Resistance to wheat rusts identified in wheat/Amblyopyrum muticum chromosome introgressions.

John P. Fellers*, Angie Matthews; Allan K. Fritz, Matthew N. Rouse, Surbhi Grewal, Stella Hubbart-Edwards, Ian P. King, and Julie King.

J.P. Fellers, USDA-ARS Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506; A. Mathews and A.K Fritz, Department of Agronomy, Kansas State University, Manhattan, KS 66506; M.N. Rouse, USDA-ARS Cereal Disease Laboratory, St. Paul, MN 55108; S. Grewal, S. Hubbart-Edwards, I.P. King and J. King, Nottingham BBSRC Wheat Research Centre, Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK. Received:__________ * Corresponding author: john.fellers@usda.gov.

Abbreviations: KASP- Kompetitive allele-specific PCR;

Abstract

Wheat rusts are a worldwide production problem. Plant breeders have used genetic resistance to combat these fungi. However, single-gene resistance is rapidly overcome due to

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi:10.1002/csc2.20120.

This article is protected by copyright. All rights reserved.
frequent occurrence of new virulent fungal strains. Thus, a supply of new resistance sources is continually needed and new resistance sources are limited within hexaploid wheat genetic stocks. Wild relatives are able to be a resource for new resistance genes, but are hindered because of chromosome incapability with domesticated wheats. Twenty-eight double haploid hexaploid wheat/Amblyopyrum muticum introgression lines, with introgressions covering the majority of the T genome, were evaluated for resistance to *Puccinia triticina*, *P. graminis* f. sp. *tritici*, and *P. striiformis* f. sp. *tritici*. At the seedling level, four lines were resistant to races of *P. triticina*, six lines were resistant to *P. graminis*, and fifteen lines were resistant to *P. striiformis*. At the adult stage, sixteen lines were resistant to *P. triticina*. Line 355 had resistance to all three rusts and line 161 had resistance to all tested races of *P. triticina*. Some of these lines will require further work to reduce the size of the introgressed segment, however, lines 92 and 355 have very small fragments and can be used directly as new resistance donors.

**Introduction**

Wheat rusts are the most formidable group of pathogens influencing production. Three species, *Puccinia triticina* Erikss., *P. graminis* Pers.:Pers. f.sp. *tritici* Erikss. & E. Henning, and *P. striiformis* Westend. f.sp. *tritici* Erikss., are the causal fungi for leaf (brown), stem (black) and stripe (yellow) rust, respectively. Each can be controlled with chemical fungicide applications. However, in many areas environmental constraints limit production resulting in low profit margins, hindering the use of fungicides.

An alternative strategy is to develop varieties carrying genetic resistance to cereal rusts. The majority of rust resistance is due to single major genes in a gene-for-gene interaction with the pathogen (Flor 1955). There are also broad spectrum resistance genes, such as the pleiotropic gene, *Lr34/Yr18/Sr57*, providing various levels of resistance to all three rusts.
Single gene resistance is often effective during the first few years after a variety release. However, large acreages of genetically similar cultivars lead to selection of rust genome mutations which can overcome resistance, resulting in the emergence of new virulent races each year. One of the most notable examples is the *P. graminis* f. sp. *tritici* race Ug99 which appeared in Uganda in 1998 and is virulent on most commercial varieties (Pretorius et al., 2000). *P. striiformis* has historically been found in cooler climates such as the U.S. Pacific Northwest, however, new races adapted to higher temperatures began appearing in the U.S. Great Plains in 2000 (Milus et al., 2008). *P. triticina* is found in all world regions of wheat production and 70+ different races are found each year in North America alone (Kolmer et al., 2005; Kolmer, 2019).

Rust resistance breeding is difficult due to the limited available sources of genetic variation within the gene pool of wheat. As a result, geneticists have turned to the wild relatives. The effort required to transfer interspecific variation into wheat depends on the closeness of the wheat-wild relative relationship. For example, transfer from closely related species *Aegilops tauschii*, the D genome progenitor, and *Triticum monococcum*, one of the A genome progenitors of wheat, can be achieved through crossing elite germplasm with synthetic wheats followed by backcrossing. Transfer of wild accession genes occurs through normal chromosome pairing and recombination (Zohary et al., 1969; Cox, 1998; Warburton et al., 2006). Linkage of target variation with undesirable traits can be resolved through further recombination with selection. The transfer of genetic variation from wild relatives carrying related but not identical genomes can be achieved using strategies like mutagenesis (Sears, 1977 and 1993) or the removal of the *Ph1* locus which restricts recombination to homologous chromosomes (Al-Kaff et al., 2008).

