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REGISTRATION

Germplasm

Registration of Hessian fly-resistant germplasm KS18WGRC65 carrying *H26* in hard red winter wheat 'Overley' background

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Abstract

Hessian fly (HF; *Mayetiola destructor* Say) causes severe damage to wheat (*Triticum aestivum* L.) worldwide. Several resistance genes have been identified in wheat and wild relatives; however, HF populations are under strong selection pressure and evolve rapidly to overcome resistance. To ensure the availability of resistance sources, HF-resistant germplasm KS18WGRC65 (TA5110, Reg. no. GP-1042, PI 688251) was developed by Wheat Genetics Resource Center at Kansas State University as a breeding stock that carries resistance gene *H26* from *Aegilops tauschii* Coss. KS18WGRC65 is a cytogenetically stable, homozygous, $BC_3F_{3:6}$ line derived from the cross between *Ae. tauschii* accession KU2147 and hard red winter wheat recurrent parent 'Overley'. KS18WGRC65 exhibited no penalty for yield or other agronomic characters, making it a suitable source of HF resistance for wheat breeding.

1 | INTRODUCTION

Hessian fly (HF; *Mayetiola destructor* Say; Diptera: Cecidomyiidae) is a serious pest of wheat (*Triticum aestivum* L.), which can cause yield loss up to 35% (El Bouhssini, Nachit, Valkoun, Abdalla, & Rihawi, 2008). Eighteen HF biotypes have been reported based on their ability to infest differential lines (Ratcliffe & Hatchett, 1997; Ratcliffe et al., 2000), and there are 34 known resistance genes. However, rapid evolution of HF biotypes to overcome resistance makes it difficult to control HF effectively.

Crop wild relatives are a useful source of novel genetic diversity for crop improvement (Greene & Warburton, 2017), and *Aegilops tauschii* is a good source of resistance genes for diseases and insects (Cox & Hatchett, 1994; Cox, Raupp, & Gill, 1994). Homology between *Ae. tauschii* genome and D-genome of wheat allows normal pairing and genetic transfer. Li, Chen, Chao, Yu, and Bai (2013) described six of the mapped HF resistance genes that have been introgressed from *Ae. tauschii*. However, working directly with wild progenitors is generally difficult due to their unadapted characteristics;

Abbreviations: GP, Great Plains; HF, Hessian fly; R, resistant; S, susceptible.

therefore, the development of more adapted germplasm carrying introgressed traits is necessary for effective use of genes introgressed from wild relatives.

Cambron et al. (2010) tested 21 known HF resistance genes and found that only five provided resistance across the southeastern United States. Among the five identified genes, *H26* was highly effective against multiple HF biotypes, which was originally transferred from *Ae. tauschii* (*Triticum tauschii*) to common wheat (Cox & Hatchett, 1994). Later, Wang et al. (2006) developed a synthetic wheat germplasm SW8 and mapped another HF resistance gene, introgressed from *Ae. tauschii* accession CIae25, at the same locus as the original *H26*. Based on allelism test, they speculated that this newfound gene could be allelic to *H26*. Although *H26* has been introgressed into wheat already, its use has been limited in breeding due to either the linkage drag from recurrent parent used or the unadapted nature of synthetic germplasm. Therefore, to facilitate its use in resistance breeding, KS18WGRC65 (TA5110, Reg. no. GP-1042, PI 688251) carrying *H26* was developed in an adapted cultivar Overley (PI 634974) background.

2 | METHODS

2.1 | Mapping population and germplasm line development

KS18WGRC65 is a $BC_3F_{3:6}$ line derived from a mapping population that was developed by directly crossing *Ae. tauschii* accession KU2147 to winter wheat cultivar Overley and has the pedigree Overley/KU2147//3*Overley. The cytoplasm donor Overley is a hard red winter wheat cultivar, and KU2147 is an *Ae. tauschii* accession collected from Gilan Province, northern Iran. Based on geographic proximity and allele matching method (Singh et al., 2019), the accession KU2147 was determined to be the same as the accession CIae25, the source of *H26*, which is maintained by the USDA-ARS National Small Grains Collection.

