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Resistance of wild wheat, *Triticum boeoticum* Boiss. to *Schizaphis graminum* (Rondani) (Hem.: Aphididae)

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Abstract. Greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), is one of the most destructive pests of wheat. Potential resistance of wild wheat, *Triticum boeoticum*, was quantified as the number of aphids, aphid load (number of aphids/plant dry biomass), percentage of alate (winged) individuals produced, and winged *S. graminum* aphids settled. Fewest aphids were on TbKu11 and TbKu1. The lowest aphid load was on TbKu7 and TbKu1. The largest percentages of winged aphids were on TbL9, TbAE2 and TbKu6. Preference tests were designed for eight populations selected based on results of performance tests. The largest number of winged aphids settled on each plant was on Pishtaz and the least was on TbKu1. The largest and smallest numbers of first-instar nymphs were produced by winged individuals on Pishtaz and TbKu1, respectively. Based on results, TbKu1 has relative resistance against *S. graminum* and could be a candidate for investigation of genomes to detect mechanisms of molecular resistance to *S. graminum*.

Key words: *Schizaphis graminum*, performance, preference, resistance, wild wheat, *Triticum boeoticum*.

Introduction

Wheat, *Triticum aestivum*, is the third-most important cereal crop throughout the world (Aradottir et al. 2017). Human population growth and increase in consumption has increased demand of wheat (Shewry 2009, Curtis & Halford 2014). Wheat like other crops is attacked by a variety of insect pests that cause serious economic damage. Among cereal pests, aphids are economically important insect pests. Cereal aphids, *Rhopalosiphum padi* (L.), *Schizaphis graminum* (Rondani), *Sitobion avenae* F. and *Diuraphis noxia* (Mordvilko) are the most destructive aphid species on wheat. They cause serious yield loss by reducing the number of grains and grain weight per spike of wheat, and indirectly by transmitting viruses (Crespo-Herrera et al. 2013). The greenbug, *Schizaphis graminum,* is Palearctic but has been introduced to other parts of the world and is now in North and South America, Europe, Asia, and Africa (Blackman & Eastop 2007). *Schizaphis graminum* feeds on several genera of Poaceae, including *Agropyron, Avena, Bromus, Dactylis, Eleusine, Festuca, Hordeum, Lolium, Oryza, Panicum, Poa, Sorghum, Triticum,* and *Zea* (Blackman & Eastop 2007). At least 60 species of grasses including cereal crops are host plants of this cosmopolitan aphid (Mc Gauley et al. 1990). *Schizaphis graminum* is a dominant cereal aphid that infests winter- and spring-sown barley crops in Iran.

Because of extensive use of insecticides, aphids have developed resistance (Wilde et al. 2001). Development of resistance to insecticides and their detrimental effects on nontarget organisms increased interest in developing alternative control methods (Panda & Khush 1995). The most viable alternative control of aphids is by genetic resistance in plant cultivars. This method is environmentally friendly, economically sound and easy for farmers to use. Plant resistance is the relative amount of heritable qualities of a plant that reduce the degree of damage by pests (Painter 1951). By incorporating resistance to aphids into wheat cultivars, farmers have access to inexpensive control in seed they obtain for planting. Incorporation of resistance into wheat is facilitated by detailed characterization and understanding of the genetic basis of such resistance (Crespo-Herrera et al. 2013). Differences in abundance of insects may be attributed to differences in antixenosis and antibiosis (e.g., precocity) because of morphological and chemical characteristics of different host plants (Panda & Khush 1995, Blackman & Eastop 2007). A resistant host plant has an indirect effect by increasing exposure of the insect to natural enemies as a result of prolonged developmental time (Dent 2000).

Plant resistance is one of the most important methods for control of wheat aphids (Porter et al. 1997, Messina & Bloxham 2004, Hesler 2005, Dogramaci et al. 2007, Elek et al. 2009, Razmjou et al. 2012, Crespo-Herrera et al. 2013, Mojahed et al. 2013, Royer et al. 2015, Xiang-Shun et al. 2016, Zeb et al. 2016, Hu et al. 2017). Use of resistant wheat cultivars is an efficient and environmentally friendly strategy to prevent infestation and has been successfully used in integrated pest management of different wheat aphids including *D. noxia*, *R. padi* and *S. graminum* (Dahms et al. 1955, Tyler et al. 1987, Porter et al. 2000, Radchenko 2000, Webster & Porter 2000, Migui & Lamb 2006, Vasicek et al. 2010, Razmjou et al. 2011, Razmjou et al. 2012, Tofangsazi et al. 2012, Crespo-Herrera et al. 2013, Mojahed et al. 2013, Suchismita et al. 2016, Aradottir et al. 2017).

