Characterization of Synthetic Wheat Line Largo for Resistance to Stem Rust

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SHORT RUNNING HEAD: Sr genes in synthetic wheat line Largo
ABSTRACT

Resistance breeding is an effective approach against wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*). The synthetic hexaploid wheat line Largo (pedigree: durum wheat ‘Langdon’ × *Aegilops tauschii* PI 268210) was found to have resistance to a broad spectrum of *Pgt* races including the Ug99 race group. To identify the stem rust resistance (*Sr*) genes, we genotyped a population of 188 recombinant inbred lines developed from a cross between the susceptible wheat line ND495 and Largo using the wheat Infinium 90K SNP iSelect array and evaluated the population for seedling resistance to the *Pgt* races TTKSK, TRTTF, and TTTTF in greenhouse conditions. Based on genetic linkage analysis using the marker and rust data, we identified six quantitative trait loci (QTL) with effectiveness against different races. Three QTL on chromosome arms 6AL, 2BL, and 2BS corresponded to *Sr* genes *Sr13c*, *Sr9e*, and a likely new gene from Langdon, respectively. Two other QTL from PI 268210 on 2DS and 1DS were associated with a potentially new allele of *Sr46* and a likely new *Sr* gene, respectively. Additionally, *Sr7a* was identified as the underlying gene for the 4AL QTL from ND495. Knowledge of the *Sr* genes in Largo will help to design breeding experiments aimed to develop new stem rust-resistant wheat varieties. Largo and its derived lines are particularly useful for introducing two Ug99-effective genes *Sr13c* and *Sr46* into modern bread wheat varieties. The 90K SNP-based high-density map will be useful for identifying the other important genes in Largo.

**Keywords:** synthetic hexaploid wheat, durum, *Aegilops tauschii*, Largo, stem rust, *Sr13*, *Sr46*
INTRODUCTION

Wheat (Triticum aestivum L., 2n = 6x = 42, AABBDD) stem rust, caused by Puccinia graminis Pers.:Pers. f. sp. tritici Eriks & E. Henn (Pgt), is a major threat to wheat production worldwide. Since the Pgt race TTKSK (also known as Ug99) was first identified in Uganda in 1999, a total of 13 variants within the Ug99 lineage, commonly known as to the Ug99 race group, have been detected across 13 countries in East Africa and the Middle East over the last two decades (Pretorius et al. 2000; Singh et al. 2015; Patpour et al. 2016; Terefe et al. 2019). Li et al. (2019) recently proposed that high genetic diversity of Ug99 races largely resulted from somatic hybridization and nuclear exchange between dikaryons, which likely is a driving force for the emergence of new pathotypes in asexual fungal populations. Additionally, there are a few non-Ug99 lineage Pgt races such as TRTTF, TTTTF, JRCQC, and TKTTF, known to carry virulence against frequently deployed stem rust resistance (Sr) genes such as Sr9e, Sr25, Sr36, SrTmp, and Sr1RSAmigo (Jin 2005; Jin and Singh 2006; Olivera et al. 2012, 2015; Olivera Firpo et al. 2017; Patpour et al. 2017). Olivera et al. (2019) also found different virulent combinations among the Pgt races collected from Georgia. Together these diverse Pgt races pose a serious threat to global food security. Development of resistant wheat varieties is an effective approach to counter these threats. To achieve this goal, the wheat research community continuously searches for new resistance genes.

The wheat primary gene pool has been considered the best resource of resistance (R) genes due to minimal deleterious effects caused by linkage drag. The hexaploid wheat D-genome progenitor Aegilops tauschii Coss. (2n = 2x = 14, DD) is known to be a great resource of R genes for various diseases and insect pests (Ogbonnaya et al. 2013; Arora et al. 2019). To utilize Ae.
tauschii accessions for the development of the resistant wheat lines/cultivars against biotic stresses, Dr. Leonard R. Joppa (USDA-ARS, retired) developed over 40 synthetic hexaploid wheat (SHW) lines by crossing durum wheat \([T. turgidium \text{ L. ssp. } durum \text{ (Desf.) Husn.}, 2n = 4x = 28, \text{ AABB}]\) ‘Langdon’ with different \textit{Ae. tauschii} accessions (Xu \textit{et al.} 2010). Among these Langdon-derived SHW germplasm, one line was released and named Largo \((\text{CI 17895})\), which carries the \(Gb3\) gene for greenbug \((\textit{Schizaphis graminum}, \text{ Rondani})\) resistance derived from \textit{Ae. tauschii} accession PI 268210 (Joppa and Williams, 1982). Since its release, Largo and its derivatives have been the primary source of greenbug resistance in the winter wheat germplasm and varieties in Texas \((\text{Lazar \textit{et al.} 1996, 1997; Rudd \textit{et al.} 2014})\). Largo was also identified to carry resistance to wheat curl mite \((\textit{Aceria tosichella} \text{ Keifer})\) \((\text{Dhakal \textit{et al.} 2018})\) and several fungal diseases, including Septoria tritici blotch \((\text{Adhikari \textit{et al.} 2015})\), Fusarium head blight \((\text{Szabo-Hever \textit{et al.} 2018})\), and stem rust \((\text{Friesen \textit{et al.} 2008})\).

The durum wheat variety Langdon was developed using a modified backcross procedure to transfer stem rust resistance from Khapli emmer \((T. turgidum \text{ subsp. } dicoccum \text{ Schrank})\) during the stem rust outbreak of the 1950s in the Northern Great Plains \((\text{Heyne, 1959})\). Previous studies indicated that Langdon carries at least four \(Sr\) genes \((\text{Salazar and Joppa 1981})\). However, besides \(Sr13c\) \((\text{Zhang \textit{et al.} 2017; Gill \textit{et al.} 2021})\), other \(Sr\) genes in Langdon have not been unambiguously identified and confirmed. Because Langdon is one of the founders for modern durum germplasm and varieties in the U.S., identification of the \(Sr\) genes it harbors will enhance our understanding of the \(Sr\) genes present in modern durum wheat germplasm. Similarly, \textit{Ae. tauschii} accession PI 268210 was previously identified to be resistant to all \(Pgt\) races tested, including TTKSK \((\text{Friesen \textit{et al.} 2008; Zhang 2013})\). However, the \(Sr\) gene(s) in PI 268210 has also not been identified.
In addition to its high values in bread wheat breeding, Largo should be a useful parental line of a permanent mapping population that can be used for identification, mapping, and marker development for the agronomically-important genes derived from durum Langdon and *Ae. tauschii* PI 268210. We conducted this study with the aim to identify the genes controlling stem rust resistance by developing, genotyping, and phenotyping a recombinant inbred line (RIL) population from a cross between Largo and the bread wheat line ND495.

**MATERIAL AND METHODS**

**Plant material and stem rust screening**

A population of 226 RILs developed from a cross between a hard spring wheat line ND495 and SHW line Largo was used for genotypic and phenotypic analysis. Largo (CI 17895) was developed from a cross between durum wheat Langdon and *Ae. tauschii* accession PI 268210 (Joppa and Williams 1982). ND495 was developed at North Dakota State University (Fargo, ND) and has a pedigree of Justin*2/3/ND 259/Conley//ND 112 (Anderson *et al.* 1999).

The RILs along with parental lines ND495, Largo, PI 268210, and Langdon were phenotyped for seedling resistance in two biological replications (5 plants/replication) with *Pgt* races TTKSK (04KEN156/04), TRTTF (06YEM34-1), and TTTTF (01MN84A-1-2). The virulence/avirulence details of the three races are listed in Table 1.

