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Identification, mapping, and marker development of stem rust resistance genes in durum wheat 'Lebsock'

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Abstract Wheat production in many wheat-growing regions is vulnerable to stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*). Several previous studies showed that most of the durum cultivars adapted to the upper Great Plains in the USA have good resistance to the major *Pgt* pathotypes, including the Ug99 race group. To identify the stem rust resistance (*Sr*) genes in the durum cultivar 'Lebsock', a tetraploid doubled haploid (DH) population derived from a cross between Lebsock and *Triticum turgidum* ssp. *carthlicum* PI 94749 was screened with the *Pgt* races TTKSK, TRTTF, and TTTTF. The stem rust data and the genotypic data previously developed were used to identify quantitative trait loci (QTL) associated with resistance. We identified one QTL each on chromosome arms 4AL, 6AS, 6AL, and 2BL. Based on marker and race-specification analysis, we postulated that the QTL on 4AL, 6AS, 6AL, and 2BL correspond to *Sr7a*, *Sr8155B1*, *Sr13*, and likely *Sr9e*, respectively. The results indicated that most of the US durum germplasm adapted to the upper Great Plains likely harbors the four major *Sr* genes characterized in this study. Among these genes, *Sr8155B1* was recently identified and shown to be unique in that it conferred susceptibility to TTKSK but resistance to variant race TTKST. Two, three, and one thermal asymmetric reverse PCR (STARP) markers were developed for *Sr7a*, *Sr8155-B1*, and *Sr13*, respectively. Knowledge of the *Sr* genes in durum germplasm and the new STARP markers will be useful to pyramid and deploy multiple *Sr* genes in future durum and wheat cultivars.

Key message Four quantitative trait loci at gene loci *Sr7, Sr9*, *Sr13*, and *Sr8155B1* were identified in durum 'Lebsock' and six new STARP markers were developed for the *Sr7*, *Sr13*, and *Sr8155B1* regions.

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Introduction

The disease stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*), constantly threatens worldwide production of bread wheat (*Triticum*

aestivum L., $2n = 6x = 42$, AABBDD) and durum wheat (*T. turgidium* ssp. *durum* (Desf.) Husnot, $2n = 4x = 28$, AABB) due to adaptation of *Pgt* populations to the deployed stem rust resistance (*Sr*) genes. Durum wheat is one of the major crops produced in the upper Great Plains in North America; North Dakota (ND) alone contributes 67% of the total US durum wheat production (Elias and Manthey 2012). Historical data indicate that durum and bread wheat crops in the upper Great Plains region are highly vulnerable to stem rust outbreaks (Roelfs 1978). Since 1904, nine outbreaks of stem rust have been reported on wheat in this region (Paarlberg et al. 2014). During the 1930s and 1950s, the epidemics caused by *Pgt* races 56 (MCCFC) and 15B (TMLKC/TPMKC) (https://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Cerealrusts/Pgtraceconversions.xls) led to significant yield losses of the durum and spring wheat crops in Minnesota and North Dakota (Dubin and Brennan 2009; Paarlberg et al. 2014; Roelfs 1978). Consequently, stem rust resistance has been one of the major targets for durum and spring wheat breeding programs in this region since the 1910s (Hayes et al. 1936). Certain *Sr* genes that are effective against the prevailing races have been deployed into durum and bread wheat cultivars. Such efforts in breeding for stem rust resistance have led to the development of wheat cultivars that carry several *Sr* genes.

The *Pgt* race TTKSK and its 12 variants (commonly known as the Ug99 race group) originated from East Africa and are currently considered a major threat to world wheat production due to their virulence on many deployed *Sr* genes (Fetch et al. 2016; Pretorius et al. 2000; Singh et al. 2011, 2015). Approximately half of the 70 known *Sr* genes are ineffective against the Ug99 race group, including the widely deployed *Sr24*, *Sr31*, *Sr36*, and *Sr38* genes (McIntosh et al. 2013, 2014; Singh et al. 2015). At present, more than 80% of worldwide wheat production is under the potential threat of the Ug99 race group (Lopez-Vera et al. 2014; Singh et al. 2011).

In addition to the Ug99 race group, several other *Pgt* races have raised concerns due to their broad virulence against frequently deployed *Sr* genes. The races TRTTF, JRCQC, and TTTTF were identified in Yemen (2006), Ethiopia (2009), and the USA (2000), respectively (Jin 2005; Olivera et al. 2012). These races were reported as virulent to *Sr9e* and/or *Sr13*, which are the major sources of resistance in North American and CIMMYT durum wheat cultivars (Jin 2005; Periyannan et al. 2014; Singh et al. 2015). However, a recent study conducted by Zhang et al. (2017) has shown that *Sr13*, which encodes coiled-coil nucleotide-binding leucine-rich repeat protein CNL13, is effective against TRTTF. In addition, TRTTF is virulent on *Sr36*, *SrTmp*, and $Sr1RS^{Amigo}$, which are present in many US winter wheat cultivars and breeding lines (Jin and Singh 2006; Olivera et al. 2012), and TTTTF is broadly virulent to *Sr* genes in the North American stem rust differential set, including *Sr36* and *SrTmp* (Jin 2005), and North American winter wheat germplasm. Another non-Ug99 lineage *Pgt* race of concern is TKTTF, which was identified in Ethiopia and Germany in 2013 (Olivera Firpo et al. 2017; Olivera et al. 2015). However, the TKTTF isolates from both locations are phenotypically different from each other (Olivera Firpo et al. 2017). TKTTF was responsible for a localized epidemic in Ethiopia, which led to nearly 100% crop loss of the popular cultivar Digalu in the southern region of the country (Olivera et al. 2015; Turner et al. 2016). Therefore, detection of highly virulent *Pgt* races in the last two decades demonstrates a vulnerability in the adapted cultivars and the need for new sources of resistance. Currently, at least three non-Ug99 lineage races (TRTTF, JRCQC, and TTTTF) are potential threats to durum and winter bread wheat production in North America.

