

# Fine Mapping a Tarspot Complex Resistance QTL in Maize

Jiafa Chen<sup>1</sup>, Xuecai Zhang, Sarah Hearne<sup>1</sup>, George Mahuku<sup>2</sup>, Prasanna Boddupalli<sup>1</sup>, and Gordon Huestis<sup>1\*</sup>

<sup>1</sup>The International Maize and Wheat Improvement Centre (CIMMYT) and <sup>2</sup>The International Institute of Tropical Agriculture (IITA)

\*corresponding author: g.huestis@cgiar.org

## INTRODUCTION

Tar Spot Complex (TSC) is a disease of maize caused by the synergistic interaction between at least two fungi, *Phyllachora maydis* and *Monographella maydis*, and is an increasingly important fungal disease in Latin America. Black "tar spots" are caused by *Phyllachora maydis*, while the halo around the tar spot indicates the presence of a second pathogen, *Monographella maydis*, forming the complex. Recently, Tar Spot was reported occurring in the USA for the first time by scientists at Purdue University. Symptoms of TSC are shown in Figure 1. To identify and characterize TSC resistance sources, GWAS was performed on a set of 890 inbred lines in seven environments. Significant QTL were detected on chromosomes 2, 7, and 8. Three bi-parental populations were developed to validate GWAS results from TSC resistant sources CML495, CML451, and CML492, and 5 QTL were identified on chromosomes 1, 6, 7, 8, and 10. A QTL located on chromosome 8 had the highest LOD value and was stable across different environments. Designated qRtsc8-1, this QTL was identified as a major resistance QTL with p-value = 3.29E-19 and it accounted for 6.92% of the phenotypic variance. We initiated fine mapping of qRtsc8-1 to develop breeder-ready markers for use in marker assisted selection.

## MATERIALS AND METHODS

Fine mapping was performed on two of the DH bi-parental populations used for QTL mapping: Population one (Pop 1) was a doubled haploid (DH) population derived from an F1 cross between white maize inbred lines CML495 (TSC resistant) and LaPostaSEQ.C7F64-2-6-2-2-B\*3 (TSC susceptible) and consisted of 201 DH lines. Population 2 (Pop 2) was a doubled haploid (DH) population derived from an F1 cross between yellow maize inbred lines CML451 (TSC resistant) and DTPYC9-F46-1-2-1-2-B\*3 (TSC susceptible) and was composed of 116 lines. Fine mapping was performed on both Pop1 and Pop2, but this poster only reports Pop1. Resistant and susceptible DH individuals from Pop 1 were crossed and then backcrossed to the recurrent susceptible parent to produce 10 BC1 populations to perform fine mapping of qRtsc8-1. These populations were artificially inoculated and screened for TSC resistance at the CIMMYT experimental station Agua Fría, Mexico, at 100M above sea level. The fine mapping work was conducted in BC1 and BC3 generation in 2014 and 2015. However, in alternating cycles each year, the conditions for disease development were not optimal for disease phenotyping. In these cycles (BC2, BC4) only genotyping was performed in order to develop the subsequent recombinant population. The same procedure was followed each cycle. For SNP genotyping we used KASP genotyping assays (LGC Genomics.)

