QTL MAPPING OF STEM RUST RESISTANCE IN BI AND MULTI- PARENTAL POPULATIONS OF DURUM WHEAT

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Stem rust, caused by the fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*), is one of the most devastating fungal diseases of durum and common wheat worldwide. The identification of sources of resistance and the validation of QTLs identified through genome-wide association studies is of paramount importance for reducing the losses caused by this disease to wheat grain yield and quality. Four segregating populations whose parents showed contrasting reactions to some *Pgt* races were assessed in the present study and 14 QTLs were identified on chromosomes 3A, 4A, 6A, and 6B, with some regions in common between different segregating populations. Several QTLs were mapped to chromosomal regions coincident with previously mapped stem rust resistance loci; however, their reaction to different *Pgt* races suggest that novel genes or alleles could be present on chromosomes 3A and 6B. Putative candidate genes with a disease-related functional annotation have been identified in the QTL regions based on information available from the reference genome of durum cv. 'Svevo'.
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Genetic materials

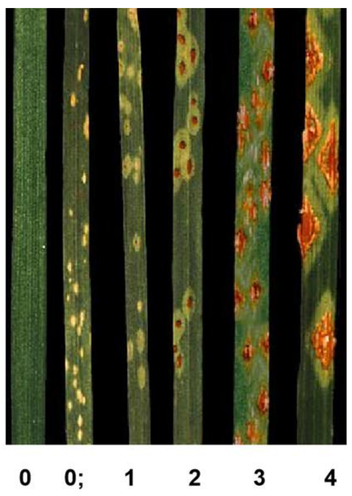
Four segregating RIL populations were evaluated for disease reaction to races of Pgt:
- two biparental populations made between durum wheat cultivars: 'Ciccio' x 'Svevo' (CicSve) with 120 RILs 120 and 'Cirillo' x 'Neodur' (CirNeo) with 148 RILs;
- one biparental population obtained by crossing the durum wheat cultivar 'Latino' to the T. dicoccum accession 'MG5323' (LatMg) with 110 RILs;
- one multiparent population from the four-way cross combination of Neodur/ Claudio// Colosseo/ (Rascon/2*Tarro) with 338 lines (NCCR).

A genetic map constructed with 398 DArT and PCR-based markers was available for the CirNeo population, while for others genetic maps previously developed based on the Illumina wheat 90K SNP array were available.

Four Pgt races with different virulence phenotypes were tested on the plant populations in a greenhouse: race **TTTTF**, avirulent only on *Sr24* and *Sr31*, and reported also in Sicily (Italy); race **TPMKC,** virulent on *Sr36*; race **JRCQC**, virulent on the two resistance genes Sr9e and Sr13 widely used in durum cultivars; race **TKTTF**, virulent on *SrTmp*. Evaluation was conducted in the Biosafety Level-3 Containment Facility on the St. Paul campus of the University of Minnesota (USA)

Phenotyping

ITs were assessed 12-14 days after inoculation using the 0–4 **scale of Stakman**, where IT = 0 represents a highly resistant (immune) reaction and IT = 4 represents a susceptible (fully compatible) reaction.
Raw IT data were converted to a 0–9 linear disease scale.