*Lr9* is one of the earliest examples of resistance genes transferred from a wild relative with a non-homoeologous genome. Using X-rays, a wheat/*Ae. umbellulata* Zhuk monosomic...
addition line was irradiated and through chromosome breakage and repair, the chromosome segment with \( Lr9 \) translocated into the wheat genome (Sears, 1956). Stem rust resistance gene \( Sr2 \) was derived from \( Triticum turgidum \) L. subsp. \( dicoccum \) (Schrank ex Schübl.)Thell (McFadden 1930). \( SrTA10171 \) and \( SrTA10187 \), with resistance to Ug99, were transferred from \( Ae. tauschii \) (Olsen et al., 2013). Resistance has been found in many relatives. Stem rust resistance has been found in hexaploid introgression lines with segments from \( Secale cereale \), \( Leymus mollis \), \( L. racemosus \), and \( Thinopyrum junceiforme \) (Rahmatov et al., 2016) and crosses of \( Triticum durum \) with \( Th. junceum \), \( Th. intermedium \), \( Th. bessarabicum \), \( Th. elongatum \), \( Th. ponticum \), \( Elymus rectisetus \), \( Ae. caudata \), and \( Ae. speltoides \) (Xu et al., 2009). Introgressions can also provide resistance to multiple pathogens. Resistance to all three rusts was obtained by the transfer of the short arm of chromosome 1R of \( Secale cereale \) (1RS) containing \( Lr26 \), \( Sr31 \), and \( Yr9 \) (Zeller, 1973) while a Robertsonian translocation from \( Thinopyrum intermedium \) carried resistance genes \( Sr44 \) and \( Bdv2 \) to \( P. graminis \) and \( Barley yellow dwarf virus \), respectively (Liu et al., 2013).

\textit{Amblyopyrum muticum} [(Boiss.) Eig.; \textit{Aegilops mutica} Boiss; \( 2n=2X=14; \) genome TT] is a wild relative of wheat originating from the Middle East and Armenia. \textit{Am. muticum} possesses a \( Ph1 \) suppressor gene(s) which facilitates recombination between \textit{Am. muticum} chromosomes and the homoeologous chromosomes of wheat (Dover and Riley, 1972). Relatively little research has previously been undertaken on \textit{Am. muticum}, but merit as a genetic resource has been shown. Lines homozygous for a 5D/5T introgression provide winter hardiness (Iefimenko et al., 2015), while a complete 7T chromosome substitution line provides resistance to powdery mildew (\textit{Blumeria graminis} f. sp. \textit{tritici}; Eser, 1998). With the development of new marker systems and higher throughput karyotyping, the majority of the \textit{Am. muticum} genome has now been introgressed into hexaploid wheat (King et al., 2017 and 2019). In this report, we begin the
characterization of the first 28 lines containing overlapping segments of the T genome by screening for resistance to cereal rusts.

**Materials and Methods**

**Introgression lines.**

The development, and characterization of the double haploid *Triticum aestivum* L./*Am. muticum* lines are described in King et al., (2017 and 2019) and in Supplementary Table 1. In summary, two *Am. muticum* accessions, JIC2130004 and JIC2130012 were obtained from the Germplasm Resource Unit (John Innes Centre, Norwich, UK) and initially crossed to either Chinese Spring (CI6223) or Pavon 76 (P1519847). The F₁s were crossed to either Paragon (Cereal Variety Handbook, 1997) or Pavon 76, then backcrossed to Paragon to the BC₃. At the BC₃ generation, double haploids were produced to stabilize the segments using maize pollination, embryo rescue, and chromosome doubling (King et al. 2019).

**Seedling infection testing.**

Five seed from each of the 28 doubled haploid (DH) introgression lines were planted in 20 x 20 x 3 cm aluminum cake pans containing BM-1 soil media (Berger Peat Moss, LLC). Five seed of the hexaploid parents Paragon and Pavon 76 along with a susceptible check Thatcher were also included and placed throughout the pan. At the 2-3 leaf seedling stage, 25 mg of *P. triticina* rust spores from each of the races BBBD, TNRJ (PRTUS35), and 97AZ103 were suspended in 2 mls of Soltrol 170 parafin oil (Phillips Petroleum) and sprayed onto the seedlings with an atomizer (Tallgrass Solutions) at 40 psi. Seedlings were transferred to a Percival humidity chamber at 100% relative humidity with wall settings of 5 °C and water basin of 40 °C for 16 h. Plants were transferred back into the greenhouse. At 14 days post inoculation
(DPI), infection types were rated on a scale of 0 - 4 (McIntosh et al., 1995); 0 - no infection; "fleck" - small focused infection points caused by hypersensitive reaction with no pustule formation; "1-4" - scale of pustule size with 1 being very small and focused with distinct hypersensitive reaction to "4" with large pustules and no plant defense reaction.

