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Resistance to multiple cereal aphids in wheat-alien substitution and translocation lines

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Abstract

Rhopalosiphum padi, *Schizaphis graminum,* and *Sitobion avenae* are three of the most destructive aphid species of wheat (*Triticum aestivum* L.). They can significantly reduce wheat yields directly by feeding and indirectly by transmitting viruses. This study aimed to search for resistance to these aphid species among lines derived from different rye (*Secale cereale*) origins and from *Aegilops speltoides*, all in the genetic background of the wheat cultivar Pavon F76. Resistance was quantified as aphid weight (*R. padi*, *S. avenae*, *S. graminum*), and number of aphids and percentage of infested leaf area exhibiting chlorosis (*S. graminum*). The most resistant genotypes reduced *R. padi* and *S. avenae* weight by 24.2% and 34.3%, respectively, at the seedling stage, compared to Pavon F76 control plants. Strong *S. graminum* resistance was found only in *Ae. speltoides*-derived lines, the most resistant of which (7A.7S-L5) sustained just 3% chlorosis and reduced *S. graminum* colony weight by 67.7%. One line carrying the 1AL.1RS$_{am}$ wheat-rye translocation from Amigo wheat (originally from Insave rye) reduced *S. avenae* weight by 23.2 and 21.8% in seedling and adult plants, respectively. Single genotypes carrying the complete 1R chromosome or the 1RS chromosome arm derived from E12165 wheat and Presto triticale proved to be resistant to both *R. padi* and *S. avenae* at the seedling stage. Further research should be conducted to unravel the genetic basis of resistance to these aphids in 1RS genotypes. The sources of resistance identified here may be useful for incorporating multiple aphid species resistance in wheat breeding programs, particularly for *R. padi* and *S. avenae*, to which no resistant wheats have been bred.

Keywords: *Rhopalosiphum pad; Schizaphis graminum; Sitobion avenae; Triticum aestivum; Secale cereale; Aegilops speltoides*

Introduction

Aphids are a major biotic constraint to wheat (*Triticum aestivum* L.) production because they inflict direct feeding damage and can transmit viruses. Among those of particular importance are: the bird cherry – oat aphid (*Rhopalosiphum padi* L.), the greenbug (*Schizaphis graminum* [Rondani]), and the English grain aphid (*Sitobion avenae* [Fabricius]), which are widely distributed in wheat producing areas, with some differentiation depending on the geographic region (Blackman and Eastop 2007). These aphids can reduce wheat yields by 30-40% (Kieckhefer and Gellner 1992; Voss et al. 1997) and by up to 60% when direct damage is combined with losses from virus infection (Riedell et al. 2003). Damage due to *R. padi* and *S. avenae* is evident mainly as reduced plant growth and grain yields, whereas *S. graminum* feeding also causes plant chlorosis and necrotic spots at the feeding site (Voss et al. 1997; Blackman and Eastop 2007; Franzen et al. 2008). Given that plant resistance to arthropod pests is an environmentally and economically sound plant protection strategy, it is important to identify sources of aphid resistance for developing aphid-resistant wheat cultivars (Berzonsky et al. 2003; Porter et al. 2009).

Wheat-alien chromosome translocations and substitutions have been successfully exploited in the development of new wheat cultivars, and alien genetic sources such as rye (*Secale cereale* L.) were shown to enhance yield potential and confer resistance to biotic stresses (Friebe et al. 1996; Kim et al. 2004). A widely utilized source is the 1R chromosome from Petkus rye, which is deployed in hundreds of cultivars, such as Kavkaz wheat (Rabinovich 1998). Resistance to various biotic stresses has also been found in *Aegilops speltoides* Tausch (Friebe et al. 1996).

Breeding for *S. graminum*-resistant wheat cultivars began in the 1950s (Porter et al. 1997; Berzonsky et al. 2003); two of the resistance genes (*Gb2* and *Gb6*) originate from the 1RS chromosome arm of Insave rye (Sebesta and Wood 1978; Lu et al. 2010), whilst *Gb5* was derived from the 7SL chromosome arm of *Ae. speltoides* (Tyler et al. 1985, 1987; Lukaszewski 1995). No *R. padi*-resistant wheat cultivars currently exist, but several triticale and rye-wheat derived lines express resistance to *R. padi* (Hesler 2005; Hesler and Tharp 2005; Hesler et al. 2007). Similarly, no wheat cultivars have been bred for resistance to *S. avenae*, though a resistance gene from durum wheat (*Triticum turgidum* L. ssp. *durum* [Desf.]) was recently mapped (Liu et al. 2012). Previous studies have searched for *S. avenae* resistance in several wild *Triticum* relatives, but not in wheat-rye derived germplasm (Di Pietro et al. 1998; Migui and Lamb 2003; Migui and Lamb 2004). One exception is Amigo wheat (Lowe 1981; Hu et al. 2004), but *S. avenae* resistance has not been attributed to the 1AL.1RS translocation carried by this cultivar.

