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Markers Linked to Wheat Stem Rust Resistance Gene *Sr11* Effective to *Puccinia graminis* f. sp. *tritici* Race TKTTF

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ABSTRACT

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Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici*, can cause severe yield losses on susceptible wheat varieties and cultivars. Although stem rust can be controlled by the use of genetic resistance, population dynamics of *P. graminis* f. sp. *tritici* can frequently lead to defeat of wheat stem rust resistance genes. *P. graminis* f. sp. *tritici* race TKTTF caused a severe epidemic in Ethiopia on Ug99-resistant 'Digalu' in 2013 and 2014. The gene *Sr11* confers resistance to race TKTTF and is present in 'Gabo 56'. We identified seven single-nucleotide polymorphism (SNP) markers linked to *Sr11* from a cross between Gabo 56 and 'Chinese Spring' exploiting a 90K Infinium iSelect Custom beadchip. Five SNP markers were validated on a 'Berkut'/'Scalavatis' population that segregated for *Sr11*, using KBioscience competitive allele-specific polymerase chain reaction (KASP) assays. Two of the SNP markers, KASP_6BL_IWB10724 and KASP_6BL_IWB72471, were predictive of *Sr11* among wheat genetic stocks, cultivars, and breeding lines from North America, Ethiopia, and Pakistan. These markers can be utilized to select for *Sr11* in wheat breeding and to detect the presence of *Sr11* in uncharacterized germplasm.

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* Erikss. & Henning, can result in significant yield losses worldwide (Singh et al. 2015). Historically, this disease has threatened wheat since Biblical times (Chester 1946), causing famines and epidemics (Bushnell and Roelfs 1984). In the United States, *P. graminis* f. sp. *tritici* caused sporadic stem rust epidemics between 1900 and 1965, resulting in wheat yield losses up to 56% across states such as North Dakota (Leonard and Szabo 2005). However, wheat stem rust has been effectively controlled in the United States for half a century by eliminating the alternate host barberry (Leonard and Szabo 2005) and growing early-maturing wheat cultivars with multiple sources of resistance (Kolmer et al. 1991). Despite these efforts, wheat stem rust remains a threat to the world's wheat production because of variability of virulence in the *P. graminis* f. sp. *tritici* population, the ability of urediniospores to spread over long distances by wind, and an exponential reproduction capacity (Leonard 2001). Emergence of *P. graminis* f. sp. *tritici* race TTKSK (also known as Ug99) and its variants from Africa raised serious concern because several resistance genes deployed in commercial production are not effective against this race group (Jin et al. 2008, 2009; Pretorius et al. 2000; Wanyera et al. 2006). This not only heightened the attempts to develop cultivars with new sources of resistance but also intensified surveillance efforts, especially in Africa, to monitor the emergence and spread of new and existing virulent races. As a result, race TKTTF was identified to be the culprit behind the 2013–14 stem rust epidemics in Ethiopia (Olivera et al. 2015). This race was highly virulent on 'Digalu' wheat, which reached 50% of the bread wheat acreage in Ethiopia because of its resistance to stripe rust and to race TTKSK, the predominant *P. graminis* f. sp. *tritici* race in Ethiopia until 2013 (Olivera et al. 2015). Though race TKTTF has been detected in Ethiopia since 2012, the 2013 epidemic is believed to be due to the narrow genetic background of stem rust resistance in widely planted Digalu, conferred by the single gene *SrTmp*, coupled with environmental conditions favoring stem rust.

Race TKTTF is genetically different from members of the Ug99 race group and it is avirulent to stem rust resistance genes *Sr11*, *Sr24*, and *Sr31* (Olivera et al. 2015). Therefore, these genes could be deployed in combination with other genes to combat race TKTTF. *Sr11* was originally mapped to chromosome 6B, more than 60 centimorgans (cM) away from the awn inhibitor gene *B2*, which is near the centromere (Sears 1966) and believed to be linked to leaf rust resistance gene *Lr3*. Diversity Arrays Technology (DArT) markers that might be linked to *Lr3* were suggested by Crossa et al. (2007). However, there is no strong evidence to substantiate the linkage of these DArT markers to either *Lr3* or *Sr11*. Despite the knowledge of chromosome location, there are no diagnostic markers currently available to identify and select for *Sr11* in wheat breeding. There is

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an urgent need to identify molecular markers closely linked to *Sr11* to allow breeders to combine *Sr11* with other resistance genes in wheat lines to provide resistance to race TKTTF. 'Gabo 56' wheat is known to possess *Sr11* (Knott and Anderson 1956; Sears et al. 1957) and is resistant to race TKTTF. Gabo 56 also has *Sr9h* (Rouse et al. 2014), which confers resistance against race TTKSK. We assessed race TKTTF response in a Gabo 56/'Chinese Spring' recombinant inbred line (RIL) population and genotyped this population with a custom Infinium iSelect 90K wheat single-nucleotide polymorphism (SNP) chip to (i) identify molecular markers that are closely linked to *Sr11* from Gabo 56, (ii) convert the closely linked 90,000 SNP markers into a more breeder-friendly KBioscience competitive allele-specific polymerase chain reaction (KASP) assay platform, and (iii) validate their ability to postulate the presence of *Sr11* in diverse germplasm from the United States, Canada, Ethiopia, and Pakistan.