A second set of plants was inoculated with a composite of two *P. striiformis tritici* isolates collected in Kansas in 2012 and 2014 (Robert Bowden, personal communication). Plants were inoculated as above, but humidity chamber conditions were wall settings of 0 °C and water basin of 29 °C for 24 h. At 14 DPI, plants were scored using a scale of 1-resistant to 9-susceptible (McNeal et al., 1971).

A third set of seedlings were inoculated with a composite of two races of *P. graminis tritici*, RKQQC and QFCFC (obtained from Robert Bowden), as above except humidity chamber conditions were with wall settings of 6 °C and water basin of 42 °C for 16 h. At 14 dpi, infection types were scored on a scale of 0, ;, 1 - 4 (see description for leaf rust; McIntosh et al., 1995). Seedlings were also tested at the USDA-ARS Cereal Disease Laboratory (St. Paul, MN) BL-3 containment facility with *P. graminis tritici* Ug99 Race TTKSK (isolate 04KEN156/04) as described in Hundie et al., (2019).

**Adult infection testing**

Two plants from each line including Paragon, Pavon 76 and the susceptible check Thatcher, were grown in a 4 L pot containing BM-1 soil media in the greenhouse as described above. At spike emergence, plants were inoculated with a field composite of *P. triticina* collected in 2017 from Thatcher growing in Alfalfa County Oklahoma and transferred to a humidity chamber as described previously. At 21 DPI, flag leaves were scored for infection using percentage of leaf coverage and infection type (McIntosh et al., 1995).

This article is protected by copyright. All rights reserved.
Twenty-one lines were tested for the presence of \( \text{Lr}_{46} \) and \( \text{Lr}_{34} \), using KASP markers. Fifty to 100 ng of DNA was dried in a 384 well plate and re-suspended using 3 µl of Kompetitive Allele-Specific PCR (KASP™) assay master mix containing primer, 2X KASP buffer and enzyme (Keygene), and MgCl₂. For \( \text{Lr}_{46} \) the primer used was \( \text{Lr}_{46}\text{-Yr29\_JF2-2}\text{-KASP} \) (Brown-Guedira, G. and Fellers, J.P., unpublished), and for \( \text{Lr}_{34} \) two separate primers \( \text{Lr}_{34}\text{Exon11}\text{-KASP} \) (Lagudah et al., 2009) and \( \text{Lr}_{34}\text{JagExon22}\text{-KASP} \) (Yan, L. unpublished) were used to check for the \( \text{Lr}_{34} \) gene and also confirm there was not a false positive gene. For \( \text{Lr}_{34}\text{Exon11} \) and \( \text{Lr}_{46} \), the thermal cycler conditions used were: 94 °C for 15 min, 97 °C 15 sec, 68 °C 1 min -0.8 °C /cycle for ten cycles, 97 °C 20 sec, 60 °C 1 min for 30 cycles, 59 °C 30 sec -1.0 °C /cycle for 34 cycles, and a final cool down of 10 °C 5 min. For \( \text{Lr}_{34}\text{JagExon22} \), the thermal cycler conditions were: 94 °C 15 min, 94 °C 20 sec, 60 °C 1 min for 30 cycles and a cool down 5 min at 10 °C. Reactions were run on a GeneAmp Systems 9700 (Applied Biosystems) and results were processed using Klustercaller (LGC).

**Results**

Three different races of \( \text{P. triticina} \) were used to screen seedlings of the introgression lines. Race 1 BBBD, the most avirulent race available with virulence only to \( \text{Lr}_{14a}, \text{Lr}_{14b}, \text{Lr}_{20}, \) and \( \text{Lr}_{50} \), was used to identify any resistance contained in the lines. Only lines 28, 161, and 355 were resistant with infection type (IT) ‘; (fleck)’ meaning a very small, hypersensitive-like point on the leaf with no pustule formation.
Figure 1. Infection types of seedling bread wheat/Am. muticum introgression lines infected with P. triticina (leaf or brown rust) races A) BBBD; B) 97AZ103; and C) TNRJ. Plants were rated as R-resistant based on infection types of 0, 1, 2 or S-susceptible with infection type of >= 3 (McIntosh et al., 1995).

