides), chromosome segments of 2S#3 (Ae. speltoides AEG357-4), 2S#4 (CS/ Ae. speltoides TA8026) or 2S#5 (CS/ Ae. speltoides TS01) against four exotic pathotypes (Yue Jin unpublished 2009). The SrR line and some 2S#4 lines were known to be segregating for the alien chromosome

	TTKSK	TTKSK	TTKST	TTTSK	TRTT
Line	04KEN156/04	04KEN156/04	06KEN19v3	07KEN24-4	06YEM34-1
SrR^{+} Sec^{-}	2-,4	;2-	2	;2-	;
2S#1/ 102 (+*Sr32*)	2-	;2-	;2-	;2-	2-
2S#1/ 122a (+*Sr32*)	2-;,3+	;2-,4	;2-	;2-,3	2
2S#1/ 122b (+*Sr2S#1*)	2-;	;2-,2	;2-	;,4	2-
2S#1/ 122c (*Sr2S#1*)	12+	23-	23-,3+	23-,;	-
2S#3 recomb #3	2+	22+	2	2	2
2S#3 recomb #16	;2--	2-	;2-	;2-	2-
2S#3 recomb #20	;2-	-	-	-	-
2S#3 recomb #27	2	2	2-	2-	2
2S#3 recomb #79	2	;2-	2	;2-	2
2S#4	;2,2+,3	2;,3	;2-,22+,23	;,23,4	22+
2S#4	2-;	;2-	;2-	0	2
2S#4 recomb 25	;,;N	;1	;1	;1	22+
2S#5	;,2-,2+	;	;	;	2-
2S#5	;1	;	;,2-	;,;2-	2
Westonia	4	4	4	4	4
Angas	2++3	3	3+	3+	2+

was developed by crossing TA5599 and TA5602. The F_3 families were evaluated for stem rust resistance by inoculation with race RKQQ at the two-leaf stage. Transmission of T5M^{g}S·5M^{g}L-5DL (23 homozygous resistant: 80 segregating: 44 homozygous susceptible; $chi^2_{1:2:1} = 7.12$; $P<0.05$) was significantly reduced, but *SrAge5* appeared to segregate as a single gene because DNA markers tagging 5M^{g}L co-segregated with resistance.

SrAsp5. TA7693, a disomic addition line with chromosome 5S of *Ae. speltoides* (21″+1″ [5S#3]) in CS background, is resistant to North American races and race TTKSK. TA7693 was crossed with CS M5D. Selected double monosomic F_1 plants were self-pollinated and ~250 F_2 progeny were screened with race RKQQ and characterized by molecular markers to identify putative Robertsonian translocation progeny. Based on marker results, resistant progeny had 5SL, whereas susceptible progeny lacked 5SL. Putative Robertsonian events were identified as progeny lacking 5SS marker alleles and having 5SL marker alleles. F_3 families of putative Robertsonian progeny were screened by C-banding. Family U5909-2-166 was identified as a Robertsonian translocation T5DS·5S#3L; the others were telosomic lines. U5909-2-166 was crossed to CS *ph1b* to develop populations for reducing the size of this translocation.

SrHv6. TA7682, a disomic addition line with chromosome 6V of *Haynaldia villosa* in CS background (21″+1″ [6V#3]), is resistant to North American stem rust races and TTKSK. Addition lines involving the other six chromosomes from the same donor were susceptible. A population was produced from the cross CS M6A (20″ + 6A′) / DA 6V in an effort to derive Robertsonian translocation chromosomes involving the 6V short and

long arms. Selected double monosomic F_1 plants were self-pollinated and their F_2 progeny were characterized by molecular markers and FGISH. C-banding analysis and molecular markers were then used to characterize and verify these Robertsonian translocation events and T6AS·6V#3L translocations were discovered. The T6AS·6V#3L translocation has been crossed to Chinese Spring *ph1b* to develop populations for reducing the size of this translocation.

