Genome-Wide Association mapping of seedling response of a durum wheat panel against stem rust

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Abstract

A panel of 283 durum wheat lines from the International Maize and Wheat Improvement Center (CIMMYT) were evaluated at the seedling stage for responses against races TTKSK, JRCQC, TKTTF and TTRTF in the BSL3 facility at the University of Minnesota. The lines were genotyped using genotyping-by-sequencing (GBS) at the USDA-ARS Eastern Regional Small Grains Genotyping Lab in Raleigh, NC. The mean linearized scale of seedling infection types and 26,439 Single Nucleotide Polymorphism (SNP) markers for 280 lines were used to undertake Genome-Wide Association Analysis (GWAS) and a False discovery rate of 5% was used as a threshold to declare significant marker-trait associations (MTAs). MLM identified 52, 47, 20 and 71 significant MTAs for responses against races TTKSK, TKTTF, JRCQC and TTRTF, respectively. Among the total MTAs, 1%, 16.6% and 30.7% were consistent between the four, three and two races, respectively and 51.7% were identified for single race. FarmCPU detected eight, nine, eleven and nine significant MTAs for responses against TTKSK, TKTTF, JRCQC and TTRTF, respectively. MLM detected a total of 17 QTL while FarmCPU detected 20 QTL and six of them were consistent between the two models. Chromosome 6A harbored the highest number of significant MTAs (70) with the largest contribution to the phenotypic variation (3.2% to 17.1%). The identified regions matched the location of *Sr7a*, *Sr11*, *Sr13*, *Sr17*, *Sr22*, *Sr49* and other previously reported loci. Novel loci on chromosomes 2B, 3A, 6A and 7A were consistent between races and the two models and can be used in marker-assisted selection after validation. Two regions on chromosome 6A (611 Mb and 615 Mb) that are postulated as *Sr13a* and *Sr13c* based on the race specificity can be used to differentiate the two alleles. The significant markers identified in the current study can be utilized to identify sources of resistance to stem rust. These results, complementing evaluations of field response to *Pgt* races, would enhance the capacity of durum wheat breeding programs to conduct a more effective effort tackling stem rust resistance breeding.

Introduction

Yield and end use product quality of Durum wheat (*Triticum turgidum* L., ssp. *Durum* (Desf.) Husnot) can be negatively affected by stem rust caused by the fungal pathogen *Puccinia graminis* Pers.f.sp. *tritici* Eriks. and Henn. (Singh et al., 2006; Bhavani et al., 2019) . Stem rust can occur in all wheat production areas where the environment is favorable for disease development and can cause a total yield loss upon growing susceptible varieties of both common and durum wheat (Singh et al., 2008; Yu et al., 2014). Continuously emerging virulent races including the Ug99 race group , TKTTF, JRCQC,TTRTF (Olivera et al., 2019) and several other races with broad virulence to commercially deployed resistance genes threatened global wheat production and food security (Olivera et al., 2012b). Therefore, evaluation and identification of sources of resistance in the germplasm pool is paramount for proper utilization of resistance sources. The objectives of the current study were to 1) evaluate seedlings of a panel of durum wheat lines against four virulent races (TTKSK, TKTTF, JRCQC and TTRTF) and 2) conduct GWAS analysis to identify genomic regions associated with seedling resistances

Material and Methods

A panel of 283 spring durum wheat lines assembled by the CIMMYT durum wheat breeding program was evaluated at the seedling stage against races TTKSK, JRCQC, TKTTF, TTRTF in two replications in the BSL3 facility of the University of Minnesota in January 2019. Seedlings were inoculated following the procedure by Rouse et al. (2011) and scored after 14 days using a 0 to 4 scale described by Stakman et al. (1962). The mean linearized scale of the two replications was used as a response to fit GWAS models. Lines were genotyped using the GBS protocol (Poland et al.,2012). After filtering, 26,439 SNPs for 280 lines were retained for analysis. GWAS analysis was done using GAPIT by fitting MLM, CMLM and FarmCPU. Results were interpreted from MLM and validated by FarmCPU. The regions of significant MTAs were compared with previously reported loci for alignment of positions on the respective chromosomes of the durum wheat assembly of cv 'Svevo' using the blastn program of IWGSC database.

Results and Discussion

The distribution of the seedling responses against the four Pgt races was skewed towards the resistant scores (linearized response <= 6 or IT <= 2^{+}) (Fig. 1). Higher percentage of resistance against races TKTTF and TTKSK than the durum virulent races, JRCQC and TTRTF was observed (Table 1). The proportion of lines that showed resistance varied from 50.88% to 58.30% for combinations of three races and 52.29% to 67.14% for combinations of two races indicating the presence of resistance against multiple races which was supported by the phenotypic correlation between the races (Table 2; Fig. 2). The broad sense heritability ranged from 0.61 for race TTRTF to 0.91 for race TKTTF (Table 1) indicating a large amount of phenotypic variation being explained by the genotypic component.

