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RESEARCH

Characterization of *Thinopyrum* Species for Wheat Stem Rust Resistance and Ploidy Level

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ABSTRACT

In the tribe Triticeae, several *Thinopyrum* species have been used as sources of resistance to stem rust (caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., abbreviated as *Pgt*) and other wheat (*Triticum aestivum* L.) diseases. To identify novel sources of resistance to *Pgt* race TTKSK (Ug99), we evaluated and characterized the stem rust resistance of 242 accessions belonging to five *Thinopyrum* species, including beach wheatgrass [*Th. bessarabicum* (Savul. & Rayss) A. Löve], diploid tall wheatgrass [*Th. elongatum* (Host) D.R. Dewey], intermediate wheatgrass [*Th. intermedium* (Host) Barkworth & D. R. Dewey], sand cough or sea wheatgrass [*Th. junceum* (L.) A. Löve], and decaploid tall wheatgrass [*Th. ponticum* (Podp.) Barkworth & D.R. Dewey]. These accessions were evaluated for seedling reactions to nine *Pgt* races (TTKSK, TTTTF, TRTTF, RTQQC, QFCSC, TCMJC, TPMKC, TMLKC, and TPPKC), genotyped with molecular markers linked to four stem rust resistance genes (*Sr24*, *Sr25*, *Sr26*, and *Sr43*) derived from *Thinopyrum* species, and examined for ploidy levels. All accessions but one (*Th. elongatum* PI 531718) were resistant to all or most races. Most of the *Th. elongatum* and *Th. ponticum* accessions showed near-immunity to all of the races while the accessions of the other three species (*Th. bessarabicum*, *Th. intermedium*, and *Th. junceum*) had varied levels of resistance ranging from near immunity to moderate resistance. Molecular marker analysis showed that most of the markers appeared to be species- or genus-specific rather than linked to a gene of interest, and thus genotyping analysis was of limited value. Comparisons of infection types of accessions based on ploidy level suggested that higher ploidy level was associated with higher levels of stem rust resistance. The results from this study substantiate that the *Thinopyrum* species are a rich source of stem rust resistance.

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Abbreviations: IT, infection type; PCR, polymerase chain reaction; *Pgt, Puccinia graminis* f. sp. *tritici*.

A major threat to wheat production has been the emergence of highly virulent races of the stem rust pathogen *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*). The most notable new *Pgt* race has been TTKSK (Ug99), with virulence to stem rust resistance (*Sr*) gene *Sr31* (Jin and Singh, 2006). TTKSK is part of a lineage of new, highly virulent races (Visser et al., 2011), with variant races TTTSK (Jin et al., 2009) and TTKST (Jin et al., 2008), virulent on *Sr36* and *Sr24*, respectively, being particularly worrisome. Additional races that are not part of the Ug99 lineage also pose risk. Race TRTTF was identified in Yemen in 2006 and it possesses virulence on four TTKSK-effective resistance genes, including *Sr13*, *Sr36*, *SrTmp*, and *Sr1RS^{Amigo}* carried

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by the wheat-rye (*Secale cereale* L.) 1AL·1RS translocation (Olivera et al., 2012). Identified from the United States in 2003, race TTTTF (Jin, 2005) is broadly virulent against *Sr* genes in the North American stem rust differential set.

In the tribe Triticeae, several wheatgrass species within the *Thinopyrum* genus have been used as sources of resistance to wheat stem rust. Five stem rust resistance genes have been derived from *Thinopyrum*. Intermediate wheatgrass [*Th. intermedium* (Host) Barkworth & D. R. Dewey, $2n = 6x = 42$, genomes $EEE^{st}E^{st}StSt$; syn. *Agropyron intermedium* (Host) P. Beauv, *Elytrigia intermedia* (Host) Nevski] was the source of *Sr44*, which has recently been transferred to a Robertsonian T7DL·7J#1S translocation chromosome (Liu et al., 2013). Decaploid tall wheatgrass [*Th. ponticum* (Podp.) Barkworth & D.R. Dewey, $2n = 10x = 70$, $EEEEEEE^{st}E^{st}E^{st}E^{st}$ or $EEEEEEStStStSt$; syn. *Agropyron elongatum* (Host) P. Beauv., *Elytrigia pontica* (Podp.) Holub, *Lophopyrum ponticum* (Podp.) A. Löve], was the source of *Sr24*, *Sr25*, *Sr26*, and *Sr43* (Kim et al., 1993; McIntosh et al., 1977; Niu et al., 2014). The gene *Sr24* is carried on a T3DS·3DL-3Ae#1L translocation chromosome (Friebe et al., 1996), although *Sr24* is also found on a noncompensating 1BL·1BS-3Ae translocation chromosome (Jiang et al., 1994; Mago et al., 2005; Olson et al., 2010). The *Sr25* gene is located on a T7DS·7DL-7Ae#1L translocation chromosome (Friebe et al., 1996) that originally conditioned yellow flour pigmentation, but pigment expression was partially reduced by ethyl methanesulfonate treatment (Knott, 1984). The *Sr26* gene is carried on a T6AS·6AL-A6Ae#1L translocation chromosome (Friebe et al., 1996). The original T6AS·6AL-A6Ae#1L translocation chromosome had reduced yield, but subsequent chromosome engineering produced a wheat line with a shortened alien segment and no yield penalty (Mago et al., 2011). Using the original *Sr43* translocation line KS10-2 (Kim et al., 1993), Niu et al. (2014) used chromosome engineering to produce lines carrying *Sr43* on a shortened T7DS·7DL-7AeL translocation chromosome. Like *Sr25*, *Sr43* is linked to a gene for yellow flour pigmentation.

