Resistance to the Ug99 Race Group of *Puccinia graminis* f. sp. *tritici* in Wheat–intra/intergeneric Hybrid Derivatives

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**Abstract**


New races of *Puccinia graminis* f. sp. *tritici*, the causal agent of stem rust, threaten global wheat production. In particular, races belonging to the Ug99 race group significantly contribute to yield loss in several African nations. Genetic resistance remains the most effective means of controlling this disease. A collection of 546 wheat–intra- and intergeneric hybrids developed by W. J. Sando (United States Department of Agriculture, Beltsville, MD) was screened with eight races of *P. graminis* f. sp. *tritici*, including races TTSSK, TTKST, TTTSK, TKTFT, TTTTF, TMPKC, RKQKC, and QTHIC. There were 152 accessions resistant to one or more races and 29 accessions resistant to TTKSK, TKTFT, and TTTSK. Of these 29 accessions, 9 were resistant to all races, 14 had infection type patterns that were indistinguishable from cultivars possessing *Sr9h* and *Sr42*, 2 were indistinguishable from accessions with *SrTmp*, and 4 did not display resistant patterns of accessions with any known *Sr* gene. Three accessions (604981, 605286, and 611952) characterized cytogenetically were disomic substitution lines, each with a single *Thiopronium ponticum* chromosome pair. One accession (606087) was a disomic substitution or addition line with two pairs of *T. ponticum* chromosomes. In total, seven accessions are postulated to contain novel stem rust resistance genes. This research indicates the value of extant collections of wheat–intergeneric hybrids as sources of disease resistance genes.

Despite more than a century of research, wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici* Erikk. & Henning, continues to threaten global wheat (*Triticum aestivum* L.) production. Although this disease has been controlled effectively via genetic resistance in Europe and North America since 1951 and 1974, respectively, localized severe epidemics of stem rust in East Africa, caused by new races of *P. graminis* f. sp. *tritici*, serve to remind wheat scientists that, indeed, “rust never sleeps” (31,62). The stem rust races responsible for these epidemics are predominantly members of the Ug99 race group, so-called due to a *P. graminis* f. sp. *tritici* isolate discovered in the highlands of Uganda in 1998 and named in 1999 (50). This isolate was typed as race TTSSK, in accordance with the international stem rust nomenclature system, and was particularly alarming due to the isolate’s virulence on the widely deployed stem rust resistance gene *Sr31*. Subsequent wheat nursery screenings at the Kenya Agricultural Research Institute in Njoro, Kenya revealed that the majority of wheat cultivars grown in countries threatened by potential TTSSK migration routes were susceptible (62). The discovery of two races in Kenya similar to TTSSK but with additional virulence to the resistance genes *Sr24* (TTKSTK) and *Sr36* (TTTSK) prompted the revision of the international nomenclature system with the addition of a fifth gene set in the differential series: genes *Sr24, Sr31, Sr38*, and *SrMcn* (19,20). Currently, the Ug99 race group is comprised of eight races: PTSSK, PTKST, TTKST, TTSSK (*Ug99*), TTKSP, TKTST, TTTSK, and TTKSF+ (48,51). One or more of these races have been found in South Africa, Zimbabwe, Mozambique, Tanzania, Rwanda, Kenya, Uganda, Ethiopia, Sudan, Eritrea, Yemen, and Iran (51,61,67).

There are at least 33 wheat stem rust resistance genes (*Sr*) that provide resistance against various members of the Ug99 race group: *Sr2, Sr9h, Sr13, Sr21, Sr22, Sr24, Sr25, Sr26, Sr27, Sr28, Sr32, Sr33, Sr35, Sr36, Sr37, Sr39, Sr40, Sr42, Sr43, Sr44, Sr45, Sr46, Sr47, Sr50, Sr51, Sr52, Sr53, Sr57 (Lr34), SrTA10171, SrTA10187, SrTA1662, SrTnp, and Sr1R8* (9,14,15,17,18,29,34,35,42,46,47,53,59,60,63).