Plant materials are rarely evaluated for resistance to more than two aphid species. However, Smith et al. (2004b) evaluated 20 *R. padi*-resistant accessions, belonging to six *Aegilops* spp. and one *T. araraticum* Jakubz. They reported that *Aegilops neglecta* Req. accession 8052 also possessed resistance to *S. avenae* and the Russian wheat aphid, *Diuraphis noxia* (Kudjumov). Similarly, Migui and Lamb (2003) evaluated 19 wild wheat relatives for resistance to *R. padi*, *S. avenae,* and *S. graminum*, and found resistance to *S. avenae* and *R. padi,* or *S. avenae* and *S. graminum* in individual accessions, but none resistant to all three aphid species. Although diploid species appear to have the highest frequency of resistance (Migui and Lamb 2003), introgressing resistance genes from multiple wild resistance sources into adapted germplasm requires considerable pre-breeding efforts.

Burd and Porter (2006) studied *S. graminum* - host resistance interactions and found considerable intraspecific plant variation and several *S. graminum* virulence patterns. A chronological review of the field deployment of *S. graminum* resistant wheat cultivars and the detection of new *S. graminum* biotypes states that "The use of greenbug [*S. graminum*]-resistant wheat cultivars could not have contributed to the development of new biotypes" (Porter et al. 1997). There is also variation in the virulence patterns of various *S. avenae* populations (Lowe 1981; Caillaud et al. 1995; Xu et al. 2011). However no *S. avenae* biotype designations have been made, since the biotype concept refers to the specific outcomes from the aphid resistance/susceptibility interaction with germplasm differing in the resistance genes they carry. No variation in *R. padi* virulence has been reported to date.

This study aimed to search for resistance to *R. padi*, *S. avenae,* and *S. graminum* in a set of wheat-alien translocation and substitution lines previously developed in the same genetic background (spring wheat Pavon F76). Resistance to multiple aphid species within a single genotype is a highly desired trait and can greatly facilitate the development of new, aphid-resistant cultivars, particularly in regions where distributions of different aphid species overlap.

Materials and methods

Plant materials

We screened germplasm previously developed in the genetic background of the spring wheat cultivar Pavon F76 for resistance to *R. padi*, *S. avenae,* and *S. graminum*. The material consisted of 54 wheat-rye centric translocation and substitution lines, seven wheat-rye, and four *Ae. speltodies*-wheat recombinant derived lines (Table 1) from the genetic resources program of the International Maize and Wheat Improvement Center (CIMMYT, Mexico). These lines were second generation offspring from self-pollinated single plants that had been analyzed by C-banding to confirm published substitution/translocation descriptions. Descriptions of the genotypes can be found in Lukaszewski (1993, 1995, 1997, 2000, 2006, 2008), Brunell et al. (1999), Kumlay et al. (2003), Kim et al. (2004), and Lukaszewski et al. (2004). This paper will follow the same nomenclature, where subscripts represent the origin of wheat chromosomes (A, B, and D) and rye (R) genomes (L=chromosome long arm; S=chromosome short arm) (Table 1).

Aphid rearing

Aphids were reared in cages under glasshouse conditions at ca. 22^{o}C, with a minimum of 16 h of light, supplemented when needed with 400W high-pressure sodium lamps. *R. padi* and *S. avenae* were maintained on oat plants in the Department of Plant Breeding and Biotechnology at the Swedish University of Agricultural Sciences (SLU). Two sources of Swedish *R. padi,* collected 600 km apart, were used in the screenings; they were collected from their winter host (*Prunus padus* L.) in order to avoid infection by barley yellow dwarf virus. *S. avenae* adults were collected from wheat fields around Alnarp, and a colony was started from nymphs born on a sugar diet to obtain virus free aphids. Virus-free starter colonies of *S. graminum* biotype E were obtained from USDA-ARS and maintained on Jagger wheat in the Department of Entomology at Kansas State University (KSU). Biotype E is

common and virulent to wheat carrying resistance genes *gb1* and *Gb2*, but non-virulent to genes *Gb3*-*Gb7* (Burd and Porter 2006; Weng et al. 2005).

Aphid resistance screening methods

All experiments included a Pavon F76 control, since the alien material was transferred into that genetic background. Screening consisted of two phases for each aphid species - Phase 1, where all lines were screened for resistance and Phase 2, where genotypes selected in Phase 1 were screened three times with *R. padi* and *S. avenae,* or once with *S. graminum,* to confirm or reject statistically significant results from Phase 1. Other lines from the stock having the same alien origin as the statistically more resistant lines in Phase 1 were also included. *R. padi* tests preceded the *S. avenae* tests and some lines resistant to *R. padi* were also included in the *S. avenae* Phase 2 test.

Evaluation of seedling plant resistance to R. padi, S. avenae, and S. graminum

Resistance to *R. padi* and *S. avenae* was measured as reduced growth rate at SLU. Candidate lines were divided into subsets in Phase 1, as it was only possible to screen a maximum of 24 entries per week. Seeds of each genotype were placed on humid filter paper in Petri dishes, cold treated at 5^{o}C for three days, maintained at room temperature for two days, and then individually planted in a plastic 10 cm-diameter pot (300 ml) in Weibull's Kronmull® potting soil with Leca. Pots were arranged in a randomized complete block design with four replicates (one plant per replicate). Seedlings were grown in a walk-in climate controlled chamber at 22^{o}C, 80% RH, and 16 h light at the intensity of 250 µmol photons $m^{-2}s^{-1}$ at plant level.