In addition to *Th. intermedium* and *Th. ponticum*, several other *Thinopyrum* species have also been hybridized with wheat (see Wang, 2011). These include *Th. bessarabicum* (Savul. & Rayss) A. Löve (2n = 2x = 14, JJ or E^bE^b; syn. *Agropyron bessarabicum* Savul. & Rayss) (Zhang et al., 2002), *Th. elongatum* (Host) D.R. Dewey (2n = 2x = 14, EE or J^eJ^e; syn. *Agropyron elongatum* (Host) P. Beauv, *Triticum elongatum* Host, *Lophopyrum elongatum* (Host) A. Löve) (Dvořák and Knott, 1974; Jauhar et al., 2009), *Th. junceum* (L.) A. Löve ($2n = 6x = 42$, JJJJEE or $E^bE^bE^bE^bE^eE^e$; syn. *Agropyron junceum* (L.) P. Beauv.) (Charpentier, 1992), and *Th. junceiforme* (A. Löve & D. Löve) A. Löve ($2n = 4x = 28$; JJEE or $E^bE^bE^eE^e$; syn. *Agropyron junceum* ssp. *boreoatlandicum* Simonet & Guinochet) (Ellneskog-Staam and A. Merker, 2002; Jauhar and Peterson, 2001). Several wheat-*Thinopyrum* species derivatives, including partial amphiploids (Turner et al., 2013; Xu et al., 2009; Zeng et al., 2013) and chromosome addition lines (Xu et al., 2009), were identified to be highly resistant to races in the Ug99 race group of stem rust. However, the wheat-*Thinopyrum* species derivatives that are currently available were derived from only a few genotypes of these *Thinopyrum* species, most of the accessions of the *Thinopyrum* species have not been characterized for their resistance to stem rust.

In the present study, our objective was to identify novel sources of stem rust resistance in accessions belonging to five *Thinopyrum* species (*Th. bessarabicum*, *Th. elongatum*, *Th. intermedium*, *Th. junceum*, and *Th. ponticum*) maintained at the USDA-ARS Western Regional Plant Introduction Station. To achieve this objective, we evaluated accessions against nine *Pgt* races of stem rust including TTKSK, TRTTF, TTTTF, and six additional local races and then we genotyped the accessions using the available molecular markers associated with *Sr* genes derived from *Th. ponticum*.

MATERIALS AND METHODS

Plant Materials

The seed samples used in this study, including 4 *Th. bessarabicum*, 61 *Th. elongatum*, 155 *Th. intermedium*, 14 *Th. junceum*, and 8 *Th. ponticum* accessions, were provided by USDA-ARS Western Regional Plant Introduction Station, Pullman, WA (Supplemental Table S1). The detailed description of these accessions are available through the online database for species records of *Thinopyrum* (USDA, ARS, National Genetic Resources Program, 2013). Wheat cultivar 'Chinese Spring' and line LMPG-6 were used as the susceptible checks in the stem rust test and the negative checks in the molecular marker analysis. Wheat cultivar 'Wheatear' with *Sr25* (Liu et al., 2010) and three wheat lines BtSr24Ag (PI 520490; http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1415426, accessed 1 May 2014), WA1 (Dundas et al., 2007), and RWG34 (Niu et al., 2014) carrying *Sr24*, *Sr26*, and *Sr43*, respectively, were used as the positive checks in the molecular marker analysis. The original seed of BtSr24Ag were provided by Dr. Harold Bockelman, National Small Grains Collection, USDA-ARS, Aberdeen, ID and the seed of Wheatear and WA1 were provided by Dr. Scott D. Haley, Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO. RWG34 (Niu et al., 2014) is a wheat line carrying *Sr43* on a shortened *Th. ponticum* chromosome segment derived from the wheat-*Th. ponticum* translocation line KS10-2 (Kim et al., 1993), which was recently verified to carry a pair of $7DS\text{-}7el_2S\cdot7el_2L$ translocation chromosomes (Niu et al., 2014).

Stem Rust Resistance Evaluation

These accessions were inoculated with nine *Pgt* races (RTQQC, QFCSC, TCMJC, TPMKC, TMLKC, TPPKC, TTTTF, TRTTF, and TTKSK) at the 1-leaf stage, 10 to 14 d post planting. The avirulence–virulence profiles of these races on the North America differentials are summarized in Table 1. Races TTTTF, TRTTF, and TTKSK were evaluated at the USDA Cereal Disease Lab, St. Paul, MN, while the remaining races

Table 1. Avirulence and virulence on the North American differentials for nine races of *Puccinia graminis* f. sp. *tritici*.

Pgt race/isolate	Avirulent	Virulent
QFCSC/370C	6 7b 9b 9e 11 24 30 31 36 38 Tmp	5 8a 9a 9d 9g 10 17 21 McN
RTQQC/72.00	9e 10 17 24 30 31 38 Tmp	5 6 7b 8a 9a 9b 9d 9g 11 21 36 McN
TCMJC/A-21	6 8a 9a 9b 11 24 30 31 38 Tmp	5 7b 9d 9e 9g 10 17 21 36 McN
TMLKC/72.41sp1	6 8a 9a 9b 17 24 30 31 38	5 7b 9d 9e 9g 10 11 21 36 McN Tmp
TPMKC/TNMK	6 9a 9b 24 30 31 38	5 7b 8a 9d 9e 9g 10 11 17 21 36 McN Tmp
TPPKC/81AC-46(2)	6 9a 9b 24 31 38	5 7b 8a 9d 9e 9g 10 11 17 21 30 36 McN Tmp
TRTTF/06YEM34-1	8a 24 31	5 6 7b 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp
TTTTF/01MN84A-1-2	24 31	5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp
TTKSK/04KEN156/04	24 36 Tmp	5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 31 38 McN