Of these 33 genes, only 5 are derived from *T. aestivum*. Source species for the remaining 27 genes include *T. turdium* Flaksb., *T. monococcum* subsp. *monococcum* L. Flaksb., *T. timopheevii* subsp. *timopheevii* (Zhuk.). *T. timopheevii* subsp. *armeniacum* (Jakubtz.) MacKey, *Aegilops comosa* Sm. in Sibth. & Sm. var. *comosa*, *A. ventricosa Tausch*, *A. speltoides Tausch* var. *speltoides*, *A. taushi* Cross, *Thiopronium ponticum* (Podp.) Barkworth and D. R. Dewey (syn. *Agropyron elongatum* (Host) Beauvois), and *T. intermedium* (Host) Barkworth and D. R. Dewey (syn. *A. intermedium* (Host) Beauvois). Singh et al. (61) listed the barriers to the large-scale deployment of these genes, including linkage with undesirable agronomic traits, known virulence in other races of *P. graminis* f. sp. *tritici*, or ineffective levels of resistance conferred under high inoculum loads. Yet despite the continued erosion of resistance and barriers to gene deployment, host genetic resistance remains the most effective form of disease control available, and several groups have reviewed the importance of alien gene transfer for disease resistance in wheat (12,21,71).

One effective strategy to discover and rapidly develop new sources of resistance is the focused stem rust screening of existing collections of wheat–intra- and intergeneric hybrids. The W. J. Sando collection of intra- and intergeneric hybrids was created by W. J. Sando (United States Department of Agriculture [USDA], Beltsville, MD) during the first half of the 20th century. Species used for hybrid breeding with *Triticum aestivum* included *Aegilops spp.*, *Secale cereale*, *Thiopronium intermedium*, *T. ponticum*, *Triticum timopheevii*, and *T. turdium* subsp. *durmum*. Early research with wheat–intergeneric hybrids, including that of W. J. Sando, was spurred by the pursuit of perennial grain and forage crops as well as disease resistance (55,64). Although perennial material of agronomic value eluded early researchers, disease-resistant material did result from this work, including material developed by W. J. Sando (55). Of note, crosses made with selections from a Sando line resulted in wheat cultivar ‘Agent’, the modern version of *Sr24* (12,65). In more recent work, accessions from the Sando collection have shown resistance to eyespot (*Oculimacula yallundae* (Wallwork & Spooner) Croas & W. Gams; synonym *Tapesia yallundae*), Cephalosporium stripe (*Hymenula cerealis* Ellis & Everh.), scab (*Gibberella zeae* (Schwein.) Petch;
synonym *Fusarium graminearum* Schwabe), Stagonospora blotch (*Para-
stagonospora nodorum* (Berk.) Quaedv.L., Verkley & Crous.; synonym
*Stagonospora nodorum* (Berk.) E. Castell. & Germaino), tan spot (*Py-
renophora tritici-repentis* (Died.) Dreschler), wheat stem mosaic
(*Wheat streak mosaic virus*), barley yellow dwarf (*Barley yellow dwarf
virus*), and stem rust (*Puccinia graminis f. sp. tritici*) (1,5,43,71).

To our knowledge, there is no published work characterizing the
Sando collection for stem rust resistance. Therefore, we screened the
546 accessions of the W. J. Sando collection available from the USDA
National Small Grains Collection in Aberdeen, ID. Our goal was to
identify accessions resistant to the Ug99 race group and characterize
these accessions cytogenetically in order to select promising
material for further manipulation and introgression.

**Materials and Methods**

**Plant material and stem rust screening.** In total, 546 accessions
of the W. J. Sando Collection were obtained from the USDA National
Small Grains Collection (Aberdeen, ID). The accessions were each
screened with eight races of *P. graminis f. sp. tritici*. Stem rust races
TTKSK, TTKST, TTTTS, and TRTTF were selected for the isolates’
broad virulence and prevalence in African stem rust epidemics
(Table 1). North American stem rust races TTTTF, TPMKC,
RKQQC, and QTHJC were also selected for screening (Table 1). All
stem rust races used are maintained at the USDA Agricultural
Research Service (ARS) Cereal Disease Laboratory in St. Paul, MN.

Urediospores were collected from infected wheat seedlings (for
race TTKST) or removed from storage in ~80°C freezers (for all other
races). Urediospores removed from storage were heat shocked at
45°C for 15 min and placed in a rehydration chamber maintained at
80% relative humidity using a KOH solution for 2 to 4 h. Newly pro-
duced urediospores (TTKSTK) were collected from plants, placed into
gelatin capsules, and immediately inoculated onto seedlings following
in suspension in a light mineral oil (Soltrol 170; Chevron Phillips
Chemical Co. LP, The Woodlands, Texas). New and stored urediospores
were inoculated onto seedlings following previously described meth-
ods (18). Plants were scored for Stakman seedling infection types
(IT) at 14 days postinoculation (66). Accessions with an IT of “0”,
“1” and “2” or a combination thereof were considered resistant.
Accessions with an IT of “3” or “4” were considered susceptible. Each
accession was sampled for eight plants of each accession. If plants of a sin-
gle accession segregated for resistance, the accession was considered
heterogeneous. All assays were performed in duplicate.