Seven to eight days after transplanting (second - third leaf stage), each plant was exposed to five nymphs born within a time span of 24 hours. These nymphs were obtained from alate females in the rearing colony maintained overnight on oat plants. Newborn nymphs were confined at the base of the test plants in transparent cylindrical acrylic cages (2 cm diameter, 5 cm length), sealed with cotton wool at the bottom and the top. Nymphs were individually weighed on a Mettler M3 microbalance four days after infestation.

S. graminum experiments were performed under glasshouse conditions (ca. 22^{o}C, 80% RH, and 16 h light) at KSU. In Phase 1, 8-10 seeds of each genotype were sown in tufts in flats (36 cm x 51 cm) filled with Pro-mix® BX (Hummert International), in a complete randomized experimental design with four replicates. The stock was divided spatially in two subsets since there was only room for 35 genotypes per flat. The *S. graminum*-resistant control, wheat line GRS-1201, containing the *Gb6* resistance gene from Insave rye (Lu et al. 2010) was included in each flat. The plants were infested at the second to third leaf stage with previously infested leaves of Jagger wheat, at an average density of 5 nymphs per tuft. We scored the percentage of chlorosis on infested leaves 14 days after infestation. For Phase 2 screening, eight genotypes were selected based on their genetic background and a low percentage of chlorosis in Phase 1. Seeds of each genotype were germinated in Petri dishes for six days, and seedlings of each entry were then planted individually in 10 cm pots. Each pot was covered with a fine-mesh of organdy fabric supported by wooden stakes allowing undisturbed growth of plants. Plants at the second to third leaf stage were then infested with two third or fourth instar nymphs. The experiment was established as a randomized

complete block design with 10 replicates. Percentage of chlorosis, number of aphids, and weight of the colony were recorded 10 days after infestation.

Evaluation of adult plant resistance to S. avenae

We selected and evaluated 12 genotypes for adult plant *S. avenae* resistance, using a similar rationale for selections as for Phase 2 seedling tests. Screening additional lines was not possible due to space and management requirements or lack of synchrony with the phenology of the control line.

Seeds were germinated as described above, transplanted singly into 16 cm plastic pots (2 L), and grown under glasshouse conditions (in a randomized complete block design with four replicates) until ear emergence (Zadoks growth Scale GS: 53-55, Zadoks et al. 1974). Plants were then infested with five newborn *S. avenae* (obtained using the above methods). Nymphs were confined to one emerging ear and its flag leaf with a perforated polyethylene bag (18 x 26 cm, Baumann Saatzuchtbedarf), and plants were then transferred to a walk-in climate controlled chamber (22°C, 80% HR, and 16 h light at the intensity of 250 µmol photons $m^{-2}s^{-1}$ at plant level), where they were kept for four days before the aphids were weighed on a Mettler M3 microbalance. The Pavon F76 control was sown weekly three more times after the test plants, so that growth stages could be matched. Entries were grouped in two subsets according to their phenology because they had different days until flowering.

Statistical analysis

We used SAS/STAT® software, version 9.2 (SAS Institute Inc. 2009) for all statistical analyses. Analysis of variance for aphid weights from Phase 1 experiments with *R. padi* and *S. avenae* was conducted using the GLM procedure, and least-square means per genotype were calculated. Statistical differences between genotype and control means were tested by the comparatively less stringent *t*-test to identify all potentially resistant lines (type 1 error was not anticipated, as Phase 2 experiments would correct). The ratio between the mean of aphids on a genotype and on a control plant was calculated as aphid weight percentage of Pavon F76. Results from Phase 2 experiments were analyzed with the MIXED procedure, where genotypes were included in the model as fixed effects, and blocks and round of evaluation as random effects. Blocks were nested within round of evaluation. Least square means were compared with those of Pavon F76 in a Dunnett's test. We analyzed both phases of the *S. graminum* evaluation with the GLM procedure. In Phase 1, plant chlorosis was scored and means were compared with *t*-tests. In Phase 2, numbers of *S. graminum* and the weight of the *S. graminum* colony were quantified and genotype means were compared with those of Pavon F76 in a Dunnett's test. One degree of freedom contrasts were made between genotypes 7A.7S-L7, 7A.7S-L5, 7A.7S-Gb5, and the resistant control (GRS-1201).

Results

In Phase 1 evaluations, *R. padi* weights ranged from 65.2 % to 119.3 % of the Pavon F76 control, though we detected statistical differences between genotypes in only the first two subsets (subset 1: $df = 15, 213$; $F = 3.11$; $p<0.0001$; subset 2: $df = 17, 273$; $F = 3.63$; $p< 0.0001$; subset 3: $df = 17, 284$; $F = 1.55$; $p = 0.0771$; subset 4: $df = 16, 282$; $F = 1.41$; $p = 0.1428$). Eight genotypes carrying rye chromatin on chromosome 1 reduced *R. padi* weight

significantly ($p \leq 0.05$; *t*-test) compared to the Pavon F76 control (Table 1). These lines, six other lines carrying rye chromatin of similar origin and the control (15 total genotypes) were selected for Phase 2 screening. Significant differences were detected with the SAS MIXED procedure ($df = 14, 146$; $F = 3.29$; $p < 0.0001$). According to the Dunnett's test, genotypes $1R_e(1A)$, $1R_e(1B)$, $1R_{pr}(1D)$, $1R_{pr}.1D_{5+10-2}(1D)$, $1AL.1RS_e$, and $1BL_v.1RS_e$ reduced *R. padi* weight significantly (23.9-14.4%; Table 2), compared to the Pavon F76 control.