were evaluated at the USDA Red River Valley Agricultural Research Center, Fargo, ND using the procedures as described by Jin et al. (2007) and Williams et al. (1992), respectively. The primary leaves of plants were scored 12 to 14 d postinoculation using the 0 to 4 scale of Stakman et al. (1962). In this scoring system, plants scored as infection type (IT) 0, ; (fleck), 1, 2, or combination thereof, are considered resistant, and plants scored as 3 or 4 are considered susceptible. For leaves exhibiting a combination of ITs, order indicated the predominant type; hence an IT 23 would have predominant IT 2 with decreasing amounts of IT 3. The plus (+) and minus (−) signs were used to indicate pustules larger or smaller, respectively, within each class. The letter C indicated that infected leaves had more chlorosis than normally observed. The letter N indicated leaf necrosis. The slash (/) symbol was used to indicate heterogeneity within an accession, with the predominant type listed first. Hence a 2/34 indicated that plants with IT 2 were predominant to plants with IT 34. For each accession–race, five to six primary leaves were scored.

Chromosome Number Analysis

To analyze chromosome number and ploidy level of the *Thinopyrum* accessions, we prepared chromosome spreads following the procedures of Kato et al. (2006) and Han and Lv (2013) with some minor modification. The seeds were germinated on moist filter paper at 23°C for 48 h. Root tips of 1 to 2 cm length were collected and pretreated with nitrous oxide (N_2O) at 10 atm pressures for 2 h in a gas chamber, as described by Kato et al. (2006). The root tips were then fixed in 90% acetic acid for 10 min and washed three times with distilled water. The apical meristem of the root tips were collected in 0.5 mL microcentrifuge tube and treated with a mixture of 1% pectinase (Yakult Pharmaceutical, Tokyo, Japan) and 2% cellulase (Yakult Pharmaceutical, Tokyo, Japan) for 45 to 50 min. Then the root sections were washed three times with 75% ethanol and crushed by steel needles to form a suspension solution of single cells. After centrifugation at 4000 × *g* for 3 min, the cell pellet was washed with 75% ethanol and resuspended in 100% acetic acid. About 8 μL of cell suspension solutions were dropped onto a clean slide. After the slide was air dried, the root cells were examined under an Olympus BX53 phase contrast microscope. A minimum of 15 metaphase cells were observed for each accession. On the basis of chromosome number, accessions were classified as diploid (2*x*), tetraploid (4*x*), hexaploid (6*x*), octoploid (8*x*), nonuploid (9*x*), or decaploid (10*x*). Accessions expressing aneuploidy were classified on the basis of clustering of chromosome number around $2n = 42, 56, 63$, or 70.

Molecular Marker Analysis

For preparation of DNA samples for molecular marker analysis, five seeds from each accession were planted in super-cell cones (Stuewe and Sons, Inc., Corvallis, OR) filled with Sunshine SB100 Mix (Sun Gro Horticulture Distribution Inc., Bellevue, WA) fertilized with Osmocote Plus 15–19–12 (Scotts Sierra Horticultural Product Company, Marysville, OH). When most of the plants had grown to 2-tiller stage, the leaf tissues were collected. For a few of the accessions with no or poor germination, five seeds from each of the accessions were first ground using a hammer to produce a coarse grind. The leaf or ground seed samples were placed in 96 well plates with the addition of a 3-mm tungsten carbide bead and samples were ground to a fine powder on a MM300 shaker (Retsch, Haan, Germany) as described in Niu et al. (2011). The remainder of the extraction process followed Niu et al. (2011).

Marker genotyping was performed using six polymerase-chain-reaction (PCR) based markers, including Sr24#12 and *Xbarc71*, which detect *Sr24*, Gb and BF145935, which detect *Sr25,* Sr26#43, which detects *Sr26,* and *Xcfa2040*, which detects *Sr43* (Ayala-Navarrete et al., 2007; Mago et al., 2005; Niu et al., 2014; Yu et al., 2010). All PCR was performed using the procedures described by Röder et al. (1998) with modification. A volume of 15 μL reaction mixture per PCR included 1× green GoTaq buffer with 1.5 mM $MgCl_2$ (Promega Corporation, Madison, WI), 0.08 mM each dNTP, 400 nM each forward and reverse primer, 1 unit *Taq* polymerase, and 100 ng DNA template. The PCR was conducted using the following profile: one cycle of 94°C for 4 min, 35 cycles of 94°C for 30 sec, 55°C for annealing for 45 sec, 72°C for extension for 45 sec, and one cycle of 72°C for final extension for 10 min. The PCR products were separated on 6% nondenaturing gels, stained with GelRed (Biotium), and visualized using a Typhoon 9410 scanner (GE Healthcare Biosciences, Pittsburgh, NJ).

RESULTS AND DISCUSSION

A total of 242 accessions of *Th. bessarabicum*, *Th. elongatum*, *Th. intermedium*, *Th. junceum*, and *Th. ponticum* were evaluated for seedling resistance to nine *Pgt* races (RTQQC, QFCSC, TCMJC, TPMKC TMLKC, TPPKC, TTTTF, TRTTF, and TTKSK), genotyped with molecular markers linked to four *Sr* genes (*Sr24*, *Sr25*, *Sr26*, and *Sr43*) from *Thinopyrum* species, and cytologically examined for their ploidy levels and chromosome number variations. Due to germination problems, only 241, 234, and 208

accessions were eventually scored from the stem rust test, genotyped with the markers, and determined for ploidy levels and chromosome number, respectively. Results from illustrative lines for stem rust infection type, ploidy levels, and molecular marker genotypes are summarized in Table 2. Complete results for stem rust evaluation and chromosome and marker analysis are shown in Supplemental Tables S1 and S2, respectively.