The resistance to TTKSK in the durum wheat cultivars and germplasm adapted to the upper Great Plains of the USA is primarily due to the presence of *Sr13* derived from Khapli emmer wheat [*T. turgidum* ssp. *dicoccum* (Schrank ex Schubler) Thell.] (Simons et al. 2011). Most recently, Nirmala et al. (2017) reported that most of the US Great Plains durum cultivars also carry an *Sr* gene (temporarily designated as *Sr8155B1*) located in the *Sr8* region of chromosome arm 6AS. This gene is unique due to its resistance against the *Pgt* race TTKST and other variants, although it was not effective against the first-detected race TTKSK in the Ug99 race group. Furthermore, it has been found out recently that all the ND durum cultivars and breeding lines tested were also highly resistant to TRTTF, indicating that they may carry additional uncharacterized *Sr* genes. As current cultivars and adapted germplasm are usually used as parents for developing new cultivars, knowledge of the *Sr* genes already present in the cultivars and adapted germplasm

is essential for the selection of donor *Sr* genes and breeding schemes suitable for gene pyramiding and deployment. It has been recognized that pyramiding, or combining more than one resistance (R) gene, is the most promising strategy to achieve broad-spectrum, durable resistance against multiple races of a single pathogen or pest (Singh et al. 2011).

Marker-assisted selection (MAS) is frequently used to pyramid multiple genes in wheat lines. Various molecular markers have been used for MAS breeding, but in the last few years, SNP genotyping technologies have made significant progress in agricultural research. Recently, Long et al. (Long et al. 2017) developed a novel SNP genotyping method known as semi-thermal asymmetric reverse PCR (STARP). This method is advantageous for MAS, due to high accuracy, low operation costs, and its adaptability to different genotyping platforms (Klindworth et al. 2017; Long et al. 2017). Therefore, development of STARP markers linked to the major *Sr* genes will facilitate gene pyramiding and deployment for improving stem rust resistance in wheat breeding.

Among the major durum cultivars in production in North Dakota, 'Lebsock' (Elias et al. 2001) was the leading cultivar in the state for 5 years from 2004 (27.9%) to 2008 (26.7%) (USDA-NASS 2006, 2009). Although Lebsock production is reduced now due to its susceptibility to Fusarium head blight (USDA-NASS 2017), it has been used as a parent for developing new durum germplasm and populations. A doubled haploid (DH) population designated as LP749 derived from a hybrid between Lebsock and *T. turgidium* ssp. *carthlicum* (Nevski in Kom.) Á. Löve & D. Löve ($2n = 4x = 28$, AABB) accession PI 94749 and its SSR-based linkage map have already been developed (Chu et al. 2010). This DH population and the linkage map were previously used for the identification of novel quantitative trait loci (QTL) for tan spot resistance (Chu et al. 2010), a novel *Vrn-B1* allele (*Vrn-B1c*) for plant growth habitat (Chu et al. 2011), and a new sensitivity gene (*Snn5*) for a necrotrophic effector (SnTox5) produced by *Parastagonospora* (syn. *ana*, *Stagonospora*; *teleo*, *Phaeosphaeria*) *nodorum* (Berk.) (Friesen et al. 2012). The parents of the LP749 population, Lebsock and PI 94749, differ in their resistance against *Pgt* races.

Considering the significance of Lebsock in the future resistance-breeding program, our first objective was to identify QTL associated with stem rust resistance derived from Lebsock. To determine that, we screened the LP749 DH population with *Pgt* races TTKSK, TRTTF, and TTTTF. Our second objective was to screen the other North Dakota leading durum wheat cultivars with these three *Pgt* races. Additionally, allele-specific STARP markers for the *Sr* regions were developed to facilitate the pyramiding of these genes in modern cultivars.

Materials and methods

Plant materials

Nine durum wheat cultivars in addition to a population of 146 tetraploid DH lines derived from the F_1 hybrids between durum wheat cultivar Lebsock and *T. turgidium* ssp. *carthlicum* accession PI 94749 were analyzed for their stem rust reaction. The nine durum wheat cultivars, including 'Joppa', 'Carpio', 'Tioga', 'Alkabo', 'Divide', 'Grenora', Lebsock, 'Maier', and 'Ben', were developed by the North Dakota Agricultural Experiment Station in cooperation with the USDA-ARS and released from 1996 to 2014 (Elias and Manthey 2007a, b, c, 2016; Elias and Miller 1998, 2000; Elias et al. 2001, 2014). In 2015, these cultivars accounted for over 40 and 76.5% of the durum acreage planted in Montana (USDA-NASS 2015a) and North Dakota (USDA-NASS 2015b), respectively. The development of the LP749 population was previously described (Chu et al. 2010).

Stem rust analysis

The nine durum wheat cultivars and the LP749 population were evaluated for reactions to three *Pgt* races: TTKSK (isolate 04KEN156/04), TRTTF (06YEM34-1), and TTTTF (01MN84A-1-2), at the USDA-ARS Cereal Disease Laboratory, St. Paul, MN. Stem rust evaluations were performed at the seedling stage using a method described by Rouse et al. (2011). The avirulence/virulence profiles of these three races on the North America differentials are listed in Table S1. The evaluation of the 146 DH lines in the LP749 population and parents (Lebsock and PI 94749) was conducted in two biological replications. For each replication, five seeds were used for individual DH lines. The F_1 plants were also tested for stem rust response. Plants were scored for infection type (IT) using the Stakman et al. (1962) scale, where basic ITs were

"0, ; , 1, 2, 3, and 4". The additional symbols "$^{+}$" and "$^{-}$" were used to represent large and small pustules within an IT, respectively (Roelfs and Martens 1988). Plants with an IT score 0–2 were considered resistant, and those with a score of 3–4 were considered susceptible. For QTL analysis, the IT scores of individual races were converted using a scale of 0 to 9 based on the method described by Zhang et al. (2014). The mean of converted scores for each line in response to individual *Pgt* races from two replications was used in the QTL analysis.

Marker analysis

The LP749 population linkage maps, composed of SSRs (Chu et al. 2010), were used for the initial QTL analysis, by which we identified one major QTL each on chromosome arms 4AL, 6AS, and 6AL. To further saturate the QTL regions in current linkage maps, additional SSRs that were previously reported from chromosome arms 4AL and 6AS were analyzed. Additionally, allele-specific STARP markers were developed for these two regions and the recently cloned *Sr13* on chromosome arm 6AL (Zhang et al. 2017). For both approaches, genomic DNA was extracted from the 146 DH lines and the parents using the method described by Faris et al. (2000). For SSR analysis, 16 molecular markers selected from different maps (Somers et al. 2004; Sorrells et al. 2011; Sourdille et al. 2004; Torada et al. 2006; Xue et al. 2008; Zhang et al. 2008) were further mapped on the linkage groups in this study. Of these newly selected markers, 11 and five were mapped on chromosomes 4A and 6A, respectively. Of the 11 molecular markers on 4A, six were SSRs *Xgwm397*, *Xgwm637*, *Xbarc135*, *Xcfd31*, *Xwmc776*, and *Xhbg452*, and the remaining five were eSTS markers *Xmag3886*, *Xmag3733*, *Xmag3092*, *Xmag1574*, and *Xmag1604*. The five newly mapped markers on chromosome 6A were SSRs *Xgpw2295*, *Xhbe302*, *Xhbg239*, *Xhbg347*, and *Xhbe297*. For marker assays, polymerase chain reactions (PCR) were performed by following the procedure in Röder et al. (1998). The amplified PCR products were electrophoresed on 6% non-denaturing polyacrylamide gels. Gels were stained with Gelred™ nucleic acid stain (Biotium Corporate, Hayward, CA), and a Typhoon 9410 variable mode imager (GE healthcare Biosciences, Waukesha, WI) was used to capture images.