**Data analysis.** Statistical analysis of accessions and their race-
specific resistance or susceptibility patterns was performed using R
version 3.0.2 (http://www.r-project.org) in RStudio (http://www.rstudio.
com/products/rstudio/#Desk). A multiple correspondence analysis
(MCA) was performed using the R package ‘ca’ version 0.53 (41).
MCA, based on the work of Benzécri and colleagues (2), can explain
underlying patterns in complex data sets and is an appropriate alterna-
tive to principal coordinate analysis when the data to be analyzed are
categorical (as in, “Resistant” or “Susceptible”) instead of quantitative (3).

MCA can be used to visualize the spatial relationships among the
“responses”—namely, susceptible (S) and resistant (R)—of accessions
to individual races of *P. graminis f. sp. tritici* (i.e., how resistance to
TTKSK correlates with resistance to all other races). In total, 152
accessions displaying a resistant IT to one or more races were ana-
lized. For simplicity, only those accessions resistant in all replications
were coded as “R”; all other accessions were coded as “S”, even if the
mixed reactions included an R and S IT. ITS of 1+3 and 2+3 were con-
sidered susceptible reactions, and were coded as “S”. The MCA was
performed using a data frame in which the qualitative variables (col-
umns) were the eight races of *P. graminis f. sp. tritici* and the observa-
tions (rows) were reactions of individual accessions to each race. The
analysis was run with the variable λ as “λ = adjusted”, in which the
analysis is based on a Burt matrix with adjusted inertias, and the num-
ber of dimensions (nd) to be included in the output as nd = 5 (41).

**Molecular marker screening of selected accessions.** Plant intro-
duction (PI) accessions 604981, PI 605057, PI 605286, and PI
611932 were screened with the molecular markers Xbarc71 (Sr24),
Gb (Sr25), and BES158799/Sr26#43 (Sr26). Leaf tissue for each
accession was collected at the seedling stage (approximately 7 days af-
after germination) and total DNA was extracted using a modified
cetyltrimethylammonium bromide method (58). Published protocols
were followed for all polymerase chain reactions (PCR) involving
these markers (32,37).

**Cytogenetic analysis of selected accessions.** Nine resistant
accessions were examined using the root squash method outlined be-
low to count the number of chromosomes present. Briefly, rootlets of
germinated seed were cut when 1.5 to 2.0 cm long, placed in 2-ml
glass vials containing double-distilled H2O, and cooled to 1°C in an
ice-water bath for 20 to 24 h. Roots were fixed in 2 ml of Carnoy’s
solution (1:3 glacial acetic acid/absolute ethanol) and stored at 4°C until
examined. For chromosome examination, roots were stained in a 1%
aceticarmine solution for 1 to 3 h. Root caps were then removed with
a razor blade and the meristematic tissue was squeezed out with a lancet
needle. Meristematic tissue was placed on a glass slide in a single drop
of 1% aceticarmine, carefully compressed, and covered with a glass
slide. Prepared slides were heated to just below boiling and final com-
pression was performed manually. A minimum of three rootlets was
examined for each accession. Observations were made using a Zeiss
Photomicroscope III (Carl Zeiss AG, Oberkochen, Germany).

Accessions resistant to race TTKSK and initially found to possess
42 chromosomes were assessed for the presence of *Thinopyrum pon-
ticum* DNA using genomic in situ hybridization (GISH), with geno-
ic DNA (gDNA) from *T. ponticum* as a probe (74). To detect the
homeologous group of *T. ponticum* chromosomes, these accessions
were submitted to combined fluorescence in situ hybridization (FISH)
and GISH procedures, using GAA and pAs1 oligonucleotide
probes to identify wheat chromosomes and a *T. ponticum*
gDNA probe to identify alien chromosomes (6). It was assumed that
the missing wheat chromosomes were substituted by *T. ponticum*
homeologs. The FISH-GISH procedure followed modified protocols

**Table 1.** Virulence or avirulence formulae of *Puccinia graminis f. sp. tritici* isolates used to screen the W. J. Sando collection of wheat-intra- and intergeneric hybrids and derivatives from the United States Department of Agriculture