Numerous studies evaluated resistance of wheat to aphids. Goldasteh et al. (2012) studied life table parameters of *S. graminum* on four wheat cultivars. Mojahed et al. (2013) evaluated three classical resistance mechanisms (antixenosis, antibiosis and tolerance) against *S. graminum* in commercial wheat cultivars and lines. Wang et al. (2004) studied effects of *R. padi* and *D. noxia,* on wheat cultivar 'Tugela' and near-isogenic lines (Tugela, Tugela-Dn1, Tugela-Dn2, and Tugela-Dn5). Aradottir et al. (2017) evaluated 338 wheat lines against *R. padi* and 340 lines for *S. avenae* from laboratory and field phenotyping of a range of wheat, including landraces from the Watkins collection, more modern cultivars from the Gediflux collection (north-western Europe), and modern UK Elite varieties. Xiang-Shun et al. (2016) investigated three classical resistance mechanisms of 14 commercial wheat cultivars and hybrids (*Triticum aestivum*×*Triticum monococcum*) to *S. avenae* under laboratory and field conditions.

Triticum boeoticum originated in Turkey (Giles et al. 1997). The wild wheat species is an invasive weed common in wheat fields of western and northwestern regions of Iran (Farhangfar et al. 2015). Potential resistance and responses of wheat aphids to wild wheat, *T. boeoticum*, are unknown. Elek et al. (2009) found reduced settlement and nymph production on diploid wheat such as *T. boeoticum* and *T. monococcum* in comparison to hexaploid (*T. aestivum*) and tetraploid (*T. durum*) wheat varieties. Based on results of Radchenko (2011), diploid species with genomes Au (*Triticum urartu*) and Ab (*T. boeoticum, T. monococcum*) were most resistant to *S. avenae* and *R. padi*. Some studies proved that diploid wild wheat species had different levels of potential resistance which may be useful for production of more resistant hybrids (Elek et al. 2009, Radchenko 2011, Crespo-Herrera et al. 2013).

In this study, resistance of *T. boeoticum* to *S. graminum* was quantified as the number of aphids, percentage of winged individuals produced, and winged aphids settled on *T. boeoticum*. Resistance to *S. graminum* as an important wheat pest is desirable for identification of wild wheat that could be used for development of resistant cultivars and studies of resistance mechanisms. This study aimed to search for resistance to greenbug, *S. graminum*, among wheat populations derived from *T. boeoticum* populations. We evaluated performance and preference of the greenbug for resistance by *T. boeoticum*.

Materials and Methods

Insect and plants

Seeds of 22 populations of wild wheat, *Triticum boeoticum* (Poaceae), which had been collected from western and northwestern regions of Iran (Table 1) were used to evaluate potential resistance against the greenbug, *Schizaphis graminum* (Hem.: Aphididae). Pishtaz cultivar of *Triticum aestivum* wheat was used as a check.

Table 1. Collection regions of 22 populations of wild wheat, *Triticum boeoticum* (Poaceae), in western and northwestern provinces of Iran.

Collection regions	Population codes
Ardabil Province	TbAr1
East Azerbaijan Province	TbAE2
Kermanshah Province	Tbke1, Tbke2, TbKe3 and TbKe4
Kurdistan Province	TbKu1, TbKu2, TbKu3, TbKu4, TbKu5, TbKu6 and TbKu7
Lorestan Province	TbL1, TbL6, TbL7, TbL8, TbL9, TbL10 and TbL11

Seeds were sown in two-liter pots filled with equal ratios of sand, clay and organic material. One seed was placed into a hole in the center of each pot and covered by perlite to germinate easily. The plants were grown at 24 and 20°C during day and night respectively, relative humidity of 50-60% and light period of 16:8 (L:D) in a greenhouse. The plants were irrigated every other day.

For mass rearing of *S. graminum*, colonies were collected from wheat fields in the countryside near Kerman City, Iran. The aphids were transferred to a greenhouse and reared on Pishtaz wheat in mesh-covered cages.