The stem rust screening experiment was performed under controlled greenhouse conditions at the USDA-ARS Cereal Disease Laboratory, St. Paul, MN using the procedure described by Hundie *et al.* (2019). Briefly, the primary leaves of the seedling plants at 7 to 9 days after planting were inoculated with the *Pgt* urediniospores. After inoculation, the plants were moved into a greenhouse maintained at 18 ± 2°C with a 16 h photoperiod. The plants were
scored for infection type (IT) at 14 days post inoculation based on the Stakman et al. (1962) 0–4 scale followed by the additional symbols (+ and −) for the pustule size (Roelfs and Martens 1988). To identify the regions harboring quantitative trait loci (QTL) associated with resistance to the three Pgt races, the IT scores of each RIL for individual races were converted to the linearized IT (LIT) scores in 0–9 scale as described by Zhang et al. (2014), where score 0 to 5 was considered as resistant and 6 to 9 considered as susceptible. To determine the repeatability of stem rust test for the RIL population, we conducted correlation analysis using LIT scores between two reps for each race. The t tests (least significant difference) were also conducted to detect the RIL lines that significantly differ from the parents. The statistical analysis was conducted by using the PROC GLM procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). The mean of the linearized IT (LIT) scores of two replications was used for the development of histograms and QTL analysis.

**Genotypic analysis**

Out of 226 RILs used for the phenotypic analysis, 188 RILs were randomly selected for the genotypic analysis to avoid the bias in the marker data set. DNA extraction of 188 RILs along with parental lines ND495, Largo, PI 268210, and Langdon was done according to the procedure described in Faris et al. (2000). For genotyping, the wheat Infinium 90K SNP iSelect array (Wang et al. 2014) was used and whole genome linkage maps were developed by using the MapDisto 1.8.2.1 software package (Lorieux 2012) with a logarithm of odds (LOD) cut-off value of 3.0, and mapping distances were measured using the Kosambi mapping function (Kosambi 1943). The order of steps used for the linkage map development was followed as described in Sharma et al. (2019a). Briefly, linkage groups were first identified and then followed by fixing the marker order within each group by using the command ‘order sequence.’ Next, ‘check
inversions,’ ‘ripple order,’ and ‘drop locus’ commands were used to generate robust linkage maps. For purposes of generating figures, linkage maps with few non-redundant loci were developed by using the software Mapchart 2.32 (Voorrips 2002).

**QTL analysis**

To detect genomic regions associated with stem rust resistance, a QTL analysis was conducted using QGENE (4.3.10) software (Joehes and Nelson 2008) and the single-trait multiple interval mapping (MIM) method (Kao et al. 1999). Based on the MIM statistical model (Kao et al. 1999), we assumed that there are m QTL \( Q_1, Q_2, \ldots, Q_m \) for controlling resistance to stem rust in the RIL population. The resistance phenotype value \( Y \) for a RIL, \( i \), can be related to the \( m \) putative QTL by the model (Kao et al. 1999)

\[
Y_i = \mu + \sum_{j=1}^{m} a_j x_{ij} + \sum_{j \neq k}^{m} \delta_{jk} (w_{jk} x_{ij} x_{ik}) + \varepsilon_i,
\]

where \( \mu \) is the mean, \( x_{ij} \) is coded as \( \frac{1}{2} (Q_j Q_j) \) or \( -\frac{1}{2} (q_j q_j) \) for the genotype of \( Q_j \), \( a_j \) is the main effect of \( Q_j \), and \( w_{jk} \) is the epistatic effect between \( Q_j \) and \( Q_k \). \( \delta_{jk} \) is the indicator for epistasis between \( Q_j \) and \( Q_k \), and \( \varepsilon_i \) is the error that is assumed to follow \( N(0, \sigma^2) \). The LOD value 3.0 was set as cut off for the QTL detection. After identification of the gene-associated regions, simple sequence repeat (SSR) markers from marker sets BARC (Song et al. 2005), CFD (Somers et al. 2004; Sourdille et al. 2003; Guyomarc’h et al. 2002), WMC (Somers et al. 2004), and GWM (Röder et al. 1998) were further used to map the specific chromosomes. Four (cfd15, cfd61, cfd72, and wmc429) and three (barc18, gwm388, and wmc154) SSR markers were mapped on chromosomes 1D and 2B, respectively. For the \( Sr46 \) gene region, 10 previously known SSRs (barc124, barc95, cfd36, cfd43, gwm102, gwm210, gwm261, gwm455, wmc112, and wmc25) were mapped on chromosome arm 2DS. Additionally, three SSRs (Xrwgs46, Xrwgs47, and...
*Xrwgs49* developed based on reference genome sequences were also mapped (Table 2). The primers of these markers were designed using the Primer-BLAST suite (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) based on sequences within the *Sr46* region of chromosome arm 2DS in *Ae tauschii* (AL 8/78) and hexaploid wheat (Chinese Spring) (IWGSC 2018). The SSR genotyping assays were performed using 6% non-denatured poly-acrylamide gels as described in Saini et al. (2018).

**RESULTS**

Stem rust screening showed that Largo exhibited low infection types to *Pgt* races TTKSK (IT 2), TRTTF (ITs 2 and 12 in replicates 1 and 2, respectively) and TTTTF (IT 22) (Table 3). ND495 was susceptible to TTKSK (IT 3), moderately susceptible to TRTTF (ITs 31 and 13 in replicates 1 and 2, respectively), and resistant to TTTTF (IT 3 and 0;13 in replicates 1 and 2, respectively). The Pearson correlation coefficients between the two replications for TTKSK, TRTTF, and TTTTF were 0.90, 0.83, and 0.95, respectively, which were highly significant ($P < 0.0001$), indicating high repeatability of the two replicates in the tests with each race. Therefore, the mean linearized infection type (LIT) scores from both reps for each race were used in the subsequent analysis.

The mean LIT scores of the RIL population for race TTKSK ranged from 4.5 to 9.0, with Largo and ND495 scoring 5.0 and 9.0, respectively (Fig. 1A, Supplementary files 1 and 2). There was no significant transgressive segregation detected even though one line (NL025) had slightly increased levels of resistance over Largo (Supplementary file 2). For TRTTF, the RILs had mean LIT scores that ranged from 0.0 to 9.0 with Largo and ND495 scoring 1.0 and 5.0, respectively (Fig. 1B). Twelve lines showed increased levels of resistance (mean LITs 0.0, and
0.5) over Largo, but the increases were not significant \((P \leq 0.05)\). However, 22 RILs (mean LITs 7.0 – 9.0) were significantly more susceptible than ND495 (Supplementary file 3), indicating presence of transgressive segregation in the population. For reactions to TTTTF, the RIL population had mean LIT scores also ranging from 0.0 to 9.0 even though both parents were in the resistant range, with ND495 being more resistant than Largo (Fig. 1C, Supplementary files 1 and 4). In the population, 35 lines (mean LITs 0.0 – 1.0) had significantly lower mean LIT scores than ND495 (2.5), whereas 44 lines (6.5 – 9.0) had significantly higher mean LIT scores than Largo (5.0), indicating a strong transgressive segregation for resistance to TTTTF in the population.

