

## 2. REPORT

### CALIFORNIA LETTUCE RESEARCH PROGRAM

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#### ISOLATE MAINTENANCE AND INOCULUM PRODUCTION IN SUPPORT OF THE *SCLEROTINIA* RESISTANCE BREEDING PROGRAM IN LETTUCE

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## ABSTRACT

Lettuce drop caused by the fungal pathogen *Sclerotinia* is a serious disease of lettuce. The use of genetic resistance as part of an integrated disease management strategy should provide a significant economic advantage in mitigating yield loss. As lettuce drop resistance is often confounded with premature bolting, previous attempts of identifying resistance were largely unsuccessful. Recently, a high level of lettuce drop resistance was identified in the slow-bolting cultivar Eruption. One hundred sixty recombinant inbred lines (RILs) were evaluated for lettuce drop resistance and bolting in a field infested with *S. minor*; genotyped by sequencing identified 840 single nucleotide polymorphism markers. Our study using the RIL population indicated that lettuce drop resistance in Eruption is quantitatively inherited; more than one gene with a minor role is involved. The association of disease reaction in the field with genetic markers identified one quantitative resistance loci (QTLs) on each of lettuce chromosomes 1 and 5, explaining 12% and 12-25% of the variation, respectively. Bolting QTLs at these locations do not have a significant effect on resistance.

## OBJECTIVES:

- A. Continue to evaluate breeding lines for *Sclerotinia minor* resistance both in the field and in the greenhouse.
- B. Develop a greenhouse screening technique to rapidly advance breeding for lettuce drop resistance.

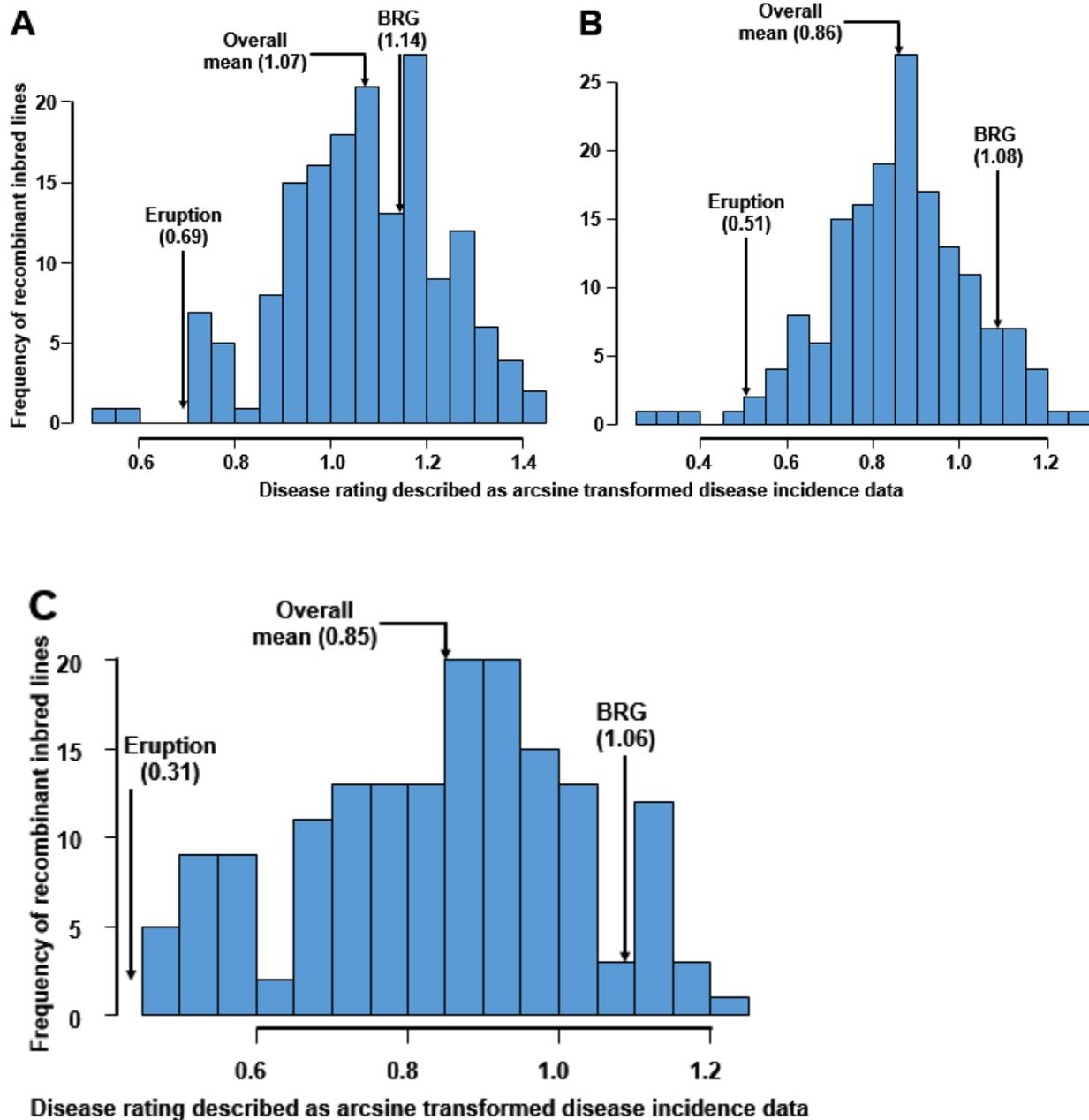
**PROCEDURES:** The RIL population and parents were evaluated for lettuce drop (LD) incidence and bolting in a field artificially infested with *S. minor* at the USDA Salinas research station in three seasons between 2016 and 2017. Tissue was collected from the 162 RIL population and parents, DNA extracted, and subjected to genotyping by sequencing (GBS) to develop genetic markers; single nucleotide polymorphism markers (SNPs) were generated. A genetic map was constructed using the SNP data. QTL mapping was conducted using the phenotypes (disease incidence and bolting) and the genetic map datasets.