To develop STARP markers for the 4AL region, SNPs were selected from a 9 K-based *Sr7a*-associated 4AL linkage map reported by Turner et al. (2016). In this map, two SNPs, *IWA1066* and *IWA1067*, were located near the *Sr7a* region. These SNPs were used to select additional SNPs from the 90 K consensus map (Wang et al. 2014). To develop STARP markers for the 6AS QTL region, SNPs were selected from a 90 K-based chromosome 6A linkage map of a tetraploid RIL population (RP883) developed from a cross between durum wheat and cultivated emmer wheat (J.D. Faris unpublished). The selection was made based on a common SSR *Xhbe302* between the chromosome 6A linkage maps of LP749 and RP883 populations. For the QTL on chromosome 6AL, the only SNP (T2200C) between the *Sr13* susceptible and resistant haplotypes in *CNL13* gene (Zhang et al. 2017) was used to develop STARP markers. Flanking region sequences for the selected SNPs (Wang et al. 2014; Zhang et al. 2017) were used as BLASTn queries (Altschul et al. 1997) to search the wheat genome scaffolds (https://urgi.versailles.inra.fr/blast_iwgsc/blast.php and/or https://urgi.versailles.inra.fr/blast/?dbgroup=wheat_all&program=blastn) to identify low copy sequences. After selection, the flanking genomic sequences of the selected SNPs were used to design the asymmetrically modified allele-specific (AMAS) and reverse primers according to the method described by Long et al. (2017). The two universal priming element-adjustable (PEA) primers were used as reported in Long et al. (2017). The program Primer-BLAST was used for the calculation of primer's T_m values (Ye et al. 2012). PCR was conducted as described in Long et al. (2017). Thirty-three cycles were used for gel-based detection of PCR products, whereas 46 cycles were used for gel-free detection. For the gel-based system, STARP markers were evaluated as described earlier for SSR markers. However, for gel-free marker analysis, the CFX384 Touch™ Real-Time PCR detection system (Bio-Rad Laboratories, Inc., Foster City, CA, USA) was used.

To evaluate the potential of the newly developed STARP markers for MAS, a validation analysis was done using a diverse set of 50 durum and bread wheat cultivars and lines. This validation panel was composed of 18 durum and 32 common wheat cultivars and lines from Australia, Brazil, Canada, China, Italy, and the USA. One of the durum lines, 8155-B1, is monogenic for stem rust resistance derived from a durum accession

C.I. 8155 (Nirmala et al. 2017; Williams and Gough 1965). In addition, the common wheat line ISr8a-Ra, which is monogenic for *Sr8a* (Jin et al. 2007), the common wheat variety 'Chinese Spring' (CS), and three CS nullisomic-tetrasomic lines involving homoeologous group 6 chromosomes (N6AT6B, N6BT6A, and N6DT6B) were used to validate the new STARP markers on chromosome arm 6AS.

Linkage and QTL analysis

The MapDisto 1.7.5 (Lorieux 2012) software package was used for linkage analysis. To find the grouping of new markers with the previously developed genetic maps, a logarithm of odds (LOD) 3.0 and an Rmax value = 3.0 were used. After confirming the grouping, the best order of the linkage groups was obtained using the 'order sequence', 'check inversions', 'ripple order', and 'drop locus' commands in consecutive order. The Kosambi mapping function (Kosambi 1943) was used to calculate genetic distances. Linkage maps of the LP749 population and stem rust screening data were used to identify genomic regions associated with resistance to *Pgt* races TTKSK, TRTTF, and TTTTF. QTL analysis was conducted using QGENE (4.3.10) (Joehanes and Nelson 2008). A significant LOD threshold of 3.0 was determined by performing a permutation test consisting of 1000 permutations, and the coefficient of determination (R^2) × 100 was calculated and used to determine the amount of phenotypic variation explained by the QTL.

Derived cleaved amplified polymorphic sequence marker analysis

The initial linkage mapping and QTL analysis showed that one of the major QTLs derived from Lebsock was located in the *Sr13* region on chromosome arm 6AL. To verify if *Sr13* is the gene underlying the QTL on 6AL, *T. turgidium* ssp. *carthlicum* PI 94749 and nine durum cultivars (Joppa, Carpio, Tioga, Alkabo, Divide, Grenora, Lebsock, Maier, and Ben) described above were genotyped using the *Sr13*-diagnostic dCAPS marker *Sr13F/R* (Zhang et al. 2017). Durum cultivar 'Langdon' carrying *Sr13* and durum line Rusty (Klindworth et al. 2007) were used as positive and negative checks for *Sr13*, respectively. PCR was performed using the *Sr13F/R* primers and a touchdown protocol reported in Zhang et al. (2017). PCR started at 65 °C, with the temperature decreasing 1 °C per cycle until reaching 55 °C, then continuing with 20 additional cycles at 55 °C. PCR products were digested at 37 °C for 1 h using restriction enzyme *Hha*I. PCR and digestion products were run on 6% acrylamide gels and were visualized on the Typhoon 9410 variable mode imager (GE healthcare Biosciences, Waukesha, WI).