MATERIALS AND METHODS

Plant materials. A population of 149 F_4-derived RIL developed from a cross between Gabo 56 (CI 14035) and Chinese Spring (CI 14108) (Rouse et al. 2014) was used for mapping of *Sr11*. The pedigree of Gabo 56 includes the source line of *Sr11*, 'Gaza'. Markers closely linked to *Sr11* identified from the Gabo 56/Chinese Spring population were validated on 93 wheat accessions and 70 F_6 RIL developed from a biparental cross between 'Berkut' and accession 'Scalavatis' (PI 210945). Berkut and the Berkut/Scalavatis RIL were obtained from the Triticeae Coordinated Agricultural project. The 93 accessions were selected as follows: 27 genetic stock lines, including several near-isogenic lines, and 66 cultivars and breeding lines from North America, Pakistan, and Ethiopia. The list of lines used, with their origin and sources, are described in Supplementary Table S1. Cultivars and breeding lines were selected for *Sr11*-linked marker validation based on high or intermediate infection types in response to races TTKSK and TRTTF in addition to either high or *Sr11*-characteristic low infection types in response to race TKTTF. The purpose of this selection procedure was to attempt to eliminate cultivars and breeding lines that possessed other major resistance genes that might mask the presence of *Sr11*.

Stem rust assays. Seedling assays were conducted at the United States Department of Agriculture–Agricultural Research

TABLE 1. Primer types used for KBioscience competitive allele-specific polymerase chain reaction (KASP) assays for single-nucleotide polymorphism markers on chromosome arm 6BL derived from the 90K iSelect assay

KASP marker, primer	Primer sequence
KASP_6BL_IWB10245	
A1	AAGGTGACCAAGTTCATGCTCCGTAACTTCAGAACGTGCTGGT
A2	GAAGGTCGGAGTCAACGGATTCGTAACTTCAGAACGTGCTGGC
C	CTCACTGATCACTTGCACACTAGTAAATT
KASP_6BL_IWB58199	
A1	GAAGGTGACCAAGTTCATGCTTTACCTGACAAAGAACCCGCTCTA
A2	GAAGGTCGGAGTCAACGGATTACCTGACAAAGAACCCGCTCTG
C	CGCCGCTGCGATTTCGGCAAA
KASP_6BL_IWB12438	
A1	GAAGGTGACCAAGTTCATGCTGGCTTTGCTTTGTGCCATGGTAT
A2	GAAGGTCGGAGTCAACGGATTGGCTTTGCTTTGTGCCATGGTAG
C	GTCTATTGAAGAGAACTTTGTTACTTTCAT
KASP_6BL_IWB10700	
A1	GAAGGTGACCAAGTTCATGCTCGCTTACTGCTGTCATGTAAATA
A2	GAAGGTCGGAGTCAACGGATTCTCGCTTACTGCTGTCATGTAAATG
C	GGATGCTATTTCAGCTAAGGTATCTCAA
KASP_6BL_IWB10724	
A1	GAAGGTGACCAAGTTCATGCTATGTAAATGTTGAGATACCTTAGCTGAAAT
A2	GAAGGTCGGAGTCAACGGATTGTAAATGTTGAGATACCTTAGCTGAAAC
C	GGAAAACCGTCATCTCGCGTATGTA
KASP_6BL_IWB73072	
A1	GAAGGTGACCAAGTTCATGCTGATGTAAATGTGTATGTAGCTTGATTTTATTA
A2	GAAGGTCGGAGTCAACGGATTGATGTAAATGTGTATGTAGCTTGATTTTATTG
C	CATAATACTTGATACTCCTACGTACCCTA
KASP6BL_IWB72471	
A1	GAAGGTGACCAAGTTCATGCTAAACTCAAAGCTAAAGGATAAACTAGATGT
A2	GAAGGTCGGAGTCAACGGATTCTCAAAGCTAAAGGATAAACTAGATGG
C	CAACTGATCTAAGTTCCTTTGTCAATTCAT
KASP_6BL_IWB9416	
A1	GAAGGTGACCAAGTTCATGCTCAACACAACTAGAGCGCTAACTGATT
A2	GAAGGTCGGAGTCAACGGATTAACACAACTAGAGCGCTAACTGATC
C	ATGTGATCGGCTAGGAACCCGTTT
KASP_6BL_IWB59006	
A1	GAAGGTGACCAAGTTCATGCTCCAATCCACATGCTTTGCCGGAT
A2	GAAGGTCGGAGTCAACGGATTCAATCCACATGCTTTGCCGGAG
C	CCTTTTGTAAGCATGTGATCTCACTCAA
KASP_6BL_IWB45581	
A1	GAAGGTGACCAAGTTCATGCTCAACAGATCCGCGGAGCCGT
A2	GAAGGTCGGAGTCAACGGATTAACAGATCCGCGGAGCCGC
C	ATGCCGTAGCGCAATGGTTCCTT
KASP_6BL_IWB3553	
A1	GAAGGTGACCAAGTTCATGCTGGAAGACCCAGTTACATTCTCATCA
A2	GAAGGTCGGAGTCAACGGATTGAAGACCCAGTTACATTCTCATCG
C	GGTAACTTCTTTCTCAGCCTCCGTT
KASP_6BL_IWB46893	
A1	GAAGGTGACCAAGTTCATGCTGGTATCGCCGATGGGAAAGAC
A2	GAAGGTCGGAGTCAACGGATTCGGTATCGCCGATGGGAAAGAT
C	CAAGCATCACCCCACCCCCAT