S. avenae mean weights in Phase 1 evaluations ranged from 69.8 % to 157.4 % of Pavon F76. However, statistical differences ($p \leq 0.05$) between genotypes were observed only in subset 1 ($df = 18, 199$; $F = 3.63$; $p \leq 0.001$), where only the $1BL_v.1RS_e$ genotype reduced *S. avenae* mean weight significantly more than Pavon F76 (Table 1). Selection of genotypes for Phase 2 was therefore less strict than before ($p \leq 0.1$) and included some lines with rye chromosome 1 chromatin resistant to *R. padi*. *S. avenae* weights were significantly reduced on 11 genotypes ($df = 22, 211$; $F = 3.52$; $p \leq 0.0001$) compared to Pavon F76 (Table 2). The weight of *S. avenae* feeding on adult plants of the $1AL.1RS_{am}$ genotype was significantly reduced ($p \leq 0.05$) compared to Pavon F76 (Table 3). This line also significantly reduced *S. avenae* weight by 23.2% in the Phase 2 seedling test compared to Pavon F76).

Genotypes differed significantly for *S. graminum*-related leaf chlorosis (subset 1: $df = 34, 101$; $F = 1.84$; $p = 0.0105$; subset 2: $df = 33, 65$; $F = 2.32$; $p = 0.0019$). However, only four lines with *Ae. speltoides* chromatin (7A.7S-L7, 7A.7S-L5, and 7A.7S-Gb5) were significantly less chlorotic than Pavon F76 (Table 1). Statistical differences were observed for the numbers of *S. graminum* developing on the plants ($df = 9, 60$; $F = 2.37$; $p = 0.023$), weight of the colony ($df = 9, 60$; $F = 3.24$; $p = 0.0029$) and chlorosis ($df = 9, 68$; $F = 7.74$; $p < 0.0001$). As in Phase 1, genotypes 7A.7S-L7, 7A.7S-L5, and 7A.7S-Gb5 showed less leaf chlorosis than Pavon F76 (Table 4), and 7A.7S-L5 also significantly reduced *S. graminum* colony weight by 67.7 % and the number of *S. graminum,* compared to Pavon F76. Genotypes 7A.7S-L7 and 7A.7S-Gb5 showed low average levels of chlorosis (0.9 % and 2.3%, respectively), but did not significantly reduce *S. graminum* numbers or colony weight. Line 7A.7S-S3 showed a susceptible response, as it also did in the Phase 1 test (Tables 1, 4). For the four genotypes with low chlorosis, one degree of freedom contrasts showed differences in the number of *S. graminum* per plant between genotypes 7A.7S-L7 and 7A.7S-L5, and between 7A.7S-L7 and GRS-1201 ($p < 0.01$), but no significant differences were observed between the four lines in terms of aphid colony weight or chlorosis (Table 5). Therefore these results indicate that genotypes 7A.7S-L5 and 7A.7S-Gb5 performed similarly to the resistant control GRS-1201 in all aspects studied (Table 5).

Discussion

Certain translocation and substitution lines with rye chromatin in chromosome 1 were resistant to both *R. padi* and *S. avenae*, but no 1R or 1RS line was strongly resistant to *S. graminum* biotype E. However, previous studies have shown that the 1RS chromosome arm carries the *Gb2* and *Gb6 S. graminum* resistance genes from Insave rye (Sebesta and Wood 1978; Porter et al. 1991; Lu et al. 2010). Chromosome 1R from the E12165 wheat substitution line incorporated into Pavon F76 carries gene(s) for *R. padi* and *S. avenae* resistance. Most genotypes with this $1R_e$ source reduced the weights of *R. padi* or *S. avenae*, and genotypes $1R_e(1A)$, $1R_e(1B)$, $1AL.1RS_e$, and $1BL_v.1RS_e$ significantly reduced the weights of both aphid species. These genetic stocks were produced by Lukaszewski (1997)

from the wheat line E12165 with a 1R(1D) substitution from a cross between Panda triticale and a CIMMYT wheat line. Both sources of resistance are very useful in breeding programs, since 1RS has previously been reported to increase yield in the Pavon F76 background (Kim et al. 2004). Of the two lines resistant to both *R. padi* and *S. avenae* (1AL.1RS$_e$ and 1BL$_v$.1RS$_e$), 1AL.1RS$_e$ is preferred because it has less of a negative effect on baking quality (Kumlay et al. 2003). Genotypes 1R$_{pr}$(1D) and 1R$_{pr}$.1D$_{5+10-2}$(1D), from Presto triticale, also expressed resistance to *R. padi* and *S. avenae*. The 1R chromosome from Presto was engineered to improve bread-making quality in hexaploid triticale, and later, genotypes 1R$_{pr}$(1D) and 1R$_{pr}$.1D$_{5+10-2}$(1D) were produced in the Pavon F76 background (Lukaszewski 2006).

