Sclerotinia stalk rot resistance in sunflower: Introgression of resistance from wild annual species and QTL mapping of resistance in cultivated sunflower

Zahirul Talukder[1], Yunming Long[1], Thomas Gulya[2], Charles Block[3], Gerald Seiler[2], Lili Qi[2]

[1]Department of Plant Sciences, NDSU

[2]USDA-ARS Northern Crop Science Laboratory, Fargo, ND

[3]USDA-ARS, Plant Introduction Station, Ames, IA

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INTRODUCTION

Sclerotinia sclerotiorum causes two serious diseases in sunflower

Stalk rot, incited by root infection (unique to sunflower)

Head rot, caused by airborne ascospores

Genetics of resistance is different for the two diseases

Resistance is polygenic, no major resistant gene is known

Resistance breeding relies on incorporating genetic factors from various partially-tolerant breeding lines

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INTRODUCTION…CONT.

Wild annual sunflower species are valuable sources of Sclerotinia resistance

Stalk rot resistance was identified in wild annual species *H. argophyllus*, *H. debilis*, *H. praecox*, and *H. petiolaris*

Wild annual species were selected to transfer Sclerotinia stalk rot resistance into cultivated sunflower

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GOAL OF THE PROJECT

Overall objective

- To improve Sclerotinia stalk rot resistance in the cultivated sunflower

Specific objectives

- Pre-breed novel Sclerotinia resistance from wild annual species (*H. argophyllus, H. praecox,* and *H. petiolaris*) into cultivated sunflower
- Investigate inheritance of Sclerotinia resistance in introgressed lines
- QTL mapping of Sclerotinia stalk rot resistance using high throughput SNP marker resource

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Introgression of Sclerotinia resistance from wild species

2009 NMS HA 89 × *H. petiolaris*, *H. argophyllus*, *H. debilis*, and *H. praecox*

↓

2010 HA 458 × F_1

↓

2010 HA 89 × BC_1

↓

2011 BC_2F_1

↓

2011-12 BC_2F_2

↓

2012-13 Resistant BC_2F_3 families

↓

2013-14 Selected BC_2F_4 families with higher levels of resistance

↓

2015 Confirm resistance of BC_2F_5 lines

The individuals in each generation were screened for resistance in the greenhouse

Families or lines were evaluated for resistance in greenhouse and/or field trials

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Table 1. Evaluation of the13 most resistant BC_2F_3 families and derived BC_2F_4 lines for Sclerotinia stalk rot resistance in the field from 2012 to 2014

Resistance donor	Disease incidence (%)				
	2012		2013		2014
	Pedigree	BC_2F_3	Pedigree	BC_2F_4	BC_2F_4
H. petiolaris ssp. *fallax* PI 435843	11-256-049	0	12F-3405-2	4.0	8.4
	11-256-053	0	12F-3406-5	5.6	-
H. argophyllus PI 494573	11-275-037	0	12F-3416-4	9.3	2.9
	11-283-037	0	12F-3424-4	0	10.0
H. praecox ssp. *runyonii* PI 468853	11-291-01	6.6	12F-3438-2	3.1	9.5
	11-291-09	4.5	12F-3442-1	6.7	4.5
	11-291-17	1.7	12F-3443-1	4.2	6.3
	11-291-45	5.3	12F-3451-4	3.9	7.9
	11-291-57	1.5	12F-3456-1	8.3	-
	11-291-65	4.2	12F-3459-1	0	0.7
	11-291-67	2.3	12F-3460-4	0	7.9
	11-292-33	0	12F-3467-1	3.3	0
	11-294-21	3.1	12F-3482-1	3.3	3.3
Susceptible checks	Cargrill 270	34.8		72.6	36.0
	HA 89	23.7		51.6	35.7
Resistant checks	Croplan 305	12.4		34.9	9.6
	HA 441	27.4		28.6	18.3