Service (USDA-ARS) Cereal Disease Laboratory and at a biocontainment facility at the University of Minnesota in St. Paul. The Gabo 56/Chinese Spring and Berkut/Scalavatis RIL populations were evaluated using *P. graminis* f. sp. *tritici* race TKTTF (isolate 13ETH18-1) according to previously described methods (Rouse et al. 2011). The additional wheat lines were evaluated with races TTKSK (isolate 04KEN156/04), TKTTF (isolate 13ETH18-1), and TRTTF (isolate 06YEM34-1). A subset of near-isogenic lines and breeding lines was also evaluated with two other U.S. races—RKRQC (isolate 99KS76A-1) and RTQQC (isolate 04MN74-1)—which are avirulent and virulent to *Sr11*, respectively. Seedling infection types were determined 14 days after inoculation following the 0-to-4 scale developed by Stakman et al. (1962). Infection types 0 to 2 were classified as "low" (incompatible interactions indicative of host resistance and pathogen avirulence) whereas infection types 3 to 4 were classified as "high" (compatible interactions indicative of host susceptibility). Symbols + and – were used to denote relatively larger or smaller pustule sizes, respectively, within each infection-type classification. When two or more infection types were observed on the same leaf, both infection types were recorded, with the most prevalent infection type listed first. For example, infection type 33+ indicated that infection type 3 was most prevalent on a leaf and infection type 3+ was also present on the same leaf. Symbol / was used to separate different infection types observed on separate leaves of the same line, indicating a heterogeneous line. Two biological replicates of seedling screening were performed and a representative infection type was reported. For lines with an inconsistent infection type across the two replicates, a null phenotype was reported.

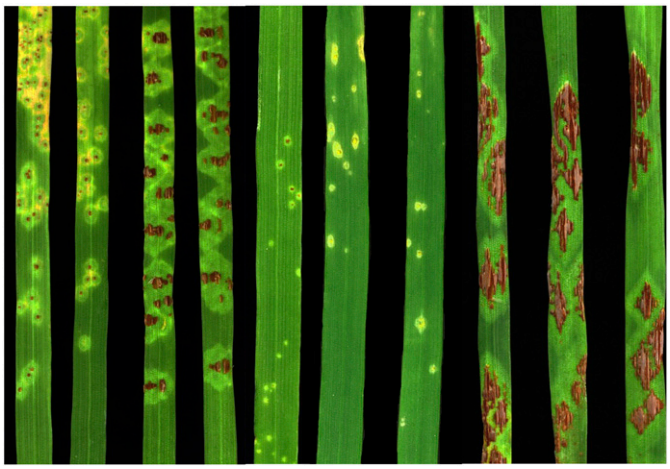

Fig. 1. Disease reaction of wheat accessions with and without *Sr11* in response to *Puccinia graminis* f. sp. *tritici* race TKTTF at the seedling stage. Wheat accessions are represented by a single seedling leaf, arranged from left to right: Trident (*Sr11* + *Sr38*), Gabo 56 (*Sr9h* + *Sr11*), ISr11-Ra (*Sr11*), Berkut (*Sr11*), Gaza (*Sr9h* + *Sr11*), Charter PI 210978 (*Sr9h* + *Sr11*), Timstein PI 16868 (*Sr9h* + *Sr11*), Chinese Spring, Scalavatis, and Bobin.

SNP genotyping and mapping. DNA was isolated from 10-day-old seedlings using a modified cetyltrimethylammonium bromide extraction method (Rouse et al. 2012) and resuspended in water. The amount and purity of DNA was determined using NanoDrop model ND-1000 (NanoDrop Products). DNA isolated from 149 RIL of the Gabo 56/Chinese Spring population was genotyped at the USDA-ARS Cereal Crops Research Unit, Fargo, ND with 90,000 gene-based SNP using a custom Infinium iSelect beadchip array and an iScanner, following the manufacturer's instructions (Illumina Inc.) (Wang et al. 2014). Allele calls were performed using Genome Studio software (v2011.1; Illumina Inc.). The SNP consensus map data were imported into Genome Studio software and alleles were called for codominant markers previously mapped to chromosome arm 6BL. Linkage maps

