QTL mapping of Sclerotinia basal stalk rot (BSR) resistance in sunflower using genotyping-by-sequencing (GBS) approach

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INTRODUCTION

- Basal stalk rot (BSR) is caused by the fungus *Sclerotinia sclerotiorum*
- It is a serious disease of sunflower in the cool and humid growing environments of the world
- Mycelia grown from germinating sclerotia causes root infection (unique to sunflower)
- The genetics of BSR resistance is complex and conditioned by multiple genes, each having a small effect
- No major gene has been identified that confers complete resistance against *S. sclerotiorum*
- Resistance breeding relies on incorporating genetic factors from various partially-tolerant breeding lines
- Little is known about the quantitative trait loci (QTL) mapping for Sclerotinia BSR in sunflower

GOAL OF THE PROJECT

Overall objective

- To improve Sclerotinia disease resistance in the cultivated sunflower

Specific objectives

- QTL mapping of Sclerotinia BSR resistance using high throughput SNP marker resource
- Identify QTL associated with Sclerotinia BSR resistance in an RIL population derived from the cross HA 441/RHA 439
- Identify novel QTL associated with Sclerotinia BSR resistance in an AB population derived from the cross HA 89/*H. argophyllus*
- Develop an AB population for novel QTL mapping derived from the cross HA 89/*H. praecox*

MATERIALS & METHODS

Plant Materials

- Parents
  - HA 441 (a maintainer line) and
  - RHA 439 (a restorer line)
- Mapping population
  - 106 F_7-derived Recombinant Inbred Lines (RIL) developed through Single Seed Descent (SSD) method from the cross HA 441/RHA 439

Genotyping

- Genotype-by-sequencing (GBS) technology was used for simultaneous discovery and genotyping of large numbers of single nucleotide polymorphism (SNP) markers
- GBS was conducted at the Genomic Diversity facility of Cornell University
- A total of 1,236 good quality polymorphic SNP markers were obtained from the GBS protocol for linkage mapping

MATERIALS & METHODS (CONT….)

Stalk rot evaluation

- Environments
  - 2012: Carrington, ND and Crookston, MN
  - 2013: Crookston, MN
  - 2014: Carrington, ND and Grandin, ND
- Field design
  - Randomized complete block design (RCBD)
  - Two replications each for 2012 and 2013 field trials
  - Four replications each for 2014 field trials
- Field inoculation
  - *S. sclerotiorum* mycelia grown on millet and 80 gm of inoculums deposited in furrows next to the rows of sunflower lines at V-6 growth stage
- Disease incidence (DI) scoring
  - Percent of plants showing BSR lesion

MATERIALS & METHODS (CONT….)

Linkage Mapping

- JoinMap 4.1 software was used for linkage analysis
- Maximum likelihood (ML) algorithm was used to determine marker order
- Final genetic map was constructed with 1,049 SNP markers placed on 17 linkage groups of sunflower genome

QTL mapping

- QTL analysis was conducted separately for each environment and also using integrated data across environments
- Composite interval mapping (CIM) option of WinQTL Cartographer v2.5 was used for QTL mapping
- QTL analysis results were confirmed using PLABQTL v1.2

RESULTS

Table 1. Analysis of variance (ANOVA) of BSR disease incidence (DI) for HA441/RHA 439 RIL population evaluated in five environments

Component	df	Variance estimate	Confidence limit (0.05)		F/Z value	Pr > F/Z
			lower	upper		
Env	4	-	-	-	164.7	<.0001
Rep (Env)	9	σ^2_r = 13.19	5.42	66.09	1.71	0.0439
Genotype	107	σ^2_g = 161.81	119.21	232.30	5.92	<.0001
Genotype x Env	428	σ^2_{ge} = 61.15	39.35	107.90	3.95	<.0001
Error	963	σ^2_e = 345.73	315.86	380.07		

Analysis was performed across all five environments using PROC MIXED of SAS version 9.3. All factors were treated as random effects except environment