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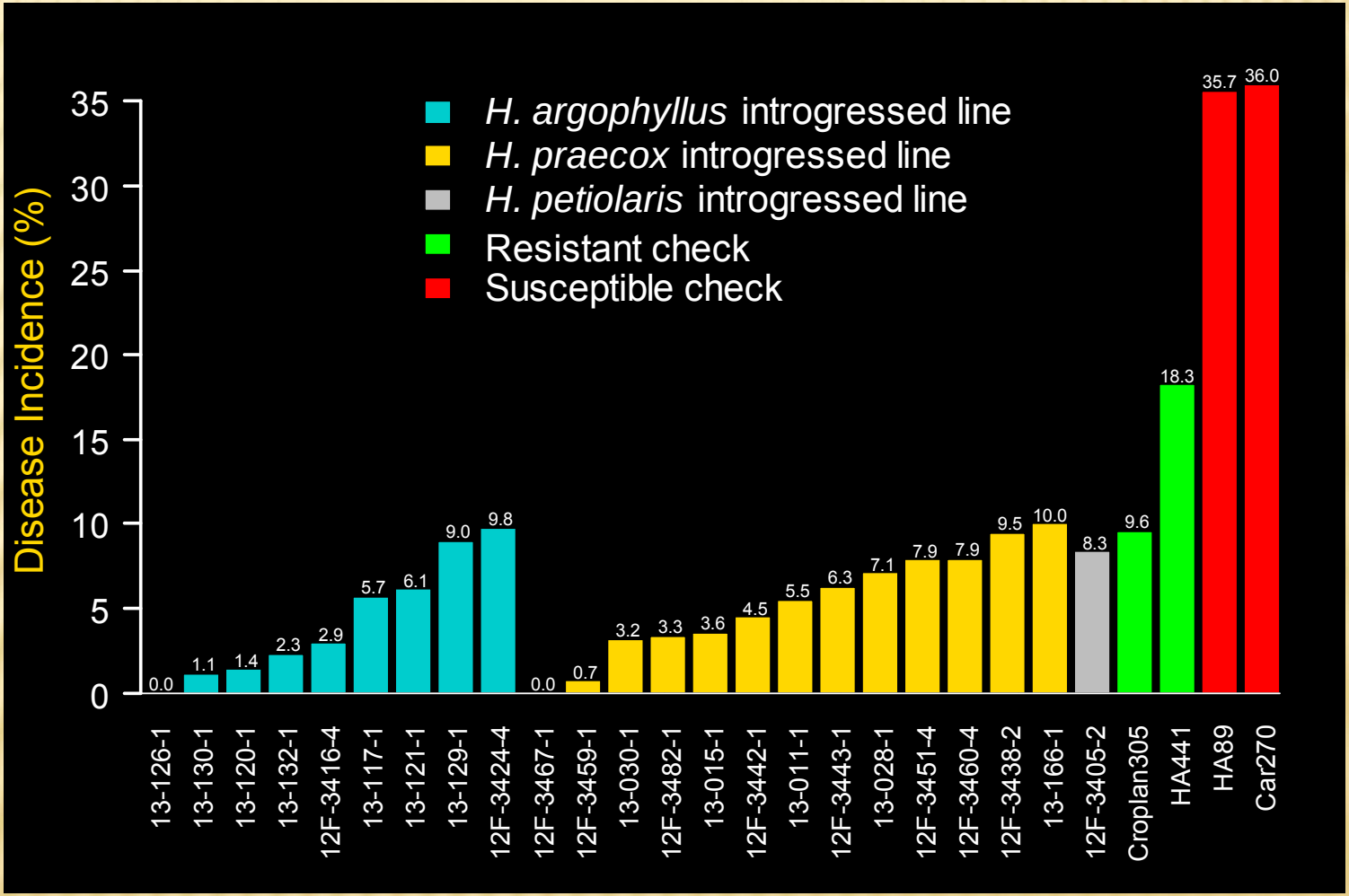
Mean performance of 23 BC_2F_4 families for Sclerotinia stalk rot resistance in two locations of North Dakota during 2014



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QTL mapping of Sclerotinia stalk rot resistance in sunflower

Mapping population

- 106 F_7-derived RILs developed through Single Seed Descent method from a cross between HA 441 (a maintainer line) and RHA 439 (a restorer line)

Table 2. Stalk rot evaluation of HA 441 and RHA 439

Materials	2009 Field		2011 Greenhouse		2013 Field		2014 Field	
	No. of replicas	Disease incidence (%)	No. of replicas	Disease incidence (%)	No. of replicas	Disease incidence (%)	No. of replicas	Disease incidence (%)
HA 89 (S-check)	10	21.0	2	56.3	4	52.7	20	35.7
CAR 270 (S-check)	12	22.0	2	79.2	4	72.5	20	36.0
Croplan 305 (R-check)	12	5.0	2	25.0	4	34.7	20	9.9
HA 441 (Parent)	6	5.43	2	16.7	6	35.2	28	19.4
RHA 439 (Parent)	6	11.48	2	35.1	2	43.1	8	11.7