Variability in Ploidy Level and Chromosome Number of *Thinopyrum* Species

There was a wide range of ploidy levels, from diploid through decaploid, among the *Thinopyrum* species (Table 2). Except for *Th. intermedium* with 153 accessions examined, all being hexaploid, the other four species all had variability in ploidy levels within species. Among the accessions examined, three and one *Th. bessarabicum* accessions were diploid and decaploid, respectively; two and six *Th. junceum* accessions were tetraploid and hexaploid, respectively; and one and six *Th. ponticum* accessions were tetraploid and decaploid, respectively. The ploidy level was even more variable among *Th. elongatum* accessions, which were hexaploid, octoploid, nonuploid, decaploid, or mixed ploidy levels. Schulz-Schaeffer et al. (1971) reported diploidy, tetraploidy, hexaploidy, octoploidy and decaploidy in *Th. elongatum* distributed in Turkey. The different ploidy levels detected within a species in the collections in this study probably resulted from cross pollination and misclassification. Cross pollination between plants with different ploidy levels could certainly generate new plants with different ploidy levels. For instance, nonuploid plants could be derived from cross pollination between octoploid and decaploid plants. The taxonomy of the various wheatgrass species has been a topic of controversy (Wang, 2011). In the past, the species *Th. elongatum* included both diploid and decaploid types. It is now widely accepted that *Th. bessarabicum* and *Th. elongatum* are diploid, *Th. intermedium* and *Th. junceum* are hexaploid, and *Th. ponticum* is decaploid (Wang, 2011). Thus, the taxonomy of the decaploid (W6 21890) accession in *Th. bessarabicum*, tetraploid accessions in *Th. junceum*, and hexaploid accession in *Th. ponticum* and all the *Th. elongatum* accessions examined in this study should be reexamined.

Chromosome counting revealed that some individuals with the same polyploidy level had a fixed number of chromosomes. For instance, the two tetraploids of *Th. junceum* (PI 414667 and PI 636522) and the tetraploid (PI 383583) from *Th. ponticum* all had 28 chromosomes. The two octoploids of *Th. elongatum* (PI 205279 and PI 401119) had 56 chromosomes, suggesting that these accessions consisted of stable euploid plants. In contrast, some polyploid accessions had both euploid plants and aneuploid plants, which formed a mixed population with a variable number of chromosomes. This phenomenon was most commonly observed in nonuploid and decaploid plants. We identified more aneuploid plants than euploid plants among these two polyploidy levels. For instance, 80% of nonuploid accessions in *Th. elongatum* and >70% of decaploid accessions in *Th. ponticum* were aneuploid. In contrast, aneuploidy was observed in only 62 of the 153 (40.5%) hexaploid accessions of *Th. intermedium*. We speculate that the high frequencies of aneuploids in the polyploid species were mainly the result of cross pollination, asexual reproduction, and perennialism. The plants that reproduce asexually tend to have variable chromosome numbers. Although a relationship between aneuploidy and perennialism has not been established in plants, we believe that perennialism could increase the frequency of aneuploids by increasing the frequency of nondisjunction of chromosomes in both mitotic and meiotic divisions due to long life span.

Among the 207 accessions examined, the chromosome number has been documented for 175 of them (http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?W6, accessed 18 Aug. 2014). Although previous studies did not report variations in chromosome numbers in polyploid accessions, comparison of the previously reported results with those of this study showed that most of the polyploid accessions had consistent ploidy levels. However, we found that *Th. elongatum*, which showed the most variable ploidy levels, had four accessions with different ploidy levels from those reported previously. That is, PI 179162 was reported to have 56 chromosomes in previous reports, whereas our results revealed that the accession has 58 to 66 chromosomes. Because the *Thinopyrum* genus has a basic chromosome number of 7, we postulated that this accession was nonuploid. In addition, PI 179169 was reported to contain 70 chromosomes, whereas our results revealed that it has 56 to 66 chromosomes. Therefore, this accession might have nonuploids as well as octoploids. PI 276709 was previously reported to contain 70 chromosomes; however, we found that it has two types among the eight plants examined: one with 60 to 64 chromosomes and the other with 68 to 70 chromosomes. This finding suggested that this accession consisted of both nonuploid and decaploid plants. In a previous study, PI 401007 was reported to have 56 chromosomes, whereas among the nine plants examined in our study, only one had 56 chromosomes and all of the others had chromosome numbers ranging from 68 to 77. Therefore, we postulated that PI 401007 was a mixed population of both octoploid and decaploid plants.

Seedling Reactions to Stem Rust

Accessions representative of the ITs observed in the populations are shown in Table 2. Across *Thinopyrum* species, all accessions except one were resistant to all or most of the *Pgt* races. Susceptible plants were observed in *Th. elongatum* accession PI 531718 tested with seven of the nine *Pgt* races. Heterogeneity for stem rust infection types was common

Table 2. Seeding reactions to nine races of *Puccinia graminis* f. sp. *tritici* and marker genotypes of illustrative accessions of the five *Thinopyrum* species.