Linkage maps were developed for the entire genome with 8,203 (90K SNP + SSR) markers representing 1,739 loci across the 21 chromosomes and map density ranging from 0.9 cM/locus for chromosome 1B to 4.4 cM/locus for chromosome 4D (Table 4, Supplementary files 5 and 6). Two QTL regions associated with TTKSK resistance were identified on chromosome arms 2DS and 6AL designated as \(QSr_{\text{rgw-2D}}\) and \(QSr_{\text{rgw-6A}}\), respectively (Table 5, Fig. 2). The \(QSr_{\text{rgw-2D}}\) QTL was positioned at 4 cM, flanked by \(X\text{rgw}46\) and \(I\text{WB}43851\) with a LOD value of 52.4 and explained 62.1% of the phenotypic variation \((R^2 \times 100)\). This region of chromosome arm 2DS is known to carry \(Sr46\). The second TTKSK-associated QTL, \(QSr_{\text{rgw-6A}}\), was positioned at 98 cM and flanked by \(I\text{WA}441\) and \(I\text{WB}51469\). It had a LOD value of 23.5 and explained 21.3% of phenotypic variation. The gene \(Sr13\) is known to lie within this genomic region. Both \(QSr_{\text{rgw-2D}}\) and \(QSr_{\text{rgw-6A}}\) had positive additive values of 1.2 and 0.7, respectively, indicating that TTKSK resistance was derived from Largo.

For TRTTF, four QTL were identified on chromosome arms 2BS, 2BL, 2DS, and 6AL, designated as \(QSr_{\text{rgw-2B.1}}\), \(QSr_{\text{rgw-2B.2}}\), \(QSr_{\text{rgw-2D}}\), and \(QSr_{\text{rgw-6A}}\), respectively (Table
5, Fig. 2). *QSr.rwg-2B.1* (42.0 cM) was flanked by *IWB7072* and *IWB2380* with a LOD value of 22.9 and it explained 33.3% of the phenotypic variation. The second TRTTF-specific QTL, *QSr.rwg-2B.2* (74.0 cM), was identified on chromosome arm 2BL and was flanked by *IWB71742* and *IWB73196*. This QTL had a LOD value of 4.0 and explained 16.2% of phenotypic variation. The third TRTTF-associated QTL, *QSr.rwg-2D* (LOD = 3.5), was located on chromosome arm 2DS and was similar to the TTKSK QTL located in the Sr46 region, however its effect for TRTTF was less compared with TTKSK with an explained 3.6% of phenotypic variation. Likewise, the fourth QTL, *QSr.rwg-6A*, also coincided with the TTKSK and TRTTF QTL and explained 18.5% of the phenotypic variation for TRTTF resistance and had a LOD value of 13.8. The positive additive values for all the QTL regions suggest that resistance was derived from Largo (Table 5).

A total of four QTL were identified for resistance to *Pgt* race TTTTF on chromosome arms 1DS, 2BS, 4AL, and 6AL and were designated as *QSr.rwg-1D*, *QSr.rwg-2B.1*, *QSr.rwg-4A*, and *QSr.rwg-6A*, respectively (Table 5, Fig. 2). Among these four QTL, only the *QSr.rwg-4A* associated resistance was derived from ND495, whereas all others were derived from Largo. The *QSr.rwg-1D* (LOD = 7.8) was located at 16.0 cM and flanked by *IWB22674* and *IWB31245*, explaining 16.8% of phenotypic variation. *QSr.rwg-2B.1* was located at 38 cM and flanked by *IWA413* and *IWA2571*. This QTL explained 4.6% of phenotypic variation and was adjacent to the TRTTF QTL located at 42 cM on chromosome arm 2BS. *QSr.rwg-4A* (LOD = 6.6), which explained 16.1% of the phenotypic variation, was identified on chromosome arm 4AL at 114 cM and flanked by *IWB9431* and *IWB5461* located in the region known to be associated with Sr7. The *QSr.rwg-6A* region was common among the three *Pgt* races tested in the current study, and
for TTTTF it has maximum LOD at position 100 cM (distorted from TTKSK and TRTTF QTL peak). It explained 17.0% of the phenotypic variation and had a LOD value of 8.7.

DISCUSSION

Synthetic hexaploid wheat line Largo and its parents (Langdon and Ae. tauschii accession PI 268210) were previously reported to be resistant to multiple races of the stem rust pathogen (Salazar and Joppa 1981; Friesen et al. 2008; Zhang 2013). In the present study, we identified six major QTL ($Qsr.rwg-1D$, $Qsr.rwg-2B.1$, $Qsr.rwg-2B.2$, $Qsr.rwg-2D$, $Qsr.rwg-4A$, and $Qsr.rwg-6A$) on chromosomes 1DS, 2BS, 2BL, 2DS, 4AL, and 6AL, respectively, using the ND495 × Largo RIL population, suggesting that it segregated for at least six Sr genes. With the exception of $Qsr.rwg-4A$, which was derived from the susceptible parent ND495, all the resistance QTL were derived from Largo.

The TTTTF-effective QTL $Qsr.rwg-1D$ was identified on the short arm of the chromosome 1D (16 cM), located proximal to SSR marker $cfd15$ (Fig. 2, Supplementary file 5). Thus far, three Ug99-effective Sr genes, Sr33, Sr45, and SrTA1662, have been identified on chromosome arm 1DS from Ae. tauschii (Arora et al. 2019; Sambasivam et al. 2008; Periyannan et al. 2013, 2014a; Olson et al. 2013). The Sr33 gene is flanked by SSR markers $barc152$ and $cfd15$. SrTA1662 also mapped in the same region (Olson et al. 2013), while Sr45 is positioned proximal to Sr33. Based on the location of SSR marker $cfd15$, $Qsr.rwg-1D$ mapped in the Sr45 region, however, it was not effective against TTKSK. Therefore, phenotypic characterization of $Qsr.rwg-1D$ suggests that the gene underlying the $Qsr.rwg-1D$ is most likely different from Sr33, Sr45, and SrTA1662 and further evaluation of this region is required.
The QTL \textit{QSr.rwg-2B.1} was located near the centromeric region of chromosome arm 2BS and was effective against TRTTF and TTTTF. There are three \textit{Sr} genes that have been reported to reside in this region and they include \textit{Sr20}, \textit{Sr36}, and \textit{Sr40} (McIntosh \textit{et al.} 1995). \textit{Sr20} is not effective against TRTTF (Y. Jin, unpublished) and \textit{Sr36} was reported to be ineffective against TRTTF (Olivera \textit{et al.} 2012) and TTTTF (Jin and Singh 2006). Because \textit{Sr40} is effective against TTKSK (Singh \textit{et al.} 2015), the possibility that it is the gene underlying \textit{QSr.rwg-2B.1} can be ruled out. In addition, both \textit{Sr36} and \textit{Sr40} are located on the alien chromosome segments 2G#1S and 2G#2S, respectively, which are derived from \textit{T. timopheevii} (Zhuk.) Zhuk (2n = 4x = 28, AAGG) (Allard and Shands 1954; Dyck 1992; Friebe \textit{et al.} 1996; Nyquist 1957, 1962), and \textit{T. timopheevii} is not present in the pedigree and parentage of Langdon (Heyne 1959). Therefore, the \textit{Sr} gene associated with \textit{QSr.rwg-2B.1} is different from any known gene in this region, indicating a minor-effect \textit{Sr} gene present in 2BS that originates from Langdon.