(Figure 1A; Table 1). Isolate 97AZ103A, originally collected from durum wheat in Arizona, is virulent on Lr2c, Lr3, LrB, Lr10, Lr14b, and Lr20 and represents one of the most virulent races on U.S. durum (tetraploid) wheat. Lines 161 and 355 were resistant to 97AZ103A (Figure 1B; Table 1).
Table 1. Disease reactions of bread wheat/\textit{Amblyopyrum muticum} introgression double haploid lines after infection with \textit{Puccinia triticina} (Leaf), \textit{P. graminis tritici} (Stem), and \textit{P. striiformis tritici} (Stripe). Resistance was evaluated at the seedling and adult stages. Lines were also tested for the presence of resistance gene, \textit{Lr34}. (NT-not tested).

| Line | Seedling | Adult | Stem Rust | Stripe Rust | \textit{Lr34} |
|------|----------|-------|-----------|-------------|--------|
|      | BBBD     | 97AZ103 | TNRJ      | OK Composite | KS Composite | KS Composite | Exon 11 |
| 1    | 3+       | 3      | 3         | 1-2C        | 4      | 9      | ins/ins |
| 11   | 3        | 3      | 3         | 1-2C        | 4      | 9      | NT      |
| 15   | 3        | 3      | 3         | 1-2C        | 4      | 9      | ins/ins |
| 16   | 3+       | 3      | 3         | 90S         | 4      | 2      | ins/ins |
| 17   | 3+       | 3      | 3         | 20R, LTN    | 4      | 5      | del/del |
| 19   | 3-       | 3      | 2         | 0R          | 4      | 3      | ins/ins |
| 20   | 3        | 3      | 3         | 40Z         | 4      | 9      | ins/ins |
| 21   | 3+       | 3      | 3         | 90S         | 1-2    | 8      | ins/ins |
| 27   | 3        | 3      | 3         | 30MR        | 2      | 2      | NT      |
| 28   | ;        | 3      | 3         | 90S         | 4      | 8      | NT      |
| 29   | 3        | 3      | 3         | 40MR        | ; 1    | 1      | ins/ins |
| 65   | 3        | 3      | 3         | 80S         | NT     | 9      | NT      |
| 86   | 3        | 3      | 3         | 30MR        | 1-2    | 9      | NT      |
| 92   | 3        | 3      | 3         | 1-2C        | 2      | 1      | NT      |
| 96   | 3        | 3      | 3         | 1          | 4      | 9      | NT      |
| 121  | 3        | 3      | 3         | 90S         | 4      | 1      | ins/ins |
| 122  | 3        | 3      | 3         | 1N          | 4      | 4      | ins/del |
| 123  | 3        | 3      | 3         | 90S         | 4      | 9      | ins/del |
| 124  | 3        | 3      | 3         | 90S         | 4      | 9      | NT      |
The third isolate, TNRJ, one of the most virulent on hexaploid bread wheat, is virulent on \( Lr \) genes \( Lr_1, \, Lr_2a, \, Lr_2c, \, Lr_3, \, Lr_9, \, Lr_{24}, \, Lr_{3ka}, \, Lr_{17}, \, Lr_{30}, \, Lr_{10}, \, Lr_{14a}, \, Lr_{28}, \, Lr_{39}, \, Lr_{14b}, \, Lr_{20}, \) and \( Lr_{28} \). Line 19 was scored as moderately resistant (IT ‘2’) having reduced pustule sizes. Line 161 was scored resistant with very small pustules surrounded by chlorosis (IT ‘1C’) (Figure 1C; Table 1).

\[ \begin{array}{cccccc}
161 & ; & ; & 1C & ;0R & 4 & 9 \text{ ins/ins} \\
191 & 3+ & 3 & 3 & 1-2C & 4 & 4 \text{ ins/ins} \\
192 & 3+ & 3 & 3 & 5R & 4 & 3 \text{ ins/ins} \\
195 & 3+ & 3 & 3 & 0R & 4 & 4 \text{ ins/ins} \\
196 & 3 & 3 & 3 & 1C,10R & 4 & 3 \text{ ins/ins} \\
198 & 3+ & 3 & 3 & 5R & 4 & 3 \text{ ins/ins} \\
202 & 3 & 3 & 3 & 60S & 4 & 3 \text{ ins/ins} \\
348 & 3 & 3 & 3 & 90S & 4 & 9 \text{ NT} \\
355 & ; & ; & 3 & 5R & 1-2 & 4 \text{ ins/ins} \\
Pavon 76 & 3 & 3 & 3 & 90S & 4 & 9 \text{ ins/ins} \\
Paragon & 3 & 3 & 3 & 1-2C & 4 & 7 \text{ ins/ins} \\
\end{array} \]

*- leaf rust infection type 0,;1,2,3, resistant-susceptible, respectively; 0R-90S, adult flag leaf based on percentage leaf coverage; LTN- leaf tip necrosis. C - chlorosis; N - necrosis; MR - medium resistance; Z - more pustules at the base, fewer at the tip (McIntosh et al, 1995)

§- stem rust infection type for seedlings, 0,;1-4, resistant-susceptible, respectively. (McIntosh et al, 1995)

‡- Stripe (Yellow) rust infection type, 1 - 9, resistant-susceptible, respectively. (McNeal et al., 1971)