For each backcross during population development, HF screening was conducted and resistant plants were selected. BC_3F_1 plants were self-pollinated and harvested in bulk to produce BC_3F_2 seed that was planted and harvested individually to produce $BC_3F_{2:3}$ lines. These $BC_3F_{2:3}$ lines were then screened for Hessian fly resistance in the greenhouse as described below. Remaining seed from these lines was grown under the field conditions in headrows for seed increase and collection of agronomic data. Each headrow was bulked and harvested separately for further agronomic evaluation. From these headrows, a single spike was selected from a line with overall Overley-type plant morphology, and its resistance was confirmed in the greenhouse using 20 seeds from the selected spike. Remaining seed from the selected spike was planted and self-pollinated in the greenhouse for two cycles and harvested in bulk to produce KS18WGRC65.

2.2 | Hessian fly screening

The HF screening was done with Great Plains (GP) biotype in in the USDA-ARS greenhouse at Kansas State University, Manhattan, KS. Lines were grown in 10-cm-deep plastic trays with temperature maintained at 20 °C. Twenty seeds for each line and four checks—'Karl 92' (PI 564245; susceptible), 'Carol' (PI 562612; gene *H3*; moderately resistant), 'Caldwell' (CItr 17897; gene *H6*; moderately resistant), and 'Molly' (PI 562619; gene *H13*; resistant)—were planted in each tray. Twenty planted seeds for each line represents one replication. Seven to 8 d after sowing, seedlings were infested with HF biotype GP. Trays were covered with white cheesecloth nets to keep humidity slightly higher than the surroundings and restrict the escape of the adult flies. Egg counts for HF were recorded periodically, and flies were removed once 10–15 eggs per leaf on average were observed. Phenotypic scoring was performed 15–17 d after infestation.

For greenhouse HF screening, KS18WGRC65, KU2147, and Overley were replicated five times, whereas $BC_3F_{2:3}$ lines were screened with single replication as described above. Lines were scored as homogeneously resistant (phenotype R; genotype RR) lines, homogeneously susceptible (S; rr) lines, or heterogeneous (H; Rr) lines, if they had 100% resistant, 100% susceptible, or mixture of resistant and susceptible plants, respectively.

2.3 | Agronomic evaluation

Due to limited seed availability in 2016–2017, we could not test KS18WGRC65 directly in the field. We therefore evaluated the agronomic performance of sister lines carrying *H26* with comparison of these resistant lines (R lines) to susceptible (S) sister lines and recurrent parent Overley. Based on the greenhouse screening of $BC_3F_{2:3}$ lines, we identified 55 each of homogeneous R and S lines and use their bulked seed harvested from the field headrows for agronomic evaluation.

Single replicates of these R and S lines and 10 replications of Overley were planted in six-row plots at Ashland Bottoms Research Station in Kansas (39.132796 lat., −96.619357 long.) in completely randomized design. Each plot measured 1.68 m wide and 2.44 m long and was planted with 60 g of seed. Data were recorded for plant height at maturity, grain yield, moisture concentration at harvesting, test weight, and days to heading from 1 January. In this design, each R and S line acted as independent replication within R and S group, respectively. Therefore, we had 55 replications for each R and S group and 10 replications of Overley. One-way analysis of

TABLE 1 Greenhouse screening for reaction to Hessian fly biotype GP. Twenty plants per replication scored as resistant (R) and susceptible (S)

Line	% R	% S	SD
KU2147	100	0	0
KS18WGRC65 (*H26*)	100	0	0
Overley	0	100	0
Karl 92	0	100	0
Carol (*H3*)	60.6	39.4	15.8
Caldwell (*H6*)	73.6	26.4	5.7
Molly (*H13*)	100	0	0

Note. Values are the average of five replications and represent percentage of plants. SD = standard deviation based on five replications.

variance (ANOVA) tests were performed for each of the five traits to evaluate any differences between R, S, and Overley groups at an alpha significance level of .05.

KS18WGRC65 was planted in the greenhouse for seed increase in 2016–2017. Sufficient seed was produced that in 2017–18, three replications of KS18WGRC65 and Overley were planted in six-row yield plots at two locations (McPherson, KS, and Republic, KS). For the 2017–2018 season, only grain yield was recorded at maturity as total grain weight per plot. Grain yields were adjusted for the plots with less than six full rows in both years and were converted to tonnes per hectare. Welch's two sample *t*-test was calculated to compare KS18WGRC65 and Overley.