TABLE 2. Disease reaction of near-isogenic lines and wheat genetic stocks against *Puccinia graminis* f. sp. *tritici* races TKTTF (*Sr11*-avirulent), TTKSK (*Sr11*-virulent), TRTTF (*Sr11*-virulent), RKRQC (*Sr11*-avirulent), and RTQQC (*Sr11*-virulent) at the seedling stage and the allele calls of five single-nucleotide polymorphism markers associated with *Sr11* based on KBioscience competitive allele-specific polymerase chain reaction (KASP) assay

	Reactions by race[a]					KASP[b]				
Lines	TKTTF	TTKSK	TRTTF	RKRQC	RTQQC	1	2	3	4	5
LcSr11 (*Sr11*)	2	3+	3+	2–	3+	C	A	G	T	G
PdSr11Lee (*Sr11*)	;2–	33+	32+	…	…	C	A	G	T	G
DK37 (*Sr11*)	22–	3+	3+	2–	3+	C	A	G	T	G
Gabo 56 (*Sr9h+Sr11*)	22–	22–	3+	;2–	;2–	C	A	G	T	G
Berkut (*Sr11*)	22+	3+	33+	2–	3+	C	H	T	T	G
ISr11-Ra (*Sr11*)	22+	3+	3+	12–	3+	C	A	G	T	G
Trident (*Sr11+Sr38*)	;2–	3+	3+	;1–	0;1	C	A	G	T	G
BtSr11Lee (*Sr11*)	22+	3+	3+	;21	…	C	A	G	T	G
PdSr11Wld (*Sr11*)	0;	3+	3+	0;2–	3+	C	A	G	T	G
Gaza (*Sr9h+Sr11*)	;1	11+	3+	;1–	11+	C	A	G	T	G
Charter PI 158406 (*Sr9h+Sr11*)	12–	2	3+	;12–	2–	C	A	G	T	G
Timstein CI 12347 (*Sr9h+Sr11*)	12–	22+	3+	2–	22–	C	A	G	T	G
Timstein PI 16868 (*Sr9h+Sr11*)	1;	22+	3+	2–	2	C	A	G	T	G
Charter PI 210978 (*Sr9h+Sr11*)	1;	2	3+	;12–	2–	C	A	G	T	G
Charter PI 155430 (*Sr9h+Sr11*)	;12–	2	3+	0;12/;2	2–	C	A	G	T	G
Flevina (*Sr11*)	12–/3+	3+	3+	0;2–/3+	3+	C	A	G	T	G
Kenya Farmer PI 187165 (*Sr11*)	;1/3–	3+	3+	2–	3	C	A	G	T	G
Kenya Farmer PI 29070 (*Sr11*)	0;1	3+	3+	;2–	33+	C	A	G	T	G
Lee (*Sr9g+Sr11+Sr16*)	2–;	3+	3+	;12–	3+	C	A	T	T	G
Yalta (*Sr11*)	12–	3+	3+	12–	3+	C	A	G	T	G
Chinese Spring	3+	3+	3+	3+	3+	T	T	T	H	T
Baart	3+	3+	3+	3+	3+	T	T	T	C	T
LMPG-6	3+	3+	3+	3+	3+	T	T	T	C	T
Little Club	3+	3+	3+	3+	3+	T	T	T	C	T
Scalavatis	33+	3+	3+	3+	3+	T	T	T	C	T
Line E	4	3+	3+	3+	3+	T	T	T	C	T
Bobin	33+/;13–	3+	3+	3+	3+	T	T	T	C	T

[a] Symbol "…" indicates null phenotype reported for inconsistent reaction across replicates.
[b] KASP 1 = KASP_6BL_IWB10724, KASP 2 = KASP_6BL_ IWB73072, KASP 3 = KASP_6BL_IWB12438, KASP 4 = KASP_6BL_ IWB46893, and KASP 5 = KASP_6BL_IWB72471.

were constructed using Map Disto. The Kosambi mapping function was used to calculate genetic distance between markers (Kosambi 1943).

Marker validation using KASP assay platform. Bajgain et al. (2015) previously identified SNP markers associated with resistance to race TKTTF in a panel of 250 North American spring wheat cultivars and breeding lines. We selected five of these markers associated with resistance to race TKTTF with map positions on chromosome arm 6BL for conversion into KASP assays. In addition, seven markers from the Gabo 56/Chinese Spring linkage map were converted to KASP assays. We evaluated these 12 KASP-based SNP markers on Gabo 56/Chinese Spring and Berkut/Scalavatis RIL populations and on *Sr11* near-isogenic lines. Two markers most closely linked to race TKTTF resistance that were diagnostic for *Sr11* in the near-isogenic lines were used in KASP assays to screen 66 breeding lines and cultivars from North America, Pakistan, and Ethiopia. The primer sequences designed for the KASP assays are provided in Table 1. Each KASP consisted of 50 ng of DNA template and 5 µl of 2× KASP buffer (LGC Genomics) and 0.14 µl of primer mixture. Thermal cycling conditions were 94°C for 15 min, followed by 10 cycles of touch-down polymerase chain reaction (PCR) of 94°C for 20 s and 65 to 57°C for 60 s (dropping 0.8°C per cycle), followed by 36 cycles of regular PCR of 94°C for 20 s and 57°C for 60 s, followed by fluorescence reading at 20°C. An additional three to nine cycles of PCR were added to obtain a good separation of clusters, if needed. Both thermal cycling and fluorescence reading were performed on an ABI Step One Plus Real Time PCR system. At least two replicates of each KASP assay were performed. If inconsistent results were observed between the two replicates, a third replicate was performed.

