# Characterizing the Genetics of Seedling Wheat Stem Rust Resistance in Two Wheat Populations

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# Background

Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is a major disease of wheat worldwide. Enhancing resistance is a major objective in wheat breeding programs due to the devastating economic losses caused during stem rust epidemics. To increase genetic diversity in U.S. spring wheat breeding programs, a nested association mapping (NAM) population was developed by crossing 25 exotic accessions selected from the USDA-ARS Spring Wheat Core Collection with 'RB07' <sup>1</sup> to develop a BC<sub>1</sub>F<sub>3:6</sub> population. The 25 exotic parents were screened for seedling resistance to *Pgt* races TTTTF, TPMKC, HKHJC, TTKSK, TTKST, TRTTF, TTKTT, and TTKSF+*Sr9h*. Two of the 25 parents (PI 519465 and PI 520033) were resistant to all the eight *Pgt* races evaluated. The goal of this study was to characterize the genetics of resistance in the PI 519465/2\*RB07 and PI 520033/2\*RB07 populations and identify *Pgt* resistance genes.

**Table 2**. Marker trait associations (MTAs) identified in the PI 519465/2\*RB07 and PI 520033/2\*RB07 populations. The most significant marker positions were included in addition to the significant haplotype including the significant SNP. The most favorable additive effects are highlighted in a green color.

| Pgt race   | MTA name         | Chr. | * Position (bp) | <sup>‡</sup> Haplotype (Mbp) | <i>p</i> -value | <b>R</b> <sup>2</sup> | § <b>RB07</b> | ¶ P24 | * <b>P26</b> |
|------------|------------------|------|-----------------|------------------------------|-----------------|-----------------------|---------------|-------|--------------|
| TTKSK      | Sr2B_139/640_UMN | 2B   | 593,792,211     | 138.9 - 640.4                | 0.0000000       | 0.35                  | 2.58          | -1.74 | -0.84        |
|            | Sr5D_175_UMN     | 5D   | 175,240,614     | 175.2                        | 0.0002042       | 0.14                  | 1.52          | -1.20 | -0.33        |
| ТТКТТ      | Sr1A_347/350_UMN | 1A   | 349,193,154     | 346.7 – 349.5                | 0.000001        | 0.29                  | 1.25          | -2.10 | 0.84         |
|            | Sr2B_223/227_UMN | 2B   | 223,367,822     | 223.3 – 227.3                | 0.0000000       | 0.34                  | 2.28          | -1.50 | -0.78        |
|            | Sr2B_386/746_UMN | 2B   | 534,770,469     | 386.2 - 745.6                | 0.0000000       | 0.31                  | 2.19          | -1.35 | -0.84        |
|            | Sr5D_175_UMN     | 5D   | 175,240,614     | 175.2                        | 0.0000126       | 0.17                  | 1.56          | -0.81 | -0.75        |
|            | Sr7B_110_UMN     | 7B   | 109,853,271     | 109.9 - 109.9                | 0.000007        | 0.30                  | 1.16          | -2.16 | 1.00         |
|            | Sr7D_16_UMN      | 7D   | 16,145,638      | 16.1                         | 0.000022        | 0.55                  | 2.15          | -4.47 | 2.33         |
| TTKST      | Sr2B_178_UMN     | 2B   | 177,975,985     | 177.9                        | 0.0000603       | 0.05                  | -0.51         | 0.88  | -0.38        |
|            | Sr2B_351_UMN     | 2B   | 351,259,098     | 351.3                        | 0.0000776       | 0.16                  | 1.56          | -0.45 | -1.11        |
|            | Sr2B_624/701_UMN | 2B   | 623,529,318     | 623.6 - 701.1                | 0.0001000       | 0.21                  | 1.79          | -0.53 | -1.26        |
|            | Sr4A_733/735_UMN | 4A   | 732,858,934     | 732.9 – 734.9                | 0.0000000       | 0.19                  | -1.11         | 1.11  | 0.00         |
| TRTTF      | Sr6A_3/6_UMN     | S6A  | 2,943,020       | 2.5 - 5.8                    | 0.0000000       | 0.31                  | 1.71          | -0.53 | -1.18        |
|            | Sr2A_76/80_UMN   | S2A  | 76,094,314      | 75.6 - 800.1                 | 0.0000263       | 0.08                  | -0.57         | -0.51 | 1.08         |
| TTKSF+Sr9h | Sr2B_139/640_UMN | S2B  | 223,367,822     | 138.9 - 640.4                | 0.0000000       | 0.24                  | 1.91          | -1.31 | -0.61        |
|            | Sr5D_175_UMN     | S5D  | 175,240,614     | 175.2                        | 0.0007079       | 0.13                  | 1.35          | -0.88 | -0.47        |