SrAse3. TA3852 is a disomic addition line with chromosome $3S^S$ of *Ae. searsii* Feldman & Kislev ex Hammer in CS background (21″+1″[$3S^S$#1]) and is resistant to North American stem rust races and TTKSK. A ditelosomic addition line with the short arm of $3S^S$ (TA7533; 21″+t″[$3S^S$#1S]) was resistant, whereas the $3S^S$#1L ditelosomic addition line (TA7534; 21″+t″) was susceptible. Thus, this gene is located on the short arm of $3S^S$#1. In order to produce compensating translocations and begin reducing the amount of alien chromatin, disomic substitution lines involving all three homoeologous wheat chromosomes (TA6555, 20″+1″ [$3S^S$#1 (3A CS)]; TA6556, 20″+1″[$3S^S$#1 (3B CS)]; TA6557, 20″+1″[$3S^S$#1 (3D CS)]) were crossed to CS *ph1b*. F_2 populations of each cross were screened for putative Robertsonian translocations by DNA markers, whereas BC_1F_1 populations were developed by crossing to CS *ph1b* to induce homoeologous recombination. Confirmation of Roberstonian translocations and identification of recombinants are underway.

Discovery of new *Sr* genes requiring cytogenetic manipulation

A number of wheat-alien species derivatives with resistance to multiple stem rust races including TTKSK were indentified from *Ae. caudata* L., *Ae. speltoides*, *Th. intermedium* , *Th. Junceum* (L.) Á. Löve, and *Th. ponticum*. Population development, molecular marker testing, and phenotypic screening are underway to further characterize these potentially new sources of resistance:

Ae. speltoides. Five *Ae. speltoides* accessions, including AEG357-4, AEG363-5, AEG818-4, AEG874-60, and AEG2106-38, were obtained courtesy of The Harold and Adele Lieberman Germplasm Bank, Tel Aviv University, Israel. Each of these accessions showed high levels of resistance to Australian pathotypes (Dundas et al. 2008) and Ug99 pathotype TTKSK, and have been targeted for introgression into hexaploid wheat. Angas*7/AEG357-4 plants with resistance to Australian races *Pgt* 34-1,2,3,4,5,6,7 and *Pgt* 343-1,2,3,5,6 were identified. *Ae. speltoides* group 2 markers for probes ABG358, ABC454 and BCD111 were found in these plants whereas markers for other homoeologous groups were not found. The *Ae. speltoides* chromosome suspected of carrying the stem rust resistance gene was named 2S#3 (Dundas et al. 2008). Crosses between a resistant wheat plant carrying the 2S#3 chromosome from AEG357-4 were made with Angas *ph1b ph1b*. A total of 214 plants were screened for dissociation of 2S#3 markers for ABG358 and BCD111; seven confirmed dissociations were found. Five of these were resistant to *Pgt* 343-1,2,3,5,6; three had the marker pattern ABG-2S#3 (+ve), BCD111-2S#3 (-ve), whereas the other two showed the reverse pattern. This indicated that *Sr2S#3* may be located between the two RFLP marker loci.

Lines carrying the 2S chromosomes (and apparently no other 'S' genome chromosome) in a cv. Angas background were derived from CS/ *Ae. speltoides* amphiploids TA8026 and TS01. The amphiploids originated from the Wheat Genetics Resource Center (Kansas) and Dr M. Feldman (Israel), respectively. Both 2S addition lines have resistance to Australian pathotypes (*Pgt* 343-1,2,3,5,6; and 34-1,2,3,4,5,6,7). Crosses were made between lines carrying the 2S chromosome from TA8026 (named 2S#4) and Angas *ph1bph1b*. A total of 51 seedlings (F_3) were screened for dissociation of RFLP probes BCD111 and ABC252 and 10 confirmed dissociation plants were identified. Only one (recombinant # 25) (BCD-2S#4, +ve; ABC252-2S#4, -ve) of eight lines tested with *Pgt* 343-1,2,3,5,6 was resistant. Recombinant #25 also shows resistance to Ug99 and derivatives.