**RESULTS AND DISCUSSION:** The lettuce drop datasets from the three experiments had good positive correlations (Table 1); RILs with low disease incidence had similar low disease levels in all the three experiments and vice versa. Lettuce drop and bolting were negatively associated (Table 1), indicating that fast bolting lines had relatively lower disease incidence. The two parents exhibited distinct levels of lettuce drop incidence. The RIL population showed a continuous phenotypic variation in reaction to lettuce drop (Figure 1); the disease rating scores ranged from 0.53 to 1.45, with a mean of 1.07 in Spring 2016 (Figure 1A). In Fall 2016, the RILs had disease ratings ranging from 0.29 to 1.30 with a mean of 0.86 (Figure 1B). The rating scores ranged from 0.45 to 1.21, with a mean of 0.85 in Spring 2017 (Figure 1C). These phenotypic distributions in the mapping population are typical of quantitative traits, indicating that Eruption has quantitatively inherited resistance genes against lettuce drop.

**Table 1.** Correlation coefficients for lettuce drop disease incidence and rate of bolting in the Reine des Glaces x Eruption recombinant inbred lines evaluated in three experiments (LD16.1, LD16.2 and LD17.1) in Salinas, CA, in 2016 and 2017

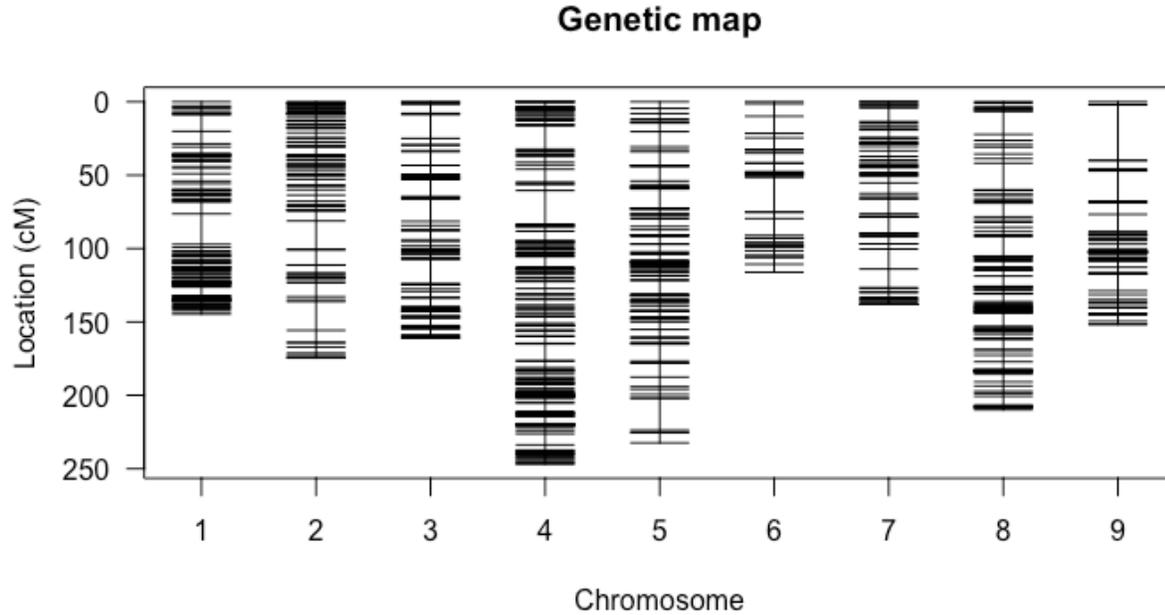
Phenotype	Drop LD16.1	Drop LD16.2	Drop LD17.1	Bolting LD16.1	Bolting LD16.2	Bolting LD17.1
Drop incidence (LD16.1)	-					
Drop incidence (LD16.2)	0.589***	-				
Drop incidence (LD17.1)	0.589***	0.650***	-			
Rate of bolting (LD16.1)	-0.298***	-0.256***	0.325***	-		
Rate of bolting (LD16.2)	-0.294***	-0.246**	0.260***	0.668***	-	
Rate of bolting (LD17.1)	-0.243**	-0.286***	0.269***	0.697***	0.634***	-

\*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; - perfect/self-correlation



**Figure 1.** Distribution of disease rating of Reine des Glaces x Eruption recombinant inbred lines and parents in *Sclerotinia minor*-infested field experiment (A) Spring 2016, (B) Fall 2016, and (C) Spring 2017