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QTL mapping of Sclerotinia stalk rot resistance in sunflower

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
  - 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 deposited in furrows next to the rows of sunflower lines (Gulya et al. 2008)
- Disease incidence (DI) scoring:
  - Percent of plants showing wilting and/or basal stem rot lesion

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Phenotypic data analysis

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

Table 3. Analysis of variance (ANOVA) of stalk rot disease incidence (DI) for HA441/RHA 439 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	21.19	<.0001

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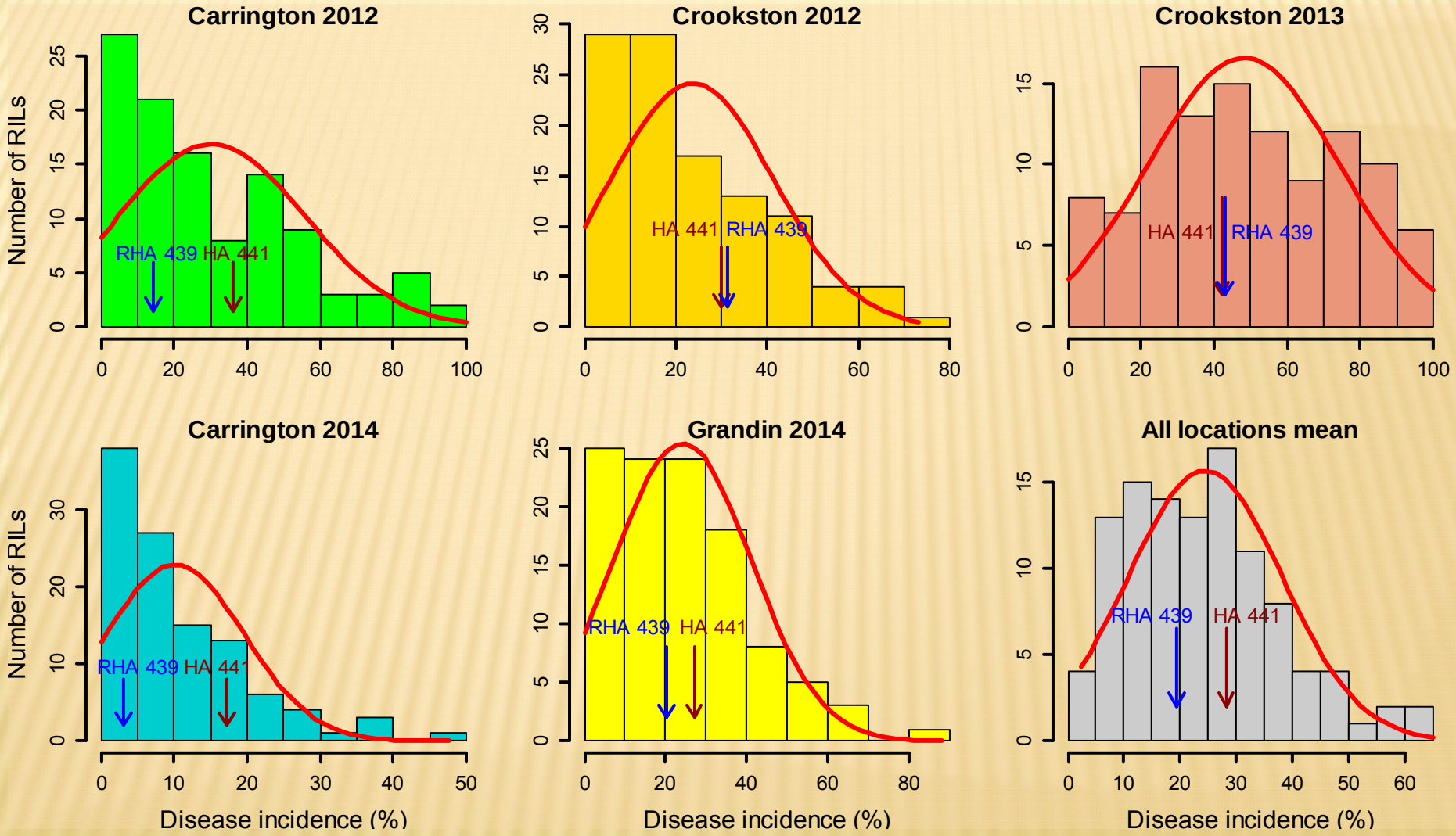
Phenotypic frequency distributions of stalk rot disease incidence (DI) for HA441/RHA 439 population evaluated in five environments