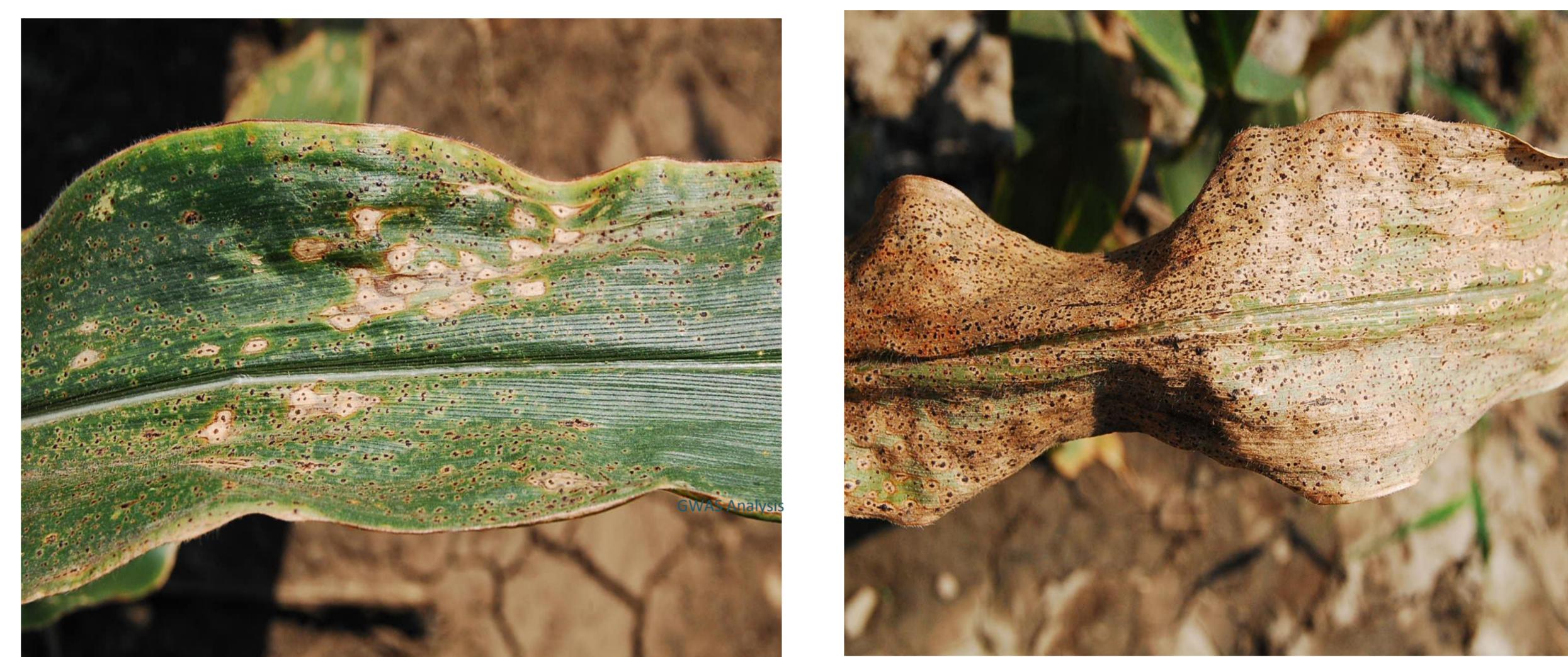


Figure 1. Symptoms of Tar Spot Complex Disease of maize.

## GWAS Analysis

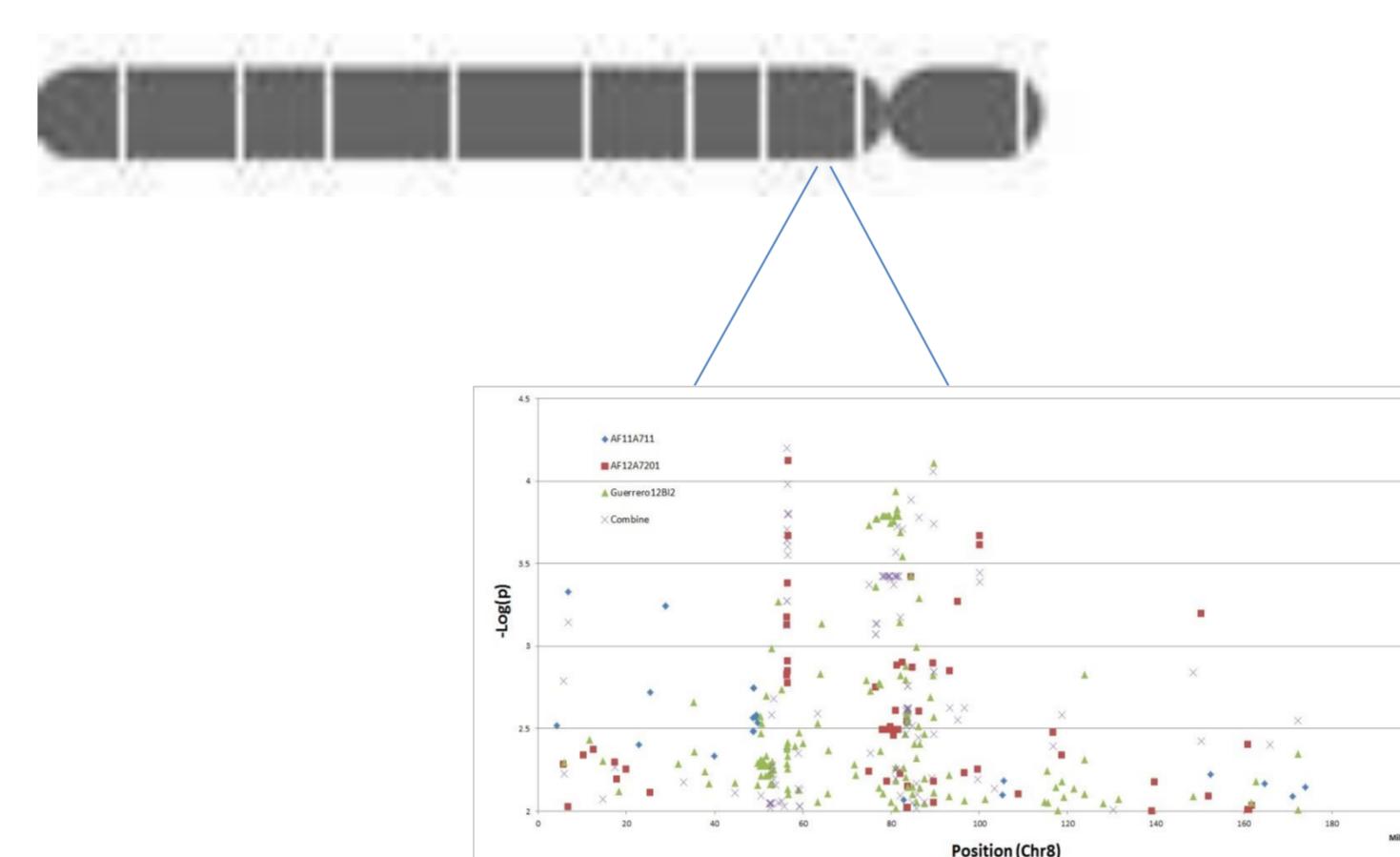


Figure 2. qRtsc8-1 located between PHM11114\_7 and PZA03135\_1 on chromosome 8 using GWAS analysis.

