RESEARCH

Wheatgrass–Wheat Partial Amphiploids as a Novel Source of Stem Rust and Fusarium Head Blight Resistance

M. K. Turner,* L. R. DeHaan, Y. Jin, and J. A. Anderson

ABSTRACT

Perennial wheatgrasses (*Thinopyrum* spp.) are recognized sources of genetic variation for annual wheat (*Triticum aestivum* L.) improvement. Amphiploid lines made by crossing *Thinopyrum* spp. and *T. aestivum* (common wheat) can increase resilience of wheat to pathogens and abiotic stress. However, lack of pairing between chromosomes of *Thinopyrum* and *Triticum* species reduces genome stability, seed set, and perenniality. Fifty-two wheat–wheatgrass amphiploids from the perennials *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey, *Thinopyrum ponticum* (Podp.) Barkworth & D. R. Dewey, and *Thinopyrum junceum* (L.) Á. Löve crossed with the annuals *T. aestivum*, *Triticum turgidum* L. subsp. *carthlicum* (Nevski) Á. Löve & D. Löve (syn. *Triticum carthlicum* Nevski), and *Triticum turgidum* subsp. *durum (Desf.) Husn*, were screened for wheat stem rust (caused by *Puccinia graminis*) and Fusarium head blight (FHB) (caused by *Fusarium graminearum*) reaction and evaluated for winter hardiness and perenniality. Twenty-four of 48 amphiploid lines were resistant to all stem rust races screened, including TTKSK (syn. Ug99), TRTTF, and common U.S. races. Of the 30 amphiploid lines point inoculated with *F. graminearum*, 21 were resistant based on the percentage of infected spikelets and the percent of visually scabby kernels. Three sources each of potentially novel stem rust and uncharacterized FHB resistance were identified and may be useful for wheat improvement. Two lines showed perenniality in Minnesota and may be valuable as cold-tolerant perennial wheat germplasm. Seven lines representing two families showed potential genetic stability based on chromosome counts and seed production.

M.K. Turner and J.A. Anderson, Department of Agronomy and Plant Genetics, University of Minnesota, St Paul, MN 55108; L.R. DeHaan, The Land Institute, Salina, KS 67401; Y. Jin, USDA-ARS, Cereal Disease Lab., Univ. of Minnesota, St. Paul, MN 55108. Received 20 Dec. 2012. *Corresponding author (turne487@umn.edu).

Abbreviations: FHB, Fusarium head blight; TKW, thousand kernel weight; VSK, visually scabby kernels.

Perennial wheatgrasses (*Thinopyrum* spp.) are recognized sources of genetic variation to improve annual wheat germplasm and as a potential perennial grain crop. Crossing *Thinopyrum* spp. and *Triticum* spp. can improve both. In wheat, two of the most destructive diseases currently threatening production are stem rust and Fusarium head blight (FHB), caused by the fungal pathogens *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. and E. Henn and *Fusarium graminearum* Schwab [teleomorph *Gibberella zeae* (Schw.) Petch], respectively. After epidemics in the early 20th century, stem rust had declined globally to insignificant levels by the 1990s with the introgression of *Sr24*, *Sr26*, *Sr31*, and *Sr38* (Singh et al., 2006). However in 1998, an aggressive new race (Ug99) of stem rust appeared in Uganda, evolving virulence to most resistance genes and spreading throughout many of the wheat growing areas of Africa and into the Middle East (Singh et al., 2006).

Fusarium head blight has been a very destructive disease in the northern Great Plains. Between 1993 and 2001, FHB decimated hard red spring wheat production in this area, resulting in 78.9% loss of hard red spring wheat with US$1.26 billion in direct yield loss (Nganje et al., 2004). Currently, there are no wheat cultivars highly resistant to FHB and fungicides are only partially effective. Therefore, finding novel sources of resistance to rapidly evolving

Published in Crop Sci. 53:1994–2005 (2013).
doi: 10.2135/cropsci2012.10.0584

races of stem rust and more effective resistance to FHB is increasingly important.

Resistance to many diseases of wheat has been identified in diverse *Thinopyrum* spp., but only a few wheatgrass genes from a limited number of accessions have been used to improve wheat (Table 1). *Thinopyrum ponticum* is the source of stem rust resistance genes and leaf rust resistance genes (caused by *Puccinia triticina*) *Sr24* linked with *Lr24* (McIntosh et al., 1977; Li et al., 2003), *Sr25* linked with *Lr19* (McIntosh et al., 1977; Zhang et al., 2005), *Sr26* (Knott, 1961), *Sr43* (Kibirige Sebunya and Knott, 1983; Friebe et al., 1996), FHB resistance (Shen and Ohm, 2007), and powdery mildew (*Blumeria graminis* f. sp. *tritici*) resistance (Sepsi et al., 2008). *Thinopyrum intermedium* is the source of *Sr44* (Friebe et al., 1996). Resistance also has been identified in *T. intermedium* to eyespot {caused by *Tapesia yallundae* (Wallwork and Spooner) and *Tapesia acuformis* (Boerma, Pieters and Hamers) Crous [anamorph *Pseudocercosporella herpotrichoides* (Fron.) Deighton]} (Cox et al., 2005) and barley yellow dwarf virus (Brettel et al., 1988; Banks et al., 1993). Fusarium head blight resistance has been mapped in *T. ponticum* to chromosome 7E (Shen and Ohm, 2007) and identified in *T. intermedium* and *Thinopyrum junceum* (L.) Á. Löve (Oliver et al., 2005). Recent studies showed high levels of resistance to FHB (Oliver et al., 2005) and stem rust (Xu et al., 2009) in several wheatgrass species. Therefore, further evaluation of *Thinopyrum* spp. as donors of disease resistance may provide additional novel genes for wheat improvement.

In addition to wheat improvement, there is a growing interest in developing perennial wheat as a grain crop. Compared to annuals, perennial species retain higher soil fertility (Culman et al., 2010), prevent loss of N and P through surface runoff (Turner and Rabalais, 2003), protect against soil erosion, and are more resilient to pathogens and abiotic stresses (Glover, 2005). Molecular markers, genetic mapping, and sequencing methodologies are being used to understand the genetic control of perenniality and to improve perennial wheat germplasm more rapidly. Cytological characterization has shown that genetically stable lines demonstrating perenniality have 42 or at least 56 chromosomes (Tsitsin and Lubimova, 1959; Scheinost et al., 2001; Hayes et al., 2012) although lines with 42 or 56 chromosomes are not always stable. Seed production can also be a good indicator of stability. Already, one measure of perenniality, post-sexual-cycle regrowth, has been mapped to the short arm of chromosome 4E in *Thinopyrum elongatum* (Host) D. R. Dewey (Lammer et al., 2004). Additional marker resources could be used to identify additional genomic regions contributing to perenniality and agronomic traits of importance.

Based on disease threats to wheat production and recent work with perennial wheat, this study investigated the potential utility of new wheat–wheatgrass partial amphiploids developed at The Land Institute for disease resistance and as a perennial crop in Minnesota. The objectives of this research were to

1. Identify resistant wheatgrass–wheat lines to stem rust and FHB,
2. Determine if stem rust resistance identified in these materials is novel, and
3. Assess perenniality and winter hardiness of wheat–wheatgrass partial amphiploids in Minnesota.

MATERIALS AND METHODS

Plant Materials

Fifty-two F_2 to F_7 families from 17 partial amphiploid wheat–wheatgrass lines were developed at The Land Institute in Salina, KS, beginning in 2001 (Table 2; Fig. 1). Lines were created by crossing perennial wheatgrass species from the *Thinopyrum* genus [*T. intermedium* (intermediate wheatgrass) [$2n = 6x = 42$; JJJ^sJ^sSS, where J and E genomes are closely related to *Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve and/or *T. elongatum* and S is related to *Pseudoroegneria libanotica* (Hack.) D. R. Dewey (Chen et al., 2001)], *T. ponticum* [syn. *Agropyron elongatum* (Host) Beauv] ($2n = 10x = 70$; $JJJJJJJ^sJ^sJ^sJ^s$ [Chen et al., 1998]), *Thinopyrum junceiforme* (Á. Löve & D. Löve) Á. Löve ($2n = 4x = 28$; $JJSS = E^eE^eE^bE^b$ [Nieto-Lopez et al., 2003]), and *Thinopyrum pycnanthum* (Godr.) Barkworth [$2n = 6x = 42$; $SSP^sP^sE^sE^s$, where the P genome is related to *Agropyron cristatum* (L.) Gaertn. (Refoufi et al., 2001)] by annual wheat [*Triticum aestivum* ($2n = 6x = 42$; AABBDD), *Triticum turgidum* subsp. *carthlicum* ($2n = 4x = 28$; AABB), *Triticum turgidum* subsp. *durum* (Desf.) Husn. (syn. *Triticum durum* Desf.) ($2n = 4x = 28$; AABB), and triticale (×*Triticosecale*) ($2n = 6x = 42$; AABBRR)]. These lines were initially developed for perenniality and high yield, with complex pedigrees involving one to two perennial wheatgrass species and one to four annual wheat lines using embryo rescue. Lines were self-pollinated resulting in F_2 to F_7 generation seed. Lines were advanced in the greenhouse. The probability of cross-pollination was low in the greenhouse due to limited pollen flow.