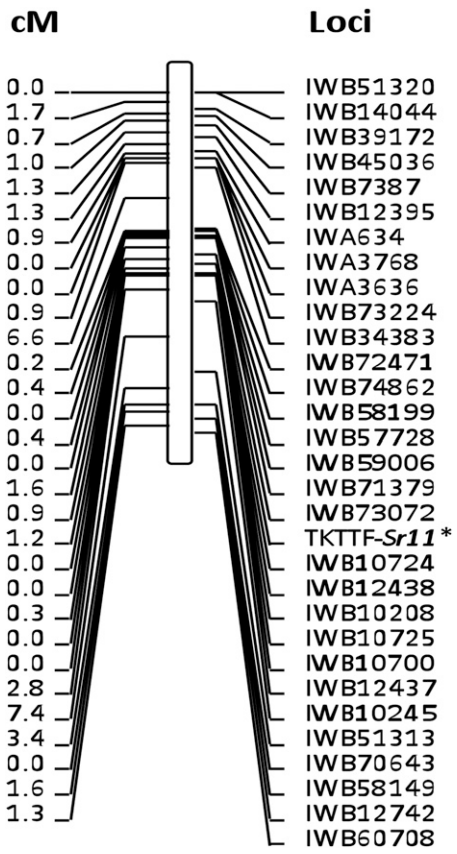

Fig. 2. Gabo 56/Chinese Spring genetic linkage map of chromosome arm 6BL including the race TKTTF resistance phenotype (*Sr11*), constructed using an iSelect 90K Infinium assay. Values to the left of the marker names are the distances in centimorgans (cM) generated using the Kosambi mapping function.

RESULTS

Stem rust assays. Gabo 56 exhibited an infection type of 22– to 22+ whereas Chinese Spring exhibited an infection type of 3+ to race TKTTF (Fig. 1). The F_4 lines of Gabo 56/Chinese Spring segregated for resistance to race TKTTF, with resistant plants exhibiting infection types 22– to 22+ and susceptible plants exhibiting 33+ to 3+ infection types. Segregation of resistance did not significantly deviate from the expected ratio for a single gene ($\chi^2 = 1.2$, $P = 0.55$). Berkut displayed a ;2 infection type whereas Scalavatis exhibited a 3+ infection type in response to race TKTTF (Fig. 1). The RIL population derived from Berkut/Scalavatis deviated from the expected 1:1 ratio for a single gene (46 resistant, 24 susceptible; $\chi^2 = 9.2$, $P = 0.01$). However, *Sr11*-linked marker KASP_6BL_IWB10724 also deviated from the expected 1:1 ratio (45 Berkut allele, 22, Scalavatis allele; $\chi^2 = 7.7942$, $P = 0.01$), indicating segregation distortion at this single locus. Both Gabo 56 and Berkut possessed infection-type patterns consistent with the presence of *Sr11* (Table 2). We refer to the single-gene-mediated race TKTTF resistance from Gabo 56 and Berkut as *Sr11*.

Genetic linkage mapping. To identify molecular markers linked to *Sr11*, we generated a linkage map spanning 36.06 cM with previously mapped chromosome arm 6BL markers identified by scoring the 90,000 SNP data from the Gabo 56/Chinese Spring population. Based on our data, resistance to race TKTTF clearly mapped to chromosome arm 6BL and was positioned between SNP markers IWB73072 (proximal) and IWB10724 (distal) (Fig. 2). The SNP names corresponding to the SNP identification numbers used are listed in Supplementary Table S2. These two flanking markers were linked to *Sr11* at 0.7- and 0.3-cM distances, respectively. Seven markers were converted into KASP-based SNP markers (namely, IWB73072, IWB12438, IWB10724, IWB58199, IWB72471, IWB10245, and IWB10700) based on distinct clustering patterns. These markers were reevaluated on 149 Gabo 56/Chinese Spring RIL to generate a linkage map encompassing the region containing the gene resistant to TKTTF (Fig. 3). Two of these markers, IWB73072 and IWB59006, were also identified as linked to race TKTTF resistance in the association mapping study by Bajgain et al. (2015).