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Race</th>
<th>Virulence or avirulence</th>
</tr>
</thead>
<tbody>
<tr>
<td>04KEN156-04</td>
<td>TTKSK</td>
<td>5,6,7b,8a,9b,9d,9e,9g,10,11,17,21,30,31,38,McN/24,36,Tmp</td>
</tr>
<tr>
<td>06KEN19-V 3</td>
<td>TTKST</td>
<td>5,6,7b,8a,9b,9d,9e,9g,10,11,17,21,24,30,31,38,McN/36,Tmp</td>
</tr>
<tr>
<td>07KEN24-4</td>
<td>TTTTS</td>
<td>5,6,7b,8a,9b,9d,9e,9g,10,11,17,21,30,31,38,McN/24,Tmp</td>
</tr>
<tr>
<td>06YM34-1</td>
<td>TRTTF</td>
<td>5,6,7b,8a,9b,9d,9e,9g,10,11,17,21,30,36,McN,Tmp/6,9a,9b,24,30,31,38</td>
</tr>
<tr>
<td>74MN1409</td>
<td>TPMKC</td>
<td>5,6,7b,8a,9b,9d,9e,9g,10,11,17,21,30,36,McN,Tmp/6,9a,24,31</td>
</tr>
<tr>
<td>01MN84A-1-2</td>
<td>TTTTF</td>
<td>5,6,7b,8a,9b,9d,9e,9g,10,11,17,21,30,36,McN,Tmp/24,31,38</td>
</tr>
<tr>
<td>75ND717-C</td>
<td>QTHJC</td>
<td>5,6,7b,8a,9b,9d,9g,10,11,17,21,McN/9a,9b,24,30,31,38,McN</td>
</tr>
<tr>
<td>99KS76A</td>
<td>RKQQC</td>
<td>5,6,7b,8a,9b,9d,21,36,McN/9e,10,11,17,24,30,31,38,McN</td>
</tr>
</tbody>
</table>

* The first two numbers indicate the year in which the isolate was collected; KEN = Kenya, YEM = Yemen, MN = Minnesota, ND = North Dakota, and KS = Kansas.
* For an explanation of stem rust race nomenclature, see Roelfs and Martens (57).
* All stem rust resistance genes listed are part of the International Wheat Stem Rust differential series (19,20). For details concerning each gene, see McIntosh et al. (40).
from Zhang et al. (74). After removing cover slips from frozen squashed preparations, slides were immersed in 100% ethanol for 5 min, dried, and UV cross-linked. The probe mixture (20 μl/slide) contained 50% formamide (Fisher Scientific, Pittsburgh), 2.75x saline-sodium citrate (SSC) buffer, 13.75% dextran sulfate, 2.4 μg of wheat-blocking gDNA, 40 ng of T. ponticum gDNA probe, 1 ng of Cy5-(GAA), and 60 ng of TEX615-pAs1-oligonucleotide probes (Integrated DNA Technologies, Inc., Coralville, IA). The mixture of probes and the slide preparations were denatured separately in 100°C water. The remainder of the FISH+GISH procedures followed the protocol of Kato et al. (22). Slides were incubated at 37°C over-night and washed twice in 2x SSC buffer (1x SSC is 0.15 M NaCl plus 0.015 M sodium citrate): 5 min at room temperature, 10 min at 42°C, and then in 1x SSC buffer for 5 min at room temperature. Chromosome preparations were mounted and counterstained with 4',6-diamidino-2-phenylindole solution or propidium iodide in Vectashield (Vector Laboratories Inc., Burlingame, CA). Images were captured with the Adobe Photoshop software (Adobe Systems Incorporated, San Jose, CA).

**Results**

**Seedling resistance to stem rust.** The W. J. Sando collection was found to harbor 152 accessions with resistance to one or more races of *P. graminis f. sp. tritici*. The number of accessions resistant to the individual races in the Ug99 race group, TTKSK, TTKST, and TTTSK, ranged from 52 to 64 (Table 2). Races TRTTF and TPMKC were virulent on the greatest number of accessions, with only 25 of 546 accessions resistant to each. Race RKQQC was the least virulent, with 79 accessions displaying resistance to this race in both replications. Full results of the screening are available in Supplementary Table S1.

The reactions of the wheat accessions to race TTKSK were correlated significantly with reactions to races TTKST *(r = 0.496 at P < 0.0001)*, TTKSK *(r = 0.480 at P < 0.0001)*, and QTHJC *(r = 0.210 at P = 0.009)*, more so than can be expected under the assumption of independence (Table 3). Reactions to race TTKST were also significantly correlated with reactions to races TTKST *(r = 0.365 at P < 0.0001)* and RKQQC *(r = 0.221 at P = 0.006)*. In comparison, reactions to TRTTF were correlated significantly with reactions to races TTKST *(r = 0.317 at P < 0.0001)* and TPMKC *(r = 0.425 at P < 0.0001)*. Reactions to races TTKST and TPMKC *(r = 0.411 at P < 0.0001)* were also highly correlated (Table 3).