Accessions TA1776-1, TA1783-1, TA1793-1, TA1955-1, TA1935-1, TA1936-2, TA2099-1, TA2771-1, TA2779-2, and TA2780-2 represent the initial set of Wheat Genetic and Genomic Resources Center *Ae. speltoides* accessions targeted for direct introgression into hexaploid wheat. Each accession is nearly immune to North American races and TTKSK, but come from dispersed geographic locations. BC_1F_1 populations have been produced for each accession by crossing with WL711, CS *ph1b*, and other stem rust susceptible backgrounds. Three durum Langdon-*Ae. speltoides* amphiploids, and two Langdon 5D(5B)-*Ae. speltoides* amphiploids showing near-immunity or high levels of resistance to North American stem rust races and Ug99 are also undergoing genetic characterization.

Ae. caudata. Stem rust resistance was found in two stocks with *Ae. caudata* chromosomes. Alcedo-*Ae. caudata* disomic addition line 'AIII' has seedling resistance to TTKSK, whereas Alcedo is susceptible, and likely has a new gene(s) for stem rust resistance because no *Sr* gene currently available is from *Ae. caudata*. The characterization of addition line 'AIII' and introgression of the stem rust resistance gene from this addition line are currently in progress. An amphiploid of wheat- *Ae. caudata* (TA3368) has resistance to stem rust

and populations derived from this line are being used to associate the rust resistance gene with molecular markers at the University of Adelaide.

***Thinopyrum* spp**. Stem rust tests have been conducted on BC_2 populations of partial amphiploid *Thinopyrum ponticum* OK7211542 (provided by Dr R. Conner, Canada). Resistance to *Pgt* 34-1,2,3,4,5,6,7 was found in these populations and characterizations are underway. A series of wheat-*Th. intermedium* partial amphiploids (Zhong 4, Zhong 5, Zhong 6, Zhong 7, Zhong 8, and 78829) and one wheat- *Th. ponticum* partial amphiploid (SS5) have high levels of seedling resistance to Ug99 and populations are under development for potentially new resistance genes.

References

Anugrahwati DR, Shepherd WK, Verlin CD, Zhang P et al (2008) Isolation of wheat-rye 1RS recombinants that break the linkage between the stem rust resistance gene *SrR* and secalin. Genome 51:341-349

Cauderon Y, Saigne B, Dauge M (1973) The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. In: Sears ER, Sears LMS (eds.) Proc. 4th Int Wheat Genet Symp, University of Missouri Agric Res Sta, Columbia, MO, U.S.A., pp401-407

Dundas IS, Shepherd KW (1994) Progress towards improving the yield of wheat cultivars carrying stem rust resistance gene *Sr26* using cytogenetical methods. In: Paull J, Dundas IS, Shepherd KJ, Hollamby GJ (eds) Proc 7th Assembly, Wheat Breeding Society of Australia, Adelaide, SA, pp129-132

Dundas IS, Shepherd KW (1996) Towards yield improvement of stem rust resistant wheat cultivars carrying *Sr26*. In: Richards RA, Wrigley CW, Rawson HM, Rebetzke GJ et al (eds) Proc 8th Assembly, Wheat Breeding Society of Australia, Canberra, ACT, Australia, pp201-203

Dundas IS, Shepherd KW (1998) Shortening the Agropyron chromosome segment carrying gene *Sr26* utilising chromosome engineering and molecular markers. In: Slinkard AE (ed), Proc 9th Int Wheat Genet Symp, University Extension Press, University of Saskatchewan, Saskatoon, Canada Vol 2:35-37

Dundas IS, Anugrahwati DR, Verlin DC, Park RF et al (2007) New sources of rust resistance from alien species: meliorating linked defects and discovery. Aust J Agric Res 58:545-549

Dundas IS, Verlin DC, Park RF, Bariana HS et al (2008) Rust resistance in *Aegilops speltoides* var. *ligustica*. In: Appels R, Eastwood R, Lagudah E, Langridge P et al (eds) Proc 11th Int Wheat Genet Symp, Sydney University Press, Sydney, Australia. pp200-202

Dyck PL (1992) Transfer of a gene for stem rust resistance from *Triticum araraticum* to hexaploid wheat. Genome 35:788-792

Faris JD, Xu SS, Cai X, Friesen TL, Jin Y (2008) Molecular and cytogenetic characterization of a durum wheat *Aegilops speltoides* chromosome translocation conferring resistance to stem rust. Chrom Res 16:1097-1105