Parental lines used to develop the partial amphiploids were obtained through the National Plant Germplasm System from the Small Grains Collection in Aberdeen, ID, and Western Region Plant Introduction Station in Pullman, WA. Additional lines were provided by Dr. Carl Griffey at Virginia Tech in Blacksburg, VA, Dr. Paul Murphy at North Carolina State University in Raleigh, NC, Ehmke Seed Co. in Healy, KS, and the Wheat Genomic Resource Center in Manhattan, KS.

Stem Rust Screening

Stem rust screenings were similar to previously described screenings of wheatgrass species by Xu et al. (2009) and were based on methods described by Jin et al. (2007). For each race, five seeds per genotype were planted in the USDA-ARS Cereal Disease Laboratory greenhouses in St. Paul, MN. Seedlings were inoculated 10 d after planting, incubated for 16 h in a dark dew chamber at 18°C, and scored 14 d after inoculation using a modified rating score of 0 to 4 (Stakman et al., 1962; Roelfs, 1988). Ratings of 0, ;, 1, 2, or any combination with these ratings were considered resistant. Ratings of 3 or 4 were

Table 1. *Thinopyrum* sources of resistance to stem rust and Fusarium head blight.

Donor species	Disease resistance	Gene	Chromosome identified	Donor line	Size of alien translocation	Reference
Thinopyrum intermedium	Stem rust	*Sr44*	7Ai#1	TAF2		Friebe et al., 1996
	Fusarium head blight		2Ai	Zhong 5		Han et al., 2003
Thinopyrum junceum	Stem rust		Not designated			Xu et al., 2009
	Fusarium head blight		Not designated			Oliver et al., 2005
Thinopyrum ponticum	Stem rust	*Sr24*	7Ae#1	Agent	1.26 µm	Friebe et al., 1996
		Sr25	7Ae#1L	Agatha	2.55 µm	Friebe et al., 1996
		Sr26	3Ae#1L	K2046	2.48 µm	Friebe et al., 1996
		Sr43	7Ae#2			Friebe et al., 1996
	Fusarium head blight		7Ae			Shen and Ohm, 2006

Table 2. Pedigrees, generation, and number of lines in partial amphiploid families.

Family	Generation	Pedigree†	No. lines
B307	F_4	Tam110/*PI 314190*//WGRC33	4
B373	F_7	Tam110/*PI 401201*//Jagger or 2137	5
B875	F_4	Do1/*PI 414667*	5
B913	F_4	PI 634318/*PI 414667*	3
B938	F_3	PI 520172/*PI 261099*//PI 520054/3/McCormick/4/*PI 401129*	10
B1016	F_2	NE422T/*PI 578681*//Presto/3/NE95T441/4/*PI 508561*	1
B1037	F_3	PI 386154/*BFC2*//PI 386154/3/*PI 380639*	2
B1085	F_3	PI 573182/*PI 314190*//McCormick/3/*PI 314189*	3
B1089	F_3	Thunderbolt/PI 573182//*PI 314190*/3/*BFC1*	2
B1094	F_3	WD48/*PI 414667*	1
B1100	F_3	Neuse/*BFC1*	1
B1107	F_3	PI 634318/*PI 414667*//Jagger or 2137	4
B1126	F_2	Tam110/*PI 401201*//Jagger or 2137/3/PI 520054/4/*PI 401168*/5/(Tam110/*PI 401201*//Jagger or 2137)	1
B1129	F_2	PI 532505/*BFC1*//Jagger/3/PI 531193/4/*IWG120*/5/(WD46/*BFC2*//*PI 314189*)	1
B1139	F_2	PI 532506/*BFC2-19*//Karl92/3/PavonF76/4/WGRC33/5/*PI 401176*/6/(Tam110/*PI 401201*//Jagger or 2137)	1
B1146	F_2	Tam110/*PI 401201*//Jagger or 2137/3/Jagger	2
B1152	F_2	PI 573182/*BFC2-4*//*BFC2-N*/3/*PI 440048*/4/(Tam110/*PI 401201*//Jagger or 2137)	2

†*Thinopyrum* spp. are indicated by italic text in the pedigree.

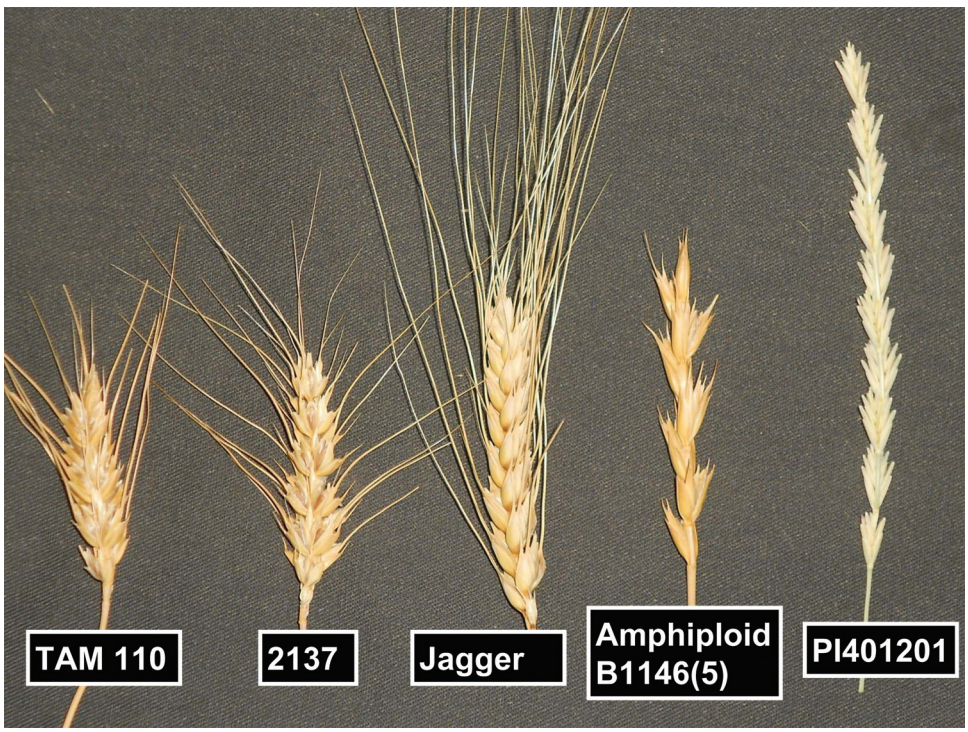

Figure 1. *Triticum aestivum* parents (TAM110, 2137, and Jagger) and *Thinopyrum intermedium* parent (PI 401201) of partial amphiploid line B1146(5).

considered susceptible. An "X" rating describes a resistant response with a mixture of infection types.

Amphiploid lines were represented by 5 to 10 seeds for each race screened. Parental lines were represented in total by 15 to 20 seeds for each race. Susceptible checks LMPG-6 and McNair 701 were used as controls in each screening. Stem rust races TTTTF, TTKSK (Ug99), and TRTTF (Yemen) were selected due to high virulence and TPMKC and MCCFC races were selected due to historic prevalence in the United States (Kolmer et al., 2009).

Fusarium Head Blight Screening and Seed Production

All lines were planted in the greenhouse in November 2009; lines producing more than five seeds per plant on average were evaluated for FHB resistance. Eight additional lines that had produced more than 100 seed per plant in past greenhouse trials in Kansas were also included.