The first dimension (x-axis) of the MCA explained 64.6% of the variance between the wheat accession reactions to the eight races of

**Table 2. Percentages of W. J. Sando wheat accessions resistant, heterogeneous, and susceptible to each of eight physiologic races of *Puccinia graminis f. sp. tritici*.**

<table>
<thead>
<tr>
<th>Raceb</th>
<th>Totala</th>
<th>Resistantd</th>
<th>Heterogeneousc</th>
<th>Susceptiblec</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTKSK</td>
<td>546</td>
<td>64 (11.72%)</td>
<td>67 (12.27%)</td>
<td>415 (76.01%)</td>
</tr>
<tr>
<td>TTKST</td>
<td>542</td>
<td>59 (10.89%)</td>
<td>70 (12.92%)</td>
<td>413 (76.20%)</td>
</tr>
<tr>
<td>TTTSK</td>
<td>544</td>
<td>52 (9.56%)</td>
<td>89 (16.36%)</td>
<td>403 (74.08%)</td>
</tr>
<tr>
<td>TRTTF</td>
<td>544</td>
<td>25 (4.60%)</td>
<td>51 (9.38%)</td>
<td>468 (86.03%)</td>
</tr>
<tr>
<td>TTTTF</td>
<td>545</td>
<td>26 (4.77%)</td>
<td>102 (18.72%)</td>
<td>417 (76.51%)</td>
</tr>
<tr>
<td>TPMKC</td>
<td>545</td>
<td>25 (4.59%)</td>
<td>37 (6.79%)</td>
<td>483 (88.62%)</td>
</tr>
<tr>
<td>QTHJC</td>
<td>536</td>
<td>50 (9.33%)</td>
<td>53 (9.89%)</td>
<td>433 (80.78%)</td>
</tr>
<tr>
<td>RKQQC</td>
<td>542</td>
<td>79 (14.58%)</td>
<td>115 (21.22%)</td>
<td>348 (64.21%)</td>
</tr>
</tbody>
</table>

a Seedlings were scored using the seedling infection type scale created by Stakman et al. (66).
b For an explanation of stem rust race nomenclature, see Roelfs and Martens (57).
c Totals are not equal among races due to the lack of seed germination in some replications.
d Accessions displayed resistant infection types (“1” to “2”) in all replications.

c Accessions displayed a mixture of resistant and susceptible infection types in one or more replication.

d Accessions displayed susceptible infection types (“3” or “4”) in all replications.

**Fig. 1.** Biplot showing relationships between resistance and susceptibility of 152 resistant W. J. Sando collection wheat accessions to eight races of *Puccinia graminis f. sp. tritici*. Races are named according to the international stem rust race nomenclature (19,20,57). Resistant accessions are those displaying resistant infection types according to Stakman et al. (66) in both replications in which a race was evaluated. Susceptible accessions are those that displayed a susceptible reaction in at least one replication of the assays. Data points were derived using a multiple correspondence analysis in the statistical platform R using the package “ca” (41,54). The x- and y-axes explained 64.64 and 11.35%, respectively, of the variation among accession reactions to the eight races of *P. graminis f. sp. tritici*. Red symbols = susceptibility to the associated race and black symbols = resistance to the associated race.

**Table 3. Significant correlations between wheat accessions of the W. J. Sando collection resistant to one or more races of *Puccinia graminis f. sp. tritici* and reactions to eight different races of *P. graminis f. sp. tritici*.**

