Evaluating Teosinte Introgressions for Resistance to Gray Leaf Spot and Southern Leaf Blight

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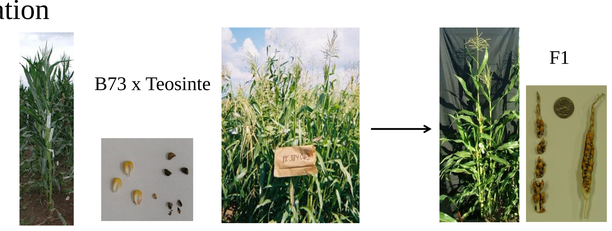
Introduction

- Two important foliar maize diseases in the US:
  - Gray Leaf Spot [(GLS), causal agent *Cercospora zeae-maydis*]
  - Southern Leaf Blight [(SLB), casual agent *Cochliobolus heterostrophus*]
  - Control methods include: using resistant cultivars, crop rotation, and conventional tillage
- Geneticists and breeders are mining germplasm for novel resistance alleles to these two diseases
  - Many studies have been published identifying chromosome regions of interest
  - Gene pool within temperate maize is relatively narrow due to genetic bottlenecks during domestication and modern breeding
- Teosinte is the wild progenitor of maize, using it as the donor parent we can bring back some of the original diversity
- Teosinte easily forms hybrids with current maize inbreds (as pictured below) allowing the exploration of its' potential utilization



- Screening teosinte introgressions in near-isogenic lines (NILs) allows us to explore small chromosomal segments in isolation
- The objectives of this research are to identify alleles from teosinte conferring resistance to each disease through:
  - Field screening
  - Identifying regions of interest using the genotypic data
  - Validating potential resistant QTL

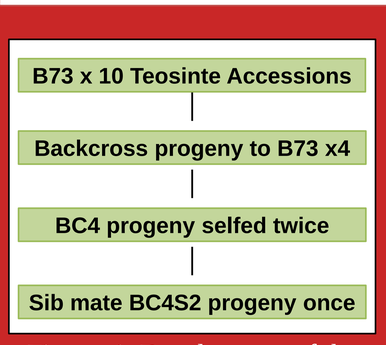


Figure 1. Development of the Ten BC4S2i NIL populations

Figure 2. Location of Teosinte Accessions parents

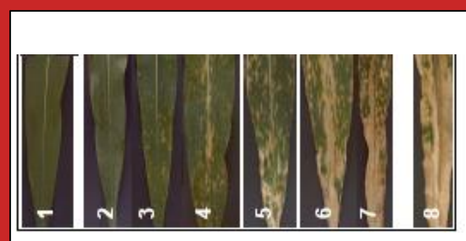


Figure 3. Score given based on ear leaf phenotype

Materials and Methods

- 775 teosinte (*Zea mays* ssp. *parviglumis)* NILs were developed by the USDA-ARS; Columbia, MO (Figure 1) by crossing 10 different teosinte accessions, collected from six areas in Mexico (Figure 2), to the maize inbred B73 (susceptible)
- Data on days to anthesis (DTA) and two-three disease scores per season (Figure 3) were collected over 2 years
- A Weighted mean disease (WMD) score was calculated (Figure 4 & 5)
- LSMEANs were obtained for WMD and DTA and all possible pairwise comparisons between the LSMEANs for WMD
- Entry Mean Heritability for each disease was calculated with the random effects of years, reps, and genotype
- Genotypic data provided by USDA-ARS; Columbia, MO
  - GoldenGate (Illumina) assay of 768 single nucleotide polymorphism (SNP) markers, 728 were informative
- A joint population stepwise regression analysis was used to determine the significant markers for each disease and DTA, using their respective LSMEANs

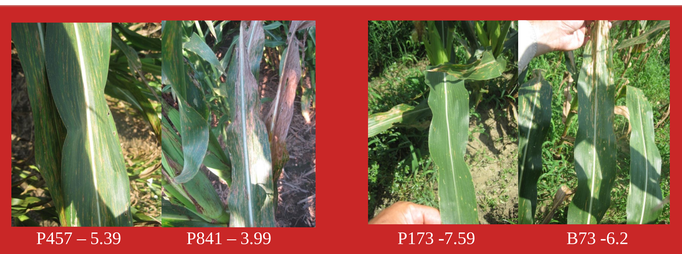


Figure 4 and 5. GLS (left) and SLB (right) WMD rating of line

Results

- The LSMEANs for GLS ranged from 2.5-6.1 (B73 = 4; Fig 6)
- 161/692 NILs for GLS were significantly different from B73
- The LSMEANs for SLB ranged 4.5-7.6 (B73 = 6.2; Fig 7)
- 144/774 NILs for SLB were significantly different from B73
- Entry Mean Heritability was 0.79 for GLS and 0.66 for SLB