Thirty amphiploid lines, 29 parental lines, resistant checks 'Alsen' (Frohberg et al., 2006) and 'BacUp' (Busch et al., 1998), and susceptible checks 'Roblin' (Campbell and Czarnecki, 1987), 'Wheaton' (Busch et al., 1984), and MN00269 were screened with a single isolate, Fg4, of *F. graminearum*. Plants were initially vernalized for 7 wk at 4.4°C and moved to the greenhouse. The greenhouse was maintained at 20°C with 16 h of light daily. In greenhouse trials during fall 2010 and spring 2011, five square pots (12.7 cm wide) with four plants per pot were planted in complete blocks over two planting dates, 1 wk apart. On average, 21 total heads per amphiploid line were inoculated over the two greenhouse seasons. Individual spikes were inoculated using the point inoculation technique described by Liu et al. (2006) by injecting 10 μL of inoculum at a concentration of 100,000 spores mL^{-1} into the central spikelet of one spike per plant at anthesis. The number of infected spikelets recorded 21 d after inoculation and visually scabby kernel counts measured disease severity. The percentage of infected spikelets was calculated to account for size of head. Since some lines showed partial sterility, seed set was measured in a noninoculated spike from each inoculated plant. Tests of significance were conducted by ANOVA and the LSD test ($\alpha = 0.05$) was used for mean separation. Greenhouse tests were combined based on Levene's test showing equal variance for number of infected spikelets ($p = 0.363$) but were not combined for percent infected spikelets ($p = 0.0162$) or percent visually scabby kernels (VSK) ($p = 0.0413$) due to significantly different variances. There was a significant interaction between lines and greenhouse test, but in figures we report averages across the two greenhouse tests for simplicity.

Marker Screening for Known Stem Rust Resistance Genes

Partial amphiploids and parents used in crosses were screened with molecular markers to assess whether stem rust resistance in the amphiploid lines differed from previously identified resistance genes from *Thinopyrum* spp. Lines were represented by three bulk samples of four individuals each. Young leaf tissue was harvested for DNA extraction with methods described by Riede and Anderson (1996) or by using a Biosprint 96 DNA Plant Kit 571 (QIAGEN Inc.) according to the manufacturer instructions. Polymerase chain reaction amplified DNA fragments associated with known wheatgrass resistance genes using markers *Sr24#12* and *Sr26#43*, described by Mago et al. (2005), and BF145935 for *Sr25*, described by Liu et al. (2010).

Field Evaluation

On 5 Oct. 2009, 52 amphiploid families were hill planted with five seeds per hill on 0.5 m centers in two adjacent randomized replications on the St. Paul campus of the University of Minnesota in St. Paul, MN. Winter survival was assessed on 7 Apr. 2010. Height was recorded on 5 Aug. 2010, when plants were harvested and characterized by head counts, seed counts, and seed weight. Survival and regrowth during spring 2011 was assessed on 12 April. Surviving plants were transplanted to a different field on campus 17 Apr. 2011, measured for height, and harvested again on 11 Sept. 2011. Measurements of disease incidence were recorded monthly throughout the growing season in 2010 and 2011.

Chromosome Counts

Twenty-four partial amphiploids demonstrating perenniality, potentially novel stem rust resistance, or previously uncharacterized FHB resistance were characterized genetically with chromosome counts. At least five seeds per line were germinated and root tips harvested after 3 to 5 d. Root tips were treated with N_2O to arrest meristematic cells in metaphase, based on modified methods described by Kato (1999). Root tips were placed inside a closed chamber with 1068.7 kPa N_2O in moist petri dishes for 2 h. Treated root tips were then fixed with 3:1 C_2H_6O (ethanol):CH_3CO_2H (acetic acid) at 4°C for a minimum of 24 h, stained with acetocarmine for a minimum of 72 h, and stored in 70% ethanol at 4°C. Root tip squashes were viewed with a microscope at 400× magnification. For each individual seed, five to six cells were counted to achieve consensus.

RESULTS AND DISCUSSION

Stem Rust Resistance in *Thinopyrum* Species

All 13 parental *Thinopyrum* accessions were highly resistant to all races of stem rust screened, with infection types of 0, ;, or ;1 (Fig. 2 and 3; Supplemental Table S1). Resistance to stem rust segregated (ranging from 0; to 3) in the majority (11 of 13) of wheatgrass lines (Supplemental Table S1). The difference in infection types within accessions indicates multiple resistance genes could be involved. Xu et al. (2009) identified *T. junceum* lines AJAP7 and AJAP8 with infection types ranging from 2 to 2++ for races TTTTF and TTKSK. The *T. junceum* accession PI 414667 tested in this study had a lower infection type (0; to 3) and was negative for *Sr24*, *Sr25*, and *Sr26* markers (Supplemental Table S1) suggesting these lines represent a different source of resistance.

Stem Rust Resistance in *Triticum* Species

Five of 20 parental annual wheats and triticales were resistant to all stem rust races screened (Fig. 2). These included wheat cultivars TAM 110 (Lazar et al., 1997),

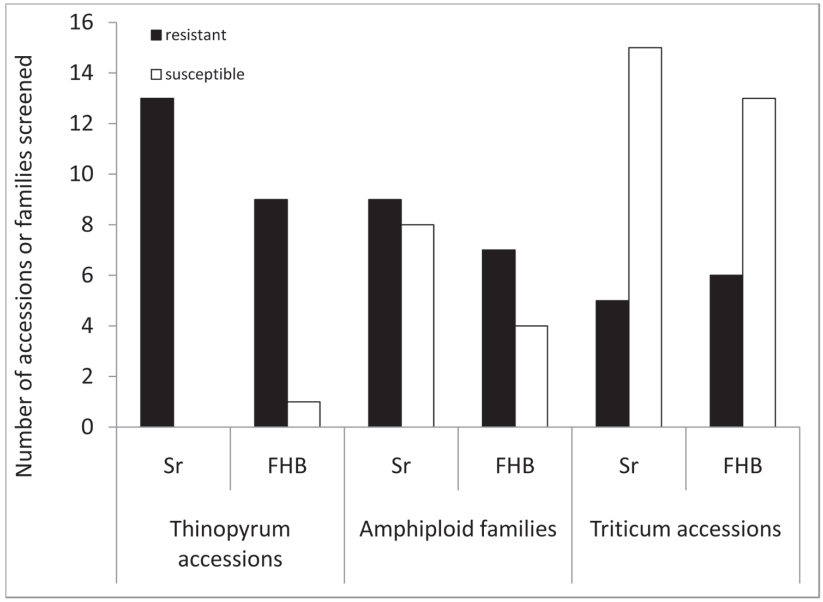

Figure 2. Number of parental accessions and amphiploid families resistant or susceptible to stem rust and Fusarium head blight (FHB). Accessions or families categorized as resistant to stem rust demonstrated resistance to all seven races used in screening. Accessions or families categorized as resistant to Fusarium head blight demonstrated resistance to all measures of severity including number of infected spikelets, percentage of infected spikelets, and percentage visually scabby kernels (VSK), with the exception of *Thinopyrum* accessions that do not include percentage of VSK kernels assessment. Susceptible lines included any line susceptible to at least one race of stem rust or one measure of FHB severity. Sr, stem rust.

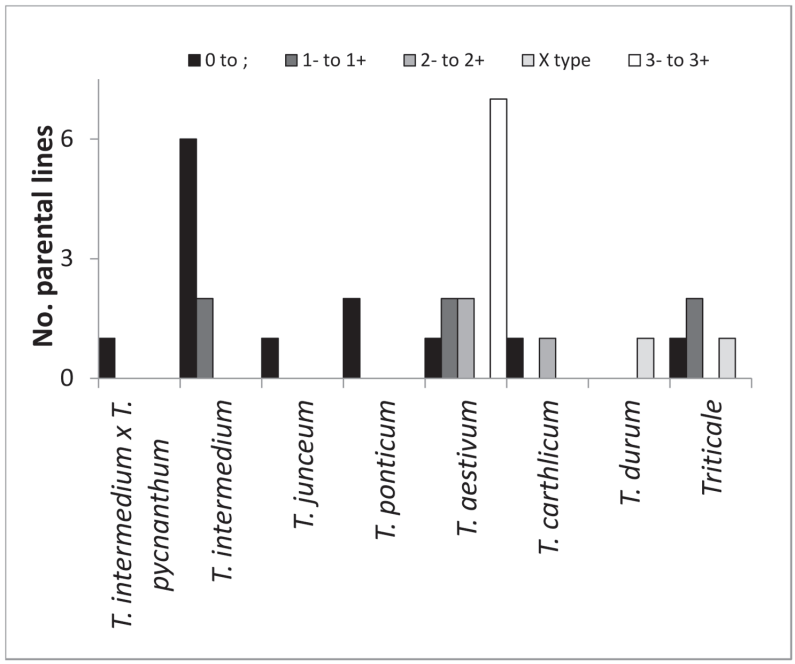

Figure 3. Number of parental lines used to create partial amphiploid lines with infection types of 0 to ;, 1- to 1+, 2- to 2+, x type, or 3- to 3+ when inoculated with stem rust race TTKSK. These infection types reflect the lowest recorded rating of multiple individuals.