Results

The nine durum cultivars, the LP749 population, *T. turgidium* ssp. *carthlicum* PI 94749, and the F_1 hybrid (Lebsock/PI 94749) were screened with *Pgt* races TTKSK, TRTTF, and TTTTF at the seedling stage. For TRTTF, the nine durum cultivars showed nearly immune to moderately resistant (IT ; to 2⁻) phenotypes (Table S2). For TTKSK and TTTTF, eight cultivars were moderately resistant and nearly immune, respectively, but one cultivar (Divide) was susceptible to both races (Table S2). Contrary to the durum cultivars, PI 94749 was susceptible to all three races. The F_1 plants (Lebsock/PI 94749) had a similar level of resistance to the three races as Lebsock, suggesting that the *Sr* genes in Lebsock are of dominant nature (Table S2). Among the 146 DH lines, 145, 143, and 139 lines were evaluated for reactions to TTKSK, TRTTF, and TTTTF, respectively (Table 1). For TTKSK, the LP749 population segregated into 76 resistant to 69 susceptible, and, for TTKSK and TRTTF, the population segregated into 124 resistant to 19 susceptible and 98 resistant to 41 susceptible, respectively (Table 1). A Chi-squared goodness-of-fit test showed that segregation for TTKSK reaction fit a 1:1 ratio ($\chi^2 = 0.34$, $P = 0.56$), indicating the presence of a single gene for TTKSK resistance (Table 1). On the other hand, segregation of reaction to TTTTF fit a two-gene ratio of 3:1 ($\chi^2 = 1.5$, $P = 0.22$), and segregation of reaction to TRTTF fit a three-gene ratio of 7:1 ($\chi^2 = 0.08$, $P = 0.78$).

Four QTL were associated with the Lebsock-derived resistance against the *Pgt* races, and they were located on chromosome arms 2BL, 4AL, 6AS, and 6AL (Fig. 1; Table 2). The 2BL QTL associated with TRTTF resistance had an LOD of 5.92 and was designated as *QSr.rwg-2B*. This QTL was flanked by SSR markers *Xgwm16* and *Xwmc175* and explained 4% of the phenotypic variation for resistance to TRTTF (Table 2). Based on the chromosomal location of SSR marker

Table 1 Chi-squared analysis of segregation of resistance to three races of the stem rust pathogen in a doubled haploid (DH) population derived from hybrid between durum 'Lebsock' and *T. turgidum* ssp. *carthlicum* PI 94749

	No. of DH lines[a]			χ^2			Probability		
Race	Total	R	S	(1:1)	(3:1)	(7:1)	(1:1)	(3:1)	(7:1)
TTKSK	145	76	69	0.34			0.56		
TRTTF	143	124	19			0.08			0.78
TTTTF	139	98	41		1.50			0.22	

[a] No. of DH line: total = total number of DH lines evaluated, R = resistant, S = susceptible

Xwmc175, *QSr.rwg-2B* resistance was most probably conferred by an allele of *Sr9* (Rouse et al. 2014). Several alleles of *Sr9*, including *Sr9a*, *Sr9b*, *Sr9d*, and *Sr9g*, are ineffective against TRTTF (Table S1), whereas *Sr9e* has a minor effect for resistance to TRTTF (M.N. Rouse, Y. Jin, unpublished). Considering the minor effect of *QSr.rwg-2B* on resistance to TRTTF and prevalence of *Sr9e* in North American durum cultivars, we speculate that the gene underlying this QTL is possibly *Sr9e*.

The QTL on chromosome arm 4AL, designated *QSr.rwg-4A*, had an LOD of 30.73 and explained 37% of the variation associated with resistance to TTTTF (Table 2). This QTL spanned a 13-cM interval between markers *Xmag3886* and *Xwmc219*. This region is

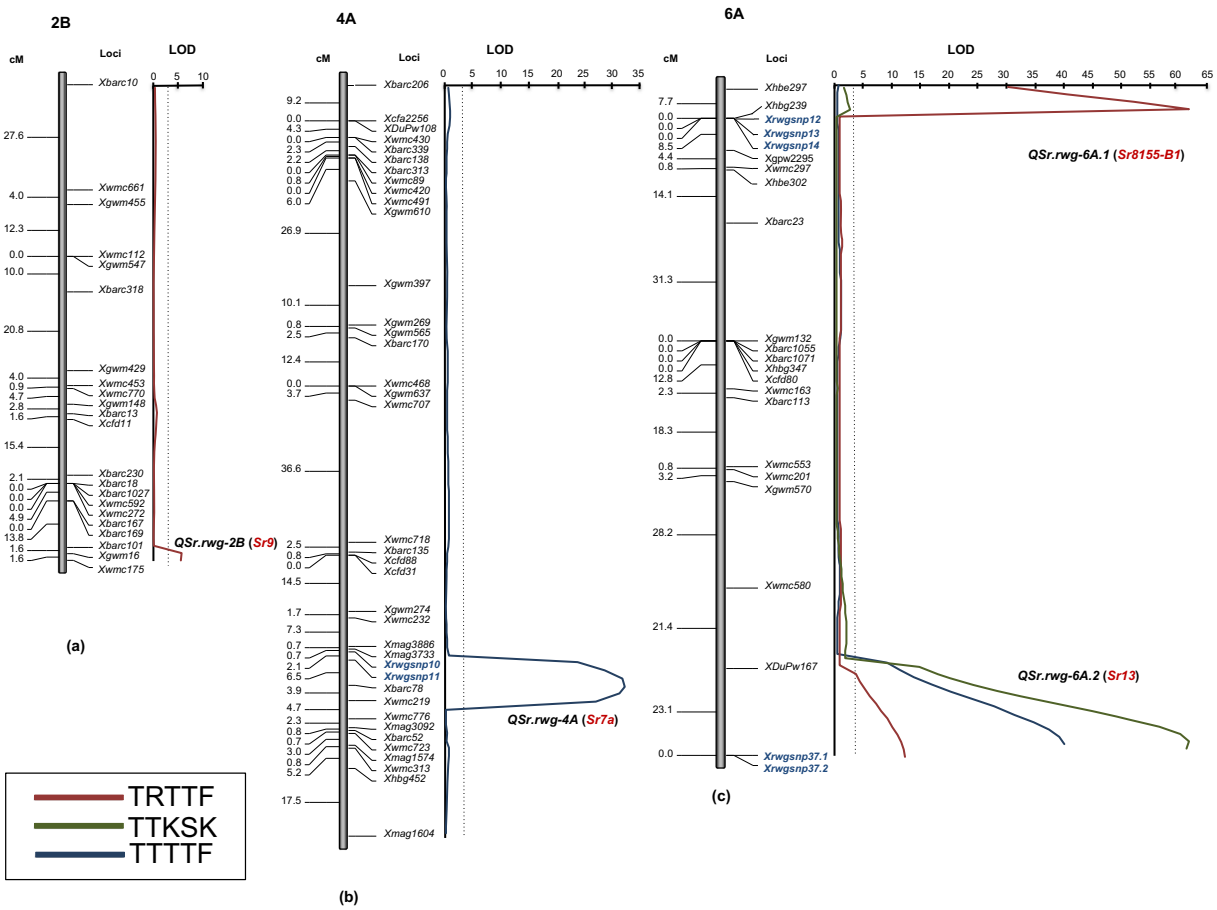

Fig. 1 Genetic map and composite interval mapping of the chromosome 2B, 4A, and 6A representing *QSr.rwg-2B*, *QSr.rwg-4A*, *QSr.rwg-6A.1*, and *QSr.rwg-6A.2*. The dashed lines represent the threshold LOD = 3.0. The putative genes associated with the QTL regions are shown in *red* font

