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REGISTRATION

Germplasm

Registration of hard white winter wheat germplasms KS14U6380R5, KS16U6380R10, and KS16U6380R11 with adult plant resistance to stem rust

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Abstract

Resistance to the Ug99 group of races of the stem rust fungus *Puccinia graminis* f. sp. *tritici* is limited in winter wheat (*Triticum aestivum* L.) germplasm adapted to the Great Plains of the United States. Our objective was to generate regionally adapted hard winter wheat germplasm with combinations of adult plant resistance genes that are expected to provide durable resistance. KS14U6380R5 (Reg. no. GP-1043, PI 689115), KS16U6380R10 (Reg. no. GP-1044, PI 689116), and KS16U6380R11 (Reg. no. GP-1045, PI 689117) were derived from backcrosses of the hard white winter wheat germplasm KS05HW14 to the stem rust-resistant Kenyan spring wheat cultivar 'Kingbird'. KS14U6380R5, KS16U6380R11, and KS16U6380R10 were developed by pedigree selection and were initially evaluated as U6380-11-2R-0A, U6380-210-2R-0A, and U6380-148-4R-2T, respectively. The germplasms were developed by the USDA-ARS and jointly released with the Kansas State University Agricultural Experiment Station. These germplasms provide parents for development of hard winter wheat cultivars with durable resistance to stem rust.

1 | INTRODUCTION

Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is a potentially devastating fungal disease of bread wheat

Abbreviations: APR, adult plant resistance; IT, infection type; KASP, Kompetitive Allele-Specific polymerase chain reaction; KSU, Kansas State University; MR, moderately resistant; MS, moderately susceptible; PCR, polymerase chain reaction; QTL, quantitative trait locus; RGON, Regional Germplasm Observation Nursery; S, susceptible.

(*Triticum aestivum* L.), durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.], triticale (\times *Triticosecale* Wittm. Ex A. Camus [*Secale* \times *Triticum*]), and barley (*Hordeum vulgare* L.). Stem rust resistance genes have provided effective control in North America since the last serious epidemic in 1974 (Leonard & Szabo, 2005). Important qualitative resistance genes in North America include *Sr6*, *Sr24*, *Sr31*, *Sr36*, *Sr38*, *SrTmp*, and *Sr1RS^{Amigo}* (Zhang, Bowden, Yu, Carver, & Bai, 2014). The emergence in eastern Africa of race TTKSK

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(commonly known as Ug99), with virulence to numerous resistance genes including *Sr6*, *Sr31*, and *Sr38*, presented a new risk to global wheat production (Singh et al., 2011). In 2006, virulence to *Sr24* was detected in race TTKST in the Ug99 race group (Jin et al., 2008). In 2007, virulence to *Sr36* was detected in TTTSK (Jin et al., 2009). Regional breeding programs then rapidly deployed cultivars with *SrTmp*, but in 2013 and 2014, severe outbreaks of stem rust were reported on *SrTmp*-carrying cultivars, and the *SrTmp*-virulent race, TTKTK, was identified as a new member of the Ug99 group (Patpour et al., 2016). Race TTKTT, with virulence to *Sr31*, *Sr24*, and *SrTmp*, was subsequently identified in 2014 collections. Of 499 U.S. hard winter wheat (*Triticum aestivum* L.) elite lines, 366 had seedling stage resistance to TTKSK and TTKST; but of these 366 resistant lines, 160 were susceptible to TTKTT (Newcomb et al., 2016). An important epidemic in Ethiopia on the cultivar Digalu, which contains *SrTmp*, was attributed to race TKTTF, which is not a member of the Ug99 race group (Singh et al., 2015). New virulence to *SrTmp* and *SrIRS^{Amigo}* was reported in TRTTF, another nonmember of the Ug99 group (Newcomb et al., 2016; Singh et al., 2015). North American hard winter wheat germplasm is generally vulnerable to one or more of the new virulent races. Improved resistance to the Ug99 races of stem rust is needed in the hard winter wheat germplasm pool to ensure the protection of the crop from this devastating disease.