For the three \textit{Pgt} races used in the current study, the QTL \textit{QSr.rwg-2B.2} located on chromosome arm 2BL was only effective against TRTTF (Fig. 2). There have been several \textit{Sr} genes reported on chromosome arm 2BL (\textit{Sr9}, \textit{Sr16}, \textit{Sr28}, and \textit{Sr883-2B}) (McIntosh 1995; Hiebert \textit{et al.} 2010; Sharma \textit{et al.} 2019b). For \textit{Sr9}, seven alleles have been identified: \textit{Sr9a}, \textit{Sr9b}, \textit{Sr9d}, \textit{Sr9e}, \textit{Sr9f}, \textit{Sr9g}, and \textit{Sr9h} (Green \textit{et al.} 1960; Knott 1966; McIntosh and Luig 1973; Loegering 1975; Rouse \textit{et al.} 2014). Among all these reported genes and their alleles, \textit{Sr9e} is known to be present in many durum wheat varieties including Langdon (Luig 1983; Singh \textit{et al.} 1992), and it has a minor effect against TRTTF (Saini \textit{et al.} 2018). \textit{Sr16} is not effective against TRTTF (Singh \textit{et al.} 2015), and the \textit{Sr28} gene is known to confer resistance against TTKSK, but \textit{QSr.rwg-2B.2} did not condition resistance to this race. Based on the consensus map location of
SNPs, the $Sr883\text{-}2B$ gene reported by Sharma et al. (2019b) is located some distance from the $QSr.rwg\text{-}2B.2$ (Wang et al. 2014). $Sr9h$ is effective against $Pgt$ race TTKSK (Singh et al. 2015), but $QSr.rwg\text{-}2B.2$ resistance was not associated with TTKSK and TTTTF. Because Largo carries the TRTTF-effective gene $Sr9e$ from Langdon and the other genes known to reside on 2BL can essentially be ruled out, it is most certain that the $Sr$ gene underlying $QSr.rwg\text{-}2B.2$ is $Sr9e$.

The TTKSK- and TRTTF-specific QTL $QSr.rwg\text{-}2D$ was located near the distal end of the chromosome arm 2DS, which is a region known to harbor $Sr32$ (Mago et al. 2013) and $Sr46$ (Yu et al. 2015; Arora et al. 2019). Both genes are effective against TTKSK and TRTTF (Olivera et al. 2012). The $Sr32$ gene was originally derived from $Ae. speltoides$ Tausch (Friebe et al. 1996) and should not be the gene underlying the QTL $QSr.rwg\text{-}2D$ because this gene had not been introduced into any of the parental lines (i.e., Langdon, ND495 and Largo). $QSr.rwg\text{-}2D$ was located proximal to $gwm210$ and distal to $cfd36$ (Fig. 2, Supplementary file 5), which corresponds to the $Sr46$ location based on the map developed in the $F_2$ population derived from the $Ae. tauschii$ cross CIae 25 × AL8/78 (Yu et al. 2015), suggesting that $Sr46$ is likely the gene underlying $QSr.rwg\text{-}2D$. $Sr46$ is effective against TTKSK, TRTTF, and TTTTF (Yu et al. 2015), however in the current analysis the $QSr.rwg\text{-}2D$ was not associated with the TTTTF resistance. Based on this phenotypic difference, we speculate that the $Sr$ gene underlying $QSr.rwg\text{-}2D$ derived from Largo may be a different allele of $Sr46$. However, $Sr46$ was mapped using the diploid $Ae. tauschii$ $F_2$ population, whereas the $QSr.rwg\text{-}2D$ was identified in the hexaploid RIL population. Because genomic interaction in allopolyploid wheat often causes the reduction or suppression of resistance of some $Sr$ genes (Hiebert et al. 2020), it is also possible that the different reactions of $Sr46$ and $QSr.rwg\text{-}2D$ to TTTTF were caused by different ploidy levels. Therefore, further study is needed to determine the identity of the gene for $QSr.rwg\text{-}2D$. 

Among all the QTL identified in this study, only \(QSr.rwg\text{-}4A\) positioned on chromosome arm 4AL was derived from ND495 (Fig. 2). This QTL conditioned resistance against \(Pgt\) race TTTTF and was located in the physical region known to be associated with the \(Sr7\) locus and a TTKSK-effective gene \(SrND643\) (Basnet \textit{et al.} 2015; Saini \textit{et al.} 2018). Because \(QSr.rwg\text{-}4A\) is not resistant to TTKSK, \(SrND643\) is apparently not the candidate gene for \(QSr.rwg\text{-}4A\). To date, two alleles, \(Sr7a\) and \(Sr7b\), have been reported at the \(Sr7\) locus (McIntosh \textit{et al.} 1995). \(Sr7b\) is not effective against TTTTF, whereas \(Sr7a\) is effective against TTTTF and it is nearly fixed in the wheat breeding germplasm in the Northern Great Plains (Jin \textit{et al.} 2007; Saini \textit{et al.} 2018; Turner \textit{et al.} 2016). As \(QSr.rwg\text{-}4A\) was located to the \(Sr7\) region and has resistance TTTTF, most likely \(Sr7a\) is the underlying gene for this region.

The QTL \(QSr.rwg\text{-}6A\) derived from Langdon located on chromosome arm 6AL, which carries three known TTKSK-effective \(Sr\) genes \(Sr13\), \(Sr26\), and \(Sr52\). Among these known genes, \(Sr26\) and \(Sr52\) were originally transferred into wheat from wild species \textit{Thinopyrum ponticum} (Podp.) Barkw. & D.R. Dewey [\textit{Agropyron elongatum} (Host) Beauv.] (Knott 1961; Dundas \textit{et al.} 2007) and \textit{Dasypyrum villosum} (L.) Candargy (Qi \textit{et al.} 2012), respectively. Because \(Sr26\) and \(Sr52\) have not been transferred into durum wheat Langdon, they can be ruled out as the causal gene for \(QSr.rwg\text{-}6A\). This QTL was known to be physically associated with \(Sr13\) and effective against all three \(Pgt\) races used in the current analysis (McIntosh 1995; Simons \textit{et al.} 2011; Periyannan \textit{et al.} 2014b; Zhang \textit{et al.} 2017; Gill \textit{et al.} 2021). Gill \textit{et al.} (2021) identified \(Sr13\) as the causal gene for the stem rust resistance in an accession PI 387696 of \textit{T. turgidum} subsp. \textit{carthlicum} (Neyski) Á. Löve & D. Löve. By comparing \(QSr.rwg\text{-}6A\) region to the \(Sr13\) region in the study by Gill \textit{et al.} (2021), we found that six SNP markers \((IWB61092, IWB50538, IWB7048, IWB28546, IWB37898, \text{and } IWB34398)\) in the two regions in
common in both 90K SNP-based high-density maps. Two of the markers, IWB37898 and IWB34398, that are tightly linked to Sr13 in the study by Gill et al. (2021) are also located in the QSr.rwg-6A region. Zhang et al. (2017) identified Sr13 as a coiled-coil nucleotide-binding leucine-rich repeat (NLR) gene. They identified three resistant (R1-R3) and 10 susceptible (S1-S10) haplotypes of this gene based on the reactions to TTKSK and designated R1/R3 and R2 as Sr13a and Sr13b, respectively, based on their resistant and susceptible reactions to JRCQC. Gill et al. (2021) re-designated the R1 and R3 haplotypes as Sr13a and Sr13c, respectively, based on their susceptible and resistant reactions to TCMJC. Among different diploid, tetraploid, and hexaploid wheat accessions that have been characterized for these haplotypes, Langdon was categorized as having the R3 haplotype of Sr13 (Zhang et al. 2017; Gill et al. 2021). Because Langdon is present in the Largo background, Sr13c is the gene underlying QSr.rwg-6A.