Experiment I: Performance assay

Thirty days after seeds were sown, two apterous (unwinged adult aphids) were placed on plant leaves and caged in a clip cage. After 24 h, adult aphids and nymphs produced were removed, except three first-instar nymphs remained on the plant. Each plant was covered by a transparent cylindrical cage of 12.5 cm diameter and 34 cm height with the top closed off by a fine-mesh cloth. After 24 hours, each plant was carefully inspected for aphids. If the number of the aphids was less than three, additional first-instar nymphs from the rearing colony were put onto the plant, so that we had the same number of aphids on each plant in the start of the experiment. After 14 days, all aphids on each plant were collected and counted (nymphs, unwinged and winged individuals) with the aid of a stereomicroscope. To measure dry weight (biomass), each plant was cut at the soil surface and put into a paper bag. The bags were placed in an oven set at 70°C for 72 hours. The dry biomass of each plant was weighed using a digital balance (A&D, HL-500i). Aphid load (number of aphids/plant dry biomass (gr)) and percentage of winged aphids produced were calculated. Because of differences among biomass of different plants, aphid load could be a relevant indicator to compare susceptibility and resistance of plants varieties (Petermann et al. 2010). A randomized complete block with seven replications was used.

Experiment II: Preference assay

Eight populations of wild wheat were selected based on results of the performance assay. Three populations had abundant aphids (TbL8, TbKu2 and TbKu3), TbKu9 had an intermediate number, and four populations had few aphids (TbKu11, TbKu1, TbL6 and TbKu7). Cultivar Pishtaz was used as a check. In each mesh cage (1.5×1.5×1.5 m), nine plants, one from each wheat population were placed on the circumference of an imaginary circle 100 cm in diameter, at the same distance apart. The position of each plant was chosen randomly. A vial containing 25 winged aphids of the same age was hung in the center of each cage, and the winged aphids were allowed to emerge and fly to plants. After 24 hours, the number of winged aphids settled on each plant was counted. After 24 h (i.e., 48 h after the start of the experiment), the number of first-instar nymphs born on each plant was counted. This experiment had 15 blocks (cages).

Statistical Analysis

Only dry biomass had normal distribution, so the other data from the performance experiment were transformed as $\log_{10}$+0.1. The data were analyzed statistically by Univariate Analysis of Variance followed by Tukey test to compare means at a probability of <0.05. A regression was used to determine how the two mathematically independent variables (number of aphids and dry biomass) are related. Because the preference assay data were not normally distributed, and did not become normal by transformation, Generalized Linear Models (GLZM) using negative binomial distribution with log link function was used, and means were compared by Mann-Whitney U Test. Data were analyzed by IBM SPSS software version 22.

Results

Experiment I: Performance assay

Total number of aphids. Mean total number of aphids differed significantly on different plant populations (F=1.84; df=22; P=0.024). Most aphids were on TbL8 (Fig. 1). Fewest were on TbKu11, TbKu1 and TbL6 while the total number of aphids on Pishtaz cultivar was intermediate (Fig. 1).

Aphid load. There was a positive relation (R^2 = 0.161) between number of aphids and dry biomass (Fig. 2). Aphid load differed significantly on the different wheat plants (F=1.87; df=22; P=0.021). The lowest aphid load was on TbKu7 and TbKu1 (Fig. 3). TbKu3, TbL1, TbKu4, TbKu12, TbL8 and TbKu2 had high aphid load while aphid load on Pishtaz cultivar was intermediate.

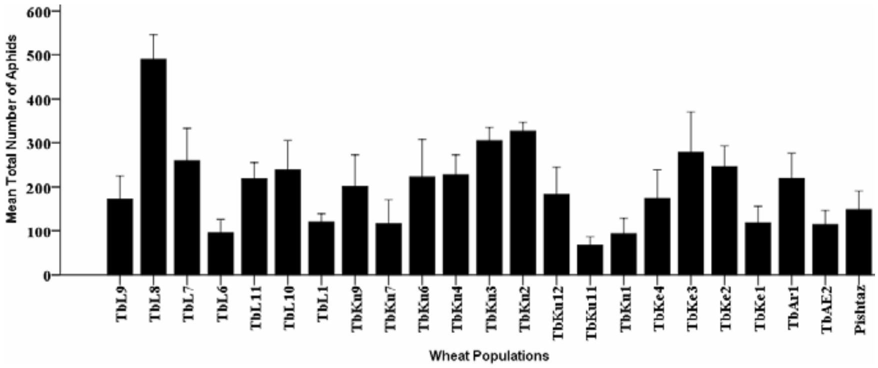

Figure 1. Mean±SE total number of aphids (*Schizaphis graminum*) on *Triticum boeoticum* populations and Pishtaz cultivar.