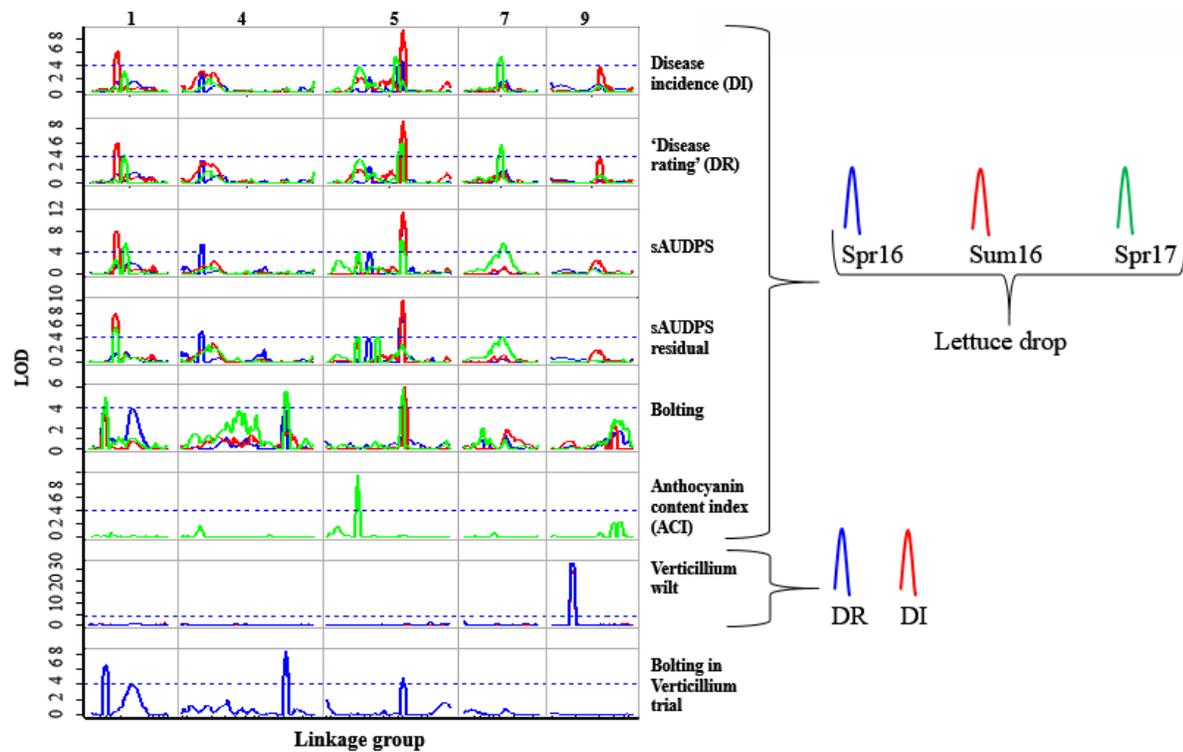
To determine the genetic basis of resistance, it was important to develop genetic markers. SNP markers were developed by analyzing the GBS data. The GBS analysis identified 840 SNPs. A genetic map was constructed using the SNP data. The SNP markers mapped to the nine linkage groups of lettuce with a good coverage (Figure 2).



**Figure 2.** Illustration of 840 single nucleotide polymorphism markers along the nine linkage groups of lettuce. Individual SNP markers are represented by a single horizontal line (marker not shown for brevity).

Mapping the genetic basis of lettuce drop resistance and determining its relationship with bolting required associating the genetic map information with disease and bolting data obtained from the field experiments through QTL mapping. The QTL mapping analysis indicated that at least two genes with small effects are responsible for lettuce drop resistance in Eruption. The genes are located on lettuce linkage groups 1 and 5 (Figure 3) and explain 9-12% and 11-25% of the variation in lettuce drop, respectively (30-41% total). Two additional QTL were also identified on linkage groups 4 and 7 (Figure 3), explaining ~9% and 11% of the variation in lettuce drop, respectively.

**Relationship between lettuce drop and bolting.** As indicated above, lettuce drop and the rate of bolting were negatively associated, confirming that fast bolting lines had relatively lower disease incidence. However, bolting has little effect on lettuce drop resistance at the resistance QTL on linkage groups 1 and 5. Likewise, the two other lettuce drop resistance QTL on linkage groups 4 and 7 were located where no bolting QTL was identified (Figure 3), indicating that bolting has little effect on drop resistance in these genomic regions. Eruption is dark red in color due to anthocyanin pigmentation. Genetic analysis identified a gene underlying anthocyanin content at a different location from resistance QTL on linkage group 5 (Figure 3), indicating that lettuce drop resistance is not associated with anthocyanin pigmentation in Eruption. Thus, Eruption could be used in developing both green and red lettuce types with lettuce drop resistance. Eruption also has complete (100%) resistance to *Verticillium* wilt (race 1). Genetic analysis identified the *Vr1* (*Verticillium* resistance 1) gene, that was mapped previously. Genetic markers identified would be useful in the marker-assisted transfer of lettuce drop and *Verticillium* wilt resistance from Eruption to commercial lettuce cultivars. Thus, Eruption can be a stable source of resistance to both lettuce drop and *Verticillium* wilt caused by race 1.



**Figure 3.** Quantitative trait loci (QTL) on linkage groups 1, 4, 5, 7 and 9 for lettuce drop resistance, rate of bolting, anthocyanin content index (ACI) and Verticillium wilt resistance identified in the Reine des Glaces x Eruption recombinant inbred lines. The blue horizontal dashed line corresponds to significant thresholds for each variable listed on the right hand side.