In summary, we mapped three known Sr genes Sr9e (QSr.rwg-2B.2), Sr13c (QSr.rwg-6A), and Sr7a (QSr.rwg-4A) in the ND495 × Largo RIL population. Additionally, there were three other genomic regions associated with stem rust resistance. Of these three Sr regions, QSr.rwg-1D (likely a new gene, temporarily designated as SrLargo1D) and QSr.rwg-2D (possibly a new allele of Sr46) were derived from the Ae. tauschii parent of Largo. The QSr.rwg-2B.1 derived from Langdon is located to a region with no known Sr genes. Therefore, QSr.rwg-2B.1 is probably associated with a new Sr gene (temporarily designated as SrLangdon2B) against Pgt races TRTTF and TTTTF. As no evaluation was previously performed to identify the Sr gene(s) in ND495, identification of Sr7a (QSr.rwg-4A) in this study suggests that ND495 carries Sr gene(s) with minor effects. The identification of these Sr genes in Largo will guide the future efforts to stack multiple resistant genes. Among the Ug99-effective Sr genes, both Sr13c and Sr46 had resistance to a broad spectrum of Pgt races. However, they are among a few genes from...
primary gene pool that have not been utilized or deployed in modern bread wheat germplasm.

Several NIL lines such as NL143, NL159, and NL193 with resistance to the three *Pgt* races were found to carry all the six genes, they may serve as the donors for simultaneously introducing *Sr13c, Sr46*, and four other genes into adapted bread wheat germplasm and varieties. Because Largo has resistance to other fungal diseases, the 90K SNP marker data set and the high-density linkage map developed in this study will be useful for identifying and mapping the genes controlling other agronomically important traits derived from durum wheat (Langdon), bread wheat (e.g., ND495), and *Ae. tauschii* (PI 268210).

**DATA AVAILABILITY**

The plant materials are available upon request. All data necessary for confirming the conclusions of the article are present within the article, figures, tables, and supplementary files. Supplemental material is provided at Figshare: https://doi.org/10.25387/g3.14450454.

Supplementary file 1 contains IT and LIT scores of all lines. Supplementary files 2, 3, and 4 present the results of LSD tests for mean LIT scores of RILs and their parental lines tested with *Pgt* race TTKSK, TRTTF, and TTTTF, respectively. Supplementary file 5 contains whole genome linkage maps and Supplementary file 6 contains genotypic data for 188 RILs genotyped with wheat Infinium 90K SNP iSelect array and SSR markers.

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CONFLICT OF INTEREST
All authors have no conflict of interest.

ETHICAL STANDARDS
The experiments were performed in compliance with the current laws of United States of America.
REFERENCES

Adhikari, T. B., S. Mamidi, S. Gurung, and J. M. Bonman, 2015 Mapping of new quantitative trait loci (QTL) for resistance to Septoria tritici blotch in spring wheat (Triticum aestivum L.). Euphytica 205:699-706.

Allard, R. W., and R. G. Shands, 1954 Inheritance of resistance to stem rust and powdery mildew in cytologically stable spring wheats derived from Triticum timopheevi. Phytopathology 44:266-274.

Arora, S., B. Steuernagel, K. Gaurav, S. Chandramohan, Y. Long et al., 2019 Resistance gene cloning from a wild crop relative by sequence capture and association genetics. Nat. Biotechnol. 37:139-143.

Anderson, J. A., R. J. Effertz, J. D. Faris, L. J. Francl, S. W. Meinhardt, et al., 1999 Genetic analysis of sensitivity to a Pyrenophora tritici-repens necrosis-inducing toxin in durum and common wheat. Phytopathology 89:293-297.

Basnet, B. R., S. Singh, E. E. Lopez-Vera, J. Huerta-Espino, S. Bhavani et al., 2015 Molecular mapping and validation of SrND643: A new wheat gene for resistance to the stem rust pathogen Ug99 race group. Phytopathology 105:470-476.

Dhakal, S., C.-T. Tan, V. Anderson, H. Yu, M. P. Fuentealba et al., 2018 Mapping and KASP marker development for wheat curl mite resistance in “TAM 112” wheat using linkage and association analysis. Mol. Breeding 38:119.

Dyck, P. L., 1992 Transfer of a gene for stem rust resistance from Triticum araraticum to hexaploid wheat. Genome 35:788-792.
Dundas, I. S., D. R Anugrahwati, D. C. Verlin, R. F. Park, H. S. Bariana, *et al.*, 2007 New sources of rust resistance from alien species: meliorating linked defects and discovery. Aust. J. Agric. Res. 58:545–549.

Faris, J. D., K. M. Haen, and B. S. Gill, 2000 Saturation mapping of a gene-rich recombination hotspot region in wheat. Genetics 154:823-835.

Friebe, B., J. Jiang, W. J. Raupp, R. A. McIntosh, and B. S. Gill, 1996 Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91:59-87.

Friesen, T. L., S. S. Xu, and M. O. Harris, 2008 Stem rust, tan spot, Stagonospora nodorum blotch, and Hessian fly resistance in Langdon durum–Aegilops tauschii synthetic hexaploid wheat lines. Crop Sci. 48:1062-1070.

Gill, B. K., D. L. Klindworth, M. N. Rouse, J. Zhang, Q. Zhang *et al.*, 2021 Function and evolution of allelic variation of *Sr13* conferring resistance to stem rust in tetraploid wheat (*Triticum turgidum* L.). Plant J. doi.org/10.1111/tpj.15263 (First published April 07, 2021).

Green, G. J., D. R. Knott, I. A. Watson, and A. T. Pugsley, 1960 Seedling reactions to stem rust of lines of Marquis wheat with substituted genes for rust resistance. Can. J. Plant Sci. 40:524-538.

Guyomarc’h, H., P. Sourdille, G. Charmet, K. Edwards, and M. Bernard, 2002 Characterisation of polymorphic markers from *Aegilops tauschii* and transferability to the D genome of bread wheat. Theor. Appl. Genet. 104:1164-1172.

Hiebert, C. W., M. J. Moscou, T. Hewitt, B. Steuernagel, I. Hernández-Pinzón *et al.*, 2020 Stem rust resistance in wheat is suppressed by a subunit of the mediator complex. Nat. Commun. 11:1123. https://doi.org/10.1038/s41467-020-14937-2
Hiebert, C. W., T. G. Fetch, and T. Zegeye, 2010 Genetics and mapping of stem rust resistance to Ug99 in the wheat cultivar Webster. Theor. Appl. Genet. 121:65-69.

Heyne, E. G., 1959 Registration of improved wheat varieties, XXIII. Agron. J. 51:689-692.

Hundie, B., B. Girma, Z. Tadesse, E. Edae, P. Olivera, et al., 2019 Characterization of Ethiopian wheat germplasm for resistance to four Puccinia graminis f. sp. tritici races facilitated by single-race nurseries. Plant Dis. 103: 2359-2366.

International Wheat Genome Sequencing Consortium (IWGSC), 2018 Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361: eaar7191. doi:10.1126/science.aar7191 pmid:30115783

Jin, Y., 2005 Races of Puccinia graminis identified in United States during 2003. Plant Dis. 89:1125-1127.