A \( P. triticina \) field composite, representing the current \( P. triticina \) population in the U.S. Great Plains, was used to screen adult plants. There were three different reaction types. First, recurrent parent Paragon exhibited an IT of ‘1-2C’ (a reduced pustule size with a surrounding...
chlorotic ring) and even pustule distribution across the flag leaf. Five of the introgression lines showed this reaction (1, 11, 15, 92 and 191 - Table 1). Second, four lines, 19, 161, 195, and 355 exhibited near immunity with few to zero pustules. The third set of lines, 17, 20, 27, 29, 86, and 196, had reduced total coverage of spores, without the chlorotic halos, which is more resistant than the parents. Interestingly, lines 191, 192, 195, 196 and 198 contain the same segment and all show some level of resistance, suggesting the presence of a resistance gene(s) on 7T. Line 202, however, which also contains the same 7T segment is susceptible (see discussion). Line 17 exhibited leaf tip necrosis (LTN; Table 1) and therefore all lines were assessed for the presence of diagnostic molecular marker alleles for Lr34 and Lr46, both broad spectrum adult plant resistance genes. Only line 17 was positive for the deletion in Exon 11 of Lr34 (del/del; Table 1) and hence was the only line containing Lr34. The Lr46 diagnostic SNP (G) was heterozygous (G/C) in all lines except 196 and 202, which were homozygous (C/C), i.e. Lr46 absent.

Seedlings were tested with a composite of two P. graminis f. sp. tritici isolates commonly found in the US Great Plains. RKQQC and GFCFC have a combined virulence to the following Sr genes 5, 6, 7b, 8a, 9a, 9b, 9d, 9g, 10, 17, 21, 36, Tmp, and McN (Jin et al. 2008). Lines 21, 27, 86, 92 and 355 had infection types indicating differing levels of resistance, while line 29 was very resistant having an IT ;1 (small hypersensitive regions, some with pustules; Figure 2a). Tests with TTKSK, for a possible new source of resistance to Ug99 race group [TTKSK
Figure 2. Infection types of seedling bread wheat/Am. muticum introgression lines infected with *P. graminis tritici* (stem or black rust) A) Composite of QFSCC and RKQC; B) Ug99 race TTKSK. Plants were rated as R-resistant based on infection types of 0, ;, 1; MR- moderately resistant 2; MS-moderately susceptible 3; or S- susceptible with infection type of >= 3 (McIntosh et al., 1995).

Resistance phenotypes to *P. striiformis tritici* have larger lesions and use a different rating scale based on lesion length and number of pustules. The composite used to inoculate the seedlings represents the current Great Plains field population and is most notable for overcoming Yr17 and the unknown “TAM111” Yr gene within QTL *QYr.tamu-2B* (Yang et al., 2019). Paragon appeared to have low level of resistance to this composite (Figure 3) and was scored as 7 out of 9 (Table 1). Fifteen of the introgression lines were scored with high to medium levels of resistance ranging from 1-5. Lines 16, 27, 29, 92, and 121 had the highest level
of resistance (Table 1, Figure 3).

![Image]

**Figure 3.** Infection types of seedling bread wheat/Am. muticum introgression lines infected with a Kansas composite of *P. striiformis tritici* (stripe or yellow rust). Plants were rated as R-resistant based on infection types of 1-3; MR-moderately resistant 4-5; MS-moderately susceptible 6-7; or S-susceptible with infection type of >= 8 (McNeal et al., 1971).

**Discussion**

The use of wheat relatives in crop improvement has been technologically limited by an inability to quickly identify and characterize introgressions. The assumed low levels of recombination between the chromosomes of wheat and those of the wild relatives have also prevented adoption of this source of variation. However, due to the presence of a Ph1 inhibitor in *Am. muticum* and the application of new marker technology, over 200 introgressions have been generated between wheat and *Am. muticum* (Dover and Riley, 1972; King et al., 2017, and 2019; Grewal et al., 2019). Twenty-four of the 28 lines evaluated here carry single introgressions with the remaining four carrying either two or three. The introgressions in two of the 28 lines are whole chromosomes or very large chromosome segments from the T genome but nine are small to telomeric in size (see karyotypes in King et al., 2019). The smaller introgressions are likely to carry fewer deleterious genes and thus can be more rapidly integrated into a breeding program. Larger introgressions, however, frequently need to be

This article is protected by copyright. All rights reserved.
reduced in size in order to minimize linkage drag. This can be done via the strategy of overlapping introgressions (Sears, 1956) or via recombination with the B and D genomes (Glèmin et al., 2019). In this report, we begin assessing whether the T genome has useful resistance to the three cereal rusts.