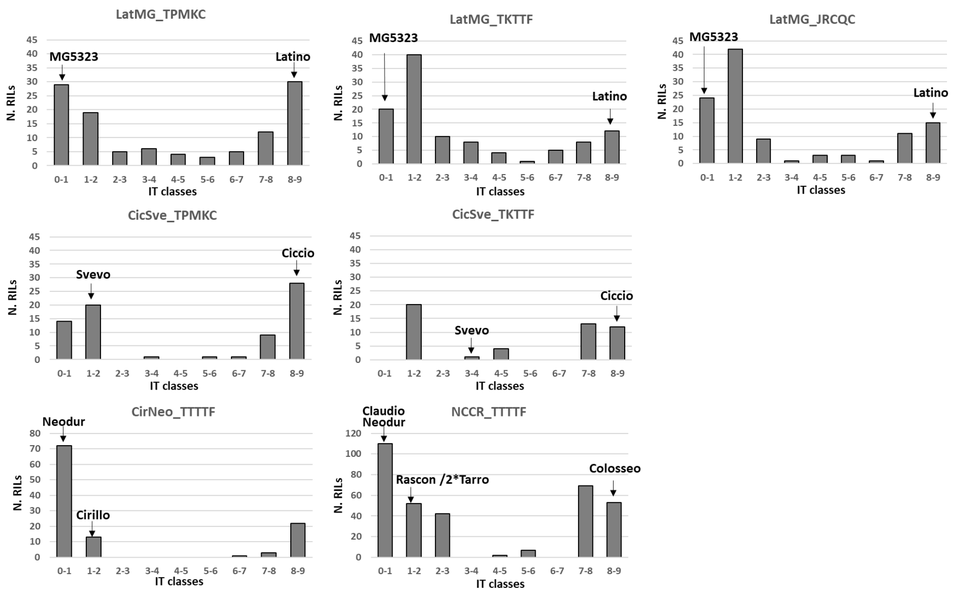


QTL analysis

For biparental populations, QTL detection was performed in QGene using **composite interval mapping**. Putative QTLs were defined as two or more linked markers that were associated with a trait at a LOD≥ 3. The 95% Confidence Intervals (CIs) for identified QTL in their original maps were calculated through the LOD-2 criterion.
For the NCCR population, a **haplotype-based QTL analysis** was performed. Haplotype blocks were determined by the "Haploview" software, then the phenotype/haplotype association analysis was firstly performed by ANOVA using the phenotype as the dependent variable and the haplotype forms at each individual block as the independent variable. Then, significantly associated haplotype blocks were subjected to forward stepwise regression using the Bayesian Information Criterion for retention or rejection of haplotype blocks in the global model. The tag-marker of a haplotype block was defined as the marker most associated with the phenotype among the markers of the same haplotype block. Confidence interval was determined on the basis of the position of the most distant markers to the left and right of the tag-marker for which a value of $r^2 > 0.5$ was observed with the tag marker itself.

Results from phenotyping analysis

In a first phenotypic evaluation, the ITs of the 9 parental genotypes was assessed with the four different *Pgt* races, then RIL populations were accordingly evaluated with the discriminating races.
In all cases the frequency distribution of IT scoring was bimodal with a majority of highly resistant and highly susceptible lines, and only few lines displaying intermediate IT values, suggesting resistance behavior based on one or a few genes segregating within each population.



A chi-square test was applied to compare the observed segregation ratios with the ones expected upon segregation of one or a few major genes.

RIL Pop. name	Stem rust race	N° of Resistant RILs (IT<=6)	N° of Susceptible RILs (IT>6)	Observed segregation	χ^2	N° of expected genes
LatMG	TPKMC	66	47	1:1	(1, N= 113) = 3.2, $p > .10$	1 gene
LatMG	JRCQC	82	27	3:1	(1, N= 109) = 0.003, $p > .90$	2 genes, both from MG5323
LatMG	TKTTF	85	25	3:1	(1, N= 110) = 0.30, $p > .50$	2 genes, both from MG5323
CicSve	TPMKC	36	38	1:1	(1, N= 74) = 0.054, $p > .80$	1 gene
CicSve	TKTTF	25	25	1:1	exact segregation	1 gene
CirNeo	TTTTF	85	26	3:1	(1, N= 111) = 0.147, $p > .50$	2 genes, one per parent
NCCR	TTTTF	213	122	5:3	(1, N= 335) = 0.167, $p > .50$	3 genes: one independent by two parents, and two dependent on one parent

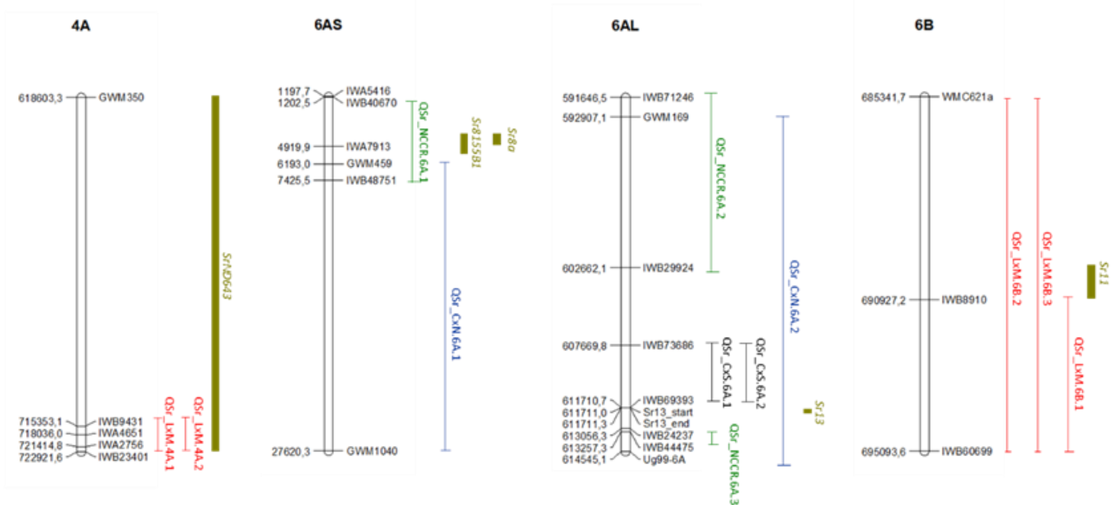
An ANOVA was carried out to assess genotype differences in the four segregating populations. Statistically significant differences ($p < 0.001$) were found across genotypes for all the populations and the Pgt races used in the study. A very good repeatability was observed for this experiment, ranging from 73% for race TKTTF in CicSve population to 99% for the NCCR population tested with the race TTTTF, highlighting the robustness of the data and the low error rate.