<table>
<thead>
<tr>
<th>Raceb</th>
<th>Race</th>
<th>r²</th>
<th>P value (&lt;0.05)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTKSK</td>
<td>TTKST</td>
<td>0.496</td>
<td>7.76 × 10⁻¹¹</td>
</tr>
<tr>
<td>TTKSK</td>
<td>TTTSK</td>
<td>0.480</td>
<td>3.75 × 10⁻⁹</td>
</tr>
<tr>
<td>TTKST</td>
<td>TTTSK</td>
<td>0.365</td>
<td>3.85 × 10⁻⁴</td>
</tr>
<tr>
<td>TTKSK</td>
<td>QTHJC</td>
<td>0.210</td>
<td>0.009</td>
</tr>
<tr>
<td>TTTSK</td>
<td>RKQQC</td>
<td>0.221</td>
<td>0.006</td>
</tr>
<tr>
<td>TRTTF</td>
<td>TTTTF</td>
<td>0.317</td>
<td>6.96 × 10⁻⁵</td>
</tr>
<tr>
<td>TRTTF</td>
<td>TPMKC</td>
<td>0.425</td>
<td>4.63 × 10⁻⁸</td>
</tr>
<tr>
<td>TTTTF</td>
<td>TPMKC</td>
<td>0.411</td>
<td>1.48 × 10⁻⁷</td>
</tr>
<tr>
<td>TPMKC</td>
<td>QTHJC</td>
<td>0.263</td>
<td>0.001</td>
</tr>
<tr>
<td>TPMKC</td>
<td>RKQQC</td>
<td>0.178</td>
<td>0.028</td>
</tr>
</tbody>
</table>

a Resistant accessions are those that displayed resistant infection types in both replications based on the seedling infection type scale developed by Stakman et al. (66). All correlations shown are positive.
b For an explanation of stem rust race nomenclature, see Roelfs and Martens (57).
c Pearson’s product moment correlations coefficient (49).
d The P value is the probability, under the null hypothesis of independence, of obtaining a result equal to or more extreme than that observed. Usually P values < 0.05 or 0.01 are considered to significantly deviate from the null hypothesis (10).
of stem rust and discriminated resistance into three clusters: (i) the Ug99 race group; (ii) races RKQQC and QTHJC; and (iii) races TTTF, TPKMC, and TRTTF (Fig. 1). The second dimension also distinguished susceptibility to races possessing virulence on Sr31 (TTSK, TKTST, and TTTSK) from susceptibility to races avirulent on Sr31 (TTTF, TPKMC, QTHJC, and RKQQC) (Fig. 1).

**Molecular markers.** None of the four accessions subjected to molecular marker screening (PI 604981, PI 605057, PI 605286, and PI 611932) amplified the Sr24 (Xbarc71) marker, PI 604981 amplified the Sr25 (Gb) marker, and PI 611932 amplified the Sr26 (BES18379/Sr26#43) marker (Table 4).

**Resistance to the Ug99 race group.** Of the 152 resistant accessions, 29 were resistant to races TTKSK (Ug99), TKTST, and TTTSK combined. The pedigrees for these accessions are listed in Supplementary Table S2. The 29 Ug99-resistant accessions clustered into distinct different race-specific IT patterns (Table 5). The most common pattern, exhibited by 14 of 29 accessions, combined resistance to the Ug99 race group with resistance to the North American race RKQC. Accessions sharing this pattern are referred to as group 1. Resistant ITs in group 1 were “2−” to “2+” (PI 605023 exhibited IT “2+3” in one replication with TTKSK). Nine accessions resistant to the Ug99 race group were also resistant to all other races used for screening. These accessions are referred to as group 2. Resistant ITs in group 2 ranged from “0−” to “2−” (PI 604981, 604986, 611887, and 611915 displayed higher ITs in some replications to some races) (Table 5). Group 3 accessions PI 605079 and PI 605321 displayed expected race-specific resistance patterns and ITs for lines possessing the resistance gene SrImp. Groups 4 to 7 displayed unique resistance or susceptibility patterns that are not readily associated with any single known Sr gene.

**Cyto genetic s of selected resistant accessions.** Nine accessions selected for resistance to race TTKSK were analyzed using the root squash method to determine the number of chromosomes present. Accessions PI 604924, PI 605132, PI 611887, and PI 611899 had chromosome counts of 2n = 54. PI 605103 had individual root tips that displayed chromosome counts of 2n = 54, 55, and 56. The remaining accessions (PI 604981, PI 605057, PI 605286, and PI 611932) had chromosome counts of 2n = 6, 22, and 74.

**Molecular markers.** None of the four accessions subjected to molecular marker screening (PI 604981, PI 605057, PI 605286, and PI 611932) had chromosome counts of 2n = 6, 22, and 74.