Useful resistance was found to all three cereal rusts within the introgression lines with some of the resistance due to new resistance genes. Line 355 had seedling resistance to the less virulent *P. triticina* races and adult resistance to the field composite but was susceptible to TNRJ. Line 355 was also resistant to Great Plains isolates of *P. graminis* f. sp. *tritici* but not Ug99. Line 355 has a small 1T fragment and due to its resistances may be useful for durum and bread wheat improvement. Line 161 had very good *P. triticina* resistance at both seedling and adult stages but contains a large segment of 1T. One *Lr* gene may be shared with Line 355, but 161 may also have a second gene that provides resistance to TNRJ. The *Am. muticum* lines also provided new resistance to *P. graminis* and *P. striiformis*. Lines 29 and 92 were highly resistant to both species with 29 also resistant to *P. graminis* Ug99 race TTKSK. No stem rust resistance gene derived from *Am. muticum* has been previously described, therefore this resistance is new. Line 29 contains a whole 7T chromosome and thus will need to be reduced in size before it can be utilized in a breeding program. Unfortunately, the lines in this study with large or small recombined segments derived from 7T did not exhibit resistance to Ug99, suggesting that they are missing the fragment of the whole chromosome with the resistance gene. Line 92 contains a very small introgression from 5T (King et al., 2019).

Several of the introgression lines tested were produced from the same original BC₃ plants (Supplemental Table 1) and were therefore expected to contain the same segments. Indeed the molecular and cytogenetic characterization appeared to confirm this. However, some of the lines tested containing the same segments did not give the same resistance results. The
most notable of these are lines 124 and 355 and line 202 compared to lines 191, 192, 195, 196 and 198. Line 124 was susceptible to all races tested while as outlined above, line 355 shows useful resistance to leaf and stem rust. Lines 191, 192, 195, 196 and 198 all show adult resistance to *P. triticina* while line 202 was susceptible. There are a number of possible explanations for these apparently conflicting results. Firstly, neither the molecular nor cytogenetic characterization would have revealed small differences in the size of the segments. The markers used for the characterization of the lines were part of the Axiom Wheat Wild Relative Array (King et al., 2019) and while they give good coverage of the chromosomes, gaps do exist. Secondly, additional very small segments might have been present in some lines but not detected. It has become clear that the level of recombination seen in the wheat/*Am. muticum* introgression lines is very extensive and the recombination occurs in the gametes of all generations and not just the *F*$_1$ hybrids as originally expected.

The hexaploid heritage is also contributing some adult plant resistance in the introgression lines. Chinese Spring has previously been shown to contain *Lr34* (McIntosh et al., 2008). Line 17 exhibited LTN and was indeed positive for the active *Lr34* allele. Chinese Spring was the first cross to *Am. muticum* for several of the introgression lines and therefore likely to be the source of *Lr34* in line 17. However, it was not maintained in the other lines developed from Chinese Spring, i.e. lines 15, 16, 19, 20, and 21. Pavon76, Paragon and 24 of the introgression lines were also found to be heterozygous for the *Lr46* allele. However, the heterozygous result for the introgression lines is probably due to the functionality of the marker as these lines were produced via a doubled haploid procedure and were therefore expected to be homozygous at all loci. The races used for all three pathogens were all virulent on Pavon76 and Paragon with the exception of the *P. triticina* field composite, which exhibited an adult *Lr12*-like reaction on Paragon (McIntosh et al., 1995). Paragon is listed as having moderate resistance
to *P. triticina* and *P. graminis* without specific resistance gene designations (Cereals Variety Handbook, 1997). *Lr12* is known to be present in Chinese Spring, but introgression lines without Chinese Spring in their parentage also expressed this phenotype, suggesting Paragon may be the source (McIntosh et al., 2008). The infection phenotype of lines 19, 86, 161, 192, 195, 196, 198 and 355 was different than that of Paragon suggesting the presence of different genes from chromosomes 1T, 2T and 4T or 7T.

In this work we have evaluated a group of *Am. muticum* introgression lines as a new source for resistance to cereal rusts. The screening has identified several lines with resistance to rust races that are representative of current field populations. Five lines, 86, 92, 96, 121, and 355 are now a useful source of germplasm with introgressions supported by GISH (King et al., 2019) and are supported by markers to follow the introgressions. From 237 *Am. muticum* specific SNPs, 137 KASP PCR markers have been derived that span the genome (Grewal et al., 2019; Supplemental Table 1 includes KASP markers that can be used for each line). Most importantly, two lines have small fragments that should have less linkage drag and can be useful as germplasm in breeding strategies. The germplasm is available through the John Innes Centre Germplasm Resource Unit or by request from USDA-ARS.

**Conflict of Interest**

The authors declare that there are no conflicts of interest.