Given the rapid development of virulence on newly deployed resistance genes, breeders have targeted combinations of adult plant resistance (APR) genes as a path to durable resistance (Hei et al., 2015; Singh et al., 2011). Five of the numerically designated wheat stem rust resistance genes confer quantitative APR to Ug99 races of stem rust: *Sr2*, *Sr55* (a pleiotropic gene also known as *Lr67/Yr46/Pm46*), *Sr56*, *Sr57* (*Lr34/Yr18/Pm38*), and *Sr58* (*Lr46/Yr29/Pm39*) (Singh et al., 2015). In addition, the seedling resistance gene, *Sr12*, which is ineffective against Ug99 races of stem rust, confers effective APR by positive epistasis when paired with other quantitative trait loci and notably, when paired with *Sr57* (Hiebert et al., 2016; Rouse, Talbert, Singh, & Sherman, 2014).

Our objective was to transfer the complex stem rust resistance of the CIMMYT spring wheat ‘Kingbird’ (syn. ‘Kenya Kingbird’) into hard winter wheat adapted to the U.S. central Great Plains. Kingbird has a particularly robust package of durable APR to stem rust (Singh et al., 2011, 2015). Stem rust resistance in Kingbird is derived from the combination of *Sr2*, *Sr9b*, *Sr12*, *Sr57*, *Sr58*, and perhaps others (Reinhart et al., unpublished data). Kingbird has a susceptible seedling reaction to Ug99 stem rust (Njau, Jin, Huerta-Espino, Keller, & Singh, 2010; Singh et al., 2011) but has seedling resistance to many races, including QFCSC. Kingbird has a long latent period relative to other cultivars (Nopsa & Pfender, 2014), which can slow the development of a stem rust epidemic. In four field seasons of trials in Kenya with the Ug99 race,

Kingbird’s field rust response ranged from 5 MSS (moderately susceptible to susceptible) to 5 MR (moderately resistant), while the susceptible (S) check reaction was 90–100 S (Njau et al., 2010). In a 2-yr field study with the Ug99 group race PTKST, mean yield loss of the susceptible check cultivar was 47.9%, while mean yield loss of Kingbird was 10.1% (Soko, Bender, Prins, & Pretorius, 2018). Kingbird’s field performance in Ethiopia against races TTKSK, TKTTF, TRTTF, and JRCQC was very good, with stem rust severities ranging from trace to 15% (Borlaug Global Rust Initiative, 2019; Hundie et al., 2019). We report here the release of three hard white winter wheat germplasm lines, KS14U6380R5 (Reg. no. GP-1043, PI 689115), KS16U6380R10 (Reg. no. GP-1044, PI 689116), and KS16U6380R11 (Reg. no. GP-1045, PI 689117) with stem rust APR derived from Kingbird.

2 | METHODS

2.1 | Plant material

KS14U6380R5, KS16U6380R10, and KS16U6380R11 were selected as U6380-11-2R-0A, U6380-148-4R-2T, and U6380-210-2R-0A, respectively from a backcross population, KS05HW14*2/Kingbird. KS05HW14 was released by the Kansas Agricultural Experiment Station and is a hard white winter wheat derived from the cross KS98HW452/CO960293//KS920709B-5-2. KS05HW14 was released for its good adaptation and baking quality, good crossability with *Aegilops tauschii* Coss., and high seedling susceptibility to stem rust, which makes it valuable for introgression studies. KS98HW452 is a hard white winter experimental line developed by the Kansas State University (KSU) wheat breeding program at Hays. CO960293 is a hard red winter germplasm line with *Wheat streak mosaic virus* resistance developed by the Colorado State University and jointly released by the Colorado and Kansas Agricultural Experiment Stations. KS920709B-5-2 is a hard red winter experimental line developed by the KSU wheat breeding program at Manhattan. Kingbird is a hard white spring wheat developed by CIMMYT and released in Ethiopia by the Ethiopian Institute of Agricultural Research for its resistance to Ug99 races of the stem rust pathogen (McCandless, 2015).