Jin, Y., and R. P. Singh, 2006 Resistance in U.S. wheat to recent eastern African isolates of Puccinia graminis f. sp. tritici with virulence to resistance gene Sr31. Plant Dis. 90:476-480.

Jin, Y., R. P. Singh, R. W. Ward, R. Wanyera, M. Kinyua et al., 2007 Characterization of seedling infection types and adult plant infection responses of monogenic Sr gene lines to race TTKS of Puccinia graminis f. sp. tritici. Plant Dis. 91:1096-1099.

Jopehannes, R., and J. C. Nelson, 2008 QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788-2789.

Joppa, L. R., and N. D. Williams, 1982 Registration of Largo, a greenbug resistant hexaploid wheat. Crop. Sci. 22:901–902.

Kao C. H., Z. B. Zeng, and R. D. Teasdale, 1999 Multiple interval mapping for quantitative trait loci. Genetics 152:1203-1216.
Knott, D.R., 1961 The inheritance of rust resistance. VI. The transfer of stem rust resistance from *Agropyron elongatum* to common wheat. Can. J. Plant Sci. 41:109-123.

Knott, D. R., 1966 The inheritance of stem rust resistance in wheat. pp. 156-166 in *Proceedings of the Second International Wheat Genetics Symposium* (Hereditas Supplementary Volume 2), edited by MacKey J. Genetics Institute, University of Lund, Sweden, 18-24 August, 1963.

Kosambi, D. D., 1943 The estimation of map distances from recombination values. Ann Eugen. 12:172-175.

Lazar, M. D., W. D. Worrall, K. B. Porter, and N. A. Tuleen, 1996 Registration of eight closely related germplasm lines differing in biotype E greenbug resistance. Crop Sci. 36:1419.

Lazar, M. D., W. D. Worrall, G. L. Peterson, K. B. Porter, L. W. Rooney *et al.*, 1997 Registration of TAM 110. Crop Sci. 37:1978-1979.

Loegering, W. Q., 1975 An allele for low reaction to *Puccinia graminis tritici* in Chinese Spring wheat. Phytopathology 65:925.

Lorieux, M., 2012 MapDisto: fast and efficient computation of genetic linkage maps. Mol. Breeding 30:1231-1235.

Luig, N. H., 1983 A survey of virulence genes in wheat stem rust, *Puccinia graminis* f. sp. *tritici*. Advances in Plant Breeding, Vol. 11. Verlag Paul Parey, Berlin.

Mago, R., D. Verlin, P. Zhang, U. Bansal, H. Bariana *et al.*, 2013 Development of wheat-*Aegilops speltoides* recombinants and simple PCR-based markers for Sr32 and a new stem rust resistance gene on the 2S#1 chromosome. Theor. Appl. Genet. 126:2943-2955.
McIntosh, R. A., and N. H. Luig, 1973 Recombination between genes for reaction to *P. graminis* at or near the *Sr9* locus, pp. 425-432, in *Proceedings of the Fourth International Wheat Genetics Symposium, 6-11 August, 1973, Columbia, Missouri, U.S.A.*, edited by Sears E. R. and L. M. S. Sears. Agricultural Experiment Station, University of Missouri, Columbia, Missouri, U.S.A.

McIntosh, R. A., C. R. Welling, and R. F. Park, 1995 *Wheat rusts: an atlas of resistance genes*. CSIRO Australia, Sydney, Kluwer Publishers, Dordrecht, the Netherlands.

Nyquist, N. E., 1957 Monosomic analysis of stem rust resistance of a common wheat strain derived from *Triticum timopheevi*. Agron. J. 49:222-223.

Nyquist, N. E., 1962 Differential fertilization in the inheritance of stem rust resistance in hybrids involving a common wheat strain derived from *Triticum timopheevi*. Genetics 47:1109-1124.

Ogbonnaya, F. C., O. Abdalla, A. Mujeeb-Kazi, A. G. Kazi, S. S. Xu *et al.*, 2013 Synthetic hexaploids: harnessing species of the primary gene pool for wheat improvement. Plant Breed. Rev. 37:35-122.

Olivera Firpo, P. D., M. Newcomb, K. Flath, N. Sommerfeldt-Impe, L. J., Szabo *et al.*, 2017 Characterization of *Puccinia graminis* f. sp. *tritici* isolates derived from an unusual wheat stem rust outbreak in Germany in 2013. Plant Pathol. 66:1258-1266.

Olivera, P., M. Newcomb, L. J. Szabo, M. Rouse, J. Johnson *et al.*, 2015 Phenotypic and genotypic characterization of race TKTTF of *Puccinia graminis* f. sp. *tritici* that caused a wheat stem rust epidemic in southern Ethiopia in 2013-14. Phytopathology 105:917-928.

Olivera, P. D., Y. Jin, M. Rouse, A. Badebo, T. Jr. Fetch *et al.* 2012 Races of *Puccinia graminis* f. sp. *tritici* with combined virulence to *Sr13* and *Sr9e* in a field stem rust screening nursery in Ethiopia. Plant Dis. 96:623-628.
Olivera, P. D., Z. Sikharulidze, R. Dumbadze, L. Z. Szabo, M. Newcomb et al., 2019 Presence of a sexual population of *Puccinia graminis* f. sp. *tritici* in Georgia provides a hotspot for genotypic and phenotypic diversity. Phytopathology 109:2152-2160.

Olson, E. L., M. N. Rouse, M. O. Pumphrey, R. L. Bowden, B. S. Gill et al., 2013 Simultaneous transfer, introgression, and genomic localization of genes for resistance to stem rust race TTKSK (Ug99) from *Aegilops tauschii* to wheat. Theor. Appl. Genet. 126:1179-1188.

Patpour, M., M. S. Hovmøller, and D. Hodson, 2017 First report of virulence to *Sr25* in race TKTTF of *Puccinia graminis* f. sp. *tritici* causing stem rust on wheat. Plant Dis. 101:1678. 

https://doi.org/10.1094/PDIS-11-16-1666-PDN

Patpour M., M. S. Hovmøller, A. A. Shahin, M. Newcomb, P. Olivera, et al., 2016 First report of the Ug99 race group of wheat stem rust, *Puccinia graminis* f. sp. *tritici*, in Egypt in 2014. Plant Dis. 100:863. http://dx.doi.org/10.1094/PDIS-08-15-0938-PDN

Periyannan, S., J. Moore, M. Ayliffe, U. Bansal, X. Wang et al., 2013 The gene *Sr33*, an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99. Science 341:786-788.

Periyannan, S., U. Bansal, H. Bariana, K. Deal, M-C. Luo et al., 2014a Identification of a robust molecular marker for the detection of the stem rust resistance gene *Sr45* in common wheat. Theor. Appl. Genet. 127:947-955.

Periyannan, S. K., Z. U. Qamar, U. K. Bansal, and H. S. Bariana, 2014b Development and validation of molecular markers linked with stem rust resistance gene *Sr13* in durum wheat. Crop Pasture Sci. 65:74-79.
Pretorius, Z. A., R. P Singh, W. W Wagoire, and T. S. Payne, 2000 Detection of virulence to wheat stem rust resistance gene Sr31 in Puccinia graminis f. sp. tritici in Uganda. Plant Dis. 84:203.

Qi, L. L., M. O. Pumphrey, B. Friebe, P. Zhang, C. Qian, et al., 2011 A novel Robertsonian translocation event leads to transfer of a stem rust resistance gene (Sr52) effective against race Ug99 from Dasypyrum villosum into bread wheat. Theor. Appl. Genet. 123:159-167.