Species and accession†	Ploidy level‡	Infection types to races§									Genotypes of markers¶					
		QFCSC	RTQQC	TPPKC	TMLKC	TCMJC	TPMKC	TTTTF	TTKSK	TRTTF	*Xbarc71*	Sr24#12	BF145935	Gb	Sr26#43	*Xcfa2040*
Th. bessarabicum																
PI 531712	2*x*	1	2	—	34	3	3	;1	0	0	–		–		+	–
W6 10232	2*x*	;	2	—	4	2	2	;	0	;	–	+	–	+	+	–
W6 21890	10*x*	;	;C	—	;1	;	;	0	0	0	–	+	+	+	+	+
Th. ponticum																
PI 340066	10*x*	;	;	;	;C	;	;	0	;	0	+	+	–	–	+	+
PI 383583	4*x*	2;C	12	;2	;C/32	;2	1	;	;1	;1		+	+	+	–	
PI 508561	10*x*	;	;	;	;	;	;	0	;	0	+	+	–	–	+	
PI 531737	10*x*	;	;1C	;	;	;	;	0	;	;		+				
PI 547312	10*x*	;	;	;	;	;	;	0	;	0		+				
PI 547313	10*x*	;	;1–	1–	;	;	;	0	;	0		+				
PI 636523	10*x*	—	;	;	;2	;	;	0	;	;		+		–		
Th. elongatum																
PI 142012	10*x*	;1–	;	;	;	;	;	0	0	0	+	+	–	+	+	–
PI 206622	10*x*	;	;	;	;	3/;	;	0	0	0	+	+	+	+	+	
PI 222958	6*x*	;1–2	;C	12	2C/3	122–	;12/34	0	;/2+	;	–	+	–	–	+	–
PI 234708	6*x*	2	2	—	3	1C	2C	—	0	;	+		–	–	–	–
PI 255148	6*x*	;1	21	34/2	;/3	;C	2	;	0	0	+	+	–	–	+	–
PI 297871	10*x*	;	;	;	;	;	;	0	0	0	+	+	+	+	+	–
PI 469212	6*x*	1–1	1	1	1C	;1–	1;	;1	;	;	–	+	–	+	+	+
PI 531718	2*x*	3/2	34	3	3/3–	3/32	34/23–	2+	0	3	+	+	–	–	–	–
PI 547326		;	1	;2	2	;	;	;	;	0	–		–	–	–	–
PI 595139	6*x*	;	1+	1	34/1	;1	12	;1/3+	0	0						
Th. intermedium																
PI 150130	10*x*	;	;12	;1	;	;1–	;	;	0	;	+		–	–	–	+
PI 206625	6*x*	1;	2;1	1	34/2	;C	1;1–	11+	;	;	–		–	–	–	–
PI 229928	6*x*	;12	23/34	12	34	12/3	1C	2+	;1	23/1	–	+	–	–	+	–
PI 273737	6*x*	;2	;	;1	;1	1	;1–	;	;	;1	–	+	–	–		–
PI 297876	6*x*	;1	12	1	;	;1	1	;/1	;	;	–	+	–	–	+	–
PI 314192	6*x*	2	34	2	2C/34	2–3	1;2	1	0/2	;	–	+	+	–		–
PI 317404	6*x*	2C	12	2	12/34	12	2/34	;1	;1	;	–	+	–	+	–	–
PI 369174	6*x*	12	;1	34	12	;1–	1–	;	;	;1	–	+	+	–	+	–
PI 401183	6*x*	;12/3	12	4	34/1C	2	2	;/23	;	1	–	+	+	–	+	–
PI 401185	6*x*	;	1;	2	34	2	;2/34	1;	2–	1		+	–		–	
PI 401200	6*x*	;1	;12/3	1	34/;C	;1C	1C2	;	;	1	–	+	–	–	–	–
PI 401204	6*x*	12	2;	3	2;	;1	12	;/1	;	0	–	+	–	–	–	–
PI 401205	6*x*	2/3	;21	2	2/34	1C/2–	;2	;	1	1/23	–	+	+	–	–	–
PI 401206	6*x*	1	2	3	;1/2	;1+C	;12	0	;	;1	–				–	
PI 401219	6*x*	12	2	2	2	1C	;1C	1	;	1	–	+	–	–	–	–
PI 440038	6*x*	;	1;	;	21	;	;1	;	;	;	–	+	–	–	–	–
PI 440039	6*x*	12/3	;	2/4	21/34	34/2	3/;	;/1	;	;1	–	+	–	–	–	+
PI 440044	6*x*	2	2	1C2	2	2	21	12	;1	12	–	+	–	–	–	–
PI 440049	6*x*	2/3	2	2	12	23	12	1+	;	;1	–	+	–	–	–	
PI 440056	6*x*	2	;21	2	2	12	2/4	;	;1	;1	–	+	–	–	–	–
PI 469214	6*x*	2	2	3	2;1	21C	21	2	;	2/3	–	+	–	–	+	–
PI 634290	6*x*	;	1;	;	1	;1C	;1–	1;	;	;	–	+	–	+	+	–
Th. junceum																
PI 119604	6*x*	;	;	—	;1	;	;	;	0	0	+	+	–	–	–	–
PI 276566	6*x*	1;2	;21/34	;1C	1C	;C	1;	;	;	;/1/2	–	+	–	–	+	–
PI 414667	4*x*	;C	21	1	2	2	1	;/23	;	0/2/23	–	+	–	–	+	–

(cont'd)

Table 2. Continued.

Species and accession†	Ploidy level‡	Infection types to races§									Genotypes of markers¶					
		QFCSC	RTQQC	TPPKC	TMLKC	TCMJC	TPMKC	TTTTF	TTKSK	TRTTF	*Xbarc71*	Sr24#12	BF145935	Gb	Sr26#43	*Xcfa2040*
PI 531727		—	;C	1	1C	—	1	—	2	0	+	+	–		+	+
PI 531728	6*x*	;C	1C	1C	23	1C	1C	1	2	1						
PI 531729	6*x*	2	2	2	2	2	1	23–	0	0/2						
PI 547330		2	1C	—	2	2	1C	;	2+	0	+	+	–	–	+	+
PI 634312	6*x*	;	21C	2/4	2	2	12/3	0	0	0	+	+	–	–	+	
PI 636521	6*x*	2;C	1C	2	2C	1	2C	0	0/3	0/2+	+	+	–	–	+	–
PI 636522	4*x*	2	2	—	2	2	12	;/1	0	0/3	–	+	–		–	

† The species name and accession number follows the designation of USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network (GRIN) (Online Database), National Germplasm Resources Laboratory, Beltsville, Maryland.