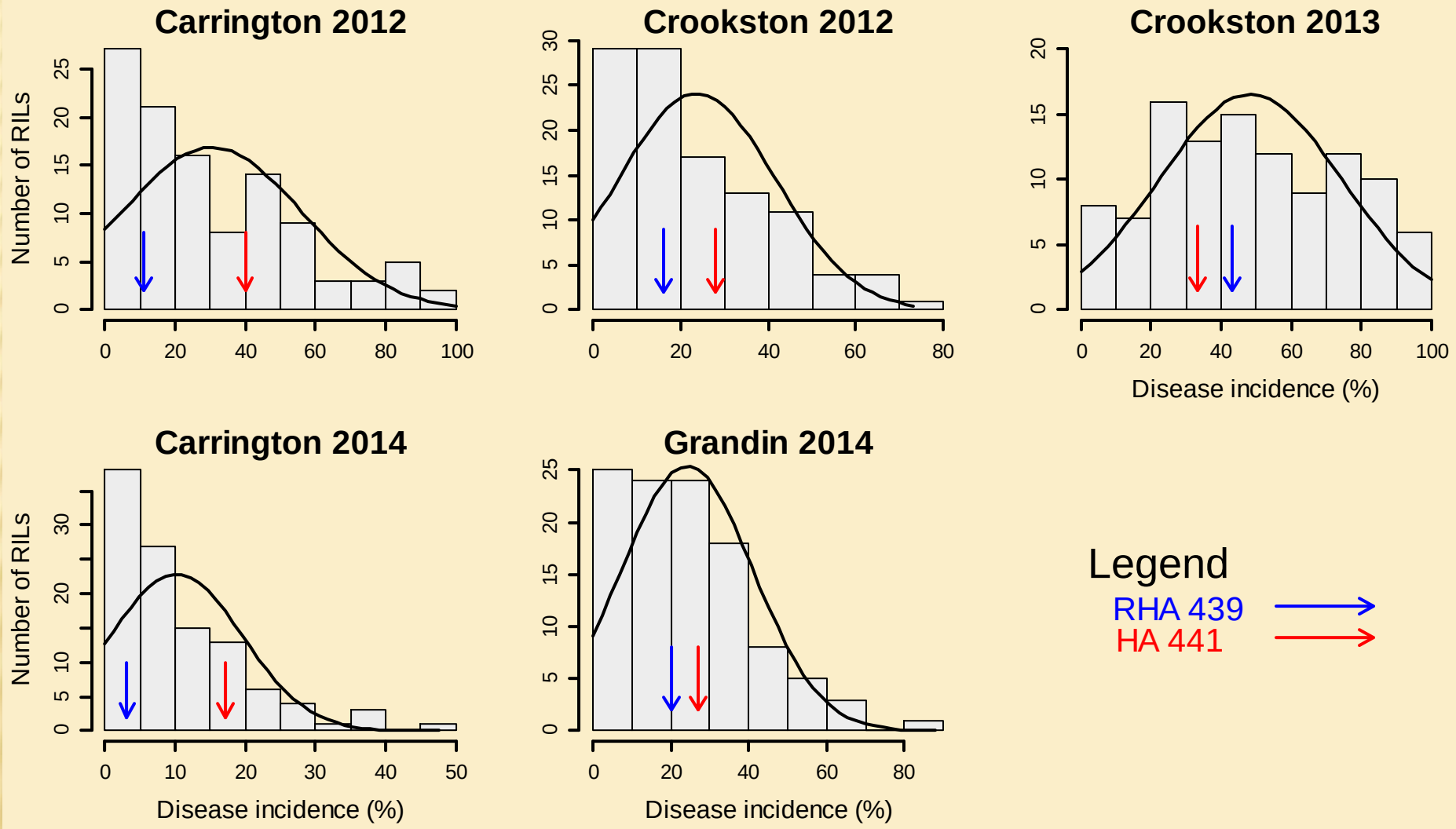


Fig. 1. Frequency distributions of BSR disease incidence (DI) in the HA441/RHA439 RIL population evaluated in five environments