Table 2 QTLs associated with seedling resistance to stem rust caused by *Puccinia graminis* races TRTTF, TTKSK, and TTTTF detected by composite interval mapping. The chromosome arm locations, putative *Sr* gene, associated markers, LOD, R^2, and additive effects are given

QTL	Chr.[a]	Marker interval	Putative *Sr* gene or allele	TRTTF			TTKSK			TTTTF		
				LOD	R^2	Add.[b]	LOD	R^2	Add.	LOD	R^2	Add.
QSr.rwg-2B	2BL	*Xgwm16-Xwmc175*	*Sr9e*	5.92	0.04	−0.51	–[c]	–	–	–	–	–
QSr.rwg-4A	4AL	*Xmag3886-Xwmc219*	*Sr7a*	–	–	–	–	–	–	30.73.	0.37	−1.86
QSr.rwg-6A.1	6AS	*Xhbe297-Xgpw2295*	*Sr8155B1*	61.89	0.79	−2.92	–	–	–	–	–	–
QSr.rwg-6A.2	6AL	*Xwmc580-Xrwgsnp37*	*Sr13*	11.68	0.05	−0.76	63.64	0.85	−3.07	39.63	0.48	−2.31

[a] Chr.: chromosome arm

[b] Add.: additive effects of the QTL, a negative value indicates resistance derived from Lebsock

[c] A symbol "–" indicates no significant association with resistance

known to be associated with the deletion bin 4AL-4 (Sourdille et al. 2004). *QSr.rwg-4A* most likely corresponded to *Sr7a* based on the chromosomal location of the *Sr7* locus, the unique avirulence of *Sr7a* to TTTTF, and the parentage of Lebsock (Elias et al. 2001). Among the other eight durum cultivars evaluated this study, only Divide was susceptible to TTTTF (IT 3⁻), indicating that Divide does not carry *Sr7a* (Table S2). However, other seven cultivars all had the same IT (;) as Lebsock, suggesting that they most likely all have this gene (Table S2).

Among the two QTL present on chromosome 6A, the QTL located on chromosome arm 6AS was specific only to *Pgt* race TRTTF (Fig. 1). This QTL, designated as *QSr.rwg-6A.1*, had a LOD value of 61.89 and explained 79% of the phenotypic variation associated with resistance to TRTTF (Table 2). *QSr.rwg-6A.1* was flanked by the SSR markers *Xhbe297* and *Xgpw2295* (Fig. 1) in the distal deletion bin 6AL-5. The map location and uniquely high level of resistance to TRTTF suggested that *QSr.rwg-6A.1* corresponds to the *Sr8155B1* gene recently reported by Nirmala et al. (2017). The second QTL located on 6AL was designated as *QSr.rwg-6A.2* and provided resistance against all three *Pgt* races used in the current study with LOD values ranging from 11.68 to 63.64 (Table 2; Fig. 1). *QSr.rwg-6A.2* was flanked by markers *Xwmc580* and *Xrwgsnp37* (Fig. 1) and explained 85, 48, and 5% of the variation for resistance associated with races TTKSK, TTTTF, and TRTTF, respectively (Table 2). Based on the molecular markers mapped in the *QSr.rwg-6A.2*-associated genomic region, this QTL is located in the distal deletion bin 6AL-8 (Fig. 1) where the *Sr13* gene resides (Simons et al. 2011). The result from dCAPS marker (*Sr13F/R*) analysis showed that Lebsock and *T. turgidium* ssp. *carthlicum* PI 94749 had the *Sr13* resistant and susceptible haplotypes, respectively (Fig. S1). Except for Divide, the other seven cultivars (Alkabo, Joppa, Ben, Carpio, Tioga, Maier, and Grenora) had the *Sr13* resistant haplotype (Fig. S1), Divide was the only cultivar that was susceptible to TTKSK and TTTTF, the other seven cultivars all had the same or similar ITs (2 or 2⁻) as Lebsock, suggesting that these TTKSK-resistant cultivars all have *Sr13* (Table S2).

Six STARP markers were developed and mapped in the current study (Table 3; Figs. 1 and 2). Out of six STARP markers, two (*Xrwgsnp10* and *Xrwgsnp11*) were mapped near the *Sr7a* region of chromosome arm 4AL (Figs. 1 and 2). Three STARP markers, *Xrwgsnp12*, *Xrwgsnp13*, and *Xrwgsnp14*, were mapped in the *Sr8155B1* region on chromosome arm 6AS (Figs. 1 and 2; Table 3). The STARP marker *Xrwgsnp37* (*Xrwgsnp37.1* and *Xrwgsnp37.2* have different reverse primers) developed from the SNP (T2200C) between susceptible and resistant haplotypes of *Sr13* was mapped on the extreme distal side of the chromosome arm 6AL (Figs. 1c and 2). All of the markers worked effectively with the gel-based system; however, only *Xrwgsnp11*, *Xrwgsnp13*, *Xrwgsnp14*, and *Xrwgsnp37.1* worked effectively with the gel-free system (Fig. 2). Of the six STARP markers linked with *QSr.rwg-4A*, *QSr.rwg-6A.1*, and *QSr.rwg-6A.2*, four (*Xrwgsnp11*, *Xrwgsnp13*, *Xrwgsnp14*, and *Xrwgsnp37*) were co-dominantly inherited, whereas *Xrwgsnp10* and *Xrwgsnp12* were

Table 3 The semi-thermal asymmetric reverse PCR (STARP) markers and their SNP source, sequence, product size, and inheritance