The 1RS chromosome was present in all of the lines that significantly reduced the weight of *R. padi* and/or *S. avenae* in Phase 2 tests, suggesting that gene(s) for resistance are located on 1RS. Interestingly, the genotype carrying the 1RS translocation from Amigo wheat (1AL.1RS$_{am}$) showed resistance to *S. avenae* both at seedling and adult stages. Other studies have reported that Amigo wheat is resistant to *S. avenae* (Lowe 1981; Hu et al. 2004), and our results suggest that this resistance is conferred by 1RS from Insave rye.

Unlike *R. padi* and *S. graminum*, which cause significant damage at early plant stages, *S. avenae* normally has a higher reproductive rate when feeding on flowering plants (Watt 1979) and causes more deleterious damage to plants in later developmental stages (Voss et al. 1997). It is therefore important to screen for *S. avenae* resistance in adult plants, but the challenges of keeping plants free of other pests and diseases, and the difficulty in matching the phenology of test plants, make adult plant testing much more demanding than seedling tests. This is perhaps the reason why few studies of adult plant resistance to *S. avenae* have been conducted under controlled conditions.

Of the lines expressing *S. avenae* resistance as seedlings, only the 1AL.1RS$_{am}$ translocation line from Amigo wheat significantly reduced *S. avenae* weights on adult plants. Similar results were obtained by Migui and Lamb (2004) when they compared resistance effects on *S. avenae* from seedlings and adult plants of *Triticum monococcum* L. accessions. They hypothesized that the lower resistance of adult plants was due to a reduction in hydroxamic acids (Hx) during wheat plant growth and development (Migui and Lamb 2004). However, Hx concentration is still relatively high in the young tissues of mature plants, such as in emerging flag leaves (Thackray et al. 1990; Copaja et al. 1999). Several studies have shown a strong negative correlation between cereal aphid performance, including *S. avenae*, and Hx concentrations in young wheat plants (Thackray et al. 1990; Leszczynski and Dixon 1992; Givovich and Niemeyer 1994; Hansen 2006), whereas other have shown no correlation between Hx concentration and *S. avenae* performance (Nicol and Wratten 1997; Castaneda et al. 2010).

Hx concentrations are unlikely to explain the resistance patterns that we found in lines with a Pavon F76 background. In Australia, this wheat cultivar is called Hartog (Skovmand et al. 1997) and is known to have low Hx levels (Nicol et al. 1992). Furthermore, the genes governing the accumulation of Hx are located on hexaploid wheat chromosome groups 4 and 5, and on rye chromosomes 5R and 7R (Niemeyer and Jerez 1997; Nomura et al. 2002; Nomura et al. 2003), whereas rye resistance to *S. avenae*, and *R. padi* was only found in chromosome 1 substitution and translocation lines. Since the plant genotypes into which the alien material was incorporated have low Hx concentrations, both seedling and adult plant resistance is most likely due to other physiological processes, unless

there are epistatic effects of resistance genes on Hx genes. Further investigation is needed to elucidate the causes of seedling resistance and the lack of resistance in the adult stage.

Tyler et al. (1985) identified a source of resistance to *S. graminum* originating from *Ae. speltoides* in several wheat substitution lines. The resistance gene in those lines was later designated as *Gb5* (Tyler et al. 1987). One substitution line (CI17884) was utilized to derive the KS90H445 wheat translocation line. This line lacked suitable agronomic performance (Friebe et al. 1991). Therefore, Lukaszewski (1995) transferred the *Gb5* chromatin from KS90H445 to Pavon 76 through homoeologous recombination with the *ph1b* mutation, resulting in, among others, the lines 7A.7S-S3, 7A.7S-L7, 7A.7S-L5, and 7A.7S-Gb5. Dubcovski et al. (1998) characterized 7A.7S-S3 and 7A.7S-Gb5 as carrying the *Lr47* and *Gb5* resistance genes, respectively. The substitution line CI17884 demonstrates moderate antibiotic effects on *S. graminum* biotype E (Tyler et al. 1985), and in our experiments, genotype 7A.7S-Gb5 gave similar results combined with low chlorosis levels. Genotype 7A.7S-L7 had significantly lower levels of chlorosis compared to Pavon F76, but significantly higher numbers of *S. graminum* per plant when compared to 7A.7S-L5 and the resistant check GRS-1201. The differences between these recombinant derived lines suggest that genotype 7A.7S-L7 is predominantly tolerant to *S. graminum* feeding, and that the chromatin transferred to Pavon F76 has more than one locus responsible for *S. graminum* resistance, which may have been separated during its transfer to Pavon F76. Further evaluations are needed to investigate the two categories of resistance in 7A.7S-L7, 7A.7S-L5, and 7A.7S-Gb5 to better clarify such differences.