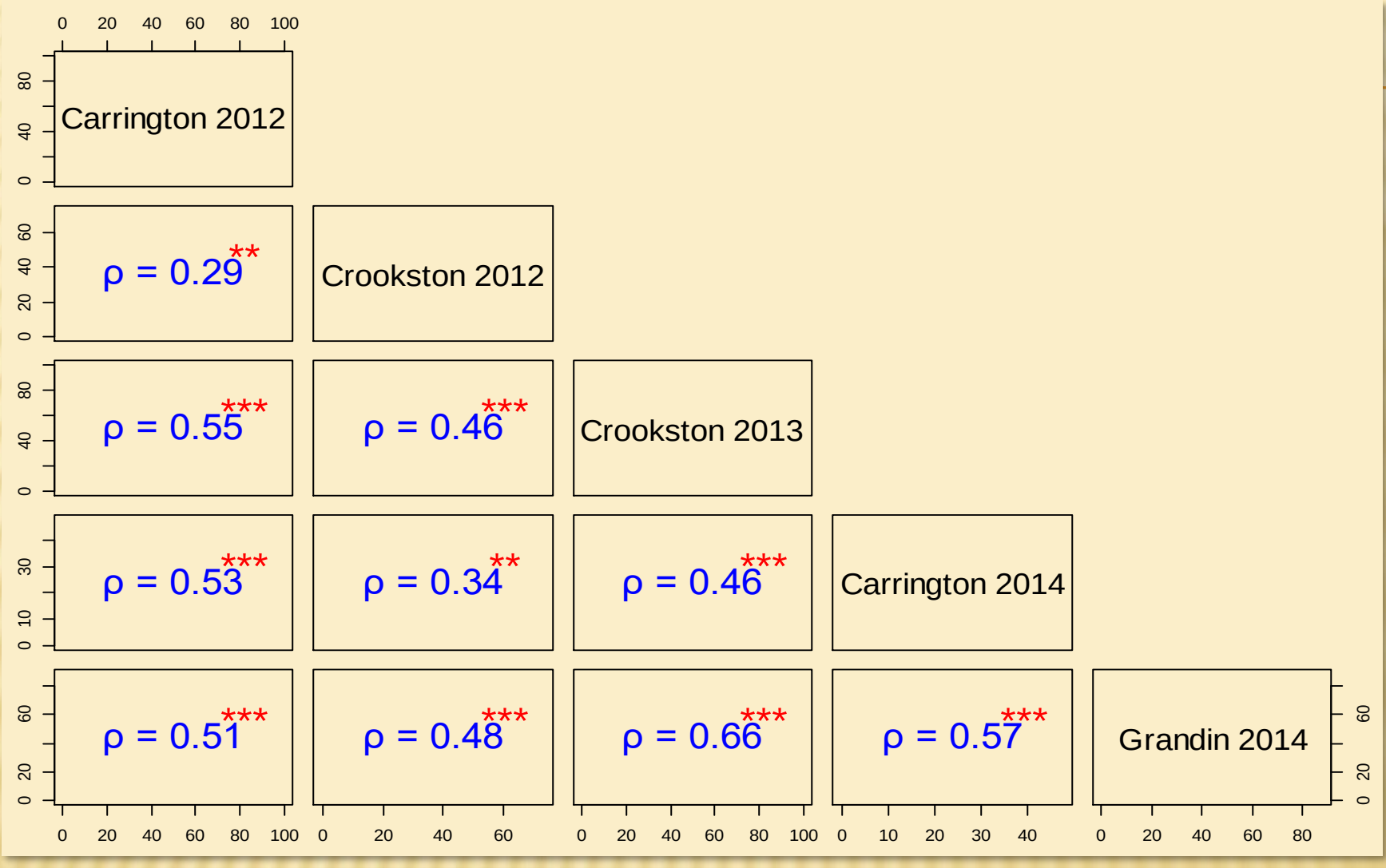


Fig. 2. Spearman's rank correlation of BSR disease incidence (DI) among environments evaluated for HA 441/RHA 439 RIL population

RESULTS (CONT.....)