KS95WGRC33, and McCormick (Griffey et al., 2005) and annual triticale lines NE95T441 and 'NET422' (Baenziger and Vogel, 2003). The Chinese winter wheat accession PI 531193 was resistant to all races screened except TTKSK and TRTTF (Supplemental Table S1). Spring wheats PI 519847 (Pavon F76) and PI 520054 (Pavon 's'), soft red winter wheat 'NC-Neuse' (Murphy et al., 2004), *T. turgidum* subsp. *carthlicum* PI 573182, Presto and PI 386154 triticales, and PI 634318 durum exhibited resistance to some races (Supplemental Table S1).

Only 3 of 23 annual wheat and triticale parents tested positive for markers for *Sr24* (McCormick) or *Sr25* (triticale lines NE95T441 and NE422T, previously characterized as moderately resistant [Baenziger and Vogel, 2003])

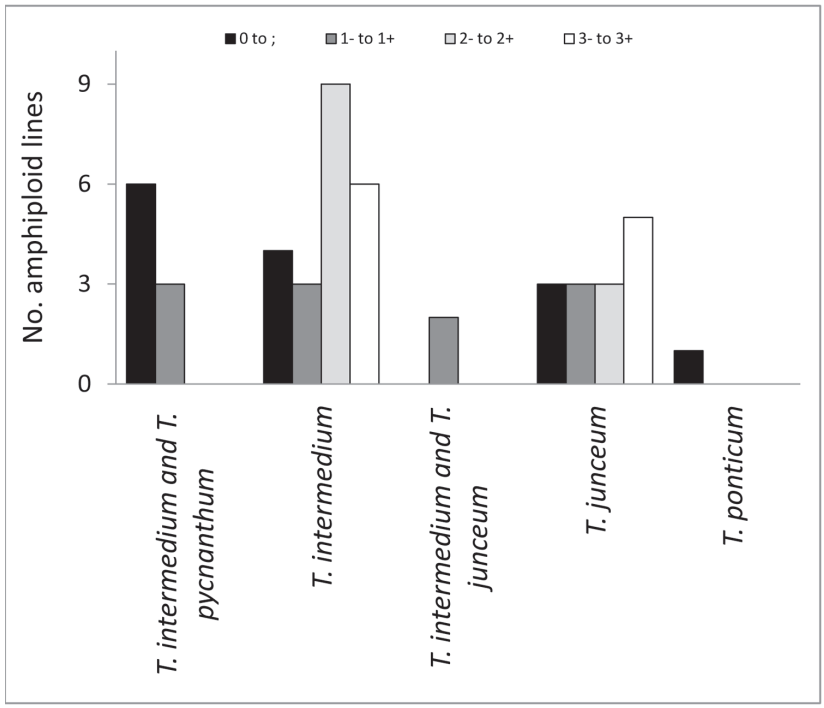

Figure 4. Number of partial amphiploid lines with infection types of 0 to ;, 1- to 1+, 2- to 2+, x type, or 3- to 3+ when inoculated with stem rust race TTKSK, grouped by *Thinopyrum* spp. present in the pedigree. These infection types reflect the lowest recorded rating of multiple individuals.

(Supplemental Table S1). Known stem rust resistance genes in McCormick include *Sr24* and *Sr1RS^{Amigo}* (Griffey et al., 2005). The gene *Sr25* has an expected infection type of 2 or 2+ when inoculated with TTKSK (Jin et al., 2007), which alone does not account for the low infection types in NE95T441 (;1 to TTKSK) and NE422T (; to TTKSK) (Supplemental Table S1). Therefore the marker for gene *Sr25* in these two lines is likely a false positive as the lines in the pedigree of NE422T have not been associated with *Sr25*. Gene *Sr36* has been identified previously in NC-Neuse (Murphy et al., 2004), explaining the observed resistance. The resistance in TAM 110 is likely contributed by a gene on chromosome 1RS (Jin and Singh, 2006). The marker data for a lack of *Sr24* (Supplemental Table S1) agreed with reported susceptibility to a leaf rust isolate avirulent on *Lr24* (Jin and Singh, 2006), suggesting that TAM 110 does not have the *Sr24* gene. No information on stem rust resistance for triticale PI 386154 was found in the literature, indicating the resistance may be uncharacterized and potentially novel.

Stem Rust Resistance in Partial Amphiploids

Thirteen of the 17 families contained one or more lines resistant to TTKSK with highly resistant ratings (0 or ;) with segregation for resistance in eight families (Supplemental Table S2). Nine of the 17 families were resistant to all races screened (Fig. 2). Of the 48 lines, 37 were resistant to TTKSK and 14 were highly resistant with ratings of ; or 0 (Fig. 4). All but two lines showed resistance to at least one race (Supplemental Table S2). None of the lines with two *Thinopyrum* spp. in their pedigree showed susceptibility (Fig. 4). *Thinopyrum intermedium* and *T. junceum* were the most prevalent *Thinopyrum* spp. in the amphiploids and showed the widest range in infection types (Fig. 4).

While almost all amphiploid lines showed resistance to stem rust, some of the resistance may be contributed by annual wheats or previously characterized genes *Sr24*, *Sr25*, and *Sr26* from wheatgrass. Three amphiploid lines may be sources of novel resistance (Table 3). In the B1107 family containing in its pedigree *T. turgidum* subsp. *durum*, *T. aestivum* ('Jagger' [Sears et al., 1997c] or '2137' [Sears et al., 1997a]), and *T. junceum*, novel resistance likely is derived from *T. junceum*. The *T. turgidum* subsp. *durum* accession PI 634318, Jagger, and 2137 are not as resistant to TTKSK, TPMKC, or TTTTF and molecular makers do not detect known stem rust resistance genes of *Thinopyrum* origin. Second, the B1089 family resembles the resistance of *T. intermedium* PI 314190, ranging in infection type from 0 to ;1- for races QTHJC, TPMKC, and TTTTF. These ratings were lower than those of the annual wheat parents Thunderbolt and PI 573182, which showed susceptibility to these races. Therefore the B1089 resistance was likely derived from the *T. intermedium* PI 314190 or the *T. intermedium* Big Flats Plant Materials Center (BFC) line developed at the Rodale Institute, which was not available for this study. Even if marker results for gene *Sr24* accurately predict the presence of the gene, there were likely additional genes contributing to resistance

Table 3. Resistance to seven stem rust races and presence of stem rust resistance gene diagnostic makers in wheat–wheatgrass partial amphiploids

Line†	Description	Stem rust infection types for races							Stem rust markers‡		
		TRTTF	TTKSK	QTHJC	MCCFC	RKQQC	TPMKC	TTTTF	*Sr24*	*Sr25*	*Sr26*
B1107(2)-14	Partial amphiploid	;1/;2-	;1	1/;	;1-	;1	0	1	–	–	–
2137	*Triticum aestivum*	4	3	3/4;	4	3	2+	3	–	–	–
Jagger	*T. aestivum*	3-	3+	;3+	2/3+	;3+	4	;3	–	–	–
PI 634318	*Triticum turgidum* subsp. *durum*	;2,2+	3+/;3+	;1-	;1-/;1	1-	3/4	3+	–	–	–
PI 414667	*Thinopyrum junceum*		0;/;2	;1-/1/3	0/1/3	0/;/3	0/;1/2/3	;1-/3	–	–	–
B1089(11)-4	Partial amphiploid	;3	;	;	0	0	0	;1-	+	–	–
Thunderbolt	*T. aestivum*	;1,3	0/0,3	3+	3	3	3	3	–	–	–
PI 573182	*Triticum turgidum* subsp. *carthlicum*	3		3+	1+	;2-	2/3	;3	–	–	–
PI 314190	*Thinopyrum intermedium*	;/2/3	0;/;2-	;/;1	;/1	;/;1	;/;1/3	;/2	+	–	–
B1016(8)	Partial amphiploid	0	;						+	+	+
PI 573182	*T. turgidum* subsp. *carthlicum*	3		3+	1+	;2-	2/3	;3	–	–	–
NE95T441	Triticale	;1-	;1	;	;	;	;	0/;1	–	+	–
NET422	Triticale	;	;	;	;	;	;	0/1-	–	+	–
Presto	Triticale	;1,3	3	;1-	1/2-/;3	;	3+	;1	–	–	–
PI 508561	*Thinopyrum ponticum*	0	0;	0/;	0/;	0/;	0/;/1	;	+	–	+
PI 578681	*T. ponticum*	0	0	0/;	0/;	0/;/1	0/1/3	0/;	+	+	+

†Amphiploid lines are designated by underline followed by *Triticum* and *Thinopyrum* parents used to generate the amphiploid.