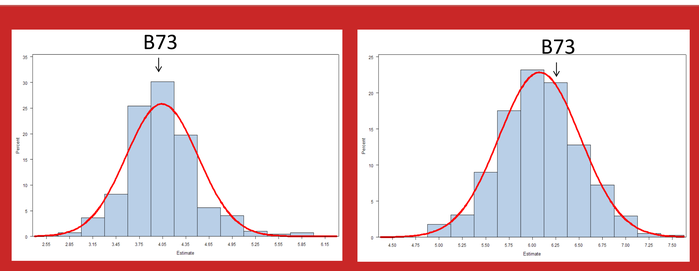


Figure 6 and 7. Distribution of GLS (left) and SLB (right) LSMEANs

- Four significant regions were identified for SLB and six for GLS
  - The effect of one allele substitution was estimated
  - Popns selected with a significant effect (Tables 1 & 2)
  - Lines within each of those popns were selected to further validate each region
- Six regions of interest were identified for DTA
  - One overlapped with SLB on Chrom 8 but not with GLS

Table 1. Effect of one allele substitution for SLB by region and popn.(with a line that was significantly different to B73)

Region/Popn	Z029	Z030	Z031	Z032	Z033	Z034	Z035	Z036	Z037	Z038
Chr2/cM67.9		0.31		0.3		0.28		0.37		
Chr3/cM50.2					0.29		0.38	0.52	0.35	
Chr3/cM70.6				-0.42	-0.37		-0.6	-0.3		
Chr8/cM66.9				0.48	0.42			0.44		

Table 2. Effect of one allele substitution for GLS by region and popn.(with a line that was significantly different to B73)

Region/Popn	Z029	Z030	Z031	Z032	Z033	Z034	Z035	Z036	Z037
Chr2/cM67.9		0.33		0.24		0.43		0.79	
Chr3/cM90.1									1.04
Chr4/cM81.7		0.5			0.92	0.53	0.61	0.45	
Chr5/cM47					-1.12				-0.36
Chr8/cM76.1				0.51	-0.61				
Chr9/cM44.5	0.39				0.57	0.68	1.06	0.45	0.85

Ongoing Research

- 2% of the NILs were selected to further validate the regions of interest associated with SLB resistance
- 4% of the NILS were selected to further validate the regions of interest associated with GLS resistance
- $F_{2:3}$ families are being developed by:
  - Crossing the selected NILs to B73
  - Selfing two generations and harvesting individual F_2 ears
- Field evaluations of each $F_{2:3}$ family occurred/will occur in:
  - 2011 (SLB-3 families, GLS-3 families)
  - 2012 (SLB and GLS-5 families)
  - 2013 (SLB-5 families, GLS-10 families)
- Leaf samples from each row pooled for DNA extraction
- Genotyping each $F_{2:3}$ family with KASPar SNP markers
- Single Marker analysis to validate association (Table 3)

Table 3. Validation of marker association with disease reduction in $F_{2:3}$ families

Trait	Family	Region	Parent score and class	$F_{2:3}$ Class Means	Significant Effect Estimate	Single Marker
SLB	Z035	C3/50.2cM	B73-5.61 (2) NIL-6.91 (0)	0-6.28 1-5.85 2-5.66	Add: 0.31 Dom: -0.12	R^2: 0.48
SLB	Z030	C2/62.2cM	B73-5.61 (0) NIL-6.67 (2)	0-5.74 1-5.98 2-6.09	Add: 0.18	R^2: 0.26
GLS	Z033	C4/90.7cM	B73-4.57 (2) NIL-5.84 (0)	0-5.28 1-5.08 2-4.93	Add:0.18	R^2: 0.06
GLS	Z036	C4/89.1cM	B73- 4.57 (2) NIL-6.64 (0)	0-6.09 1-5.83 2-5.58	Add: 0.25	R^2: 0.17
GLS	Z036	C9/42.8cM	B73-4.57 (2) NIL-6.64 (0)	0-6.01 1-6.01 2-5.66	Add: 0.18 Dom: 0.17	R^2: 0.19

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