Table 2. Significant Sclerotinia BSR resistance QTL identified in the HA 441/RHA 439 RIL sunflower population

QTL	LG	Peak position (cM)	Flanking markers		R^2	1-LOD interval
			Left	Right		
Qbsr-4.1	4	32.0	S4_147688288	S4_135190076	6.4	6.5
Qbsr-9.1	9	45.0	S9_153762438	S9_158145790	9.3	1.5
Qbsr-10.1	10	66.5	S10_288646223	S10_281294015	31.6	1.6
Qbsr-11.1	11	83.2	S14_148877201	S14_148877253	7.7	3.5
Qbsr-16.1	16	87.3	S16_157591485	S16_137964301	10.5	10.4
Qbsr-17.1	17	42.5	S17_228661362	S17_238998311	14.6	11.5

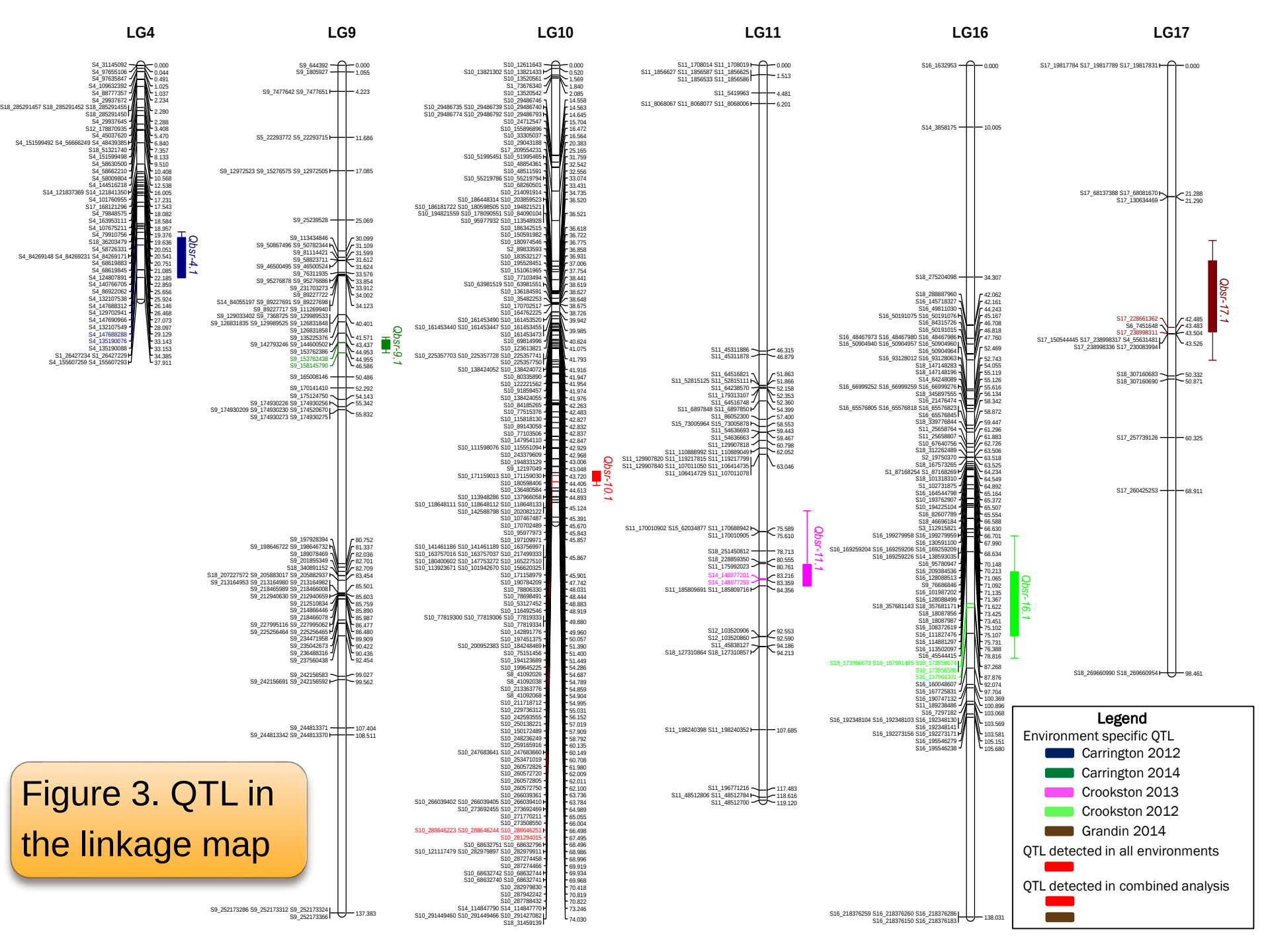