‡ Ploidy levels were verified or determined on the basis of chromosome counting in this study except that the *Th. elongatum* diploid accession PI 531718 ($2n = 2x = 14$) was based on the study by Culumber et al. (2011).

§ Infection types follow Stakman et al. (1962), where 0, ;, 1, 2, or combinations thereof, were considered low infection types and 3 and 4 were considered high infection types. The plus (+) and minus (–) signs were used to indicate pustules larger or smaller, respectively, within each class. For combinations, order indicates predominant types. The symbols C, — , and / indicate chlorosis, missing data, and heterogeneity with an accession, respectively.

¶ Symbols indicate the same (+) or different (–) genotypes as the checks carrying the resistance genes.

Table 3. Number of *Thinopyrum* accessions classified as positive or negative for markers useful in detecting stem rust resistance genes in wheat.

Marker	Gene	Number of accessions positive/negative for marker				
		Th. bessarabicum	*Th. elongatum*	*Th. intermedium*	*Th. ponticum*	*Th. junceum*
Sr24#12	*Sr24*	3/0	49/0	146/0	7/0	13/0
Xbarc71	*Sr24*	0/4	42/13	1/144	2/0	9/4
BF145935	*Sr25*	1/3	18/37	18/135	1/2	0/13
Gb	*Sr25*	3/0	30/24	10/141	1/3	0/9
Sr26#43	*Sr26*	4/0	42/11	68/77	2/1	11/2
Xcfa2040	*Sr43*	1/3	7/41	12/132	1/0	3/4

within accessions, especially within *Th. intermedium*. This included accessions with highly resistant and susceptible ITs. On the basis of the high level of heterogeneity observed within accessions, testing of larger populations within accessions may prove that additional accessions are heterogeneous for rust resistance genes. A role for aneuploidy in the heterogeneous rust reactions is possible, though it cannot be definitively established without chromosome counts of susceptible plants. Race-specific susceptibility, especially within *Th. intermedium*, indicated that the resistance was conditioned by multiple genes. For example, PI 229928, PI 401185, and PI 401200 were susceptible to TMLKC, PI 314192 was susceptible to RTQQC, and PI 369174 and PI 401183 were susceptible to TPPKC.

Comparisons of ITs of lines based on ploidy level suggested that higher ploidy level was associated with higher levels of stem rust resistance (Table 2). Notably, accessions classified as decaploid produced ITs of either 0 (immune) or fleck (;). Diploid, tetraploid, and hexaploid accessions had ITs that were predominantely IT 1 or IT 2. The higher level of resistance in decaploid accessions suggests that they may carry multiple stem rust resistance genes, which had additive effects of different genes, or dosage effects of the same gene.

Genotypes of the Markers Linked to *Th. ponticum*-Derived Stem Rust Resistance Genes

Since stem rust resistance was so prevalent among the *Thinopyrum* accessions, a method to determine which accession may carry unique genes is needed. Marker genotyping may identify those lines carrying known genes. We tested six markers that are used to detect *Sr24*, *Sr25*, *Sr26*, and *Sr43* (Table 3). Based primarily on markers detecting *Sr24* and *Sr25*, we concluded that genotyping analysis was useful but with some limitations. One problem was that some markers appeared to be species or genus specific rather than linked to a gene of interest. For example, all *Thinopyrum* accessions reported in Table 3 were positive for the 500 bp amplicon produced by marker Sr24#12. This conclusion agrees with the results of Turner et al. (2013), who tested 12 of the *Thinopyrum* accessions included in the present study and found that 11 were positive for the 500 bp amplicon produced by Sr24#12. At the same time, we also tested *Xbarc71*, another marker that also detects *Sr24* in wheat. We found that in *Th. bessarabicum* and *Th. intermedium*, the *Xbarc71* amplicons detecting *Sr24* were nearly absent (Fig. 1; Table 3). The only *Th. intermedium* accession that tested positive for the *Xbarc71* amplicons was PI 150130, which was determined