Evaluation of SNP markers to postulate the presence of *Sr11*. To identify potential markers that can discriminate lines with and without *Sr11*, we evaluated a total of 12 KASP markers—7 derived from the Gabo 56/Chinese Spring linkage map and 5 derived from Bajgain et al. (2015)—on a set of near-isogenic lines and genetic stock lines. The allele calls for five of these markers along with their disease reaction to races TKTTF, TRTTF, TTKSK, RTQQC, and RKRQC are shown in Table 2. Lines resistant to races RKRQC and TKTTF but susceptible to races RTQQC, TTKSK, and TRTTF displayed an infection-type pattern consistent with the presence of *Sr11* (Fig. 1). Of the 12 markers tested, 5 markers

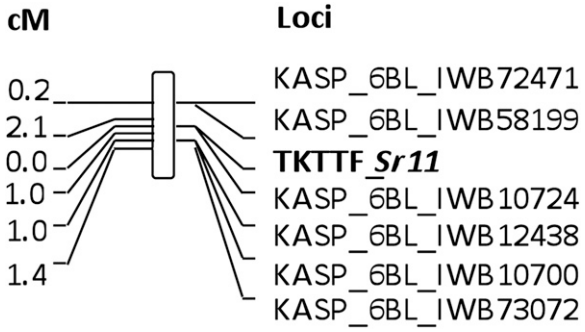

Fig. 3. Genetic linkage map of the *Sr11* gene on chromosome arm 6BL constructed from Gabo 56/Chinese Spring F_4 recombinant inbred lines using KBioscience competitive allele-specific polymerase chain reaction (KASP) assays. Values to the left of the marker names are the distances in centimorgans (cM) generated using the Kosambi mapping function.

TABLE 3. Disease reaction of cultivars and breeding lines from Ethiopia, Pakistan, and North America against *Puccinia graminis* f. sp. *tritici* races TKTTF (*Sr11*-avirulent), TRTTF (*Sr11*-virulent), and TTKSK (*Sr11*-virulent) and allele calls of two single-nucleotide polymorphism markers using KBioscience competitive allele-specific polymerase chain reaction (KASP) assays

	Reactions by race				KASP[a]	
Lines, cultivars[b]	TKTTF	TRTTF	TTKSK	Gene[c]	1	5
ETBW7872	;2–	3+	22–	*Sr11*	C	G
Tay	12–	2	33+	*Sr11*	C	H
CIMMYT-ETH-53	2–	22+	33+	*Sr11*	C	G
Digalu	3+	22+	22–	No *Sr11*	T	T
Kubsa	33+	3+	3+	No *Sr11*	T	T
Kingbird	33–;	2	33–	No *Sr11*	T	T
Danda'a	32+	3+	3+	No *Sr11*	T	T
CIMMYT-ETH-4	12	2–	33+	*Sr11*	T	T
V-13318	2–	3+	3+	*Sr11*	C	G
V-13311	2–	3+	3+	*Sr11*	C	G
V-13324	2–	3	3	*Sr11*	C	G
12FJ06	2	3	2+	*Sr11*	C	G
CCRI-50	2	3	3	*Sr11*	…	G
QUEETA-2	2	3	3	*Sr11*	C	…
NIA-9/5	2	3–	3	*Sr11*	C	G
DANI-16	2–;	3+	2	*Sr11*	C	G
DN-93	2–	3+	4	*Sr11*	C	G
DANI-17	3+	23	3	No *Sr11*	H	H
RCA-1	3+	2	3	No *Sr11*	T	T
99172	3+	2	3	No *Sr11*	T	T
PR-106	3+	2	3	No *Sr11*	T	T
H0900081	;2–	33+	3+	*Sr11*	C	G
Macon	;2–	22–	3+	*Sr11*	C	H
Scarlet	;2–	3+	3+	*Sr11*	C	G
WA8034	;2–	3+2	3+	*Sr11*	C	G
9248	;2–	22+	3+	*Sr11*	C	G
Berkut	22+	22+	3+	*Sr11*	C	G
Alturas	12–	33+	3+	*Sr11*	C	T
Cataldo	;2–	33+	3+	*Sr11*	C	T
9247	;2	–2	3+	*Sr11*	T	T
SD4215	12–	33+	3+	*Sr11*	T	T
WA8016	;2–	3+	3+	*Sr11*	C	…
UC1551	33+	22+	3+	No *Sr11*	T	T
CDC Utmost	33+	3+	3+	No *Sr11*	T	T
HR07005-3	33+	3+	3+	No *Sr11*	T	T
Kelse	33+	;2–	3+	No *Sr11*	T	T
Tara2002	3+	22/33+	3+	No *Sr11*	…	T
WA8123	33+	22–	3+	No *Sr11*	T	T
9228	3+	22–	3+	No *Sr11*	T	T
9241	33+	22+	3+	No *Sr11*	T	T
9260	33+	3+	3+	No *Sr11*	T	…
Jubilee	3+	3+	3+	No *Sr11*	T	T
Lolo	3+	2	3+	No *Sr11*	T	T
Summit 515	33+	33+	3+	No *Sr11*	T	…
Blanca Grande 515	3+	3+/2	3+	No *Sr11*	T	T
UC1603	3+	3/22+	3+	No *Sr11*	T	T
UC1554	33+	3 /22+	3+	No *Sr11*	T	T
UC1599	33–	33+	3+	No *Sr11*	T	T
UC1601	33+	33+/2+	3+	No *Sr11*	T	T
UC1602	3+	2	3+	No *Sr11*	T	T
UC1616	3+	2	3+	No *Sr11*	C	T
10013-1	33+	22–	3+	No *Sr11*	T	T
UC1683	33+	2	3+	No *Sr11*	T	T
10014/7	33+	33+	3+	No *Sr11*	T	T
RIL203	33+	;2–	3+	No *Sr11*	T	T
RIL 29	33+	22–	3+	No *Sr11*	T	…
SD4218	3	3+	3+	No *Sr11*	T	T
WhiteBird	33+	3+	3+	No *Sr11*	T	T
AC Andrew	33+	3+	3+	No *Sr11*	T	T
Selkirk	3+	3+	3+	No *Sr11*	T	T
Park	33+	3+	3+	No *Sr11*	T	…
Marquis	33+	3+	3+	No *Sr11*	T	T
MT0945	33+	2	3+	No *Sr11*	T	…
Garnet	3+	3+	3+	No *Sr11*	T	G
UI Pettit	33+	3+/ 22+	3+	No *Sr11*	C	T
MT1053	33+	3+	3+	No *Sr11*	C	T