Röder, M. S., V. Korzun, K. Wendehake, B. S. Gill, and M. W. Ganal, 1998 The physical mapping of microsatellite markers in wheat. Genome 41:278-283.

Roelfs, A. P. and J. W. Martens, 1988 An international system of nomenclature for Puccinia graminis f. sp. tritici. Phytopathology 78:526-533.

Rouse, M. N., J. Nirmala, Y. Jin, S. Chao, T. G. Jr., Fetch et al., 2014 Characterization of Sr9h, a wheat stem rust resistance allele effective to Ug99. Theor. Appl. Genet. 127:1681-1688.

Rudd, J. C., R. N. Devkota, J. A. Baker, G. L. Peterson, M. D. Lazar et al., 2014 ‘TAM 112’ wheat: A greenbug and wheat curl mite resistant cultivar adapted to the dryland production system in the Southern High Plains. J. Plant Regist. 8:291-297.

Saini, J., J. D. Faris, Q. Zhang, M. N. Rouse, Y. Jin et al., 2018 Identification, mapping, and marker development of stem rust resistance genes in durum wheat ‘Lebsock’. Mol. Breed. 38:77. doi: 10.1007/s11032-018-0833-y

Salazar, G. M., and L. R. Joppa, 1981 Use of substitution-monosomics to determine the chromosomal location of genes conditioning stem rust-resistance in Langdon durum. Crop Sci. 21:681-685.

Sambasivam, P. K., U. K. Bansal, M. J. Hayden, J. Dvorak, E. S. Lagudah et al., 2008 Identification of markers linked with stem rust resistance genes Sr33 and Sr45, pp. 351-353,
Sharma, J. S., K. L. D. Running, S. S. Xu, Q. Zhang, A. R. P. Haugrud et al., 2019a Genetic analysis of threshability and other spike traits in the evolution of cultivated emmer to fully domesticated durum wheat. Mol. Genet. Genomics 294:757-771.

Sharma, J. S., Q. Zhang, M. N Rouse, D. L. Klindworth, T. L. Friesen et al., 2019b Mapping and characterization of two stem rust resistance genes derived from cultivated emmer wheat accession PI 193883. Theor. Appl. Genet. 132:3177-3189.

Simons, K., Z. Abate, S. Chao, W. Zhang, M. Rouse et al., 2011 Genetic mapping of stem rust resistance gene Sr13 in tetraploid wheat (Triticum turgidum ssp. durum L.). Theor. Appl. Genet. 122:649-658.

Singh, R. P., E. Bechere, and O. Abdalla, 1992 Genetic analysis of resistance to stem rust in ten durum wheats. Phytopathology 82:919-922.

Singh, R. P., D. P Hodson, Y. Jin, E. S. Lagudah, M. A. Ayliffe et al., 2015 Emergence and spread of new races of wheat stem rust fungus: continued threat to food security and prospects of genetic control. Phytopathology 105:872-884.

Stakman, E. C., D. M. Stewart, and W. Q. Loegering, 1962 Identification of physiologic races of Puccinia graminis var. tritici. USDA ARS E-617. U.S. Gov. Print. Off., Washington, DC.

Somers, D.J., P. Isaac, and K. Edwards, 2004 A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.). Theor. Appl. Genet. 109:1105-1114.

Song, Q. J., J. R. Shi, S. Singh, E. W. Fickus, J. M. Costa et al., 2005 Development and mapping of microsatellite (SSR) markers in wheat. Theor. Appl. Genet. 110:550-560.
Sourdille, P., T. Cadalen, H. Guyomarc'h, J. W. Snape, M. R. Perretant et al., 2003 An update of the Courtot × Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor. Appl. Genet. 106:530-538.

Szabo-Hever, A., Q. Zhang, T. L. Friesen, S. Zhong, E. M. Elias et al. 2018 Genetic diversity and resistance to Fusarium head blight in synthetic hexaploid wheat derived from Aegilops tauschii and diverse Triticum turgidum subspecies. Front. Plant Sci. 9:1089. Doi: 10.3389/fpls.2018.01829.

Terefe, T., Z. A. Pretorius, B. Visser, and W. H. P. Boshoff, 2019 First report of Puccinia graminis f. sp. tritici race PTKSK, a variant of wheat stem rust race Ug99, in South Africa. Plant Dis. 103:1421. https://doi.org/10.1094/PDIS-11-18-1911-PDN

Turner, M. K., Y. Jin, M. N. Rouse, and J. A. Anderson, 2016 Stem rust resistance in ‘Jagger’ winter wheat. Crop Sci. 56:1719-1725.

Voorrips, R. E., 2002 MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 93:77-78.

Wang, S., D. Wong, K. Forrest, A. Allen, S. Chao, et al., 2014 Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol. J. 12:787-796.

Xu, S. S., K. Khan, D. L. Klindworth, and G. Nygard, 2010 Evaluation and characterization of high-molecular weight glutenin subunits from Aegilops tauschii in synthetic hexaploid wheat. J. Cereal Sci. 52:333-336.

Yu, G., Q. Zhang, T. L. Friesen, M. N. Rouse, Y. Jin et al., 2015 Identification and mapping of Sr46 from Aegilops tauschii accession Clae 25 conferring resistance to race TTKSK (Ug99) of wheat stem rust pathogen. Theor. Appl. Genet. 128:431-443.
Zhang, D., R. L. Bowden, J. Yu, B. F. Carver et al., 2014 Association analysis of stem rust resistance in U.S. winter wheat. PLoS One 9:e103747. doi: 10.1371/journal.pone.0103747

Zhang, Q. J. 2013 Development and characterization of wheat germplasm for resistance to stem rust Ug99 in wheat. Ph. D. Dissertation. North Dakota State University, Fargo, North Dakota.

Zhang, W., S. Chen, Z. Abate, J. Nirmala, M. N. Rouse et al., 2017. Identification and characterization of Sr13, a tetraploid wheat gene that confers resistance to the Ug99 stem rust race group. Proc. Natl. Acad. Sci. U. S. A. 114:E9483-E9492.
**Figure Legends**

**Figure 1.** Histograms representing the mean distribution of the linearized infection type (LIT) score of two replications for each recombinant inbred line (RIL) of the ND495 × Largo population against the races TTKSK, TRTTF, and TTTTF of *Puccinia graminis* f. sp. *tritici*.

**Figure 2.** QTL regions identified across five chromosomes in the ND495 × Largo recombinant inbred line (RIL) population against the races of TTKSK (A), TRTTF (B), and TTTTF (C) of *Puccinia graminis* f. sp. *tritici*. The critical LOD threshold is indicated by the **dotted line**. The confirmed stem rust resistance genes (*Sr*) associated with QTL regions are presented in brackets and *blue font*. The temporary designated genes are presented in orange color.