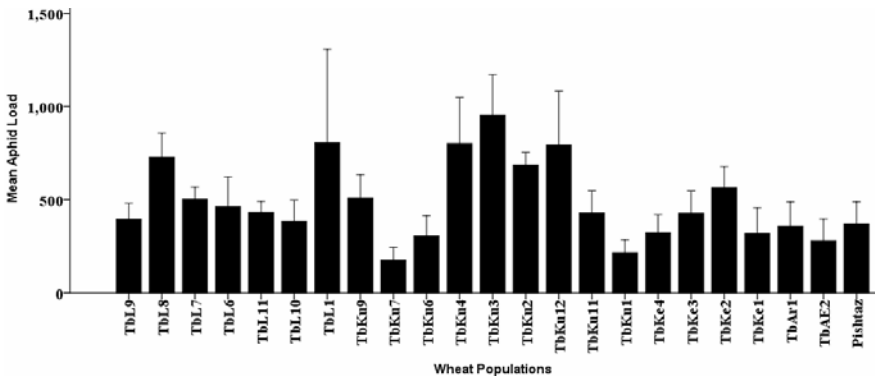

Figure 3. Mean±SE aphid load of *Schizaphis graminum* on *Triticum boeoticum* populations and Pishtaz cultivar.

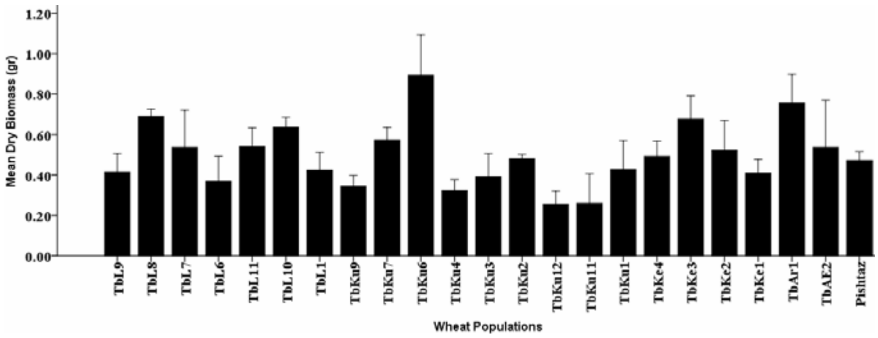

Figure 4. Mean±SE dry biomass of *Triticum boeoticum* populations and Pishtaz cultivar.

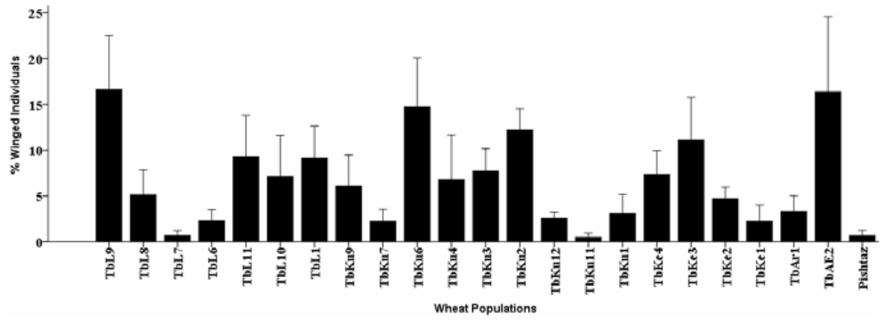

Figure 5. Mean±SE percentage of winged adult aphids produced on *Triticum boeoticum* populations and Pishtaz cultivar.

Plant dry biomass. Mean weight of dry biomass of different wild populations was significantly different (0.2 — 1.0 gr) (F=2.67; df=22; P=0.001). Dry biomass of TbKu6 was greatest, while the biomasses of TbKu12 and TbKu11 were least (Fig. 4). Dry biomass of Pishtaz was intermediate (Fig. 4).