[a] KASP 1 = KASP_6BL_IWB10724, KASP 5 = KASP_6BL_IWB72471, and symbol "…" indicates null allele.
[b] CIMMYT = International Maize and Wheat Improvement Center.
[c] Gene postulation.

(KASP_6BL_IWB73072, KASP_6BL_IWB72471, KASP_6BL_IWB10724, KASP_6BL_IWB46893, and KASP_6BL_12438) were able to discriminate the wheat lines based on presence or absence of *Sr11*, with few exceptions (Table 2). Two KASP markers originating from the Gabo 56/Chinese Spring linkage map (KASP_6BL_IWB10724 and KASP_6BL_IWB72471) were found to clearly discriminate the lines with and without *Sr11* (Table 2).

Validation of two *Sr11*-linked SNP markers on diverse germplasm. Markers KASP_6BL_IWB10724 and KASP_6BL_IWB72471 were subsequently evaluated on 66 selected breeding lines from Ethiopia, Pakistan, and North America (Table 3). Of these 66 lines, 14 possessed the *Sr11* alleles, 34 possessed the non-*Sr11* alleles, and 14 possessed mixed alleles for the two markers or heterozygous alleles. Of the 14 lines with the *Sr11*-alleles, all displayed infection-type patterns consistent with the presence of *Sr11*. Of the 34 lines with the non-*Sr11* alleles, 31 displayed infection-type patterns consistent with the absence of *Sr11,* though 3 displayed *Sr11* infection-type patterns.

Validation of *Sr11*-linked SNP markers in the Berkut/Scalavatis population. Five SNP markers were found to be polymorphic between Berkut and Scalavatis. These markers were evaluated on 70 F_6 RIL from Berkut/Scalavatis and the data generated were used to make a linkage map (Fig. 4). KASP_6BL_IWB10724 cosegregated with *Sr11* while KASP_6BL_IWB46893 was positioned 0.3 cM distal to *Sr11*. Marker KASP_6BL_IWB58199, though polymorphic, was not linked to the race TKTTF phenotype.

DISCUSSION

Segregation of resistance and linked markers revealed that a single resistance gene conferred resistance to *P. graminis* f. sp. *tritici* race TKTTF in both Gabo 56 and Berkut. We mapped resistance to race TKTTF to chromosome arm 6BL (Fig. 2). The map location of resistance to race TKTTF is in agreement with the location of *Sr11* (Sears 1966). Two markers (KASP_6BL_IWB10724 and KASP_6BL_IWB72471) that were linked to *Sr11* in two mapping populations were further validated by haplotype analysis of diverse germplasm. The KASP_6BL_IWB10724 and KASP_6BL_IWB72471 markers accurately predicted the presence of *Sr11*. The presence of additional unknown genes might explain why three lines possessed the *Sr11* phenotype but the non-*Sr11* marker haplotype. Alternatively, recombination may have separated *Sr11* from the *Sr11*-linked SNP marker haplotypes in these lines.

The *Sr11*-linked SNP markers predicted the presence of *Sr11* in 'Trident' wheat (Table 2) that was previously used as a stem rust differential line for detecting virulence or avirulence of *P. graminis* f. sp. *tritici* isolates in response to *Sr38* (Olivera et al. 2012) although, initially, the source of *Sr38*, 'VPM1', was used as the differential for *Sr38* (Jin et al. 2008). Screening race TKTTF resulted in a low infection type to Trident (2–) and a high infection