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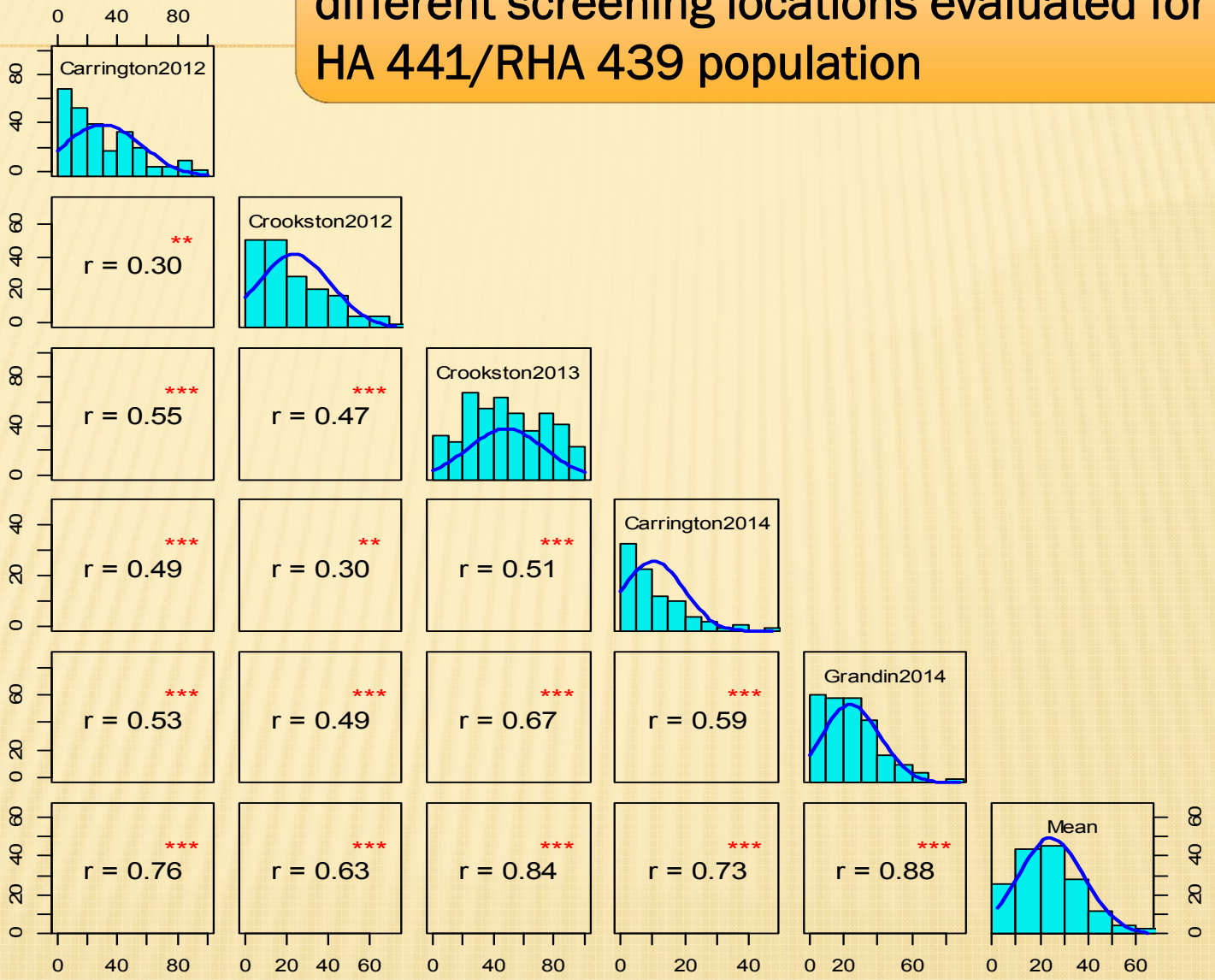
Correlations between stalk rot DI scores among different screening locations evaluated for HA 441/RHA 439 population



QTL mapping of Sclerotinia stalk rot resistance in sunflower

Genotyping of RILs

- 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
- GBS was performed simultaneously using 106 RILs, 140 F_2 progenies, 32 advance breeding lines and six parents of the respective populations
- A total of 21,059 SNP markers were generated from the GBS protocol