## AF14A BC1 population

Population	Markers on Chromosome (Order by physical map position)	Allele Count	Mean	P-value	R2
BC146-5	1 1 1 1 1 1 1 1 1 N 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1	51	3.24	9.22E-07 0.24
BC146-6	1 1	2	38	4.24	
BC146-7-8	1 1	1	48	4.17	3.74E-04 0.13
BC146-9	1 1	2	43	4.72	
Bc146-10	1 1	1	94	3.17	1.06E-10 0.21
BC146-9	1 1	2	88	4.05	
BC146-11-12	1 1	1	43	3.49	2.74E-02 0.05
BC146-16	1 1	2	48	3.96	
BC146-17-18	1 1	1	40	3.06	6.09E-05 0.17
BC146-20	1 1	2	50	3.50	
BC146-11	1 1	1	169	2.82	2.78E-11 0.12
BC146-17	1 1	2	183	3.13	
BC146-18	1 1	1	40	2.74	8.55E-09 0.33
BC146-19	1 1	2	44	3.35	
BC146-20	1 1	1	68	3.24	0.28 0.01
BC146-20	1 1	2	66	3.30	
BC146-20	1 1	1	48	3.44	2.30E-06 0.22
BC146-20	1 1	2	44	4.27	

Figure 3. Summary of GWAS (red icon), bi-parental (yellow icon), and fine mapping of BC1 population BLUE ICON), AF14A, to located the position of qRtsc8-1.

## AF15A Fine Mapping

Name	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24	M25	
AF15A-BC146-1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
AF15A-BC146-2	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
AF15A-BC146-17	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
AF15A-BC146-18	2	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
AF15A-BC146-3-4	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
AF15A-BC146-5	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
AF15A-BC17-1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
AF15A-BC17-3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
AF15A-BC146-12	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
AF15A-BC146-20	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
AF15A-BC146-9	2	2	2	1	1	1	1	1	1	1	N	N	2	R	40/28	0.52	4.0E-09	41.0%								
AF15A-BC146-13	2	2	2	1	1	1	1	1	1	1	2	2	2	R	42/40	0.45	2.5E-10	39.6%								
AF15A-BC146-21	2	2	2	2	2	2	1	1	1	1	1	1	S	27/25	0.03	0.74	0.2%									

Figure 4. Fine mapping in BC5, AF15A, showing the genotypes of informative individuals from populations segregating for resistance. The best estimate for the location of qRtsc8-1 is indicated. AF14A is summarized for reference.

## RESULTS AND DISCUSSION

GWAS results are shown in Figure 2. GWAS data placed qRtsc8-1 between markers PHM11114\_7 (indicated as red M8 in Figure 3) and PZA03135\_1 (red M9 in Figure 3), a 29.6M bp window. Twenty markers on the reference map were polymorphic between the two parents and converted to KASP assays for the first round of fine mapping. In the BC1 generation, AF14A, overlapping donor chromosomal segments indicated the location of qRtsc8-1 between SNP markers PZA00379\_2 (blue M7 in Fig.3.) and PHM3978\_104 (blue M10 Fig.3.) Our best estimate using all data sources placed qRtsc8-1 between M8 and PZA02683\_1 (black box in Fig. 3) a 19.9M BP window. In BC3 AF15A, using the selected recombinant population from BC2, we performed similar analysis of phenotypic and genotypic data allowing us to narrow the location of qRtsc8-1 between SNP markers MD1 and MD2, a 5M bp window. Based on all the evidence our best estimate of qRtsc8-1 is a 2M bp window closest to SNP marker MD2. The BC3 alignment is shown in expanded view in Figure 4 and maintains the color scheme of Figure 3. This region is near the centromere on chromosome 8 and there is reduced recombination slowing the progress of fine mapping. BC5 is currently in the field with a larger population to capture more recombinants, and will be genotyped and screened for resistance to fine map qRtsc8-1 to a smaller window.

## CONCLUSIONS

We have confirmed qRtsc8-1 was a major QTL for TSC resistance, which can explain around 40% of the phenotypic variation. QTL qRtsc8-1 is located on chromosome 8 in the centromere region, within a 5Mb interval. Our best estimate places qRtsc8-1 in a 2Mb regions. We increased the population size of BC5 to compensate for reduced recombination near the centromeric region where qRtsc8-1 is located.

## ACKNOWLEDGEMENTS

The research was supported by funding from the project 'MasAgro Maiz', part of 'Modernización Sustentable de la Agricultura Tradicional (MasAgro)', funded by SAGARPA.