As expected, the line 1AL.1RS$_{am}$ containing *Gb2* was susceptible to *S. graminum* biotype E, whereas the GRS-1201 control containing *Gb6* was resistant. Both genotypes are derived from the rye cultivar Insave. Lu et al. (2010) used molecular mapping to show that they are non-allelic genes and that Insave must be genetically heterogeneous.

The usefulness of the resistant genotypes we found, especially those with resistance to *R. padi* and *S. avenae*, will be largely determined by the genetic variation in aphid populations. It is well known that aphid virulence can vary in different geographic areas, as demonstrated for the Russian wheat aphid (Haley et al. 2004; Smith et al. 2004a; Weiland et al. 2008). Several unique *S. graminum* biotypes in the USA differ in virulence patterns to resistant cultivars (Burd and Porter 2006), and were present before resistant cultivars were grown (Porter et al. 1997). Variation in host responses also exist in different *S. avenae* populations (Lowe 1981; Caillaud et al. 1995; Xu et al. 2011), but to our knowledge, no studies have compared virulence patterns among different *R. padi* populations.

Our results indicate that resistance to *R. padi* and *S. avenae* can be transferred from rye into wheat depending on the chromosome arm of wheat that is substituted and the source of the alien chromatin. We used reduced aphid growth in no-choice assays as an estimate of resistance levels. How this translates into reduced population growth remains to be determined. There may also be a component of antixenosis that would be expressed more if aphids were not confined. Future field evaluations need to be conducted to determine the degree of aphid infestation and to estimate yield losses due to aphid damage in this resistant germplasm.

Chromosome arm-specific mapping populations are being developed to unravel the genetic basis of resistance to *R. padi* and *S. avenae* in 1AL.1RS$_e$ and 1AL.1RS$_{am}$ translocation lines. The high levels of *S. graminum* resistance found in the *Ae. speltoides* recombinant-derived lines require further investigation, since it is advantageous to combine

resistance mechanisms to reduce damage levels and to improve the durability of resistance. The sources of resistance to *R. padi*, *S. avenae,* and *S. graminum* identified in this study have great potential for use by breeding programs aiming to transfer aphid resistance into cultivars. Resistance is already present in adapted spring wheat germplasm, and, in the case of *R. padi* and *S. avenae*, single genotypes can be used as resistance sources.

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Table 1. *R. padi* and *S. avenae* weights, and percent leaf chlorosis response to *S. graminum* relative to the control Pavon F76 in Phase 1 seedling experiments, and subset number in which lines were tested for each aphid species.

Genotype[†]	*R. padi*			*S. avenae*			*S. graminum*	
	% Pavon F76[‡]		Subset	% Pavon F76[‡]		Subset	%Pavon F76[¶]	Subset
SUBSTITUTION LINES								
$1R_e(1A)$	81.8		1	127.8	(+)	1	89.5	1
$1R_e(1B)$	105.7		1	69.8	+	1	121.3	1
$1R_e(1D)$	91.5		1	128.4	(+)	1	110.2	1
$MA1S.1RL_e(1A)$	88.9		1	119.4		1	111.7	1
$MA1S.1RL_e(1B)$	90.9		1	130.4	(+)	1	83.6	1
$MA1S.1RL_e(1D)$	87.3		1	90.7		1	120.6	1
$1R_{pr}(1D)$	74.2	**	1	89.2		1	160.5	1
$1R_{pr}.1D_{5+10-2}(1D)$	71.2	**	1	73.8		1	90.2	1
$1R_{rec}(1A)$	105.1		1	108.5		1	84.3	1
$1R_{rec}(1B)$	115.7		1	137.7	(+)	1	85.1	1
$1R_{rec}(1D)$	85.6		1	107.0		1	114.6	1
$1R_{inv}(1A)$	83.4		1	80.7		1	56.2	1
$2R_{rec}(2B)$	91.6		1	137.8	(+)	1	156.1	1
TRANSLOCATION LINES								
$1AL.1RS_e$	75.7	**	2	79.5	+	2	116.9	1
$1AL.1RS_{am}$	104.1		2	107.7		1	95.4	1
$1AL.1RS_v$	95.6		2	82.0		2	117.6	1
$1AL.1RS_{rh}$	85.5		2	77.0	+	2	100.6	1
$1BL.1RS_e$	70.6	**	2	78.8	+	2	124.3	1
$1BL_v.1RS_e$	71.5	**	3	73.0	*	2	85.8	1
$1BL.1RS_{cim}$	117.4		2	133.2	(*)	1	162.7	1
$1BL.1RS_{gnr}$	92.4		2	130.1	(+)	1	62.1	1
$1BL.1RS_v$	95.6		2	103.2		2	85.1	1
$1BL.1RS_i$	92.3		2	96.7		2	91.0	1
$1BL.1RS_i$	101.2		4	86.7		3	125.1	2
MA1	76.8	**	2	100.4		2	123.1	1
MA2	84.7		2	110.1		2	105.0	1
Te1	91.6		2	81.9		2	81.4	1
Te2	90.3		2	82.0		2	99.1	1
$1B_i$	99.0		4	86.7		3	101.8	2
$1R_i(1B)$	110.8		1	125.5	(+)	1	86.5	1