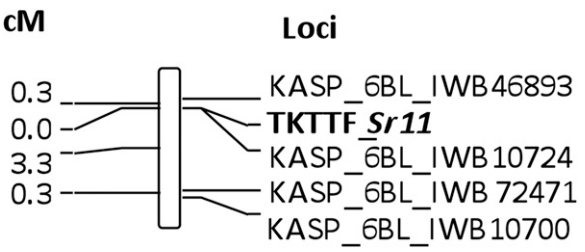

Fig. 4. Genetic linkage map of the *Sr11* gene on chromosome arm 6BL from Berkut/Scalavatis F_6 recombinant inbred lines using KBioscience competitive allele-specific polymerase chain reaction (KASP) assays. Values to the left of the marker names are the distances in centimorgans (cM) generated using the Kosambi mapping function.

type to VPM1 (3–), which indicated that Trident has *Sr11* in addition to *Sr38* (Olivera et al. 2015). These data suggest that *P. graminis* f. sp. *tritici* race analyses should avoid the use of Trident as a differential line because both *Sr11* and *Sr38* are present.

Assessing *Sr11* genetic stocks with multiple *P. graminis* f. sp. *tritici* isolates, races, and SNP markers not only confirmed the linkage of SNP markers to *Sr11* but also elucidated the source of *Sr9h* and the relationship between *Sr9h* and *SrCharter.* Varieties previously characterized as possessing *Sr11* such as 'Timstein' and 'Charter' (Knott and Anderson 1956; Luig and Watson 1965) also possessed resistance to *Sr11*-virulent races TTKSK and RTQQC (Table 2). The pedigree of Gabo 56 includes Timstein and *Sr11* source *Triticum turgidum* var. *durum* Gaza: Timstein/'Kenya 58'// Gabo. The pedigree of Gabo is 'Bobin sel.'/Gaza//'Bobin'. Though the pedigree of Timstein has been reported as 'Steinwedel'/ *T. timopheevii*, multiple studies suggest that Timstein was actually derived from a cross between Bobin and Gaza (Boasso and Levine 1951; Levine et al. 1951; Rouse et al. 2014; Watson and Stewart 1956). Susceptibility of Kenya 58 and Bobin to race TTKSK combined with the results of resistance of Gabo, Gaza, and Timstein to race TTKSK (Rouse et al. 2014) (Table 2) suggest that *Sr9h* originates from durum Gaza. Resistance of Gaza, Timstein, and Gabo 56 not only to *Sr11*-avirulent race RKRQC but also to *Sr11*-virulent race RTQQC suggests that *Sr9h* confers resistance to North American race RTQQC. Though Charter was previously demonstrated to possess *Sr11* (Luig and Watson 1965), Charter possessed resistance to isolates of *P. graminis* f. sp. *tritici* that could not be explained by *Sr11* alone (Luig 1983). This led researchers in India to include Charter as a differential line (Nagarajan et al. 1986) and led to the temporary designation of a second stem rust resistance gene in Charter as *SrCharter* (McIntosh et al. 1995). We observed Charter to possess the same infection-type pattern as Gabo 56, Timstein, and Gaza, suggesting that Charter possesses *Sr9h* in addition to *Sr11* (Table 2). Charter was developed in New South Wales, similar to Gabo and Timstein. Similar to *Sr9h*, *SrCharter* avirulence in isolates virulent to *Sr11* was detected in both North America and Kenya (Luig 1983). Though not definitively demonstrated by these data, the available evidence is consistent with the hypothesis that *SrCharter* and *Sr9h* are the same gene.

Sr11 is present in a number of U.S., Australian, Kenyan, and International Maize and Wheat Improvement Center cultivars (Roelfs and McVey 1979). Virulence to *Sr11* is present in the United States (Roelfs et al. 1991), Australia (Zwer et al. 1992), South Africa (Le Roux and Rijkenberg 1987), Canada (Harder and Dunsmore 1990), and North Africa (Huerta-Espino 1992; Singh et al. 2015). This gene was highly effective when first deployed in Australia but its widespread use resulted in an increase in virulence frequency and, subsequently, the *P. graminis* f. sp. *tritici* population became fixed for virulence to *Sr11* in Australia. Though *Sr11* has been defeated by several *P. graminis* f. sp. *tritici* races throughout the world, it may be a valuable resistance gene for particular race groups of *P. graminis* f. sp. *tritici*. The current situation in Ethiopia highlights the importance of *Sr11*, which can be exploited by breeding programs to combat race TKTTF epidemics. The unusual occurrence of the race TKTTF epidemic may be due to the widespread use of *Sr11* in breeding programs throughout the world, making many deployed wheat varieties resistant to race TKTTF. *Sr11* was transferred to spring wheat lines from Gaza durum wheat. The SNP marker haplotype predictive of *Sr11* was conserved in Gaza (Table 2), further validating the utility of the markers. The KASP assay-based markers developed for *Sr11* in this study could be used for at least three methods in selecting for stem rust resistance in the United States and around the world: (i) to select for wheat lines with *Sr11* in order to select for resistance to race TKTTF and other *Sr11*-avirulent races, (ii) to postulate the presence or absence of *Sr11* in uncharacterized germplasm, and (iii) the markers could be used in combination with field studies to select for wheat lines without *Sr11* but with adult plant resistance.

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