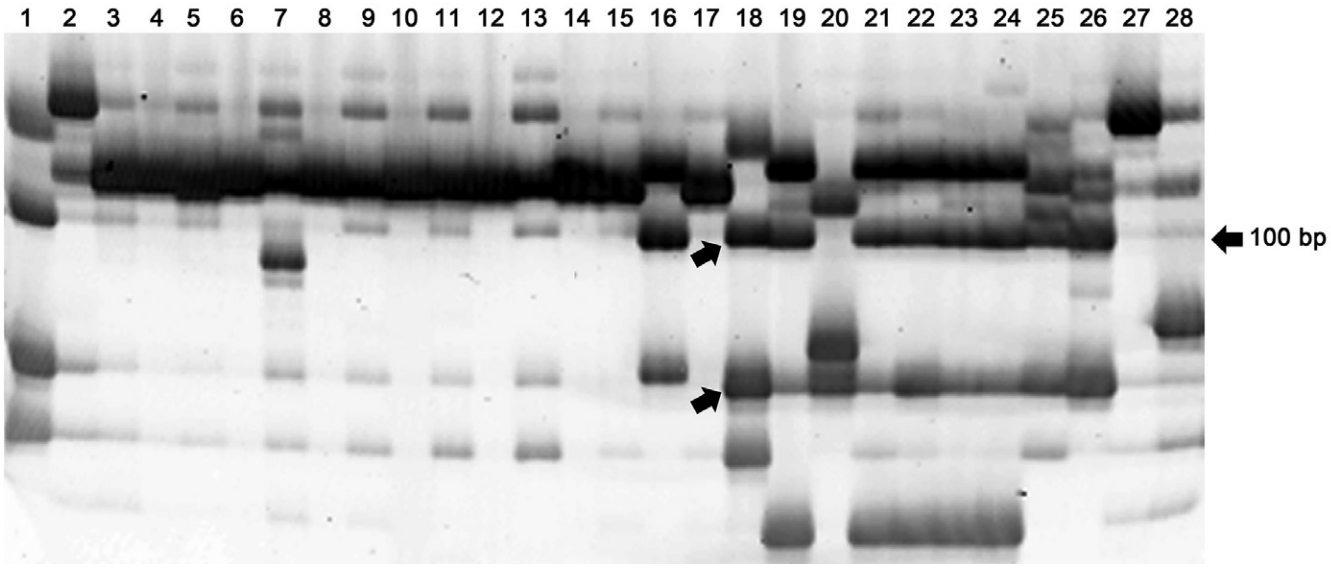

Figure 1. Screening for *Sr24* in *Thinopyrum* accessions using the marker *Xbarc71*. Lane 1, BtSr24Ag (check carrying *Sr24*); lanes 2 through 15, *Th. intermedium* (2, PI 440042; 3, PI 440043; 4, PI 440055; 5, PI 440056; 6, PI 440057; 7, PI 469214; 8, PI 562527; 9, PI 578698; 10, PI 578699; 11, PI 578700; 12, PI 634290; 13, PI 634505; 14, PI 634506; 15, W6 21796); lanes 16 through 17 and 19 through 24, *Th. junceum* (16, PI 119604;17, PI 276566; 19, PI 277184; 20, PI 414667; 21, PI 547329; 22, PI 547330; 23, PI 634312; 24, PI 636521); lane 18, BtSr24Ag; lanes 25 through 26, *Th. ponticum* (25, PI 340066; 26, PI 508561); lane 27, Chinese Spring; lane 28, LMPG6. Arrows in lane 18 point to 103 bp and 85 bp amplicons diagnostic for *Sr24* (Mago et al., 2005).

by chromosome counts as decaploid (67 chromosomes) rather than the expected hexaploid chromosome number. We concluded that the conflicting results for Sr24#12 and *Xbarc71* indicated that the reliability of Sr24#12 to detect *Sr24* is in doubt for analysis of *Thinopyrum* accessions. This conclusion does not mean that Sr24#12 is unreliable for detection of the wheat–*Thinopyrum* translocation chromosome in a wheat background.

A second problem contributing to nonreliability of the markers when tested among *Thinopyrum* accessions is crossing over and gene–marker map distance. Wheat and *Thinopyrum* homoeologues do not pair and crossover; therefore these markers reliably tag the alien segment carrying the stem rust resistance gene even if the marker locus is physically remote from the resistance gene in a wheat background. But among *Thinopyrum* accessions, physical remoteness of the marker and the resistance gene would allow for crossing over and result in the markers being unreliable. This problem can be resolved either by mapping the markers and rust resistance genes in *Thinopyrum* and showing that they are tightly linked or by developing functional markers for the rust resistance genes.

Marker genotyping did yield some useful information. There were four *Th. bessarabicum* accessions in the study. From our chromosome counts, we found that accession W6 21890 was decaploid rather than the expected diploid constitution. When the *Th. bessarabicum* accessions were screened with the six markers included in this study, the three diploid accessions produced identical banding patterns for four of the markers, while W6 21890 had a banding pattern similar to *Th. elongatum* accessions (Fig. 2). On the basis of both chromosome number and marker genotyping analysis, our results suggest that the taxonomy of W6 21890 should be re-evaluated. Similarly, in PI 150130, the unexpected chromosome number and positive score for *Xbarc71* indicated that the taxonomy of this accession should also be reevaluated.

Disregarding marker Sr24#12, the remaining five markers provided some useful information concerning lines that should be investigated for stem rust resistance. Accessions positive for several markers, such as PI 469212 or PI 297871 of *Th. elongatum* (Table 2), are likely to carry one or more of the known *Sr* genes and should have lower priority for study. Accessions negative for all five markers, such as PI 206625, PI 401204, and PI 440038 (Table 2), may carry unique *Sr* genes. These accessions may have a higher priority for further study. The presence of additional and likely new genes in the accessions is also suggested by the differential reactions of accessions within a species. For example, within *Th. intermedium*, PI 273737, PI 314192, PI 369174, and PI 401185 illustrate different stem rust resistance genes (Table 2). PI 273737 was resistant to all *Pgt* races tested, while the other three accessions showed susceptibility to one race, with PI 314192 being susceptible to RTQQC, PI 369174 susceptible to TPPKC, and PI 401185 susceptible to TMLKC. These four accessions are hexaploid and therefore the differences were not the result of higher ploidy level.