‡Polymerase chain reaction-based marker results for stem rust resistance genes *Sr24*, *Sr 25*, and *Sr26*. "+" indicates the same band as the positive check.

other than *Sr24*, which has an expected infection type of 2 or 2- to TTKSK (Jin et al., 2007). Amphiploid B1016(8) demonstrated a third source of potentially novel resistance either from *T. ponticum* PI 508561 or PI 578681 or triticale NE422T or NE95T441, as Presto did not have resistance to TTKSK. Positive marker results for all three genes *Sr24*, *Sr25*, and *Sr26* from *T. ponticum* accessions indicate probable contribution of some resistance to B1016(8). However, the *Sr24*, *Sr25*, and *Sr26* genes alone would not account for low infection types (0 and ;) in the B1016(8) family, again indicating the presence of additional genes. The three potentially novel sources of resistance identified in this study from 13 wheatgrass accessions, indicate great potential of these species for further wheat improvement.

Fusarium Head Blight Resistance in *Thinopyrum* Species

Differences were observed among lines for number of infected spikelets, percentage of infected spikelets, and percentage VSK ($p < 0.001$). All three measures of severity (number of spikelets infected, percentage of infected spikelets, and percentage of VSK) were generally consistent across amphiploid lines indicating any of these measures alone could be used to measure resistance. Resistance based on percentage of spikelets infected was reported because it was the most discriminatory among amphiploid lines. Of the 10 *Thinopyrum* accessions screened, nine were resistant based on the number and percentage of spikelets infected (Fig. 2). Because these outcrossing *Thinopyrum* accessions produced no seed in the greenhouse, the percentage of visually scabby kernels could not be assessed. All *T. intermedium* and *T. junceum* lines were resistant to FHB with percentage of infected spikelets ranging from 9 to 38% (Fig. 5; Supplemental Table S1). Fusarium head blight resistance has been mapped in *T. ponticum* to chromosome 7E (Shen and Ohm, 2007) and identified in *T. intermedium* and *T. junceum* (Oliver et al., 2005). Amphiploid lines selected for FHB screening did not contain *T. ponticum* parents and therefore *T. ponticum* lines were not screened. *Thinopyrum* accessions in this study differed from the ones screened by Oliver et al. (2005) and may contain different resistance genes.

Fusarium Head Blight Resistance in *Triticum* Species

Six of 19 *Triticum* lines screened were resistant based on all three measures of disease severity (Fig. 2). Within each *Triticum* spp., there was a range in percentage of infected spikelets from less than 20% infected in some accessions to greater than 50% infected in other accessions, with the exception of *T. turgidum* subsp. *durum* that was only represented by one accession (Fig. 5). Lines considered resistant based on number of spikelets infected, percentage of infected spikelets, and percentage of VSK included 'Karl 92' (Sears et al., 1997b), NC-Neuse, Pavon spring wheat PI 519847, *T. turgidum* subsp. *carthlicum* lines PI 532505 and PI 573182, and triticale Presto (Supplemental Table S1). Although not statistically different from resistant checks, the percentage of infected spikelets was high in Karl 92 (40%) and NC-Neuse (44%) (Supplemental Table S1). Results were consistent with other reports of moderate FHB resistance in Karl 92 (Sears et al., 1997b) and NC-Neuse (Murphy et al., 2004) and FHB resistance in Presto (Arseniuk et al., 1999), PI 532505, PI 532506, and PI 573182 (Oliver et al., 2008). Resistance to FHB in Pavon F76 spring wheat PI 519847 was not found in the literature.

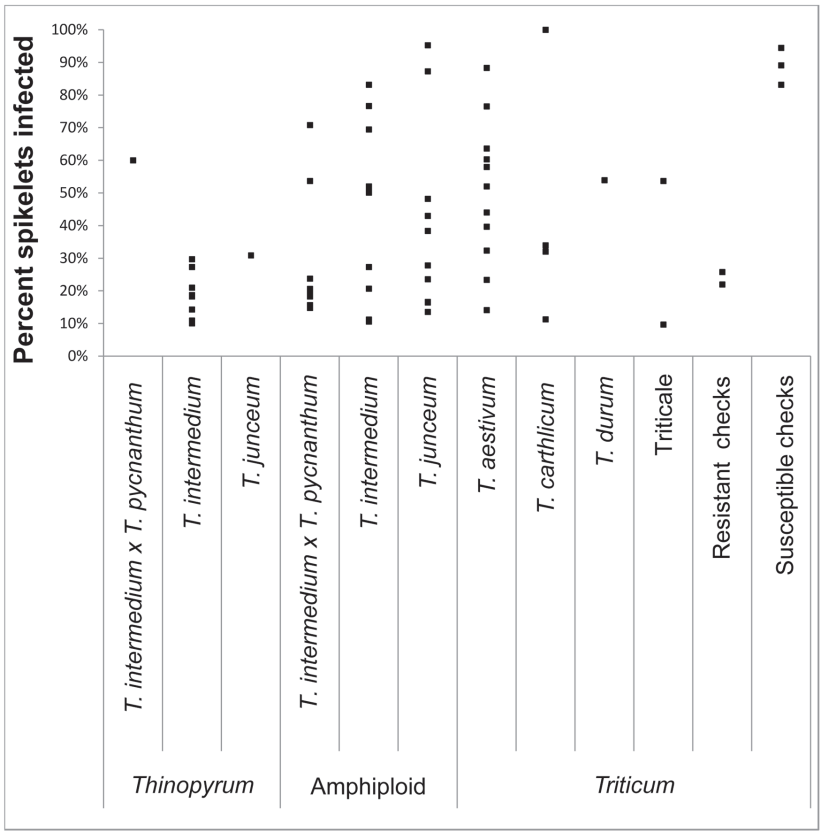

Figure 5. Percentage of infected spikelets after inoculation with *Fusarium graminearum* of parental accessions and amphiploid families. Accessions grouped by *Thinopyrum* spp. present in the pedigree. LSD(0.05) = 31%.

Fusarium Head Blight Resistance in Partial Amphiploids

Of the 30 amphiploid lines inoculated with *F. graminearum*, 18 were resistant, six intermediate, and six susceptible (Supplemental Table S2). These 30 lines represented 11 families, seven of which were resistant based on all measures (Fig. 2). Lines within a family were similar in resistance or susceptibility (Supplemental Table S2). The level and range of resistance conferred by different *Thinopyrum* spp. was similar among amphiploid lines (Fig. 5) with all three species showing lines with low infection types that could be targeted for future breeding efforts to isolate resistance genes.

Three *Thinopyrum* accessions showed previously uncharacterized resistance (Table 4; Supplemental Table S2). Resistance in the families B875 (17–48% infected), B913 (10–72%), and B1107 (8–46%) was probably at least partially derived from PI 414667 (38% in 2010 and 27% in 2011). Other parents of these crosses, *T. turgidum* subsp. *carthlicum* Do1 (100% infected), *T. turgidum* subsp. *durum* PI 634318 (62% infected in 2010 and 50% in 2011), 2137 (88%), and Jagger (48 and 76%) were susceptible or intermediate (Table 4). Additionally, *T. intermedium* PI 401201 likely contributed the majority of the resistance in amphiploid families B373 and B1146. Plant Introduction 410201 (31% infected spikelets in 2010 and 27% in 2011) had higher resistance than the susceptible wheat lines in the pedigree, 2137 (88%), Jagger (48 and 76%), and TAM110 (60%). *Thinopyrum intermedium* accession PI 401129 likely contributed the FHB resistance in B938. While *T. aestivum* PI 520054 showed low percent infection (23%), it did not show low VSK, which was observed in the amphiploid line. McCormick winter wheat is also present in the background of B938 but was not screened in this study. McCormick is moderately resistant to FHB (Griffey et al., 2005) but alone probably could not account for the highly resistant lines in the B938 family.

Wheat is most susceptible to FHB at anthesis (Osborne and Stein, 2007). Therefore, infertility could slow or prevent spread of infection in an otherwise susceptible line. Sterility was assessed by counting the number of seeds in noninoculated spikes of inoculated plants. No amphiploid lines were completely sterile, but 8 of 30 had low seed production (on average less than five seeds per spike) (Supplemental Table S3). Low fertility, however, did not prevent spread of infection. Susceptible lines with low fertility included *T. turgidum* subsp. *carthlicum* Do1 (one seed per spike on average) and winter wheat PI 531193 (four seeds per spike on average).