Results from QTL mapping

A total of 14 QTLs were identified considering all the populations and the Pgt races assessed, most of them on chromosomes 6A and 6B.
On the distal portion of **6AS**, a QTL for response to race TTTTF was identified in the CirNeo and in the NCCR, in both cases with resistant allele carried by Neodur. While the confidence interval was quite larger for CirNeo due to the low number of polymorphic SNPs in this map, this a region has extended polymorphism between Neodur and the other NCCR parents, thus these results can help in reducing the size of the 6AS region in which the resistance gene is located. Comparative analysis with other mapping studies for SR wheat resistance genes suggested that this QTL could be very close but different with respect to those already mapped, or possibly a new allele with a different race specificity.

QTL name	Trait	Population	Peak marker	Chr	LOD	R^2	Left pos. Mb	Right pos. Mb	Resistant parent
QSr_CxS.3A.1	TKTTF	CicSve	IWB66101	3A	3.5	0.28	97.5	151.0	Svevo
QSr_CxS.3A.2	TPMKC	CicSve	IWB66101	3A	2.2	0.13	66.3	151.0	Svevo
QSr_LxM.4A.1	JRCQC	LatMG	IWB72220	4AL	15.9	0.35	715.4	721.4	MG5323
QSr_LxM.4A.2	TKTTF	LatMG	IWB72220	4AL	17.1	0.42	715.4	721.4	MG5323
QSr_NCCR.6A.1	TTTTF	NCCR	IWB48751	6AS	39.1	0.27	1.2	7.4	Neodur
QSr_CxN.6A.1	TTTTF	CirNeo	Xgwm459	6AS	13.3	0.42	6.2	27.6	Neodur
QSr_NCCR.6A.2	TTTTF	NCCR	IWB29924	6AL	54.9	0.36	591.6	602.7	Claudio-Rascon/2*Tarro
QSr_NCCR.6A.3	TTTTF	NCCR	IWB60184	6AL	70.9	0.46	613.1	613.3	Claudio-Rascon/2*Tarro
QSr_CxN.6A.2	TTTTF	CirNeo	Ug99-6A	6AL	9.9	0.34	592.9	614.5	Cirillo
QSr_CxS.6A.1	TKTTF	CicSve	IWB69393	6AL	27.9	0.92	607.7	611.7	Svevo
QSr_CxS.6A.2	TPMKC	CicSve	IWB69393	6AL	40.4	0.92	607.7	611.7	Svevo
QSr_LxM.6B.1	TPKMC	LatMG	IWB60699	6BL	23.3	0.68	690.9	695.1	MG5323
QSr_LxM.6B.2	JRCQC	LatMG	IWB60699	6BL	18.5	0.44	685.3	695.1	MG5323
QSr_LxM.6B.3	TKTTF	LatMG	IWB58435	6BL	16.9	0.41	685.3	695.1	MG5323

A second region for resistance to TTTTF was identified on **6AL** that included a QTL with a large confidence interval found in CirNeo with the resistant allele contributed by Cirillo, and two distinct QTLs detected in NCCR, with resistant alleles provided by Claudio and Rascon/2*Tarro in both cases. In addition, in a very close position but for resistance to races TKTTF and TPKMC, two more QTLs were mapped in CicSve, both contributed by Svevo. The resistance gene *Sr13* (611,8 Mb) is within or close to all these QTLs.
Other chromosomal regions with high LOD values were identified on **4AL** and **6BL** in LatMG. Interestingly they gave resistance to two and three races, and in all regions, the *T. dicoccum* accession MG5323 contributed the alleles with positive effect. The QTL on chromosome 6B mapped to a position coincident with *Sr11, however the resistance spectrum indicated that MG5323 likely* carries a different allele.



The gene content of QTLs investigated by projecting the QTL confidence interval to the genome of durum wheat cultivar 'Svevo'. Their annotation was inspected to identify genes potentially involved in disease resistance. Since gene families implicated in disease response often contain many members, the probability of identifying one of these genes in a QTL interval by chance is high. Nevertheless, finding clusters of such genes at the position of the peak marker or in a very close position can help in identifying interesting candidate genes for further research. In this study, this was the case for the two likely novel SR regions, on 4A, for which a cluster of 6 genes annotated as NBS-LRR class disease resistance was found, and for the overlapping regions of QTLs on 6AS where two genes for protein kinases are present.

Conclusions

- Four segregating RILs populations whose parents showed contrasting reactions to some Pgt races were assessed
- 14 QTLs were identified on chromosomes 3A, 4A, 6A, and 6B, with some regions in common between different segregating populations.
- Several QTLs were mapped to chromosomal regions coincident with previously mapped stem rust resistance loci; however, their reaction to different *Pgt* races suggest that novel genes or alleles could be present on chromosomes 3A and 6B.
- Putative candidate genes with a disease-related functional annotation have been identified in the QTL regions based on information available from the reference genome of durum cv. 'Svevo'.