1DL.1RS$_{bb}$	85.7		2	77.5	+	2	97.6		1
1DL.1RS$_{w}$	65.2	**	2	86.4		2	90.2		1
1DL.1RS$_{e}$	90.2		2	92.1		2	101.3		1
1DL.1RS$_{v}$	101.2		2	103.4		2	118.3		1
1AS.1RL$_{e}$	95.0		3	86.2		2	84.90		2
1BS.1RL$_{e}$	88.8		3	93.0		2	69.71		2
1DS.1RL$_{e}$	75.6	**	3	96.7		2	130.4		2
1DS.1RL$_{bb}$	89.9		3	92.0		2	80.4		2
1AS.#2L	96.8		4	89.2		3	58.1		2
1AL.1RS+1DL.1RS	96.0		4	104.6		3	76.8		2
2BL.2RS$_{cs}$	94.2		3	103.6		2	103.6		2
2AS.2RL$_{cs}$	115.4		4	111.7		4	105.4		2
2BS.2RL$_{cs}$	96.8		3	107.4		2	50.0		2
2BS.2RL$_{bl}$	84.3		3	103.5		2	113.4		2
2BS.2RL$_{cs}$	102.7		4	109.4		3	88.4		2
3DL.3RS$_{rh}$	103.0		3	111.5		3	84.0		2
3DL.3RS$_{cs}$	92.7		3	86.5		3	62.5		2
3DS.3RL$_{cs}$	81.1		3	105.2		3	120.6		2
4A$_{ril}$	106.0		1	157.4	(**)	1	150.1		1
5AL.5RS$_{cs}$	98.8		3	95.6		3	85.7		2
5BL.5RS$_{e}$	90.8		3	114.1		3	114.3		2
5DL.5RS$_{rh}$	111.3		4	108.3		3	133.1		2
6BS.6RL$_{bb}$	97.3		4	115.2		3	114.0		2
7DS.4RL$_{m}$	94.3		3	111.3		3	95.6		2
RECOMBINANT DERIVED LINES									
1D+9	107.8		4	136.5	(*)	4	44.6		2
T-9	119.3	(*)	4	117.0		4	108.1		2
1D+4	94.6		4	98.4		4	96.5		2
2D(s)+2	95.8		3	97.6		3	67.0		2
2D(s)+4	92.5		3	93.2		3	108.1		2
2B.2R	104.6		4	123.6	(+)	4	75.0		2
5D.5R-1	92.7		3	103.1		3	77.7		2
7A.7S-S3	101.3		4	107.1		3	113.4		2
7A.7S-L7	98.1		4	98.7		3	31.2	*	2
7A.7S-L5	92.3		4	90.2		3	0.0	**	2
7A.7S-Gb5	110.3		4	88.5		3	31.2	*	2

[†]Subscripts for genotypes: e=E12165; pr=Presto triticale; rec=Reconstructed from Kavkaz; inv=Inverted E12165 long arm; am=Amigo wheat; v=Veery; cim=E12169; gnr=Genaro; i=inserted segment; bb=BH1146/Blanco rye; w=Wheaton, cs=Chinese Spring; ril=reduced internode length mutation in cs; bl=Blanco; rh=Rhino; cim=CIMMYT E12169 m=*Secale montanum* Guss.

[‡]Mean aphid weight on the genotypes divided by the mean aphid weight on Pavon F76 times 100

[¶] Mean % leaf chlorosis of the genotypes relative to the mean % chlorosis of Pavon F76 times 100

+ P≤0.1, * P≤0.05; ** P≤0.01 significance levels compared to control Pavon F76 in *t-test*s, () = significantly increased aphid weight relative to the control

Table 2. *R. padi* or *S. avenae* weights relative to the control Pavon F76 and mean *R. padi* or *S. avenae* weight ±SE in Phase 2 seedling experiments.

Genotype[†]	*R. padi*				*S. avenae*			
	% Pavon F76[‡]	Weight (mg)	±SE		% Pavon F76[‡]	Weight (mg)	±SE	
$1R_e(1A)$	85.3	0.251	0.0402	*	73.7	0.166	0.0169	**
$1R_e(1B)$	83.3	0.245	0.0403	**	73.7	0.166	0.0168	**
$1R_e(1D)$	87.4	0.257	0.0402		70.6	0.159	0.0170	***
$MA1S.1RL_e(1A)$	-	-	-		85.3	0.192	0.0167	
$MA1S.1RL_e(1B)$	-	-	-		79.1	0.178	0.0170	
$MA1S.1RL_e(1D)$	-	-	-		90.2	0.203	0.0166	
$1R_{pr}(1D)$	77.9	0.229	0.0404	***	65.7	0.148	0.0178	***
$1R_{pr}.1D_{5+10-2}(1D)$	80.3	0.236	0.0402	**	75.5	0.170	0.0170	**
$1R_{inv}(1A)$	-	-	-		68.4	0.154	0.0170	***
$1AL.1RS_e$	81.0	0.238	0.0402	**	74.2	0.167	0.0168	**
$1AL.1RS_{am}$	-	-	-		76.8	0.173	0.0170	*
$1AL.1RS_{rh}$	-	-	-		69.7	0.157	0.0166	***
$1BL.1RS_e$	89.7	0.264	0.0403		71.1	0.160	0.0170	***
$1BL_v.1RS_e$	75.8	0.223	0.0404	***	69.7	0.157	0.0170	***
MA1	99.3	0.292	0.0404		84.4	0.190	0.0238	
$1DL.1RS_{bb}$	-	-	-		90.2	0.203	0.0169	
$1DL.1RS_w$	102.3	0.301	0.0403		91.1	0.205	0.0258	
$1DL.1RS_e$	94.2	0.277	0.0404		95.5	0.215	0.0170	
$1AS.1RL_e$	91.5	0.269	0.0403		87.1	0.196	0.0171	
$1BS.1RL_e$	92.5	0.272	0.0402		91.5	0.206	0.0170	
$1DS.1RL_e$	95.5	0.281	0.0401		84.0	0.189	0.0172	
$1DS.1RL_{bb}$	-	-	-		82.6	0.186	0.0170	
Pavon F76	100.0	0.294	0.0403		100.0	0.225	0.0169	