Agronomic Characterization

A total of 58 of the 104 perennial wheat replicated hill plots survived through their first winter from October 2009 to April 2010. Fifteen of the 52 amphiploid lines did not survive in 2010 in either replication (Supplemental

Table 4. Previously uncharacterized resistance to Fusarium head blight (FHB) in wheat–wheatgrass partial amphiploid accessions.

Line†	Pedigree or species	FHB ratings						
		Infected spikelets				Percent VSK‡		
		2010	2011	2010	2011	2010	2011	FHB rating§
		——no.——		——%——				
B875-12-1-13	Do1/PI 414667	1.2		17		17		R
B913(3)-12-7	PI 634318/PI 414667	1.9	1.7	14	21	5	2	R
B1107(2)-14	PI 634318/PI 414667//Jagger or 2137	1.2	1.5	11	17	0	3	R
PI 414667	*Thinopyrum junceum*	1.6	1.3	38	27			R
Do1	*Triticum turgidum* subsp. *carthlicum*	9	9.5	100	100	100	100	S
PI 634318 (Afuwan)	*T. turgidum* subsp. *durum*	8.4	3.9	62	50	53	41	I
2137	*Triticum aestivum*		8.8		88		91	S
Jagger	*T. aestivum*	5	5.5	48	76		56	I
B373-4-30-3-6-1-1	Tam110/PI 401201//Jagger or 2137	2.1		27		14		R
B1146(5)	Tam110/PI 401201//Jagger or 2137/3/Jagger	5.8	1	57	12	0	5	R
PI 401201	*Thinopyrum intermedium*	2.1	2	31	27			R
2137	*T. aestivum*		8.8		88		91	S
Jagger	*T. aestivum*	5	5.5	48	76		56	I
TAM 110	*T. aestivum*		3.9		60		60	I
B938(15)-12	PI 520172/PI 261099//PI 520054/3/McCormick/4/PI 401129	1.4	1.8	14	23	17	3	R
PI 261099	*T. intermedium* × *Thinopyrum pycnanthum*	3		60				I
PI 401129	*T. intermedium*	1	1.5	13	19			R
PI 520054	*T. aestivum*	3		23		74		I
PI 520172	*T. aestivum*	5.5		58		45		I

†Amphiploid lines are designated by underline followed by *Triticum* and *Thinopyrum* parents used to generate the amphiploid; the most resistant lines within each family are represented..

‡VSK, visually scabby kernels.

§Mean disease severity ratings. Fusarium head blight rating characterizes resistant (R) lines not statistically different from resistant, susceptible (S) lines not statistically different from susceptible checks, and intermediate lines (I) with statistically higher disease severity than resistant checks but lower severity than susceptible checks or inconsistent between measures of severity.

Table S3). Nine of the 15 amphiploid lines not surviving the first winter contained spring *T. aestivum*, *T. turgidum* subsp. *carthlicum*, or triticale parents, indicating that part of the observed winter kill could be attributed to lack of cold tolerance. Variability among amphiploid lines was apparent with height ranging from 31 to 135 cm and number of heads from 0 to 86 per hill plot. Thousand kernel weights (TKWs) ranged from 3 to 40 g, with a mean of 21 g. The higher TKW of the amphiploid lines were comparable to TKW of contemporary winter wheat cultivars grown in an adjacent field, ranging from 25 to 37 g (data not shown).

Of the 52 amphiploid lines planted in 2009, two partial amphiploids B1016(8) and B1146(5) (Fig. 1) demonstrated both winter hardiness and perenniality as of November 2011 (Supplemental Table S3). Line B1016(8) was agronomically poor, with large variation in height among hill plots (78 and 110 cm). The line was largely infertile, averaging 34 heads with only two seeds per plot in 2010. Line B1146(5) was comparatively high yielding with an average of 65 heads and 403 seeds per plot in 2010. Neither line produced any seed in 2011 although B1016(8) produced five heads and B1146(5) produced four heads. The production of seed only in the first year and none in the field in the second year or in greenhouse seed production trials indicate that these lines may be self-incompatible. The parents of these lines, *T. intermedium* and *T. ponticum*, are self-incompatible (Wang et al., 2003). Wind pollination in the 2010 field, with plants in 58 surviving hill plots, would have been more likely than in the 2011 field season, with only two hill plots surviving. Lack of seed production also could be attributed to linkage between sterility and perenniality, unbalanced gametes in meiosis, or environmental conditions. Lack of fertility may be expected in early generation lines due to genomic instability. Survival over multiple years was not observed in lines more advanced than the F_2 generation. Given the instability of early generation lines and loss of wheatgrass chromatin through selfing, genes conferring perenniality may be less likely to be present in advanced generations.

Genomic Stability

Genetically characterized partial amphiploid lines ranged in chromosome number from 40 to 60, with the majority of families differing in chromosome number and seed set between lines and individual plants (Fig. 6; Supplemental Table S3). Chromosome counts from F_3 and F_4 generation families varied between individuals, but the later generation F_7 B373 family were more consistent (Fig. 6; Supplemental Table S3). Lines from the B373 family had 56 chromosomes (7 individuals) and 54 (1 individual) (Fig. 7) and showed high seed set (Supplemental Table S3). This family has been independently characterized as stable in addition to other families with $2n$ = 42, 44, 52, 54, or 56 chromosomes at The Land Institute (S.

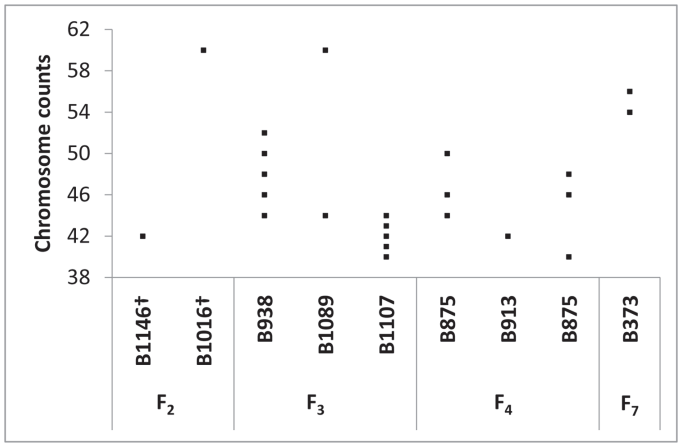

Figure 6. Chromosome counts of F_2 to F_7 amphiploid lines showing perenniality or potentially novel disease resistance. †Line demonstrated perenniality.

Wang, personal communication, 2011). The B913 family had 42 chromosomes (Fig. 7) based on six individuals counted from two lines and also showed high levels of seed production (Supplemental Table S3). These results indicate this line may be stable although the number of chromosomes and seed production would need to be confirmed over multiple generations. Fifteen of 24 lines were considered unlikely to be stable based on varying chromosome numbers among individuals, chromosome counts differing by more than two from the previously characterized stable counts of 42 or 56, and seed production of less than five seed per head. Genome instability has been attributed to the low chromosome pairing frequency of 4.6% between *Triticum* and *Thinopyrum* genomes (Chen et al., 2001). Unstable lines could be self-pollinated and selected to achieve stability for use as chromosome addition lines.

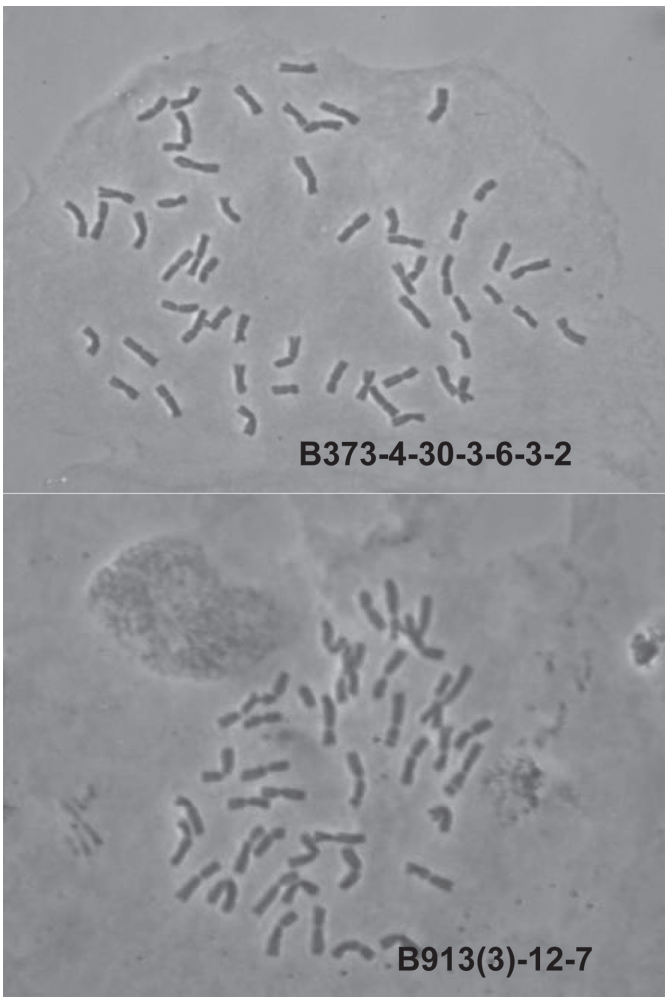

Figure 7. Mitotic chromosome squashes of potentially stable partial amphiploid lines.