Marker	Chr.[a]	Source SNP		Primers			
		ID	Name	Type	Sequence (5′-3′)[b]	Product ~size (bp)	Inheritance/phase
Xrwgsnp10	4AL	*IWB71467*	Tdurum_contig43961_607	*Xrwgsnp10*-F1	[tail-1]TCAGGTCAGCAAACCCGT	36, 50	Dominant/repulsion
				Xrwgsnp10-F2	[tail-2]TCAGGTCAGCAAACTTGC		
				Xrwgsnp10-R	CGCCGACCGTGCCTTC		
Xrwgsnp11	4AL	*IWB73323*	Tdurum_contig75819_1471	*Xrwgsnp11*-F1	[tail-2]GATGCCTCTGAAGATATATCA	43, 53	Co-dominant
				Xrwgsnp11-F2	[tail-1]GATGCCTCTGAAGATATGCCG		
				Xrwgsnp11-R	CAGTTTCAGATGAAAAGGCCAG		
Xrwgsnp12	6AS	*IWB11274*	BS00082812_51	*Xrwgsnp12*-F1	[tail-1]GTTCTCAGCATACACTTCTG	100, 125	Dominant/coupling
				Xrwgsnp12-F2	[tail-2]GTTCTCAGCATACACTTTCA		
				Xrwgsnp12-R	TTCGAGGTGCCGATGGTGC		
Xrwgsnp13	6AS	*IWB53755*	RAC875_c13610_822	*Xrwgsnp13*-F1	[tail-2]AATCATCAGATGCTGTTTGC	105, 110	Co-dominant
				Xrwgsnp13-F2	[tail-1]AATCATCAGATGCTGTCCGT		
				Xrwgsnp13-R	TTGTTACGTGTATTAGAGGGGC		
Xrwgsnp14	6AS	*IWB47842*	Kukri_c80373_786	*Xrwgsnp14*-F1	[tail-1]GCCTTCTTTTCCTTGGAAACTT	105, 110	Co-dominant
				Xrwgsnp14-F2	[tail-2]GCCTTCTTTTCCTTGGAACTTC		
				Xrwgsnp14-R	GCTCTCAGGAACAAGTTAATTGG		
Xrwgsnp37.1	6AL	*CNL13*	T2200C[c]	*Xrwgsnp37*-F1	[Tail2]AAACCTTTGTTCTCTAACTCTGC	91, 87	Co-dominant
				Xrwgsnp37-F2	[Tail1]AAACCTTTGTTCTCTAACTACGT		
				Xrwgsnp37.1-R	GCGTCAGCAAGAAGTCATCATCA		
Xrwgsnp37.2	6AL	*CNL13*	T2200C[c]	*Xrwgsnp37*-F1	[Tail2]AAACCTTTGTTCTCTAACTCTGC	98, 94	Co-dominant
				Xrwgsnp37-F2	[Tail1]AAACCTTTGTTCTCTAACTACGT		
				Xrwgsnp37.2-R	CACCATGTATTCAGCAAGAAGTCA		

[a] Chr.: chromosome arm

[b] Tail-1 and 2 universal sequences are 5′-GCAACAGGAACCAGCTATGAC-3′ and 5′-GACGCAAGTGAGCAGTATGAC-3′, respectively

[c] T2200C: an SNP associated with *Sr13*-resistant haplotype (Zhang et al. 2017)