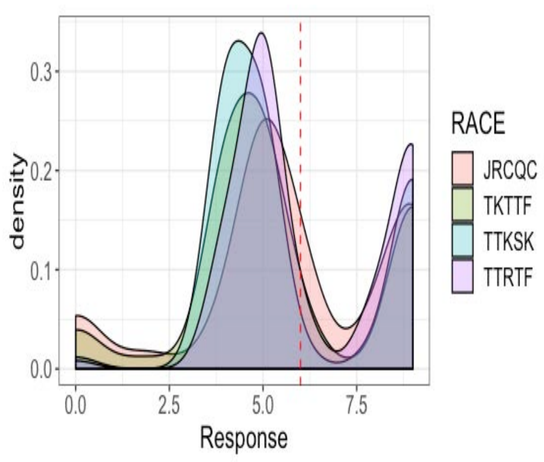

Figure 1. Distribution of seedling responses of lines against four *Pgt* races. Data was the linearized scale of the 0-4 IT score to 0-9 scale.

Table 1: Summary of resistances versus susceptible against the four Pgt races and broad sense heritability. Values are percentages and counts in parenthesis.

Race	Resistant	Susceptible	Heritability (H^2)
TTKSK	70.6(197)	29.4 (82)	0.86
TKTTF	73.1(204)	26.9 (75)	0.91
JRCQC	67.1(188)	32.8 (92)	0.90
TTRTF	56.4(159)	43.6(123)	0.61

Table 2. Number and percentage of lines resistant to different combinations of the four races

Race combination	Number of lines	Percentage of lines
TTKSK +TKTTF+JRCQC	165	58.3
TTKSK +JRCQC+TTRTF	144	50.88
TTKSK +TKTTF+TTRTF	145	51.12
JRCQC+TKTTF+TTRTF	148	52.29
TTKSK +TKTTF	190	67.14
TTKSK +JRCQC	168	59.36
TTKSK +TTRTF	148	52.29
JRCQC+TKTTF	176	62.19
TKTTF+TTRTF	151	53.36
JRCQC+TTRTF	151	53.36

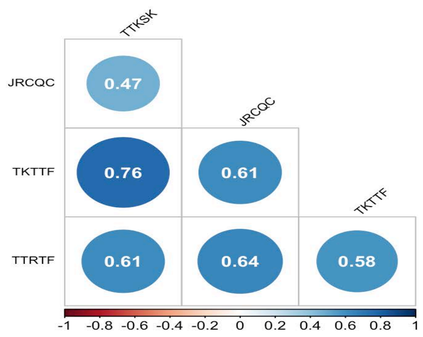

Figure 2. Correlation between responses of lines to the four races

MLM identified a total of 114 significant MTAs (52 for TTKSK, 47 for TKTTF, 20 for JRCQC and 71 for TTRTF) (Fig. 3). Among the total, 1%, 16.6%, 30.7% and 51.7% were associated with all the four, three, two and single races, respectively (Fig.4). FarmCPU detected 34 significant MTAs (8 for TTKSK, 9 for TKTTF, 11 for JRCQC and 9 for TTRTF) (Fig. 5). A total of 17 and 20 QTL were identified using MLM and FarmCPU, respectively. Six QTL on chromosomes 2B (89 Mb to 97 Mb), 3A (565 Mb and 614 Mb), 6A (205Mb, and 602 Mb to 615 Mb) and 7A (686 Mb to 721 Mb) were consistent between the two models and all the six except the one on 2B and the 614 Mb locus on 3A were consistent between two to four races indicating the reliability of the MTAs.

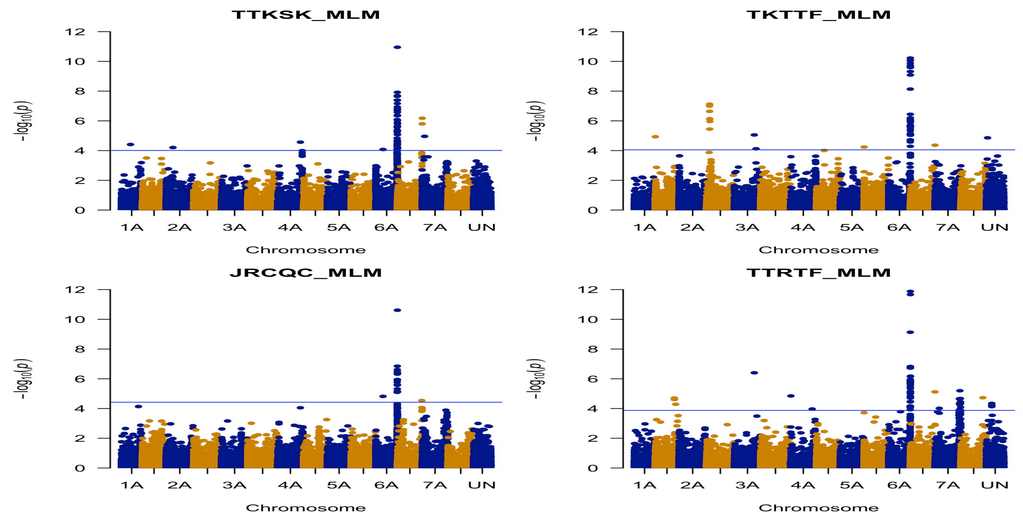

Figure 3. Manhattan plots of GWAS analyses for seedling response of lines to the four *Pgt* races identified using MLM.