#### Approach

- Experiments were conducted in the Biosafety Level-3 (BSL-3) Containment Facility.
- The respective parents and recombinant inbred lines (RILs) of PI 519465/2\*RB07 (89 lines), and RILs of PI 520033/2\*RB07 (85 lines) were evaluated for resistance to five Pgt races TTKSK, TTKTT, TTKST, TRTTF, and TTKSF+Sr9h.
- Twelve days after inoculation, infection types (ITs) were assessed on plants using the 0 to 4 scale developed by Stakman<sup>2</sup>.
- For quantitative trait loci (QTL) analyses, the raw stem rust ITs on progeny lines were converted to a 0 to 9 linear scale.
- Single nucleotide polymorphism (SNP) markers were generated by the genotypingby-sequencing platform. SNP reads were aligned against the new Chinese Spring

† The most significant SNP in the significant haplotype ‡; § The additive genetic effect for RB07; ¶ The additive genetic effect for PI 519465; \* The additive genetic effect for PI 520033

## **Genetic mapping results**

- Sixteen significant MTAs were identified in response to the five *Pgt* races (Table 2; Figure 1).
- MTA Sr2B\_139/640\_UMN was associated

**Figure 1.** Circular Manhattan plots displaying the MTAs for resistance to the five *Pgt* races



wheat genome assembly V2 to obtain physical distances for all markers.

- To identify Pgt resistance genes, QTL mapping was performed with 10,000 markers for both populations by using mixed linear model that utilized family stratification and genetic kinship as covariates, while treating markers as random effects using the empirical Bayes approach implemented in the NAM package in R<sup>3</sup>.
- Major background genetic effects were corrected for by using a sliding window algorithm by fitting all markers outside a 10 Mbp window as covariates.
- A false discovery rate at 0.05 was used to correct for Type I errors.

## **Phenotypic results**

- RB07 was susceptible to all five *Pgt* races tested (Table 1).
- PI 519465 (P24) and PI 520033 (P26) were resistant to all five Pgt races (Table 1).

**Table 1.** Seedling infection types of the parents to five races of thestem rust pathogen, *Puccinia graminis* f. sp. *tritici*.

| Parent          | TTKSK | ТТКТТ | TTKST | TRTTF | TTKSF+ <i>Sr9h</i> |
|-----------------|-------|-------|-------|-------|--------------------|
| RB07            | 33+   | 33+   | 3+3+  | 4     | 4                  |
| PI 519465 (P24) | 0;    | 0;    | 0;1   | 12    | 0;                 |
| PI 520033 (P26) | 1     | 0;    | 0;1-  | 10;   | 0;                 |

with *Sr36* on chromosome 2B and identified in response to the four *Pgt* races TTKSK, TTKTT, TTKST, and TTKSF+*Sr9h* (Table 2; Figure 1).

- Sr4A\_733/735\_UMN was associated with resistance to TTKST where the positive allele was contributed by RB07. This association is likely due to *Sr7a* on the long arm of 4A<sup>4</sup> in RB07.
- A putative new gene was identified on chromosome 5D that provided resistance against TTKSK, TTKTT, and TTKSF+Sr9h.

#### Conclusions

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- Across the two populations, we identified 35 lines that are resistant to all five Pgt races tested in the study.
- Differences in the distribution of resistance in the two populations to the same respective Pgt races indicate different resistance genes.
- In the PI 519465/2\*RB07 (FAM24) population, the frequency of resistance varied between 16.9% (TRTTF) and 62.9% (TTKSF+ Sr9h).
- In the PI 520033/2\*RB07 (FAM26) population, the frequency of resistance varied between 36.5% (TRTTF) and 84.7% (TTKTT).

Distribution of the transformed ITs varied between the two populations (Figure 1).

- PI 519465 (P24) has the largest number of favorable resistance alleles at 10 MTAs including a putative novel gene Sr5D\_175\_UMN on the long arm of chromosome 5D.
- PI 520033 (P26) carries the most favorable alleles at three MTAs associated with resistance to Pgt races TTKST and TRTTF.
- Gene annotations based on the Chinese Spring genome assembly is underway.

#### **References:**

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