435 †Subscripts for genotypes: e=E12165; pr= Presto triticale; inv=Inverted E12165 long arm; am=Amigo wheat;
436 v=Veery; bb=BH1146/Blanco rye; w=Wheaton; rh=Rhino.
437 ‡Mean aphid weight on the genotypes divided by the mean aphid weight on Pavon F76 times 100
438 * = P≤0.05; ** = P≤0.01; *** = P≤0.0001 significance levels compared to Pavon F76 in Dunnett's test
439

440 **Table 3.** *S. avenae* weight relative to the control Pavon 76 in the adult plant test.

Genotype†	% Pavon F76‡
$1R_e$(1A)	99.1
$1R_e$(1B)	92.3
$1R_{rec}$(1A)	119.9
$1R_{rec}$(1B)	125.7
$1AL.1RS_e$	104.0
$1AL.1RS_{am}$	78.2*
$1AL.1RS_v$	108.1
$1BL.1RS_e$	100.6
$1BL_v.1RS_e$	119.2
$1BL.1RS_v$	130.0
$1AS.1RL_e$	107.4
$1BS.1RL_e$	131.3

441 †Subscripts for genotypes: e=E12165; rec=Reconstructed; am=Amigo wheat; v=Veery
442 ‡Mean aphid weight on the genotypes divided by the mean aphid weight on Pavon F76 times 100
443 * P≤0.05 significance level compared to Pavon F76 in *t-tests*

444

445 **Table 4.** Resistance to *S. graminum* measured as number of *S. graminum* developing on plants, percentage of colony
446 weight relative to Pavon F76, colony weight, and percent leaf chlorosis in response to *S. graminum* feeding.

Genotype†	No. *S. graminum*	±SE	% Pavon F76‡	Colony weight (mg)	±SE	Chlorosis (%)	±SE
$1R_{rec}$(1A)	39.7	8.04	62.9	5.1	0.94	60.6	8.44
$1R_{inv}$(1A)	44.5	8.66	64.8	5.2	1.01	61.2	8.45
$2BS.2RL_{cs}$	47.2	7.53	82.0	6.6	0.88	43.0	7.97
1D+9	43.6	9.43	77.3	6.2	1.10	84.5	9.02
7A.7S-S3	45.4	7.09	75.3	6.1	0.83	61.0	7.97

7A.7S-L7	48.6	8.05		59.5	4.8	0.94		0.9	8.46	***
7A.7S-L5	22.7	7.09	**	32.3	2.6	0.83	**	3.0	7.97	***
7A.7S-Gb5	31.9	8.05		54.6	4.4	0.94		2.3	9.02	***
GRS-1201	20.3	8.67	**	32.3	2.6	1.01	**	1.4	9.70	***
Pavon F76	62.9	9.42		100.0	8.1	1.10		57.6	9.70	

[†]Subscripts for genotypes: inv= Inverted E12165 long arm; rec=Reconstructed from Kavkaz; cs=Chinese Spring

[‡]Weight percentage of Pavon F76 obtained by dividing the mean colony weight of genotypes by the mean colony weight of the control times 100

** = P≤0.01; *** = P≤0.0001 significance levels compared to Pavon F76 in Dunnett's test

Table 5. One degree of freedom contrasts for number of *S. graminum* per plant, aphid colony weight and leaf chlorosis between genotypes carrying *Ae. speltoides* chromatin and between the resistant control GRS-1201 carrying the *Gb6* resistance gene.

Contrast	**No. *S. graminum***	**Colony Weight**	**Chlorosis**
7A.7S-L7 vs. 7A.7S-L*5*	**	NS	NS
7A.7S-L7 vs. 7A.7S-*Gb5*	NS	NS	NS
7A.7S-L7 vs. GRS-1201	**	NS	NS
7A.7S-L*5* vs. 7A.7S-*Gb5*	NS	NS	NS
7A.7S-L5 vs. GRS-1201	NS	NS	NS
7A.7S-*Gb5* vs. GRS-1201	NS	NS	NS

** P≤0.01; NS = Not significantly different