### Table 4. Results of molecular marker screening of W. J. Sando wheat accessions selected for cytogenetic characterization using genomic in situ hybridization and fluorescence in situ hybridization (GISH-FISH) a

<table>
<thead>
<tr>
<th>Accession</th>
<th>Xbarc71</th>
<th>Gb</th>
<th>BE518379/Sr26#43</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI 604981</td>
<td>− d</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>PI 605057</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PI 605286</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PI 611932</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

a For GISH-FISH methods, see Materials and Methods section and references 6, 22, and 74.

b Symbols: / = separates different IT within a replication, C = excessive chlorosis, N = excessive necrosis, LIF = low infection frequency, + = excessive pustule size for category, and − = diminished pustule size for category (57).

c Accessions are grouped according to shared infection type patterns.

d − and + indicate that the accession was negative or positive, respectively, for the stem rust resistance gene marker.

### Table 5. Infection types (IT), grouped by race-specific pattern, of 29 W. J. Sando collection wheat accessions resistant to Puccinia graminis f. sp. tritici races TTKSK, TKTST, and TTTSK b

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<th>TTKSK</th>
<th>TKTST</th>
<th>TTTSK</th>
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</table>

a Seedlings were scored using the seedling IT scale created by Stakman et al. (66). For an explanation of stem rust race nomenclature, see Roelfs and Martens (57).

b Symbols: / = separates different IT within a replication, C = excessive chlorosis, N = excessive necrosis, LIF = low infection frequency, + = excessive pustule size for category, and − = diminished pustule size for category (57).

c Accessions are grouped according to shared infection type patterns.
PI 611932 had chromosome counts of \( n = 42 \) in initial examinations and were selected for analysis using GISH and FISH (Fig. 2). The ITs of these accessions are displayed in Table 6. The alien parent in the four selected accessions is the decaploid tall wheatgrass, *T. ponticum* \( [2n = 10x = 70; \text{genome} \ JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ...
Only two of four accessions analyzed using GISH-FISH amplified alleles associated with known stem rust resistance genes derived from *T. ponticum*. Recent work with *Thinopyrum* spp. or *Thinopyrum*–wheat partial amphiploids have found that false positives are common when using molecular markers designed from hexaploid bread wheat to screen *Thinopyrum* material (69,75). Zheng et al. (75) found that some markers were species or genus specific when used to screen five *Thinopyrum* spp. PI 604981 amplified the Sr25-associated allele when screened with marker Gb. Marker Gb has been verified in multiple studies as amplifying a 130-bp product only in wheat cultivars known to possess Sr25 (32,73). However, no cultivars tested in these studies possessed whole chromosomes from a *Thinopyrum* sp., as does PI 604981. Additionally, all species of *Thinopyrum* tested by Zheng et al. (75), except *T. juncum*, possessed some accessions that were positive for the Sr25 amplicon. Because Sr25 has been shown to be derived from *T. ponticum*, this may indicate that Gb detects a common *Thinopyrum* locus regardless of the presence of Sr25 (24,39). Phenotypic evidence indicates that the resistance observed in PI 604981 (“1” to “3”) differs from that expected of Sr25 (“1” to “23”) (40). The variability in both molecular and phenotypic evidence indicates that PI 604981 either does not carry Sr25 or possesses a unique allele of this resistance gene. Accession PI 611932, which amplified the Sr26-associated amplicon, was discussed above and is discussed below.

Cytogenetic analysis in this study of selected W. J. Sando accessions confirmed previous reports of the mixed ploidy and chromosome complements present in this collection (5,43). Each accession analyzed using FISH possessed *T. ponticum* as the alien species in the listed pedigree. Stem rust resistance genes Sr24, Sr25, Sr26,
and Sr43 are all derived from *T. ponticum* (12,27,40). Both Sr25 and Sr43 are derived from *T. ponticum* group 7 chromosomes (12,27). Only PI 604981 amplified the expected product when screened with Sr25 marker Gb. Sr25 is located on a *T. ponticum* group 7 chromosome and, although only chromosome pair 2D is missing from PI 604981, each plant examined of this accession also possessed telosomes and dicentric or translocation chromosomes involving 7A and an unknown D chromosome (11). The unknown chromosome involved was identified as belonging to the D genome; the only *T. ponticum* genetic material in this accession is the chromosome pair replacing 2D. Because marker Gb has only been tested in adapted *Triticum aestivum* lines, it is possible that Gb amplifies non-Sr25 loci located in other regions of the *Thinopyrum ponticum* genome. Also, see the section described above on recent work with *Thinopyrum* germplasm and molecular markers. Cytogenetic evidence supports the likelihood that PI 604981 does not possess Sr25.