Figure 3. QTL in the linkage map

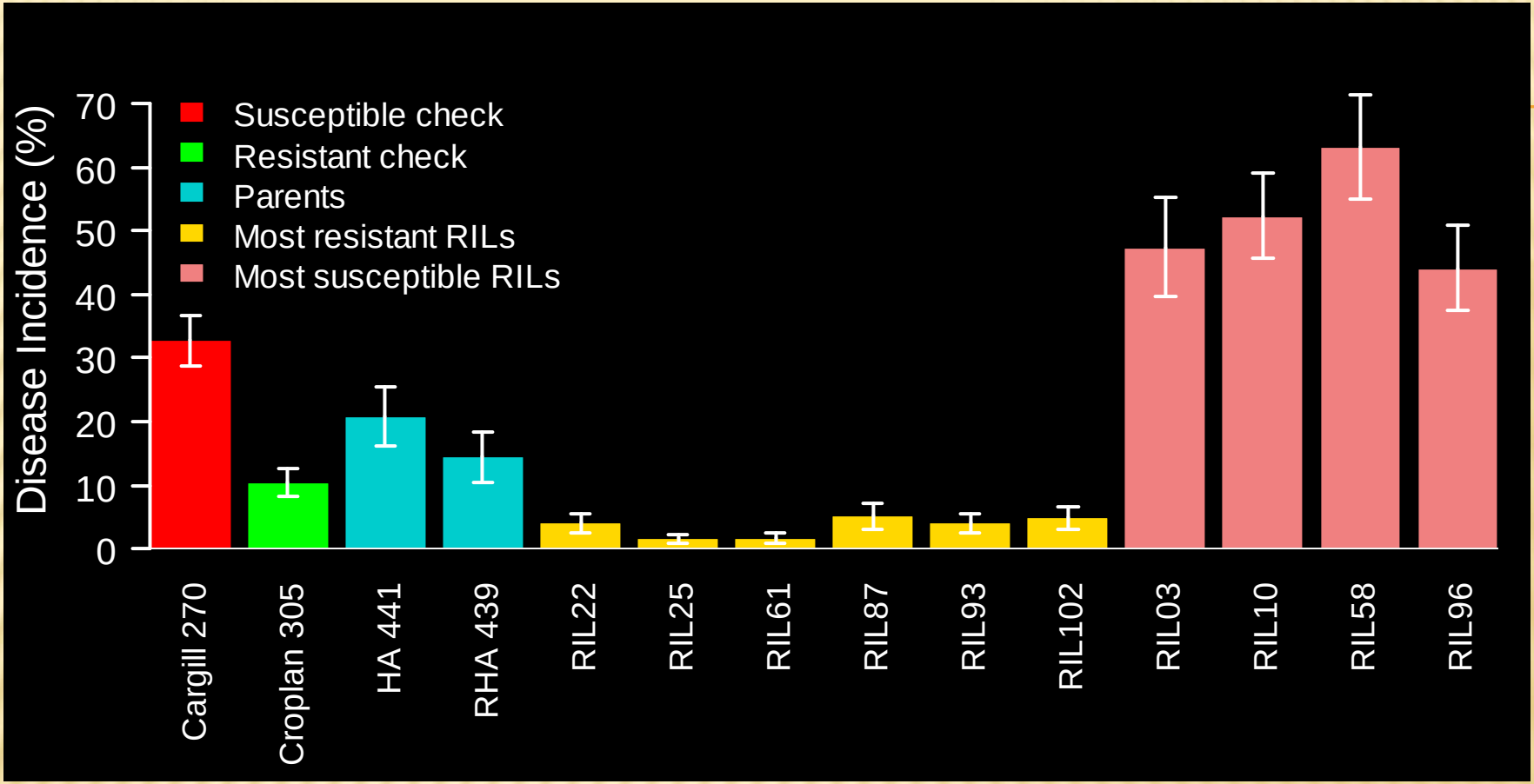


Fig. 4. Mean BSR disease incidence (DI) of the most resistant and most susceptible RILs in seven environments during 2012-2015

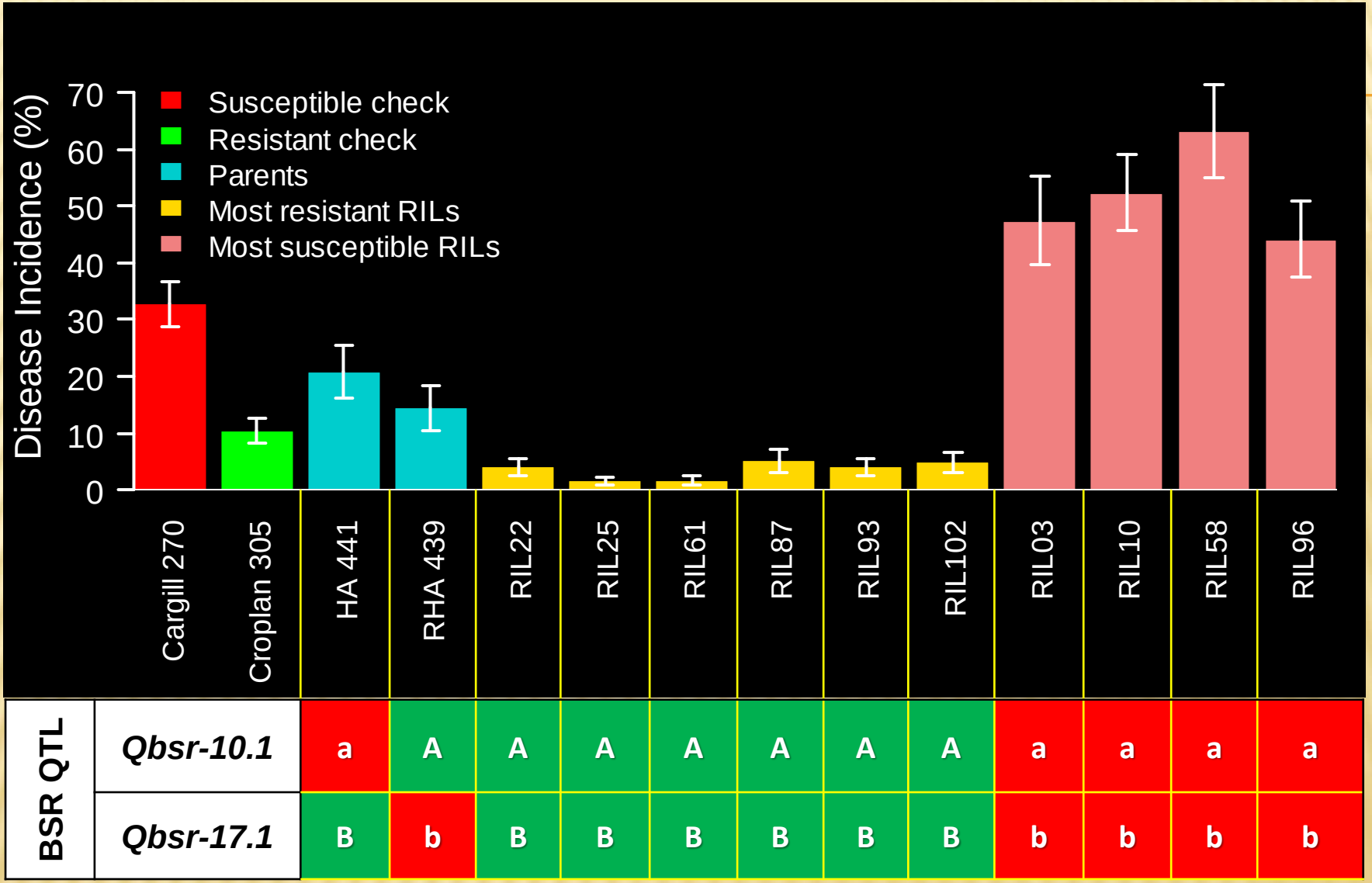
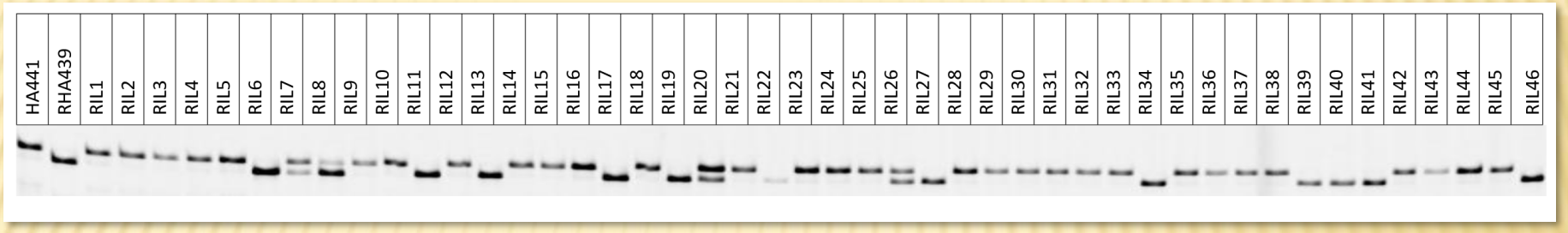