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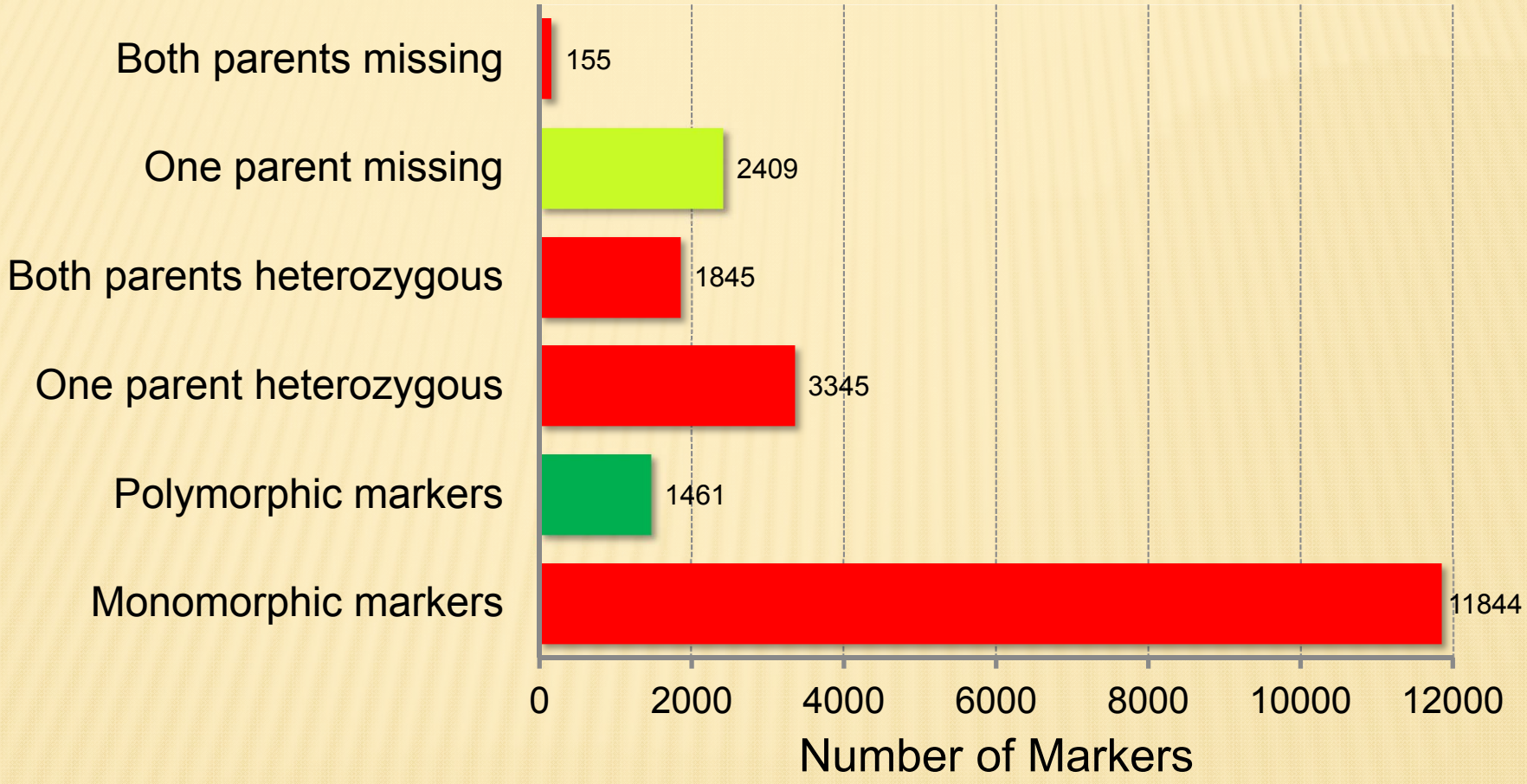
Distribution of SNP markers generated by GBS technology for HA 441/RHA 439 RIL population



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FUTURE PLAN

Complete linkage map construction and QTL mapping in HA 441/RHA 439 population

Field evaluation of stalk rot resistance and QTL mapping in a AB-RIL population derived from a cross of HA 89 and *H. argophyllus*

Genotype stalk rot resistant introgression lines by GBS and identify introgressed wild segments associated with stalk rot resistance

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