Percentage of winged aphids produced. The percentage of winged aphids produced differed significantly on the different wheats (X^2=37.59; df=22; P=0.02). Most winged aphids were produced on TbL9, TbAE2 and TbKu6, in contrast with the fewest winged individuals on Pishtaz, TbKu11 and TbL7 (Fig. 5).

Experiment II: Preference assay

Winged aphids chose certain plants (X^2=64.94; df=8; P<0001) and produced significantly different number of nymphs (X^2=79.30; df=8; P<0001) on the different wheats. Winged aphid individuals preferred and produced most nymphs on Pishtaz and wild TbKu7 (Figs. 6 and 7). Fewest winged aphids settled and fewest nymphs were produced on TbKu1 (Figs. 6 and 7).

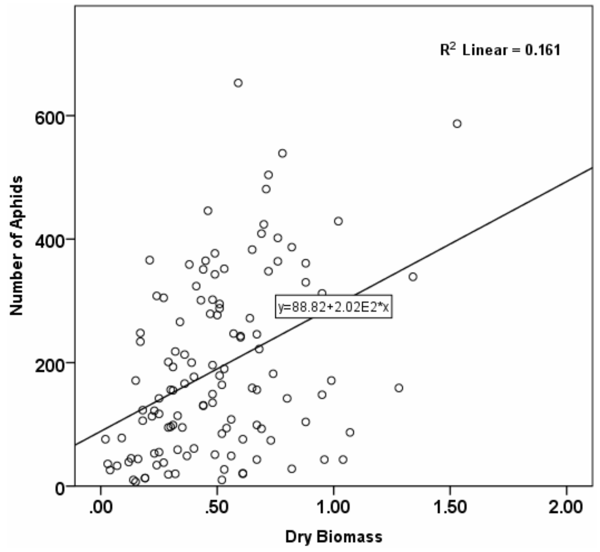

Figure 2. Relation between total number of aphids, *Schizaphis graminum,* and plant, *Triticum boeoticum,* dry biomass.

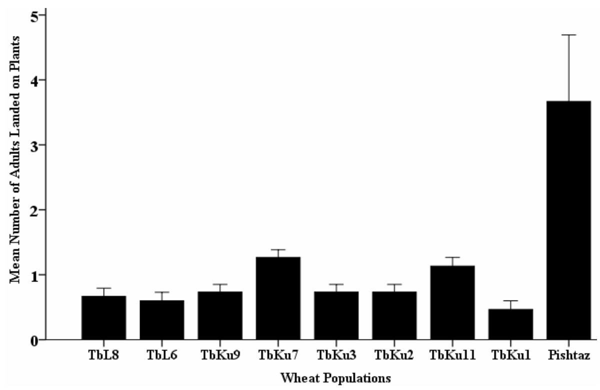

Figure 6. Mean±SE number of winged *Schizaphis graminum* individuals landed on each plant of wild *Triticum boeoticum* and Pishtaz cultivar.

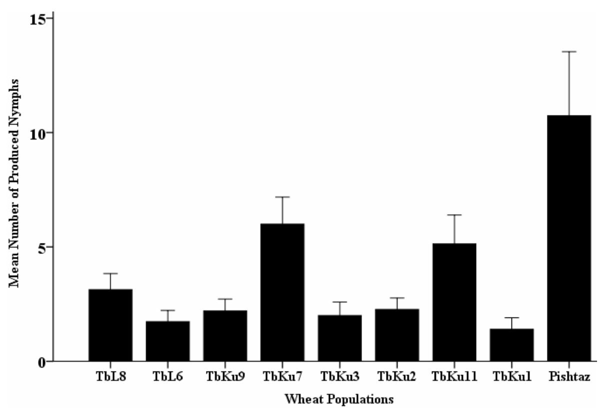

Figure 7. Mean±SE number of *Schizaphis graminum* nymphs produced on *Triticum boeoticum* populations and Pishtaz cultivar.