CONCLUSIONS

This study identified three sources of potentially novel stem rust resistance and three previously uncharacterized accessions with FHB resistance, demonstrating the potential further utility of *Thinopyrum* spp. to benefit wheat. To use this resistance in wheat improvement, isolation of small introgression segments containing resistance genes would be necessary. A strategy similar to the one described by Niu et al. (2011) could be used by backcrossing alien lines to wheat lines containing the *ph1* mutant to induce recombination of homeologous chromosomes.

Two of 52 lines showed perenniality in Minnesota after two winters. The lack of seed production of the two surviving lines in 2011 warrants further investigation. Seven lines representing two families showed potential genetic stability. The vast majority of amphiploid lines screened were resistant to stem rust (46/48) and FHB (21/30), indicating that ample variation is present for selection during the development of perennial wheat. The two amphiploid lines exhibiting stem rust resistance and perenniality may be targeted for future perennial wheat breeding efforts for cold climates.

Supplemental Information Available

Supplemental material is available at http://www.crops.org/publications/cs.

Stem rust infection types, DNA marker profile, and Fusarium head blight (FHB) ratings of wheat and wheatgrass for parental lines (Supplemental Table S1), partial amphiploids (Supplemental Table S2), and agronomic data (Supplemental Table S3) are available in supplemental tables.

Aknowledgments

We would like to thank Bob Stupar for his insight on improving data presentation and the discussion, Shuwen Wang for contribution to the discussion, Cindy Cox for her expertise and help with the cytological analysis, and Don Wyse and Kevin Betts for assisting with field plot layout and management.

References

Arseniuk, E., E. Foremska, T. Goral, and J. Chelkowski. 1999. Fusarium head blight reactions and accumulation of deoxynivalenol (DON) and some of its derivatives in kernels of wheat, triticale and rye. J. Phytopathol. 147:577–590. doi:10.1046/j.1439-0434.1999.00433.x

Baenziger, P.S., and K.P. Vogel. 2003. Registration of 'NE422T' winter triticale. Crop Sci. 43:434–435. doi:10.2135/cropsci2003.0434

Banks, P.M., S.J. Xu, R.R.C. Wang, and P.J. Larkin. 1993. Varying chromosome composition of 56-chromosome wheat *Thinopyrum intermedium* partial amphiploids. Genome 38:395–405. doi:10.1139/g95-051

Brettel, R.I.S., E.M. Banks, Y. Cauderon, X. Chen, Z.M. Cheng, J. Larkin, and P.M. Waterhouse. 1988. A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. Ann. Appl. Biol. 113:599–603. doi:10.1111/j.1744-7348.1988.tb03337.x

Busch, R.H., D.V. McVey, G.L. Linkert, J.V. Wiersma, D.D. Warnes, R.D. Wilcoxson, R. Dill-Macky, G.A. Hareland, I. Edwards, and H.J. Schmidt. 1998. Registration of 'BacUp' wheat. Crop Sci. 38:550–551. doi:10.2135/cropsci1998.0011183X003800020073x

Busch, R.H., D.V. McVey, T. Rauch, J. Baumer, and F. Elsayed. 1984. Registration of 'Wheaton' wheat. Crop Sci. 24:622. doi:10.2135/cropsci1984.0011183X002400030054x

Campbell, A.B., and E. Czarnecki. 1987. Roblin hard red spring wheat. Can. J. Plant Sci. 67:803–804. doi:10.4141/cjps87-108

Chen, Q., R.L. Conner, A. Laroche, and F. Ahmad. 2001. Molecular cytogenetic evidence for a high level of chromosome pairing among different genomes in *Triticum aestivum-Thinopyrum intermedium* amphiploids. Theor. Appl. Genet. 102:847–852. doi:10.1007/s001220000496

Chen, Q., R.L. Conner, A. Laroche, and J.B. Thomas. 1998. Genome analysis of *Thinopyrum intermedium* and *Thinopyrum ponticum* using genomic in situ amphiploidization. Genome 41:580–586.

Cox, C.M., K.A. Garrett, T.S. Cox, W.W. Bockus, and T. Peters. 2005. Reactions of perennial grain accessions to four major cereal pathogens of the Great Plains. Plant Dis. 89:1235–1240. doi:10.1094/PD-89-1235

Culman, S.W., S.T. DuPont, J.D. Glover, D.H. Buckley, G.W. Fick, H. Ferris, and T.E. Crews. 2010. Long-term impacts of high-input annual cropping and unfertilized perennial grass production on soil properties and belowground food webs in Kansas, USA. Agric. Ecosyst. Environ. 137:13–24. doi:10.1016/j.agee.2009.11.008

Friebe, B., J. Jiang, W.J. Raupp, R.A. Mclntosh, and B.S. Gill. 1996. Characterization of wheat-alien translocations conferring resistance to diseases and pests: Current status. Euphytica 91:59–87. doi:10.1007/BF00035277

Frohberg, R.C., R.W. Stack, and M. Mergoum. 2006. Registration of 'Alsen' wheat. Crop Sci. 46:2311–2312. doi:10.2135/cropsci2005.12.0501

Glover, J.D. 2005. The necessity and possibility of perennial grain production systems. Renew. Agr. Food Syst. 20:1–4. doi:10.1079/RAF200499

Griffey, C.A., W.L. Rohrer, T.H. Pridgen, W.S. Brooks, J. Chen, J.A. Wilson, D. Nabati, D.E. Brann, E.G. Rucker, H.D. Berl, M.E. Vaughn, W.L. Sisson, T.R. Randall, R.A. Corbin, J.C. Kenner, D.W. Dunaway, R.M. Pitman, H.E. Bockelman, C. Gaines, D.L. Long, D.V. McVey, S.E. Cambron, and L. Whitcher. 2005. Registration of 'McCormick' wheat. Crop Sci. 45:417–419. doi:10.2135/cropsci2005.0417

Han, F.P., A. Fedak, K. Armstrong, and T. Ouellet. 2003. Characterization of six wheat × *Thinopyrum intermedium* derivatives by GISH, RFLP, and multicolor GISH. Genome 46:490–495. doi:10.1139/g03-032

Hayes, R.C., M.T. Newell, L.R. DeHaan, K.M. Murphy, S. Crane, M.R. Norton, L.J. Wade, M. Newberry, M. Fahim, S.S. Jones, T.S. Cox, and P.J. Larkin. 2012. Perennial cereal crops: An initial evaluation of wheat derivatives. Field Crops Res. 133:68–89. doi:10.1016/j.fcr.2012.03.014

Jin, Y., and R. Singh. 2006. Resistance to recent eastern African stem rust isolates with virulence to *Sr31* in U.S. wheat. Plant Dis. 90:476–480. doi:10.1094/PD-90-0476

Jin, Y., R.P. Singh, R.W. Ward, R. Wanyera, M. Kinyua, P. Njau, T. Fetch, Z.A. Pretorius, and A. Yahyaoui. 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. doi:10.1094/PDIS-91-9-1096

Kato, A. 1999. Air drying method using nitrous oxide for chromosome counting in maize. Biotech. Histochem. 74:160–166. doi:10.3109/10520299909047968

Kibirige Sebunya, I., and D.R. Knott. 1983. Transfer of stem rust resistance to wheat from an *Agropyron* chromosome having a gametocidal effect. Can. J. Genet. Cytol. 25:215–221.

Knott, D.R. 1961. The inheritance of rust resistance. VI. The transfer of stem rust resistance from *Agropyron elongatum* to common wheat. Can. J. Plant Sci. 41:109–123. doi:10.4141/cjps61-014

Kolmer, J.A., X. Chen, and Y. Jin. 2009. Diseases which challenge global wheat production - The cereal rusts. In: B.F. Carver, editor. Wheat: Science and trade. Wiley Press, Hoboken, NJ. p. 84–124.