Sr43 is derived from a *T. ponticum* 7el2 chromosome and was transferred to wheat chromosome 7D (23,28). Because all accessions examined were fertile, it is assumed, along with Knott et al. (28), that the *T. ponticum* chromosomes found in these accessions are homoeologous with the chromosomes they replaced. Because no accession was missing wheat group 7 chromosomes, it is unlikely that the *T. ponticum* chromosome carrying Sr43 is the source of TTKSK resistance in these accessions.

Sr24, while effective against TTKSK (Ug99), is ineffective against TTKST, to which these accessions, except PI 605057, were resistant. However, PI 605286 exhibited a mixed reaction, “0/3”, to race TTKST. In contrast, PI 604981 and PI 611932 exhibited highly resistant ITs when inoculated with TTKST. Sr24 was transferred from a group 3 *T. ponticum* chromosome to chromosome 3D of wheat and has also been transferred to the short arm of wheat chromosome 1B (16,37,68). PI 605286 did not amplify the Sr24-associated fragment when screened with PCR marker Xbarc71 identified by Mago et al. (37). Yu et al. (73) have successfully used this marker to genotype 228 wheat lines from the International Maize and Wheat Improvement Center, the International Center for Agricultural Research in Dry Areas, China, and othermiscellaneous origins for the presence of Sr24. However, despite the absence of molecular data, screening results remain inconclusive regarding whether PI 605286 possesses Sr24 due to potential false negatives when using molecular markers designed from a specific *T. ponticum* translocation (69,75).

PI 605057 was susceptible to races TTKST, TTKSK, and TPMKC and exhibited a resistant reaction in one replicate test and a susceptible reaction in another replicate test to race RKQC. All known Sr genes derived from *T. ponticum* are resistant to these races (Sr24 is not effective against TTKST) (32,72). To our knowledge, no known Sr gene shares this resistance and susceptibility pattern, which may indicate either novel Sr genes or new alleles of known Sr genes, or a heterogeneous structure of the PI 605057 population.

Sr26 was transferred from the long arm of a *T. ponticum* group 6 chromosome to wheat chromosome 6A (25,26). In GISH analysis, PI 611932 was shown to possess a single pair of *T. ponticum* chromosomes and lacked wheat chromosome 6D, indicating that the *T. ponticum* chromosomes may be group 6 chromosomes possessing Sr26. PI 611932 also possessed a possible T6AS-6AL/6DL translocation. Phenotypic, molecular, and cytogenetic data in this study indicated that Sr26 is the resistance gene in PI 611932 but no allelism tests have been conducted to confirm this. Accessions PI 605057 and PI 605286, and possibly PI 604981, may have uncharacterized stem rust resistance genes effective against the Ug99 race group. Chromosome engineering efforts are currently underway to reduce the size of alien chromatin in these accessions using a homozygous phl1b line developed at Kansas State University Wheat Genetics Resource Center (13).

The W. J. Sando collection is known to harbor valuable resistance genes to multiple diseases effecting wheat production. Though individual lines had been characterized for their reaction to stem rust, to our knowledge, no published data existed characterizing the entire collection. This study resulted in characterizing the entire collection for stem rust resistance using eight races of _P. graminis f. sp. tritici_. Furthermore, the 29 accessions identified with resistance to three races within the Ug99 race group are a valuable resource in the fight against stem rust. Future work with this germplasm should proceed more efficiently with the aid of this screening. Of the 29 accessions resistant to Ug99 races, 25 could not be distinguished from known Sr genes; however, future work may show that some accessions possess new genes or alleles. Cytogenetic techniques identified promising resistant accessions, three of which are postulated to contain new sources of resistance. Introgresion of these resistance genes into adapted wheat germplasm will provide additional tools for breeding resistant wheat cultivars. Altogether, seven accessions were identified in this study as candidates possessing novel stem rust resistance.

**Acknowledgments**

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**Literature Cited**


30. Krattinger, S. G., Lagudah, E. S., Spielmeyer, W., Singh, R. P., Huerta-
1324 Plant Disease / Vol. 99 No. 10
34. Oliver, R. E., Xu, S. S., Stack, R. W., Friesen, T. L., Jin, Y., and Cai, X.
33. Olivera, P. D., Jin, Y., Rouse, M., Badeo, A., Fetch, T., Jr., Singh, R. P., and
32. Olson, L. E., Rouse, M. N., Pumphrey, M. O., Bowden, R. L., Gill, B. S., and
31. Olson, E. L., Rouse, M. N., Pumphrey, M. O., Bowden, R. L., Gill, B. S., and
30. Olson, E. L., Rouse, M. N., Pumphrey, M. O., Bowden, R. L., Gill, B. S., and