Discussion

Different populations of wild wheat, *T. boeoticum*, affected greenbug, *S. graminum* differently; performance (number of aphids per plant, aphid load, and percentage of winged individuals produced) and preference (winged individuals settled and nymph production) of the aphid differed. This indicated differences in factors that cause resistance to the aphid, such as quality of phloem composition and amounts and composition of amino acids and hydroxamic acid (Migui & Lamb 2004, Crespo-Herrera et al. 2014). In some plants that had low means of total aphids, aphid load was also low and vice versa. Some populations, for example TbL1, supported the highest aphid number but this was in large parts explained by its high biomass, while population of TbKu12, despite its lower biomass, had a significantly higher aphid load, suggesting higher nutritive quality of TbKu 12. Several studies researched resistance sources in common wheat and wild species for reduction of damage by wheat aphids. Goldasteh et al. (2012) showed that based on comparison of life table parameters, wheat cultivar Tajan was most susceptible to *S. graminum.* In contrast wheat cultivar Zagros was most resistant among four wheat cultivars studied. Mojahed et al. (2013) demonstrated that based on results of antibiosis and tolerance assays, ERWYT 87-16 and ERWYT 87-7 lines were unsuitable hosts for development of *S. graminum* compared to other cultivars and lines.

Elek et al. (2009) investigated the possibility of exploiting natural resistance in wheat varieties against insect pests. They tested a wide range of hexaploid (*T. aestivum*), tetraploid (*T. durum*) and diploid (*T. boeoticum* and *T. monococcum*) wheat for resistance to bird cherry-oat aphid (*Rhopalosiphum padi*), Russian wheat aphid (*Diuraphis noxia*), greenbug (*S. graminum*), Hessian fly (*Mayetiola destructor* (Say)) and orange wheat blossom midge (*Sitodiplosis mosellana* (Gehin)). They found less settlement and nymph production by *S. graminum* on diploid varieties such as *T. boeoticum* and *T. monococcum* (Elek et al. 2009). Similar to the Elek et al. (2009) study, our findings indicated least settlement of winged individuals and fewest nymphs produced on TbKu1, a diploid *T. boeoticum* wild wheat. Wild wheat TbKu1 has potential resistance against *S. graminum*. Xiang-Shun et al. (2016) showed that diploid wheat *T. monococcum*, and common wheat cultivars Astron, Xanthus and Batis attracted fewer winged *S. avenae* than did other varieties. Similarly, our preference assay showed that winged individuals of *S. graminum* were significantly attracted to (chose) *T. boeoticum* wild wheat less than Pishtaz, a common wheat cultivar. Wild wheat, *T. boeoticum*, has potential resistance against wheat aphids such as *S. graminum* and *S. avenae*.

Radchenko (2011) studied resistance of *Triticum* spp. to English grain aphid (*S. avenae*) and bird cherry-oat aphid (*R. padi*) in different regions of Russia and the former Soviet Union. Tyler et al. (1985) identified a source of resistance to *S. graminum* originating from *Aegilops speltoides* in several wheat substitution lines. Crespo-Herra et al. (2013) showed that two wheat genotypes 7A.7S-L5 and GRS-1201 had significantly fewer *S. graminum* per plant and lower colony weight than on other genotypes.

Based on results of preference assay, Pishtaz cultivar was most preferred by winged aphids because the number of adults that settled per plant was more than three times greater compared with eight other wild wheat populations. Different cues (visual or chemical or both) contribute to attractiveness or repellency of host plants to insects (especially aphids) to choose and colonize (Guerrieria & Digilio 2008).

The percentage of winged individuals produced was significantly different on different wheat populations. Producing more winged individuals (dispersal morph) might be because unsuitable food quality in a host plant prompts aphids to 'spread the risk' by emigration of winged individuals and colonization on other plants (Mehrparvar et al. 2014). The percentage of winged individuals produced on TbL9, TbAe2 and TbKu6 was more than on other populations (Fig. 5). Interestingly, the populations had relatively low aphid load (Fig. 3). Because the aphid load and percentage of winged individuals on different wild wheats were significantly different, the response of each wild wheat to herbivory was different. TbKu1 was least preferred and Pishtaz cultivar and wild TbKu7 were most preferred by winged aphids. TbKu1 showed resistance, because few aphids, winged aphids, and nymphs were produced per plant. This was an unsuitable wild wheat and resistant against *S. graminum*.

In conclusion, the genome of TbKu1 should be studied to detect molecular resistance against *S. graminum*. The research provides baseline information as the basis for breeding resistant wheat varieties for integrated management to reduce impact of wheat aphids. This allows evaluation of relatively large germplasm sets to identify potential resistance against other destructive wheat aphids.

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