Lammer, D., X. Cai, M. Arterburn, J. Chatelain, T. Murray, and S. Jones. 2004. A single chromosome addition from *Thinopyrum elongatum* confers a polycarpic, perennial habit to annual wheat. J. Exp. Bot. 55:1715–1720. doi:10.1093/jxb/erh209

Lazar, M.D., W.D. Worrall, G.L. Peterson, K.B. Porter, L.W. Rooney, N.A. Tuleen, D.S. Marshall, M.E. McDaniel, and L.R. Nelson. 1997. Registration of TAM110. Crop Sci. 37:1978–1979. doi:10.2135/cropsci1997.0011183X003700060055x

Li, H., Q. Chen, R.L. Conner, B. Guo, Y. Zhang, R.J. Graf, A. Laroche, X. Jia, G. Liu, and C. Chu. 2003. Molecular characterization of a wheat-*Thinopyrum ponticum* partial amphiploid and its derivatives for resistance to leaf rust. Genome 46:906–913. doi:10.1139/g03-053

Liu, S., X. Zhang, M.O. Pumphrey, R.W. Stack, B.S. Gill, and J.A. Anderson. 2006. Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Funct. Integr. Genomics 6:83–89. doi:10.1007/s10142-005-0007-y

Liu, S., L. Yu, R.P. Singh, Y. Jin, M.E. Sorrells, and J.A. Anderson. 2010. Diagnostic and co-dominant PCR markers for wheat stem rust resistance genes *Sr25* and *Sr26*. Theor. Appl. Genet. 120:691–697. doi:10.1007/s00122-009-1186-z

Mago, R., H.S. Bariana, I.S. Dundas, W. Spielmeyer, G.J. Lawrence, A.J. Pryor, and J.G. Ellis. 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. doi:10.1007/s00122-005-2039-z

McIntosh, R.A., P.L. Dyck, and G.J. Green. 1977. Inheritance of leaf rest and stem rust resistance in wheat cultivars Agent and Agatha. Aust. J. Agric. Res. 28:37–45. doi:10.1071/AR9770037

Murphy, J.P., R.A. Navarro, S. Leath, D.T. Bowman, P.R. Weisz, L.G. Ambrose, M.H. Pate, and M.O. Fountain. 2004. Registration of 'NC-Neuse' wheat. Crop Sci. 44:1479–1480. doi:10.2135/cropsci2004.1479

Nganje, W.E., D.A. Bangsund, F.L. Leistritz, W.W. Wilson, and N.M. Tiapo. 2004. Regional economic impacts of Fusarium head blight in wheat and barley. Rev. Agr. Econ. 26:332–347. doi:10.1111/j.1467-9353.2004.00183.x

Nieto-Lopez, R.M., C. Soler, and P. Garcia. 2003. Genetic diversity in wild Spanish populations of *Thinopyrum junceum* and *Thinopyrum junceiforme* using endosperm proteins and PCR-based markers. Hereditas 139:18–27. doi:10.1111/j.1601-5223.2003.01662.x

Niu, Z., D.L. Klindworth, T.L. Friesen, S. Chao, Y. Jin, X. Cai, and S.S. Xu. 2011. Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics 187:1011–1021. doi:10.1534/genetics.110.123588

Oliver, R.E., X. Cai, T.L. Friesen, S. Halley, R.W. Stack, and S.S. Xu. 2008. Evaluation of Fusarium head blight resistance in tetraploid wheat (*Triticum turgidum* L.). Crop Sci. 48:213–222. doi:10.2135/cropsci2007.03.0129

Oliver, R.E., X. Cai, S.S. Xu, X. Chen, and R.W. Stack. 2005. Wheat-alien species derivatives: A novel source of resistance to Fusarium head blight in wheat. Crop Sci. 45:1353–1360. doi:10.2135/cropsci2004.0503

Osborne, L.E., and J.M. Stein. 2007. Epidemiology of Fusarium head blight on small-grain cereals. Int. J. Food Microbiol. 119:103–108. doi:10.1016/j.ijfoodmicro.2007.07.032

Refoufi, A., J. Jahier, and M.A. Esnault. 2001. Genome analysis of *Elytrigia pycnantha* and *Thinopyrum junceiforme* and of their putative natural amphiploid using the GISH technique. Genome 44:708–715.

Riede, C.R., and J.A. Anderson. 1996. Linkage of RFLP markers to an aluminium tolerance gene in wheat. Crop Sci. 36:905–909. doi:10.2135/cropsci1996.0011183X0036000400015x

Roelfs, A.P. 1988. Genetic control of phenotypes in wheat stem rust. Annu. Rev. Phytopathol. 26:351–367. doi:10.1146/annurev.py.26.090188.002031

Scheinost, P.L., D.L. Lammer, X. Cai, T.D. Murray, and S.S. Jones. 2001. Perennial wheat: The development of a sustainable cropping system for the U.S., Pacific Northwest. Am. J. Alternative Agric. 16:147–151. doi:10.1017/S0889189300009115

Sears, R.G.., T.J. Martin, T.S. Cox, R.K. Bequette, S.P. Curran, O.K. Chung, W.F. Heer, J.H. Long, and M.D. Witt. 1997a. Registration of '2137' wheat. Crop Sci. 37:628.

Sears, R.G., T.J. Martin, T.S. Cox, O.K. Chung, S.P. Curran, W.F. Heer, and M.D. Witt. 1997b. Registration of Karl 92 wheat. Crop Sci. 37:628.

Sears, R.G., J.M. Moffatt, T.J. Martin, T.S. Cox, R.K. Bequette, S.P. Curran, O.K. Chung, W.F. Heer, J.H. Long, and M.D. Witt. 1997c. Registration of 'Jagger' wheat. Crop Sci. 37:1010. doi:10.2135/cropsci1997.0011183X003700030062x

Sepsi, A., I. Molnar, D. Szalay, and M. Molnar-Lang. 2008. Characterization of a leaf rust-resistant wheat-*Thinopyrum ponticum* partial amphiploid BE-1, using sequential multicolor GISH and FISH. Theor. Appl. Genet. 116:825–834. doi:10.1007/s00122-008-0716-4

Shen, X., and H. Ohm. 2006. Fusarium head blight resistance derived from *Lophopyrum elongatum* chromosome 7E and its augmentation with Fhb1 in wheat. Plant Breed. 125:424–429. doi:10.1111/j.1439-0523.2006.01274.x

Shen, X., and H. Ohm. 2007. Molecular mapping of *Thinopyrum*-derived Fusarium head blight resistance in common wheat. Mol. Breed. 20:131–140. doi:10.1007/s11032-007-9079-9

Singh, R.P., D.P. Hodson, Y. Jin, J. Huerta-Espino, and M.G. Kinyua. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Res. 54:1–13.

Stakman, E.C., W.Q. Loegering, and D.M. Stewart. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritic*. Minnesota Agric. Exp. Stn., St. Paul, MN.

Tsitsin, N.V., and V.F. Lubimova. 1959. New species and forms of cereals derived from amphiploidization between wheat and couch grass. Am. Nat. 93:181–191. doi:10.1086/282073

Turner, R.E., and N.N. Rabalais. 2003. Linking landscape and water quality in the Mississippi river basin for 200 years. Bioscience 53:563–572. doi:10.1641/0006-3568(2003)053[0563:LLAWQI]2.0.CO;2

Wang, Z.Y., J. Bell, and A. Hopkins. 2003. Establishment of a plant regeneration system for wheatgrasses (*Thinopyrum*, *Agropyron* and *Pascopyrum*). Plant Cell Tissue Organ Cult. 73:265. doi:10.1023/A:1023044913155

Xu, S.S., Y. Jin, D.L. Klindworth, R.R.-C. Wang, and X. Cai. 2009. Evaluation and characterization of seedling resistances to stem rust Ug99 races in wheat-alien species derivatives. Crop Sci. 49:2167–2175. doi:10.2135/cropsci2009.02.0074

Zhang, W.J., A.J. Lukaszewski, J. Kolmer, M.A. Soria, S. Goyal, and J. Dubcovsky. 2005. Molecular characterization of durum and common wheat recombinant lines carrying leaf rust resistance (*Lr19*) and yellow pigment (Y) genes from *Lophopyrum ponticum*. Theor. Appl. Genet. 111:573–582. doi:10.1007/s00122-005-2048-y