KS14U6380R5, KS16U6380R10, and KS16U6380R11 were developed by pedigree selection. Winter-habit F₂ plants were selected in 2011 in the field under stem rust disease pressure at Rocky Ford, KS, from three independent BC₁F₁-derived families constructed in the greenhouse at Manhattan, KS. Single plant selections were advanced through the greenhouse in 2012 and field in 2013. U6380-11-2R-0A and U6380-210-2R-0A were harvested from Ashland Bottoms, KS, in 2014, each as a single row of F_{4:6} seed. Seed was increased in the field in 2015, and U6380-11-2R-0A was

distributed in the 2015 Regional Germplasm Observation Nursery (RGON) nursery as KS14U6380R5. U6380-148-4R-2T was selected as a single spike from an F_5 plant in Castroville, TX, in 2014 and increased to $F_{5;7}$ at Ashland Bottoms in 2015. Individual F_7 spikes of the selections were harvested at Ashland, KS, in 2015 and grown as $F_{7;8}$ headrows in Hutchinson, KS, in 2016. Headrows were selected for uniformity, and $F_{7;9}$ seed was harvested in bulk and advanced to replicated yield trials in 2017. U6380-210-2R-0A was tested in the 2017 RGON nursery as KS16U6380R11, and U6380-148-4R-2T was tested as KS16U6380R10.

Agronomic performance of the three lines and the recurrent parent, KS05HW14, was evaluated in replicated yield trials in Saline, McPherson, and Sumner County, KS, and in Mead, Lincoln, Sydney, and North Platte, NE. Trials were conducted by the KSU breeding program and the University of Nebraska, Lincoln, breeding program using standard agronomic practices for yield trials at these locations. Trials had three replications and were harvested by small plot combines with automated weighing systems. Grain volume weight was measured gravimetrically at five trial locations. Grain protein concentration was determined from five locations by near-infrared spectrometry (Perten DA7250) calibrated to combustion analysis. Flowering time was recorded at five locations, and height was recorded at eight locations. Statistical significance of differences of line performance from the recurrent parent, KS05HW14, was evaluated using Dunnett's test.

Resistance to stem rust was evaluated in the Njoro, Kenya, main season stem rust nursery in 2017. Stems were scored at the dough development stage (Zadok's 83–87) for percentage severity using the modified Cobb scale (Peterson, Campbell, & Hannah, 1948), and infection response was scored as susceptible (S), moderately susceptible (MS), moderately resistant (MR), or resistant (R). KS16U6380R10 and KS16U6380R11 were evaluated twice, as entries in the RGON and as breeder entries. KS14U6380R5 was evaluated in Kenya in 2015 in the RGON and in 2017 as a breeder entry. Seedling reactions to Ug99 races TTKSK and TKTF were evaluated at St. Paul, MN, when lines were included in the RGON using the Stakman scale for infection type (IT) where 0 is immune and 4 is fully susceptible. The seedling reaction of KS05HW14 to Ug99 and North American races of *P. graminis* f. sp. *tritici* was evaluated in St. Paul in 2018 and in the 2006 RGON nursery. Resistance to race QFCSC was evaluated in the field at Manhattan in 2015, 2016, and 2017, and to a bulk of U.S. races in St. Paul. Stems were scored at the dough development stage (Zadok's 83–87) for percentage severity using the modified Cobb scale and for infection response using the McNeal scale (McNeal, Konzak, Smith, Tate, & Russell, 1971), where 0 is immune and 9 is fully susceptible. The Kansas field data were analyzed by analysis of variance, and Dunnett's test was used to test separation of means of the three germplasm lines from the recurrent parent.