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

Jin Y, Singh RP, Ward RW, Wanyera R et al (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

Kerber ER, Dyck PL (1990) Transfer to hexaploid wheat of linked genes for adult-plant leaf rust and seedling stem rust resistance from an amphiploid of *Aegilops speltoides* X *Triticum monococcum*. Genome 33:530-537

Kibirige-Sebunya I, Knott DR (1983) Transfer of stem rust resistance to wheat from an *Agropyron* chromosome having a gametocidal effect. Can J Genet Cytol 25:215-221

Kim N-S, Armstrong K, Knott DR (1993) Molecular detection of *Lophopyrum* chromatin in wheat-*Lophopyrum* recombinants and their use in the physical mapping of chromosome 7D. Theor Appl Genet 85:561-567

Klindworth DL, Miller JD, Xu SS (2006) Registration of Rusty durum wheat. Crop Sci 46:1012-1013

Koebner RMD, Shepherd KW (1986a) Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis. I. Isolation of recombinants. Theor Appl Genet 73:197-208

Koebner RMD, Shepherd KW (1986b) Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis. II. Characterisation of recombinants. Theor Appl Genet 73:209-217

Koebner RMD, Shepherd KW (1988) Isolation and agronomic assessment of allosyndetic recombinants derived from wheat/rye 1DL.1RS carrying reduced amounts of rye chromatin. In:. Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Institute of Plant Science Research, Cambridge Laboratory, Trumpington, England, pp343-348

Knott DR, Dvorak J, Nanda JS (1977) The transfer to wheat and homoeology of an *Agropyron elongatum* chromosome carrying resistance to stem rust. Can J Genet Cytol 19:75-79

Kuraparthy V, Chhuneja P, Dhaliwal HS, Kaur S et al (2007) Characterization and mapping of a cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance genes *Lr57* and *Yr40* in wheat. Theor Appl Genet 114:1379-1389

Mago R, Bariana HS, Dundas IS, Spielmeyer W et al (2005) Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and *Sr26* in diverse wheat germplasm. Theor Appl Genet 111:496-504

McIntosh RA, Gyarfas J (1971) *Triticum timopheevii* as a source of resistance to wheat stem rust. Z Pflanzenzucht 66:240-248

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

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers J, Appels R (2008) Catalogue of gene symbols for wheat. MacGene 2008. http://www.shigen.nig.ac.jp/wheat/komugi/genes/download.jsp. Accessed on 02 Jan 2009

Mello-Sampayo T (1971) Genetic regulation of meiotic chromosome pairing by chromosome 3D of *Triticum aestivum*. Nature New Biol 230:22-23

Qi L, Friebe B, Zhang P, Gill BS (2007) Homoeologous recombination, chromosome engineering and crop improvement. Chrom Res 15:3-19

Riley R, V Chapman (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713-715

Roberts MA, SM Reader, C Dalgliesh, TE Miller et al (1999) Induction and characterization of *Ph1* wheat mutants. Genetics 153:1909-1918

Rogowsky, PM, Guidet FLY, Langridge P, Shepherd KW, Koebner,RMD (1991) Isolation and characterisation of wheat-rye recombinant involving chromosome arm 1DS of wheat. Theor Appl Genet 82:537-544

Rogowsky PM, Liu JY, Manning S, Taylor C, Langridge P (1992) Structural heterogeneity in the R173 family of rye-specific repetitive DNA sequences. Plant Mol Biol 20:95-102

Sears ER (1977) An induced mutant with homoeologous pairing in common wheat. Can J Genet Cytol 19:585-593

Singh RP, Hodson DP, Jin Y, Huerta-Espino J et al (2006) Current status, likely migrations and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of the stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 1 No. 054, 13pp

Villareal RL, Toro ED, Mujeeb-Kazi A, Rajaram S (1995) The 1BL/1RS chromosome translocation effect on yield characteristics in a *Triticum aestivum* L. cross. Plant Breed 114:497-500

Xu SS, Jin Y, Klindworth DL, Wang RR-C, Cai X (2009) Evaluation and characterization of seedling resistance to stem rust Ug99 races in wheat-alien species derivatives. Crop Sci 49:2167-2175