**Author Contributions**

J. Fellers conceived and conducted the screening experiments, provided funding, and co-wrote the manuscript. A. Matthews and A.K. Fritz provided marker analysis and co-wrote the manuscript. M. Rouse conducted the Ug99 screening and revised the manuscript. S. Grewal, J.
King and I.P. King provided the lines, verified the introgressions with GISH and markers and co-wrote the manuscript.

**Acknowledgements**

Research for this work was funded by Biotechnology and Biological Sciences Research Council (BBSRC Grant No. BB/P016855/1) as part of the Developing Future Wheat (DFW) programme and USDA-CRIS projects 3020-21000-011-00D and 5062-21220-023-00-D. This manuscript is a joint contribution of the US Department of Agriculture and Kanas Agricultural Experiment station, contribution number 20-182-J. Mention of a trademark of a proprietary product does not constitute a guarantee of warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable. The USDA is an equal opportunity provider and employer.

**References**

Al-Kaff, N., E. Knight, I. Bertin, T. Foote, N. Hart, S. Griffiths, and G. Moore. 2008. Detailed dissection of the chromosomal region containing the Ph1 locus in wheat *Triticum aestivum*: With deletion mutants and expression profiling. Ann. Bot. 101: 863-872.

Cereals Variety Handbook, 1997. NIAB recommended lists of cereals. National Institute of Agricultural Botany, Cambridge, UK. pp 153.

Cox, T.S. 1998. Deepening the wheat gene pool. J. Crop Production. 1:1–25.

Dover, G.A., and R. Riley, 1972. Variation at two loci affecting homoeologous meiotic pairing in *Triticum aestivum x Aegilops mutica* hybrids. Nature New Biol. 235:61-62.

This article is protected by copyright. All rights reserved.
Eser, V. 1998. Characterization of powdery mildew resistant lines derived from crosses between *Triticum aestivum* and *Aegilops speltoides* and *Ae. mutica*. Euphytica. 100:269-272.

Flor, H.H. 1955. Host-parasite interaction in flax rust – its genetics and other implications. Phytopathology 45: 680–685

Glémin, S., C. Scornavacca, J. Dainat, C. Burgarella, V. Viader, M. Ardisson, G. Sarah, S. Santoni, J. David, and V. Ranwez. 2019. Pervasive hybridizations in the history of wheat relatives. Sci. Adv. 5:eaaav9188.

Grewal, S., S. Hubbart-Edwards, C. Yang, U. Devi, L. Baker, J. Heath, S. Ashling, D. Scholefield, C. Howells, J. Varde, P. Isaac, I.P. King, and J. King. 2019. Rapid Identification of homozygous, and site of wild relative introgressions in wheat through chromosome-specific KASP genotyping assays. Plant Biotech. J. In Press

Hundie, B., B. Girma, Z. Tadesse, E. Edae, P. Olivera, E.H. Adera, W. D. Bulbula, B. Abeyo, A. Badebo, G. Cisar, G. Brown-Guedira, S. Gale, Y. Jin, and M.N. Rouse. 2019. Characterization of Ethiopian wheat germplasm for resistance to four *Puccinia graminis* f. sp. tritici races facilitated by single-race nurseries. Plant Dis. 103:2359-2366.

Iefimenko, T.S., Y.G. Fedak, M.Z. Antonyuk, and T.K. Ternovska. 2015. Microsatellite analysis of chromosomes from the fifth homoeologous group in the introgressive *Triticum aestivum/Amblyopyrum muticum* wheat lines. Cytol. Genet. 49:183-191.

Jin, Y., L.J. Szabo, Z.A. Pretorius, R.P. Singh, R. Ward, and T. Fetch Jr. 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 92:923-926.

King, J., C. Newell, S. Grewal, S. Hubbart-Edwards, C. Yang, D. Scholefield, S. Ashling, A. Stride, and I.P. King. 2019. Development of stable homozygous wheat/*Amblyopyrum muticum* (*Aegilops mutica*) introgression lines and their cytogenetic and molecular characterization. Front. Plant Sci. 10:34
King, J., S. Grewal, C. Yang, S. Hubbart, D. Scholefield, S. Ashling, KJ. Edwards, A.M. Allen, A. Burridge, C. Bloor, A. Davassi, G.J. da Silva, K. Chalmers, and I.P. King. 2017. A step change in the transfer of interspecific variation into wheat from Amblyopyrum muticum. Plant Biotech. J. 15:217-226.

Kolmer, J.A. 2019. Virulence of Puccinia triticina, the wheat leaf rust fungus, in the United States in 2017. Plant Dis. 103:2113-2120

Kolmer, J.A., D.L. Long, and M.E. Hughes. 2005. Physiological specialization of Puccinia triticina on wheat in the United States in 2003. Plant Dis. 89:1201-1206

Lagudah E.S., H. McFadden, R.P. Singh, J. Huerta-Espino, H.S. Bariana, and W. Spielmeyer. 2006. Molecular genetic characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat. Theor. Appl. Genet. 114:21-30.