2.2 | Marker evaluation

DNA was extracted from seedlings grown from seed produced from rows grown from eight individual spike selections of each genotype. DNA extraction was conducted using the Qiagen BioSprint 96 bead-based extraction system as directed by the manufacturer. DNA was diluted fourfold prior to use as templates for marker evaluation. The presence of *Lr34/Yr18/Sr57/Pm38/Ltn1* was evaluated using the STS marker csLV34 (Lagudah et al., 2009). Polymerase chain reactions (PCRs) were conducted with M13-tailed primers and a fluorophore-labeled M13 common primer to generate PCR products for electrophoresis on an ABI3730 DNA fragment analyzer. The presence of *Sr12* was evaluated using Kompetitive Allele Specific PCR (KASP, LGC Biosearch Technologies) with markers IWA610 and NBS-LRR3, described in Hiebert et al. (2016). The presence of *Sr2/Yr30* was evaluated using the associated KASP marker, wMAS000005 (Integrated Breeding Platform, 2019a). The presence of *Lr46/Yr29/Sr58/Pm39* was evaluated using the KASP marker, Lr46Yr29JF2, associated with the 'Pavon' allele of *Lr46/Yr29/Sr58/Pm39* (Integrated Breeding Platform, 2019b). The presence of *Sr9b* was evaluated using the KASP markers IWA226, IWB26191, and IWB55526 (primer sequences available at http://www.polymarker.info/designed_primers). The KASP assays were conducted in either 3- or 4- μ L reactions by adding KASP mix (standard ROX) containing 0.167 μ M each sequence-specific primer and 0.417 μ M common primer to PCR plates in which approximately 50 ng of DNA had been previously dried. The amplification conditions were 94°C for 15 min; 10 cycles of 94°C for 20 s, 65°C for 1 min, decreasing 1°C each cycle; and 40 cycles of 94°C for 20 s, 57°C for 1 min.

3 | CHARACTERISTICS

3.1 | Agronomic characteristics

KS14U6380R5, KS16U6380R10, and KS16U6380R11 are similar in appearance to KS05HW14, with winter habit, awned spikes, white chaff, semidwarf stature, and hard white grain. Plant heights were similar to KS05HW14 (Table 1). KS16U6380R11 flowered 4 d later than KS05HW14. In three Kansas yield trials, KS16U6380R10 yielded 83% of the recurrent parent, KS05HW14. Grain yields of KS14U6380R5 and KS16U6380R11 were 94 and 87% of KS05HW14, but these differences were not statistically significant. Grain volume weights were not significantly different from KS05HW14. Grain protein concentrations of KS14U6380R5 and KS16U6380R10 were indistinguishable from the recurrent parent, and protein concentration of KS16U6380R11 was

TABLE 1 Agronomic performance of wheat germplasm KS14U6380R5, KS16U6380R10, and KS16U6380R11 and recurrent parent KS05HW14 in replicated yield trials in Kansas and Nebraska in 2017

Genotype	Height cm	Flowering date Julian d	Grain yield, KS kg ha ⁻¹	Grain yield, NE kg ha ⁻¹	Grain volume weight kg hl ⁻¹	Grain protein concentration g kg ⁻¹
KS05HW14	87	128	4960	4950	75.4	134
KS14U6380R5	91	131	4670	NT ^a	74.1	132
KS16U6380R10	85	129	4100*	5320	74.4	132
KS16U6380R11	84	132**	4310	4400	74.6	141**
Trials	8	5	3	4	5	5
SE	1	1	180	290	0.3	1

^aNT, not tested.

*, ** Significant differences from KS05HW14 according to Dunnett's test at $p < .05$ and $p < .01$, respectively.

7 g kg⁻¹ greater than the recurrent parent. KS16U6380R10 consistently displayed a moderate physiological black chaff phenotype associated with *Sr2*.