**Table 1.** Avirulence and virulence profile of three *Puccinia graminis* f. sp. *tritici* (*Pgt*) races TTKSK, TRTTF, and TTTTF for the North American differentials

| *Pgt* race (isolate) | Avirulent | Virulent |
|----------------------|-----------|----------|
| TTKSK (04KEN156/04)  | *Sr24 36 Tmp* | *Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 31 38 McN* |
| TRTTF (06YEM34-1)    | *Sr8a 24 31* | *Sr5 6 7b 9a 9b 9d 9eᵃ 9g 10 11 17 21 30 36 38 McN Tmp* |
| TTTTF (01MN84A-1-2)  | *Sr24 31*  | *Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp* |

ᵃVirulence of TRTTF to 9e is variable due to the minor effect.
Table 2. The newly developed simple sequence repeat (SSR) markers mapped on the chromosome arm 2DS in ND495 × Largo recombinant inbred line population.

| Marker name | Primer name | primer sequence<sup>a</sup> | Tm (°C) | Position_length in reference genomes<sup>b</sup> |
|-------------|-------------|-----------------------------|---------|-----------------------------------------------|
| Xrwgs46     | Xrwgs46F    | [Tail1]TGGAGCAAGCTAGTAGGGTT | 58.05   | 7.178 M_152 bp 8.601 M_148 bp               |
|             | Xrwgs46R    | GATGCTCTTAGGTGACAGTG       | 56.08   |                                                |
| Xrwgs47     | Xrwgs47F    | [Tail1]ATCACCGCTGCTAGTTCTTG| 57.98   | 6.535 M_266 bp 7.640 M_415 bp               |
|             | Xrwgs47R    | CAAAGTCGAAGGTAGAGA         | 57.26   |                                                |
| Xrwgs49     | Xrwgs49F    | [Tail1]GGACTGTTGTTTCTCCTTG | 56.69   | 8.870 M_212 bp 10.065 M_173                 |
|             | Xrwgs49R    | TGTACTTGGGTGTTTGGAGG       | 57.35   | bp                                             |

<sup>a</sup>Tail1 = GCAACAGGAAACCAGCTATGAC-3.

<sup>b</sup>Position coordinates and length on the *Aegilops tauschii* (AL 8/78) and Chinese Spring reference genomes (IWGSC 2018).
Table 3. Infection types scored on ND495, Largo, and parents of Largo tested using races TTKSK, TRTTF, and TTTTF of stem rust pathogen (*Puccinia graminis* f. sp. *tritici*)

| Line        | TTKSK  | TRTTF  | TTTTF  |
|-------------|--------|--------|--------|
|             | Rep 1  | Rep 2  | Rep 1  | Rep 2  | Rep 1  | Rep 2  |
| ND495       | 3+     | 3+     | 31+    | 1+3+   | ;3     | 0;13+  |
| Largo       | 2      | 2      | ;2;    | ;12;   | 22;    | 22;    |
| PI 268210   | 2;     | 2;     | 2;     | 2;     | 2;     | 2;     |
| Langdon     | 22+    | 22+    | ;2;    | ;2;    | 2;     | 2;     |

*Infection types (Its) were scored based on the Stakman *et al.* (1962) where 0, ;, 1, or 2, are considered resistant, and 3 or 4 are considered susceptible. For leaves exhibiting combinations of ITs, order indicates predominant types. Symbols “-” and “+” indicated small or large pustules, respectively, within a class."
population, number of markers, loci, and other genetic parameters

| Chromosome | No. of markers | Length | cM/locus |
|------------|----------------|--------|----------|
|            | SSR | SNP | Total | (cM) |        |
| 1A         | -   | 662 | 662   | 77   | 80.3  | 1.0   |
| 1B         | -   | 750 | 750   | 96   | 87.8  | 0.9   |
| 1D         | 4   | 258 | 262   | 69   | 120.7 | 1.7   |
| 2A         | -   | 425 | 425   | 79   | 124.3 | 1.6   |
| 2B         | 3   | 663 | 666   | 77   | 109.0 | 1.4   |
| 2D         | 13  | 140 | 153   | 84   | 142.4 | 1.7   |
| 3A         | -   | 442 | 442   | 97   | 124.2 | 1.3   |
| 3B         | -   | 775 | 775   | 155  | 145.2 | 0.9   |
| 3D         | -   | 164 | 164   | 58   | 120.2 | 2.1   |
| 4A         | -   | 394 | 394   | 88   | 123.6 | 1.4   |
| 4B         | -   | 426 | 426   | 68   | 83.3  | 1.2   |
| 4D         | -   | 54  | 54    | 26   | 114.9 | 4.4   |
| 5A         | -   | 515 | 515   | 155  | 155.9 | 1.0   |
| 5B         | -   | 613 | 613   | 104  | 130.6 | 1.3   |
| 5D         | -   | 150 | 150   | 76   | 123.9 | 1.6   |
| 6A         | -   | 399 | 399   | 55   | 101.0 | 1.8   |
| 6B         | -   | 478 | 478   | 61   | 83.7  | 1.4   |
| 6D         | -   | 131 | 131   | 59   | 122.3 | 2.1   |
| 7A         | -   | 270 | 270   | 79   | 131.5 | 1.7   |
|       | 7B  | 7D  | A genome | B genome | D genome | Total |
|-------|-----|-----|----------|----------|----------|-------|
|       | -   | -   | -        | -        | -        | -     |
|        | 289 | 185 | 3,107    | 3,994    | 1,082    | 8,183 |
|        | 289 | 185 | 3,107    | 3,997    | 1,099    | 8,203 |
|        | 90  | 86  | 630      | 651      | 458      | 1,739 |
|        | 87.1| 146.9| 840.8    | 726.7    | 891.3    | 2,458.7 |
|        | 1.0 | 1.7 | 1.3      | 1.1      | 1.9      | 1.4   |
|       | 20  | 8,183 | 8,203 | 1,739 | 2,458.7 |
Table 5. Quantitative trait loci (QTL) identified in the ND495 × Largo recombinant inbred line population tested with races TTKSK, TRTTF, and TTTTF of stem rust pathogen

| QTL       | Putative gene | Flanking markers | Chr. | Pos. | TTKSK | TRTTF | TTTTF |
|-----------|---------------|------------------|------|------|-------|-------|-------|
|           |               |                  |      |      | LOD   | Add.  | R² × 100 | LOD   | Add.  | R² × 100 | LOD   | Add.  | R² × 100 |
| QS.rw-1D  | * *           | IWB22674 – IWB31245 | 1D   | 16   | -     | -     | -     | 7.8   | 0.9   | 16.8   |       |       |         |
| QS.rw-2B.1| *             | IWA413 – IWA2571  | 2B   | 38   | -     | -     | -     | 3.0   | 0.5   | 4.6    |       |       |         |
| QS.rw-2B.1| *             | IWB7072 – IWB2380 | 2B   | 42   | -     | -     | -     | 4.6   | 0.5   | 16.2   |       |       |         |
| QS.rw-2B.2| Sr9e          | IWB71742 – IWB73196| 2B   | 74   | -     | -     | -     | -     | -     | -      |       |       |         |
| QS.rw-2D  | Sr46          | Xrws46 – IWB43851| 2D   | 4    | -     | -     | -     | 3.5   | 0.4   | 3.6    |       |       |         |
| QS.rw-4A  | Sr7a          | IWB9431 – IWB5461 | 4A   | 114  | -     | -     | -     | 6.6   | -0.8  | 16.1   |       |       |         |
| QS.rw-6A  | Sr13c         | IWA441 – IWB51469/IWB25644 | 6A  | 98-  | 23.5 | 0.7  | 21.3 | 13.8 | 0.9  | 18.5   | 8.7   | 0.9   | 17.0   |
Chr. = Chromosome
Pos. = Position in centimorgan (cM)
Add. = Additive effect of the QTL, positive values indicate resistance derived from Largo and negative values indicates resistance derived from ND495
Symbol'*' indicates no known stem rust resistance gene
Symbol'-.' indicates no QTL identified