Lagudah, E.S., S.G. Krattinger, S. Herrera-Foessel, R.P. Singh, J. Huerta-Espino, W. Spielmeyer, G. Brown-Guedira, L.L. Selter, and B. Keller. 2009. Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. Theor Appl Genet. 2009 119:889-898.

Liu, W, T.V. Danilova, M.N. Rouse, R.L. Bowden B. Friebe, B.S. Gill, and M.O. Pumphrey. 2013. Development and characterization of a compensating wheat-Thinopyrum intermedium Robertsonian translocation with Sr44 resistance to stem rust (Ug99). Theor Appl Genet 126:1177.

Mcintosh, R.A., C.R. Wellings, and R.F. Park. 1995. Wheat Rusts. An atlas of resistance genes. CSIRO, Australia.

Mcintosh R.A., Y. Yamazaki, J. Dubcovsky, W.J. Rogers, C. Morris, R. Appels, and X.C. Xia. 2008. Catalogue of gene symbols for wheat. 11th Int. Wheat Genet. Symp. 24-29 August 2008, Brisbane, Australia.

This article is protected by copyright. All rights reserved.
McNeal, F.H., C.F. Konzak, E.P. Smith, W.S. Tate and T.S. Russell. 1971. A Uniform System for Recording and Processing Cereal Research Data. Agricultural Research Service Bulletin 34-121.

McFadden, E.S. 1930. A successful transfer of emmer characters to vulgare wheat. J. Am. Soc. Agron. 22:1020–1034

Milus, E.A., K. Kristensen, and M.S. Hovmøller. 2009. Evidence for increased aggressiveness in a recent widespread strain of Puccinia striiformis f. sp. tritici causing stripe rust of wheat. Phytopathology 99:89-94.

Olson E.L., M.N. Rouse, M.O. Pumphrey, R.L. Bowden, B.S. Gill, and J.A. Poland. 2013. Introggression of stem rust resistance genes SrTA10187 and SrTA10171 from Aegilops tauschii to wheat. Theor. Appl. Gen. 126:2477-2484.

Pretorius, ZA, R.P. Singh, W.W. Wagoire, and T.S. Payne. 2000. Detection of virulence to wheat stem rust resistance gene Sr31 in Puccinia graminis f. sp. tritici in Uganda. Plant Dis. 84:203

Rahmatov, M., M.N. Rouse, B.J. Steffenson, S.C. Andersson, R. Wanyera, Z.A. Pretorius, A. Houben, N. Kumarse, S. Bhavani, and E. Johansson. 2016. Sources of stem rust resistance in wheat-alien introgression lines. Plant Dis. 100:1101-1109.

Sears, E.R. 1956. Transfer of leaf-rust resistance from Aegilops umbellulata to wheat. Brookhaven Symp. Biol. 9:1:21.

Sears, E.R. 1993. Use of radiation to transfer alien chromosome segments to wheat. Crop Sci. 33: 897:901.
Sears, E.R. 1977. An induced mutant with homoeologous pairing in common wheat. Can. J. Genet. Cytol. 4: 585-593.

Wan A., X. Chen, and J. Yuen. 2016. Races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2011 and 2012 and comparison with races in 2010. Plant Dis. 100: 966-975.

Warburton M.L., J. Crossa, J. Franco J. M. Kazi, R. Trethowan, S. Rajaram, W. Pfeiffer, P. Zhang, S. Dreisigacker S, and M. van Ginkel. 2006. Bringing wild relatives back into the family: Recovering genetic diversity in CIMMYT improved wheat germplasm. Euphytica. 149: 289-301.

Xu, S.S, Y. Jin, D.L. Klindworth, R.R-C. Wang, and X. Cai. 2009. Evaluation and characterization of seedling resistance to stem rust in wheat-alien species derivatives. Crop Sci. 49: 2167-2175.

Yang, Y., B. R. Basnet, A. M.H. Ibrahim, J. C. Rudd, X. Chen, R. L. Bowden, Q. Xue, S. Wang, C. D. Johnson, R. Metz, R. E. Mason, D. B. Hays, and S. Liu. 2019. Developing KASP markers on a major stripe rust resistance QTL in a popular wheat TAM 111 using 90K array and genotyping-by-sequencing SNPs. Crop Sci. 59: 165-175.

Zeller, F.J. 1973. 1B/1R wheat-rye chromosome substitutions and translocations. In Proceedings of the Fourth International Wheat Genetics Symposium. Eds. E.R. Sears and L.M.S. Sears. pp. 209-221.

Zohary D., J.H. Harlan, and A. Vardi. 1969. The wild diploid progenitors of wheat and their breeding value. Euphytica. 18: 58-65.