3 | CHARACTERISTICS

3.1 | Hessian fly resistance

KS18WGRC65, KU2147, and Overley were tested for their reaction to HF biotype GP maintained by USDA-ARS facility in Manhattan, KS (Table 1). Twenty individual plants of each line were evaluated for resistance. KS18WGRC65 and KU2147 expressed complete resistance to GP biotype, with each having all resistant plants, whereas Overley was completely susceptible with all plants susceptible. Phenotypically, KS18WGRC65 was similar to Overley and had similar maturity period. All checks expressed expected reaction, with Karl 92 completely susceptible, Carol (*H3*) and Caldwell (*H6*) moderately resistant, and Molly (*H13*) completely resistant. We did not observe HF-susceptible plants within KS18WGRC65, suggesting that the germplasm is cytogenetically stable and homozygous for the *H26* gene.

3.2 | Agronomic evaluation

KS18WGRC65 is free-threshing hard red winter wheat germplasm line, which is phenotypically similar to cultivar Overley with similar maturity period. KS18WGRC65 also inherited black glumes characteristic from Overley. In the 2016–2017 season, one-way ANOVA did not reveal any significant differences between R, S, and Overley group for plant height, grain yield, moisture concentration, test weight, or days to heading (Table 2).

To confirm equal yield performance, KS18WGRC65 and Overley were planted at two locations in 2017–2018 in six-row yield plots. KS18WGRC65 had significantly higher grain yield than Overley at Republic, KS, as revealed by Welch's *t*-test, but had equivalent yield at McPherson, KS (Table 3). This two-location yield testing established that KS18WGRC65 is equivalent to or better performing than its recurrent parent Overley.

4 | DISCUSSION

Wheat cultivars with genetic resistance to Hessian fly are the key to control this devastating pest in an economic and environmentally friendly manner. Wild relatives, such as *Ae. tauschii*, are a valuable source of untapped genetic diversity for insect pest resistance. Based on the geographic proximity and allele matching results, we could determine that KU2147 and CIae25 are same accessions, and we introgressed *H26*

TABLE 2 Agronomic data for resistant (R) and susceptible (S) groups and Overley planted at Ashland, KS, during 2016–2017 for extrapolated grain yield, test weight, moisture concentration, plant height, and days to heading

Trait	R $n = 55$	S $n = 55$	'Overley' $n = 10$	*P*-value
Grain yield (Mg ha^{-1})	4.14 (0.53)	3.99 (0.49)	4.10 (0.39)	.321
Test weight (kg m^{-3})	712 (1.48)	709 (3.79)	714 (1.39)	.750
Moisture (%)	11.3 (0.27)	11.3 (0.36)	11.3 (0.31)	.959
Plant height (cm)	91.9 (3.02)	93.0 (3.54)	94.4 (4.77)	.051
Days to heading (d)	113.9 (0.8)	113.7 (0.8)	113.4 (0.9)	.281

Note. Grain yield initially recorded as grams of grain per plot, and then calculated and projected as Mg ha^{-1}. Test weight initially recorded as kg hl^{-1} by HarvestMaster, and then calculated and projected as kg m^{-3}. Moisture concentration (%) reported as recorded by HarvestMaster. Days to heading are relative to 1 Jan. 2017. *P*-values are calculated using one-way ANOVA tests for each trait with R, S, and Overley as three groups.

TABLE 3 Grain yields (Mg ha^{-1}) and *t*-test *P*-values for KS18WGRC65 and Overley planted at McPherson and Republic, KS, 2017–2018

Location	KS18WGRC65	Overley	*P*-value
	Mg ha^{-1}	Mg ha^{-1}	
McPherson	3.73 (0.24)	3.25 (0.35)	.133
Republic	2.68 (0.16)	2.32 (0.14)	.039*

Note. Values in parentheses are standard deviations based on three replications. *P*-values calculated from Welch's two sample *t*-test.
*Significant at the probability level .05.

gene from *Ae. tauschii* accession KU2147 into hard red winter wheat Overley background. Hessian fly screening in the greenhouse confirmed the resistance in KS18WGRC65, and field trials showed that KS18WGRC65 has agronomic and yield performance equivalent to or better than its recurrent parent Overley. The availability of this germplasm will facilitate the use of genetic diversity from wild relatives and insect resistance breeding for breeding improved wheat cultivars.

5 | AVAILABILITY

For the first five years following publication, seed in small quantities can be requested from the Wheat Genetics Resource Center at Kansas State University. After five years, seed stock will be maintained by and can be requested from the USDA National Plant Germplasm System (NPGS). We request reference of this germplasm as KS18WGRC65 when used in research or in the development of any new germplasm or cultivar.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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