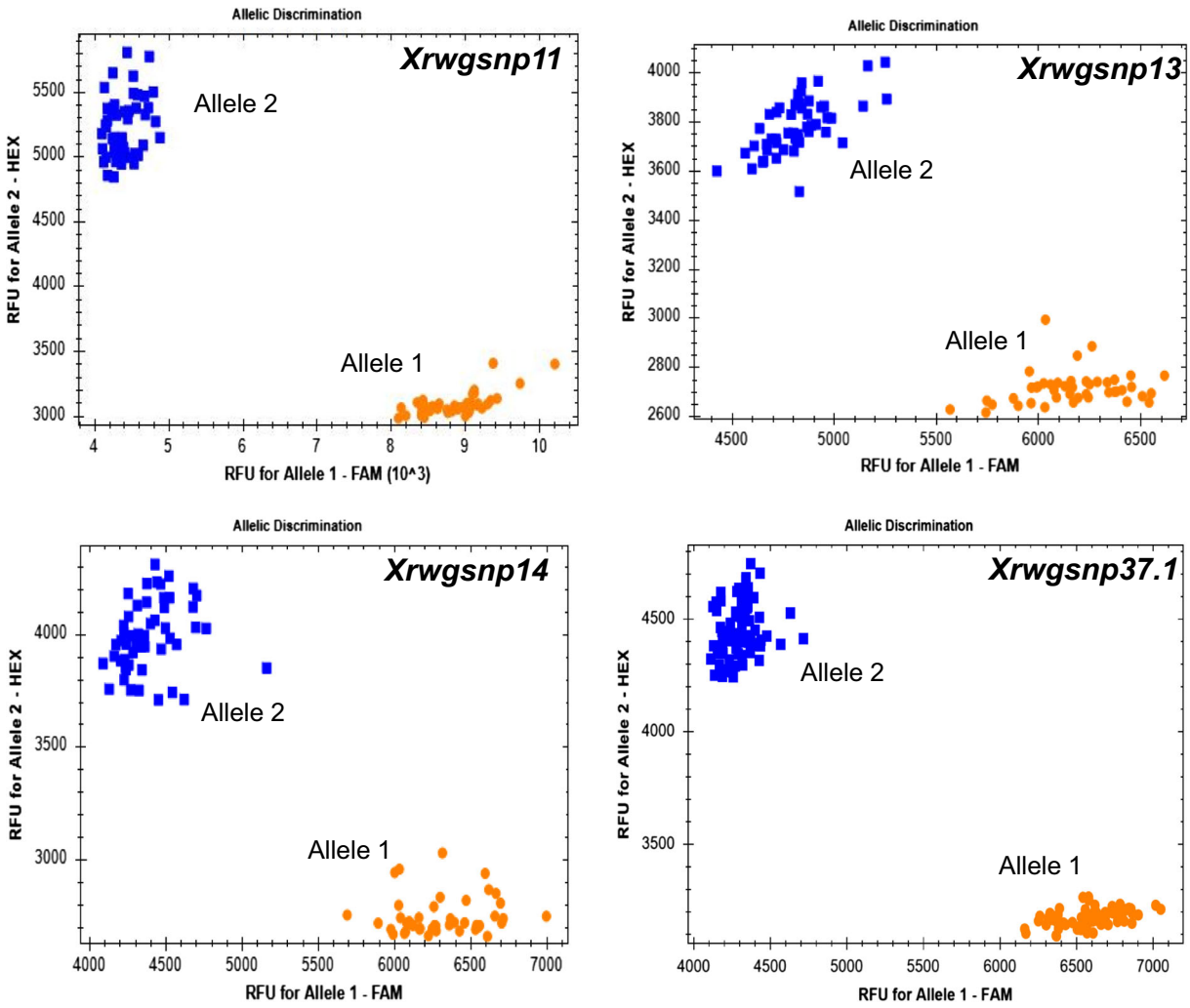

Fig. 2 Plots showing the clusters of Lebsock, *T. turgidum* ssp. *carthlicum* PI 94749, and a subset of doubled haploid (DH) lines (LP749-1 through in LP749-94) in the LP749 (Lebsock × PI 94749) population for four STARP markers *Xrwgsnp11*, *Xrwgsnp13*, *Xrwgsnp14*, and *Xrwgsnp37.1* analyzed with the CFX84 Touch™ Real-Time PCR Detection system. Alleles 1 and 2 in marker loci *Xrwgsnp11*, *Xrwgsnp13*, and *Xrwgsnp14* are associated with Lebsock and PI 94749, respectively, whereas the alleles 1 and 2 for *Xrwgsnp37.1* are associated with PI 94749 and Lebsock, respectively. All 94 DH lines were shown in the *Xrwgsnp11* and *Xrwgsnp14* plots, but one (LP749-9) and two (LP749-51 and LP749-85) DH lines were not included in the *Xrwgsnp13* and *Xrwgsnp37.1* plots, respectively, due to PCR amplification failures

dominant in nature, with *Xrwgsnp10* being in the repulsion phase and *Xrwgsnp12* in the coupling phase (Table 3). The Lebsock and PI 94979 alleles were differentiated in durum and common wheat by three (*Xrwgsnp11*, *Xrwgsnp12*, and *Xrwgsnp13*) of the STARP markers (Table S3). Two STARP markers *Xrwgsnp10* and *Xrwgsnp14* were polymorphic only in durum cultivars (Table S3). Additionally, *Xrwgsnp14* was able to differentiate between the different durum cultivars for the *Sr8155B1* alleles but it could not differentiate *Sr8a* and *Sr8155B1* alleles (Fig. S2).

Haplotypes for STARP marker *Xrwgsnp37* matched well with those of the dCAPS marker *Sr13F/R* (Table S3; Fig. S1). The durum cultivars (Alkabo, Joppa, Ben, Carpio, Maier, Grenora, and Langdon) carrying *Sr13* detected by *Sr13F/R* all had the Lebsock allele, whereas both Rusty and Divide which lacked *Sr13* had the PI 94749 allele, suggesting that *Xrwgsnp37* is diagnostic for *Sr13*. Therefore, the other durum wheat cultivars 'Strongfield', 'Transcend', 'Svevo', 'ND Grano', and 'ND Riveland' and breeding lines 15FAR344 and D101073 which had the Lebsock allele likely carry *Sr13*

(Table S3). The absence of the Lebsock allele of *Xrwgsnp37* in the hexaploid wheat cultivars and lines indicates that these hexaploid wheat genotypes do not carry *Sr13*.

Discussion

North Dakota is the major producer of durum wheat in the USA. Although this region is particularly vulnerable to stem rust, most of the durum wheat cultivars grown in this region are highly resistant (Elias et al. 2001; Olivera et al. 2012). Among the nine durum cultivars evaluated in this study, eight are highly resistant to the US race TTTTF and moderately resistant to the African race TTKSK. All nine cultivars are either highly or moderately resistant to the Yemen race TRTTF. These results strongly indicated the presence of multiple *Sr* genes in ND durum cultivars. It is well known that emmer wheat (*T. turgidum* ssp. *dicoccum*) lines Khapli and Vernal and the Ethiopian durum landrace ST464 (PI 191365) were used to introduce resistance into modern durum cultivars (Klindworth et al. 2007; Simons et al. 2011). Several previous studies indicated that Khapli carries *Sr7a*, *Sr13*, and *Sr14*, Vernal has *Sr9e* and *Sr13*, and ST464 has *Sr9e* and *Sr13 (*Klindworth et al. 2007*;* Knott 1962, 1996*;* Simons et al. 2011; Williams and Gough 1965*)*. In addition, both Khapli and ST464 carry additional undetermined *Sr* genes (Klindworth et al. 2007; Simons et al. 2011). However, except for *Sr13* and *Sr8155B1*, other *Sr* genes present in modern ND durum cultivars have not been previously identified.

In the current study, four QTL associated with resistance to *Pgt* were identified in the ND durum cultivar Lebsock. Among these QTL, *QSr.rwg-2B*, associated with a minor effect for resistance to TRTTF, was located in the region associated with *Sr9h* and *Sr28* (Rouse et al. 2014*)*. These genes confer resistance to TTKSK, but in the current study, *QSr.rwg-2B* was not associated with the TTKSK resistance. Based on the avirulence/virulence profile (Table S1), race TRTTF is virulent on *Sr9a*, *Sr9b*, *Sr9d*, and *Sr9g*. Therefore, *Sr28* and any of these *Sr9* alleles can be ruled out as the genes underlying *QSr.rwg-2B*. Although *Sr9e* is classified as susceptible to TRTTF, it does have some minor effect that is noticeable (M.N. Rouse, Y. Jin unpublished). *Sr9e* is derived from the emmer wheat line Vernal, which is in the parentage of Lebsock and many other currently grown North American durum cultivars (Simons et al. 2011; Elias et al. 2001). Because *Sr9e* has been extensively deployed in North American durum cultivars (Jin 2005; Olivera et al. 2012), it is most likely the gene underlying *QSr.rwg-2B*. However, we cannot rule out the possibility that this QTL was controlled by an uncharacterized new *Sr9* allele, or a new gene linked to the *Sr9* locus. If *Sr9e* is the gene underlying *QSr.rwg-2B* in Lebsock, its presence in all other cultivars (Table S2) investigated in this study could not be determined due to its minor effect on resistance to TRTTF (Fig. 1; Table 2).

The race TTTTF-specific QTL *QSr.rwg-4A* was identified in the *Sr7* region of chromosome arm 4AL. The *Sr7* locus is known to have two alleles, *Sr7a* and *Sr7b* (McIntosh et al. 1995). The three *Pgt* races used in this study are virulent against *Sr7b* (Table S1). The *Sr7a* reaction response to TRTTF is not yet characterized; however, it was reported to be ineffective against TTKSK (Jin et al. 2007) and confers resistance against TTTTF in hard red winter wheat 'Jagger' (Turner et al. 2016). Additionally, the presence of *Sr7a* in the old ND durum wheat cultivar 'Langdon' suggests that it might be present in modern durum cultivars. Therefore, *QSr.rwg-4A* most likely corresponds to *Sr7a* in Lebsock. Basnet et al. (2015) recently mapped *SrND643* to the *Sr7* region of hexaploid wheat, but *QSr.rwg-4A* and *SrND643* differ in their reaction to TTKSK, so these two genes are clearly not the same.