CONCLUSIONS

In the tribe Triticeae, the genus *Thinopyrum* comprises several diploid species and many different polyploid species,

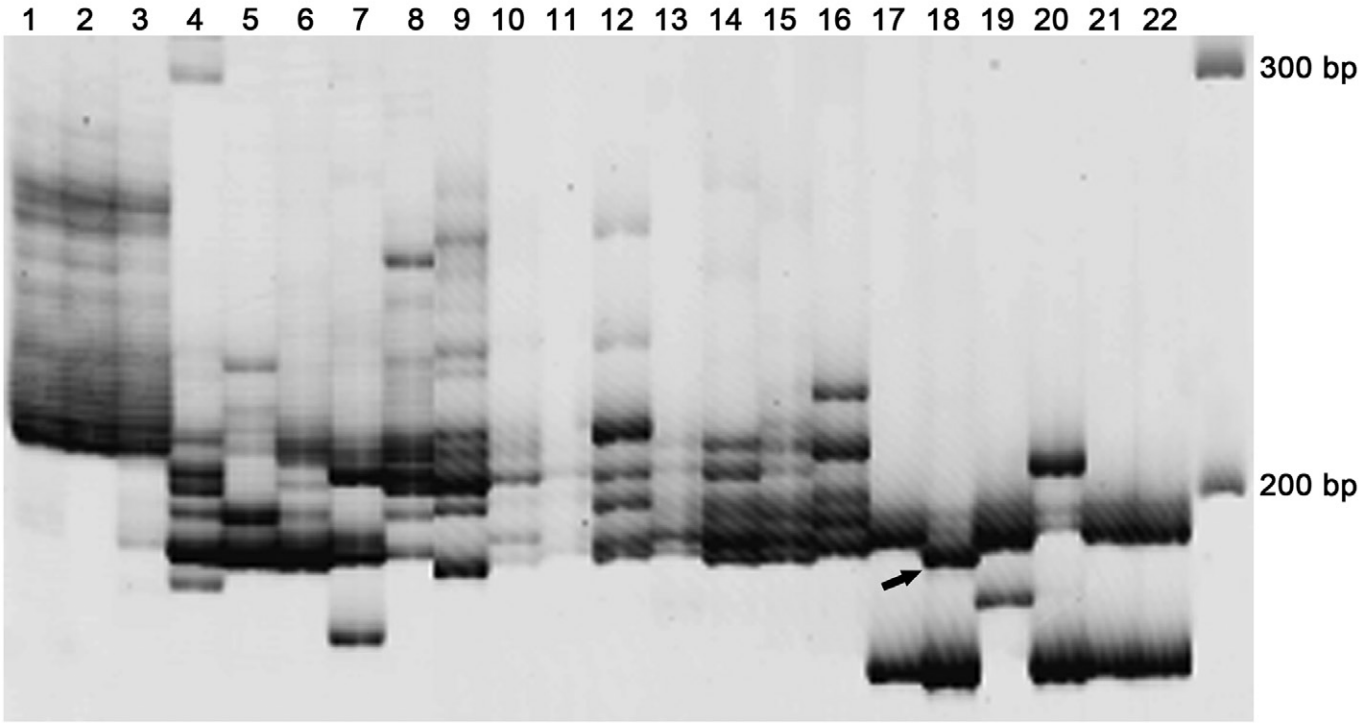

Figure 2. Screening for the presence of *Sr25* in *Thinopyrum* accessions using marker BF145935. Lanes 1 through 4, *Th. bessarabicum* (1, PI 531711; 2, PI 531712; 3, W6 10232; 4,W6 21890); lanes 5 through 16, *Th. elongatum* (5, PI 98526; 6, PI 109452; 7, PI 142012; 8, PI 179162; 9, PI 401117; 10, PI 401118; 11, PI 401119; 12, PI 401120; 13, PI 442631; 14, PI 442633; 15, PI 442635; 16, PI 469212); lane 17, BtSr24Ag (check carrying *Sr24*); lane 18, Wheatear (*Sr25*); lane 19, WA-1 (*Sr26*); lane 20, RWG34 (*Sr43*); lane 21, Chinese Spring; lane 22, LMPG6 (wheat line lacking any major *Sr* genes). The arrow in lane 18 points to the 198 bp amplicon diagnostic for *Sr25* (Yu et al., 2010). *Th. bessarabicum* accessions in lanes 1 through 3 all had the expected 14 chromosomes, but the accession in lane 4 (W6 21890) had variable chromosome number between 69 and 72. The bands in W6 21890 were more similar to those found in *Th. elongatum* than in *Th. bessarabicum.*

which collectively possess many genes that are potentially useful for wheat improvement. A number of the useful genes, mainly for resistance to major wheat diseases, have been transferred from *Th. elongatum*, *Th. intermedium*, and *Th. ponticum* into the wheat genome and a few of them have been successfully used in wheat production (see review by Mujeeb-Kazi et al., 2013). Although *Thinopyrum* species have traditionally been considered a source of unique genes for stem rust resistance, major collections of the *Thinopyrum* species to stem rust had not been evaluated for reactions to stem rust until this study. A striking finding of this study is that almost all of the accessions in the five species (*Th. bessarabicum, Th. elongatum, Th. intermedium, Th. junceum,* and *Th. ponticum*) tested showed resistance to Ug99 and other *Pgt* races and many of the accessions showed immunity or near immunity to Ug99, indicating that the collections of these species are a rich source of genes for resistance to stem rust. Among the 57 *Sr* genes in wheat (McIntosh et al., 2012), however, only five were transferred from *Thinopyrum* species. The infection types, ploidy levels, and marker genotypes revealed in this study provide preliminary information for further efforts to transfer novel *Sr* genes from the *Thinopyrum* species into wheat. This study revealed for the first time that there is variability in ploidy levels in four of the five species and a high frequency of aneuploids in the polyploid species. Therefore, it is particularly important to examine individual plants during genetic analyses and in breeding practice when using wild *Thinopyrum* species.

Supplemental Information Available

Supplemental information is included with this article.

Supplemental Table S1. Seeding reactions of 242 accessions of the five *Thinopyrum* species to nine races of *Puccinia graminis* f. sp. *tritici*.

Supplemental Table S2. Chromosome number and marker genotypes of accessions of five *Thinopyrum* species

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