Fig. 5. QTL allele distribution in the parents and in the RILs

Conversion of flanking SNPs into AS-PCR primers

SNP marker: S10_288646223



SNP marker: S10_281294015

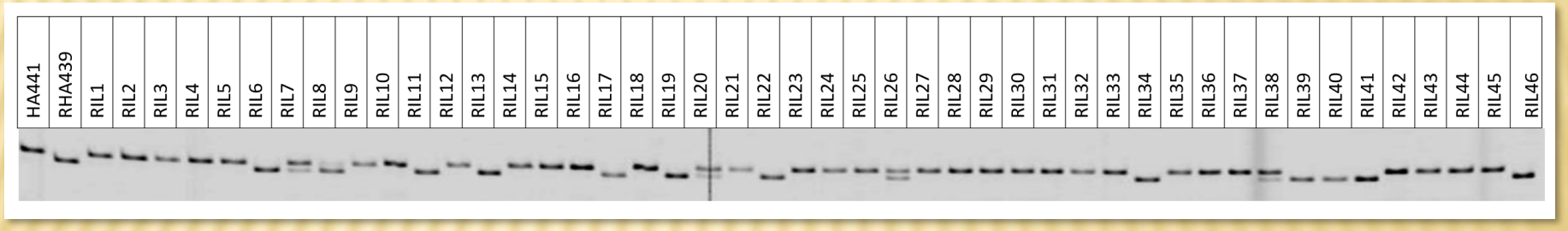


Fig. 6. PCR gel picture of two SNP markers linked to the BSR resistance QTL amplified using allele-specific primers designed for length polymorphism

FUTURE PLAN

Conduct 2nd year field evaluation of HA 89/*H. argophyllus* advanced backcross (AB)-QTL population for BSR resistance

Conduct 1st year field evaluation of HA 89/*H. praecox* AB-QTL population for BSR resistance

Genotyping and linkage map construction of AB-QTL population derived from the cross of HA 89/*H. argophyllus*

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