The TRTTF-specific QTL *QSr.rwg-6A.1* was identified at the distal end of chromosome arm 6AS. Among the known *Sr* genes in wheat, only *Sr8* is located in this region (Bhavani et al. 2008; Chhetri et al. 2016; Dunckel et al. 2015; Knott and Anderson 1956; McIntosh 1972; Sears et al. 1957; Singh and McIntosh 1986). Two alleles (*Sr8a* and *Sr8b*) were previously identified at the *Sr8* locus (McIntosh et al. 1995). However, Nirmala et al. (2017) recently reported the mapping of a putative new allele designated as *Sr8155B1*. The two *Sr8* alleles and *Sr8155B1* are known to be ineffective against TTKSK, but alleles *Sr8a* and *Sr8155B1* are effective against TRTTF (Jin et al. 2007; Nirmala et al. 2017) (Table S1). *Sr8a* and *Sr8b* phenotypically produce infection types of 2 and X (an IT type with random distribution of uredia of different sizes, see Roelfs and Martens 1988), respectively (McIntosh et al. 1995; Bhavani et al. 2008; Jin et al. 2007). These ITs were not observed in Lebsock, LP749, or the other durum cultivars tested with the three *Pgt* races, which suggests the absence of *Sr8a* and *Sr8b* alleles in the current study.

Sr8155B1 was derived from the durum wheat line 8155-B1 and carries resistance against the Ug99 variant TTKST. However, due to its ineffectiveness against race TTKSK, this gene is unique compared to other known *Sr* genes. Nirmala et al. (2017) did not report whether *Sr8155B1* was effective against TTTTF, but did report that it was ineffective against TTKSK and effective against TRTTF, which is the same as QTL *QSr.rwg-6A.1*. Furthermore, Nirmala et al. (2017) validated the *Sr8155B1* allele in 11 durum cultivars, indicating its presence in Grenora, Divide, and Alkabo, and absence in Rusty and Tioga. Interestingly, in the present study, the validation analysis of two STARP markers (*Xrwgsnp12* and *Xrwgsnp14*) associated with *QSr.rwg-6A.1* produced similar results (Table S3; Fig. S2). Even though *Xrwgsnp13* did not differentiate the *Sr8155B1* allele in Rusty, it was effective for differentiating other durum wheat cultivars (Fig. S2).

The marker *Xrwgsnp14* linked to *QSr.rwg-6A.1* was developed based on 90 K SNP marker *IWB47842* (Table 3), which co-segregated with *Sr8155B1* in the Rusty × 8155-B1 F_2 population (Nirmala et al. 2017). Therefore, mapping of the same SNP in both populations suggests that *IWB47842* is predicting the *Sr8155B1* allele in both studies. Additionally, in the validation analysis, Lebsock had the same sized amplicons as 8155-B1 for all three STARP markers mapped in the *QSr.rwg-6A.1* region (Fig. S2). Hence, based on these analyses, we conclude that *QSr.rwg-6A.1* corresponds to *Sr8155B1* derived from Lebsock, and *Xrwgsnp14* detects the presence and absence of this allele in the different durum cultivars (Table S3; Fig. S2). In this study, we tested eight additional durum cultivars using rust tests and new STARP markers. Nirmala et al. (2017) had previously tested four of these and found *Sr8155B1* to be present in Alkabo, Divide, and Grenora, but absent in Tioga; our results agreed with their conclusion. For the four additional cultivars, the rust tests and STARP markers indicated that *Sr8155B1* was present in Maier and Ben, absent in Carpio, and heterogeneous in Joppa (Table S2; Fig. S2). Because Tioga and Carpio do not carry *Sr8155B1*, they exhibited only moderate resistance against TRTTF (Table S2).

The second chromosome 6A QTL, *QSr.rwg-6A.2*, was located in the same region known to associate with the previously mapped (Simons et al. 2011) *Sr* gene *Sr13* on chromosome arm 6AL. In the current study, *QSr.rwg-6A.2* spanned a 21.4 cM interval (Fig. 1) between markers *Xwmc580* and *Xrwgsnp37.1*. The markers associated with *QSr.rwg-6A.2* were also reported to span the region containing *Sr13* in the genetic map developed from the durum UC1113 × 'Kofa' population (Simons et al. 2011). In addition, marker analysis with dCAPS marker *Sr13F/R*, which is diagnostic for *Sr13*, confirmed that *Sr13* is the gene underlying *QSr.rwg-6A.2* in Lebsock. *Sr13* typically has ITs ranging from 1 to 2 for most of the *Pgt* races at the seedling stage (McIntosh et al. 1995; Periyannan et al. 2014; Simons et al. 2011; Zhang et al. 2017). *QSr.rwg-6A.2* showed a minor (5% of the phenotypic variation) but a significant (LOD ~ 12) effect on resistance to TRTTF (Fig. 1). The low phenotypic variation associated with *QSr.rwg-6A.2* (*Sr13*) was probably due to the presence of *QSr.rwg-6A.1*, whose gene conditioned IT 0; and thus masked, the TRTTF resistance effects of *Sr13* and other *Sr* genes in Lebsock.

In conclusion, we identified *Sr7a*, *Sr13*, *Sr8155B1*, and likely *Sr9e* in the durum cultivar Lebsock. This study showed that the existing *Sr* genes in most North Dakota durum cultivars provide adequate protection from the threat of significant *Pgt* races including TTKSK, TRTTF, and TTTTF. Therefore, these cultivars are not only highly valuable in current durum production, but they are also useful genetic resources for future durum breeding. To further diversify breeding germplasm, additional *Sr* genes for resistance to TTKSK, TRTTF, and TTTTF should be introduced from other sources. For example, *Sr13* provides a moderate level of resistance against TTKSK and resistance of cultivars carrying *Sr13* could be enhanced if it were combined with other effective *Sr* genes like *Sr2*, *Sr26*, *Sr39*, *Sr47*, *Sr56*, etc. (Singh et al. 2015). Stacking of *Sr* genes will play a significant role in development of future breeding lines with improved resistance against *Pgt* races, and this process could be expedited by using STARP or similar PCR-based SNP genotyping technologies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments were performed in compliance with the current laws of the USA.

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