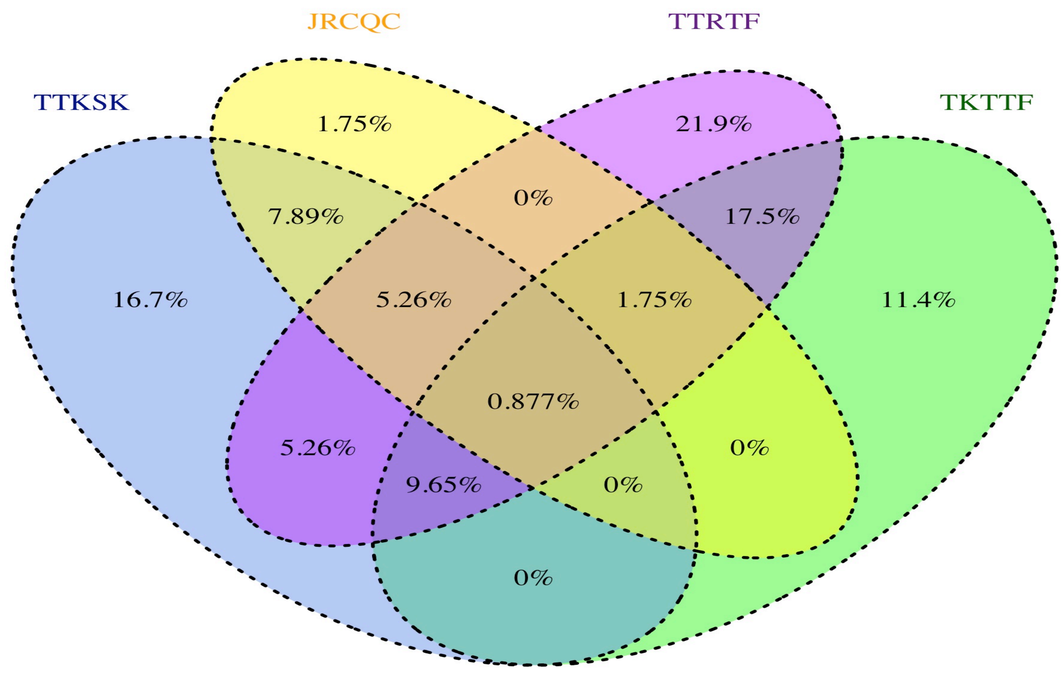

Figure 4. Percentage of common significant markers among seedling responses to the four *Pgt* races.

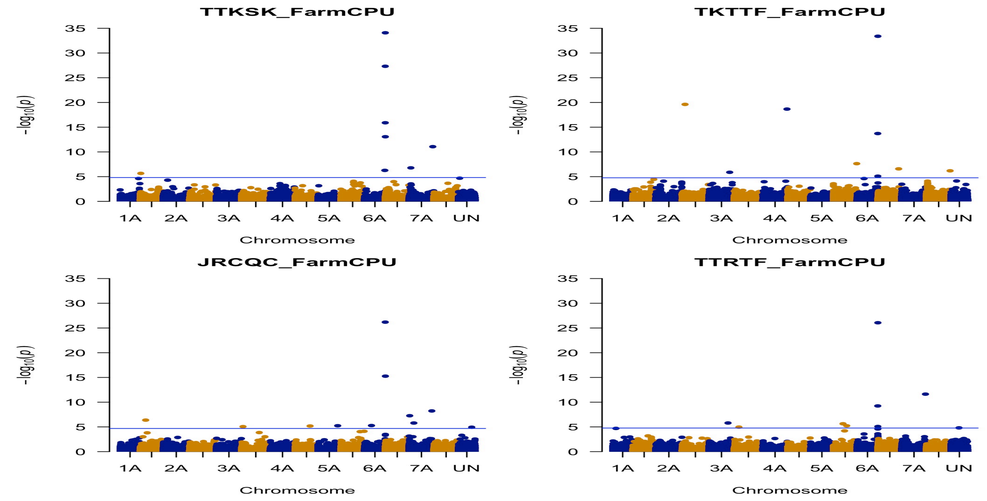

Figure 5. Manhattan plots of GWAS analyses for seedling response of panel of durum wheat lines to four *Pgt* races identified using FarmCPU.

Conclusion

The result of the current study revealed that the CIMMYT durum wheat breeding lines confer race-specific and multiple-race resistance to virulent *Pgt* races at the seedling stage. Lines consistently resistant is the seedling assay can be used as sources of resistance in the durum wheat breeding program after field evaluation. Consistent markers between races and models can be used in MAS to identify sources of resistance after being validated. Further study on allelic variation in the *Sr13* region is needed. Sr49 was rare in the population and future selection needs to be done cautiously to retain this gene in the selection process. Studies on minimizing the deleterious effect on using *Sr22* is needed for wide utilization as this gene is a multiple-race specific resistance gene.