3.2 | End use quality

Milling and baking quality data were collected by the USDA-ARS Hard Winter Wheat Quality Laboratory in Manhattan, KS. Quality of KS05HW14, KS16U6380R10, and KS16U6380R11 was evaluated from the four Nebraska trials and the Saline County, KS, trial. Flour extraction from KS16U6380R11 was 12 g kg⁻¹ less than from KS05HW14 (Table 2). KS16U6380R10 and KS16U6380R11 had significantly shorter mixing times (2.5 and 2.8 min) than KS05HW14 (5.5 min). KS16U6380R10 and KS16U6380R11 also had poor mixing tolerance (1.4 and 1.8) relative to KS05HW14 (4.2). Loaf volume of KS16U6380R10 (797 cm³) was inferior to KS05HW14 and KS16U6380R11 (885 and 860 cm³, respectively) and failed to meet the wheat industry's quality target of 850 cm³. The inferior mixing and baking quality of KS16U6380R10 and KS16U6380R11 is a concern that will need to be addressed through breeding.

3.3 | DNA marker information

KS14U6380R5, KS16U6380R10, and KS16U6380R11 are distinguished from KS05HW14 by molecular markers associated with disease resistance traits. KS14U6380R5, KS16U6380R10, and KS16U6380R11 were positive for the *csLV34* marker associated with *Lr34/Yr18/Sr57/Pm38/Ltn1*. KS14U6380R5 and KS16U6380R11 were positive for *Sr12*-associated markers IWA610 and NBS-LRR3. KS16U6380R10 was positive for the *Sr2/Yr30*-associated marker, *wMAS000005*. KS14U6380R5, KS16U6380R10, and KS16U6380R11 all were positive for the

Lr46/Yr29/Sr58/Pm39. Both parents also were positive for this marker. All three lines were positive for the Kingbird alleles of markers IWA226, IWB26191, and IWB55526, which are tightly linked to *Sr9b* (Reinhart et al., unpublished) and span a region of 2BL from 587 to 683 Mb. Edae, Pumphrey, and Rouse (2018) postulated the presence of *Sr9b* in North American spring wheat germplasm using markers IWB1190 (682 Mb) and IWB28807 (687 Mb). A previous quantitative trait loci (QTL) mapping study in the PBW343 × Kingbird population detected both *Sr2/Yr30* and a second QTL on 3BS, which may be *Sr12* (Li et al., 2015). No QTLs were detected in the genomic regions of *Lr34/Yr18/Sr57/Pm38/Ltn1* and *Lr46/Yr29/Sr58/Pm39*, although these were significant QTLs in the PBW34 × 'Kenya Swara' population.

3.4 | Disease resistance

KS14U6380R5, KS16U6380R10, and KS16U6380R11 were distinguished from KS05HW14 by their resistance to the stem rust pathogen (Table 3). In the 2017 main season stem rust nursery in Njoro, Kenya, with Ug99 races of stem rust, KS05HW14 was evaluated as 50S. In contrast, KS14U6380R5 was evaluated as 10S. KS16U6380R10 and KS16U6380R11 were evaluated twice in 2017 and averaged 5 S-MS, and 10S, respectively. KS14U6380R5 also was evaluated in Kenya in 2015 as 10MSMR. In RGON nursery seedling evaluations in St. Paul, KS14U6380R5, KS16U6380R10, and KS16U6380R11 were all susceptible (Stakman scores 3, 3+) to Ug99 race TTKSK but were resistant to moderately resistant to the North American race QFCSC. In 3 yr of field trials in Manhattan, KS14U6380R5, KS16U6380R10, and KS16U6380R11 all had resistant reactions, most commonly an infection response ≤3 using the McNeal 0–9 scale, and reduced severity (average ≤10%), relative to KS05HW14, which most commonly had IT = 8

TABLE 2 Milling and baking quality of wheat germplasm KS05HW14, KS16U6380R10, and KS16U6380R11 grown in five trials

Genotype	Flour extraction g kg ⁻¹	Mixograph peak time min	Tolerance min	Bread loaf volume cm ³	Flour protein concentration g kg ⁻¹
KS05HW14	672	5.5	4.2	885	117
KS16U6380R10	677	2.5***	1.4**	797**	118
KS16U6380R11	660*	2.8***	1.8**	860	126**
Standard error	3	0.1	0.4	17	1

, * Significant differences from KS05HW14 according to Dunnett's test at $p < .01$ and $p < .001$, respectively.

