

# Genome-Wide Association mapping of *Septoria Tritici* Blotch Resistance in a collection of Mediterranean durum wheat landraces via High-Density Marker Arrays

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### Introduction

Septoria tritici blotch (STB), caused by the fungus *Zymoseptoria tritici*, is currently the most damaging leaf disease on wheat in Europe and is one of the most significant diseases on wheat around the world. (Savary et al., 2019) The frequent use of fungicides causes long-term severe negative effects on human health and the environment (Gikas et al. 2022) hence the characterization of new resistance sources and the development of resistant wheat cultivars is crucial. Wheat landrace collections contain wider genetic diversity than most breeding programs and constitute an easily transferable and valuable source of genetic variation for agronomical, morphological, adaptive, and quality traits (Giraldo et al., 2016). Genome-wide association mapping (GWAS) using a large number of markers with high genome coverage is a powerful tool for detecting the resistance loci associated with diverse germplasm possessing natural variation of resistance genes (Bartoli and Roux 2017).

• The frequency of resistance level varied between years and countries of origin(Fig1). Accessions from Tunisia and Portugal showed good levels of resistant lines, but also showed slight frequency changes between years. Landraces from Egypt, Italy showed a lower frequency of resistant lines . The latter was the most variable, showing resistant, intermediate, and susceptible accessions.



The present study relies on genotyping and phenotyping a core set of 315 Mediterranean durum wheat landraces, explore the genetic diversity and identify possible novel QTLs associated with STB resistance.

## **Materials and Methods**

#### Phenotyping

A collection of durum landraces from the Mediterranean region, provided by ARS-USDA National Plant Germplasm System repository, originated from 11 countries. The landraces were screened for STB resistance in two replicated field trials at the Wheat Septoria Precision Phenotyping Platform–experimental station of Kodia, during 2017-2018 and 2018-2019 cropping seasons.



Fig1. Collection of durum wheat landraces planted in Tunisia

• STB progression was visually scored by measuring incidence and severity based on the Saari and Prescott double digit scale (00-99) (Eyal et al. 1987;

*Fig3. Adult growth-stage frequency distribution (%) of disease response based on the country of origin.* 

 Principal component analysis showed two dimensions explaining 67% of data variance. The first dimension accounted for 50.2% of the variance, while the second dimension accounted for 18.49% of the variance. Accessions were separated into 3 groups: cluster 1(red) comprised 184 accessions, cluster 2 (green) contained 88 accessions, while cluster 3 (blue) contained 29 accessions from Italy, Tunisia, Turkey and Egypt.



Fig4. PCA of the 315 durum wheat landraces

• The markers were well distributed across all the 14 chromosomes of durum wheat genomes based on the consensus bread wheat genetic map obtained from the International Wheat Genome Sequencing Consortium database.

Saari and Prescott 1975.). The percentage of disease severity (%DS), the Area under the Disease Progress Curve (AUDPC), sAUDPC, plant height and heading were also calculated.

#### DNA extraction and genotyping

During seed multiplication, a small leaf sample of each plant per entry was collected and bulked. DNA was extracted from the ground leaves using a modified cetyltrimethylammonium bromide (CTAB) protocol described in Dreisigacker et al., (2016). All entries were fingerprinted using the DArTSeq<sup>®</sup> technology at the Genetic Analysis Service for Agriculture (SAGA) in Mexico.

• Data analysis were carried out in R software.

## Results

Pearson's correlation coefficient (Fig.2) was highly significant for adult plant resistance between both years (r = 0.439; p  $\leq$  0.001). A positive correlation was also observed between plant height and heading date (r = 0.415). Plant height was found to have a negative significant effect on adult-stage resistance (r = -0.388 and -0.369) for both years, implying the effect of plant height on the severity of the disease. It also showed that the shorter the plant, the higher the STB infection.



- The array yielded 68,928 scorable Single Nucleotide Polymorphism (SNP) markers. 26,439 SNPs were retained after filtering using a 25% cutoff for missing data in either loci or accessions.
- ADMIXTURE analysis defined a minimum of seven distinct genetic clusters (k) and showed distinct clustering of genotypes according to the geographical origin.

## **Discussion and Conclusions**

The results of this study showed that durum wheat landraces provided by the USDA constitutes a good and diverse source of resistance to STB disease. Some entries had similar resistance levels for adult stage indicating that these accessions may harbor potential novel QTLs. Genome-wide association analysis (GWAS) are currently underway using Genomic Association and Prediction Integrated Tool (GAPIT). We expect that new genomic regions associated with STB resistance will be identified and resistant haplotypes could be potentially selected for KASP (Kompetitive allele specific PCR) marker development.

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**CGIAR** 

logos of the companies involved and the University of Bologna