TABLE 3 Reaction of wheat germplasm KS14U6380R5, KS16U6380R10, and KS16U6380R11 and the recurrent parent KS05HW14 to stem rust

Genotype	Adult plant reaction, Kenya main season rust ^a	Seedling reaction, St. Paul, MN ^b			Field reaction, Manhattan, KS, 2015–2017	
		Race QFCSC	Race TTKSK	Race TKTF	Infection response	Severity
KS05HW14	50S	3 ⁺	3/2 ⁺	3 ⁺	7.9	81
KS14U6380R5	10S, 10MSMR	;	3	2 ⁺	3.3***	10***
KS16U6380R10	1MS, 10 SMS	2	3 ⁺	3	2.6***	2***
KS16U6380R11	0, 20 SMS	0;/2	3	2 ⁺ 3	2.3***	3***

^aEvaluated in 2017 except for one evaluation of KS14U6380R5, which was made in 2015. KS14U6380R10 and KS16U6380R11 were evaluated as two independent entries in 2017; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

^bKS14U6380R5 evaluated 2015; KS14U6380R10 and KS16U6380R11 evaluated in 2017; KS05HW14 evaluated in 2019.

*** Significantly different from KS05HW14 by Dunnett's test ($p < .001$).

and average severity of 81%. The lower infection response in Kansas compared with Kenya may be due to the effectiveness of *Sr9b* and *Sr12* seedling resistance against race QFCSC. In a field stem rust nursery inoculated with a bulk of North American races, all three lines exhibited moderately resistant responses. Among the three lines, KS16U6380R10 had the lowest disease severity (5%) and exhibited a pseudo-black chaff phenotype that is associated with *Sr2* (Njau et al., 2010).

In nine field trials with significant infection (either natural or a composite of local races) of stripe rust (caused by *Puccinia striiformis* var. *tritici*), KS05HW14 was moderately susceptible to stripe rust (mean IT = 6.8, mean severity = 39%), while KS14U6380R5, KS16U6380R10, and KS16U6380R11 were moderately resistant (mean IT = 4.8, 3.3, and 4.5, respectively, and mean severity = 2, 8, and 11%; Table 4). In Njoro, all genotypes, including KS05HW14, were evaluated as moderately resistant to stripe rust. The presence of *Lr34/Yr18* in all three germplasms likely contributed to the improved resistance to stripe rust. The presence of *Sr2/Yr30* in KS16U6380R10 likely contributed to its moderate resistance, and KS14U6380R5, KS16U6380R10, and KS16U6380R11 had slightly improved resistance, consistent with the action of *Lr34/Yr18* derived from Kingbird. However, the presence of *Lr34/Yr18* in these three germplasms also may have reduced yield of the germplasms relative to the recurrent parent. In the absence of disease, yield reduction of 4.3–4.4% (Johnston et al., 2017) and 5% (Singh & Huerta-Espino, 1997) has been

TABLE 4 Reaction of wheat germplasm KS14U6380R5, KS16U6380R10, and KS16U6380R11 and the recurrent parent KS05HW14 to stripe rust. Data are the means of nine trials

Genotype	Infection type	Severity
KS05HW14	6.8	39
KS14U6380R5	4.8*	2***
KS16U6380R10	3.3***	8***
KS16U6380R11	4.5**	11***

*, **, *** Significantly different from KS05HW14 by Dunnett's test ($p < .05$, $p < .01$, and $p < .001$, respectively).

reported with the presence of *Lr34* in sib-pair lines and isolines.

4 | AVAILABILITY

Seed of KS14U6380R5, KS16U6380R10, and KS16U6380R11 is available from the USDA National Small Grains Germplasm Collection. Seed has also been deposited with the USDA-ARS National Laboratory for Genetic Resources, where it will be available immediately upon publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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