2012 Progress for Molecular Mapping of the Downy Mildew Resistance Genes in Sunflower

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Project Objectives (2012-2014)

1. Phenotype an $F_{2:3}$ population of the cross HA 89 × RHA 464 with downy mildew (DM) race 734
2. Investigate inheritance of DM resistance genes in RHA 428, HA 458, and 803-1, and a putative new gene from *H. argophyllus* accession PI 494573
3. Identify SSR markers linked to DM resistance genes in these lines
4. Use SNP markers to saturate the regions where the new DM resistance genes reside

Background

♦ Sunflower downy mildew (DM) is one of the most serious sunflower diseases

♦ A total of 18 DM resistance genes (R-genes) have been reported (*Pl1* to *Pl16*, *Pl21*, and Pl_{ARG})

♦ As of 2010, five North American (NA) races and at least eight new races in France were identified as virulent on Pl_6 and Pl_7 widely used *R*-genes in sunflower production

♦ The USDA-released inbred lines RHA 419/420 and RHA 464 (Pl_{ARG}), RHA 340 (*Pl8*), HA 458, and RHA 428, and TX16R, were found to be downy mildew-resistant to all five of NA new races

Table 1 Progress of molecular mapping of the DM *R*-genes in sunflower

Gene	Linkage group	Source line	R-gene origin	Reference
Pl13	1	HA-R 5		Sujatha et al. 2009
Pl14	1	HA-R 4		Bachlava et al. 2011
Pl16	1	HA-R 4		Liu et al. 2012
Pl_{ARG}	1	RHA 419	*H. argophyllus*	Duβle et al. 2004
Pl1	8	RHA 266, RHA 274	Wild *H. annuus*	Mouzeyar et al. 1995
Pl2	8	AMES 3235, PI 497250, RHA 274	Wild *H. annuus*	Vear et al. 1997
Pl6	8	HA 335, HA 336	Wild *H. annuus*	Roeckel-Drevet et al. 1996
Pl7	8	HA 337, HA 338, HA 339,	*H.praecox*	Bert et al. 2001
Pl15	8	RNID		De Romano et al. 2010
Pl5	13	RF-S11-5566-74-10, Novinka and Progress	*H.tuberrosus*	Bert et al. 2001
Pl8	13	RHA 340	*H. argophyllus*	Radwan et al. 2003, 2004
Pl21	13	HA61		Vicourt et al. 2012

Table 2 List of materials used in the project

Lines	Genes	Origin of DM resistance
RHA 464	Pl_{ARG}	*H. argophyllus*
HA 458	Unknown	Wild *H. annuus* collected in Idaho
RHA 428	Unknown	Wild *H. annuus* collected in New Mexico
803-1	Unknown	*H. tuberosus*
New cross	Unknown	*H. argophyllus*

The Goals for 2012

1. Phenotype the $F_{2:3}$ population of the cross HA 89 × RHA 464 with race 734 and identify SNP markers linked to Pl_{ARG}

2. Advance F_2 populations of four crosses, HA 234 × RHA 428, HA 234 × HA 458, HA 89 × 803-1, and HA89 × *H. argophyllus* PI 494573, to the F_3 generations

3. Screen the polymorphism between each pair of parents, susceptible and resistant, with 860 previously mapped SSR markers

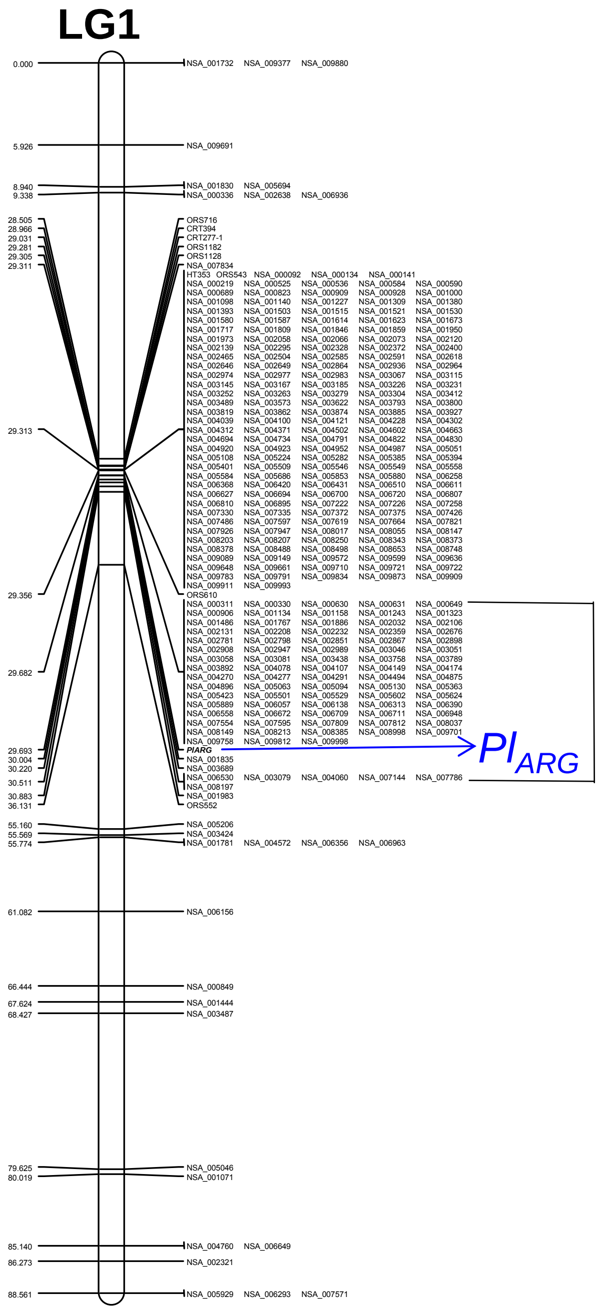
4. Phenotype the $F_{2:3}$ population of the cross HA 458 × HA 234 and perform bulked segregant analysis

Outcomes Achieved in 2012

I. Identification of SNP markers linked to *Pl_{ARG}*

a. Phenotyping of 140 F_3 families (~4, 000 individuals) derived from the cross of HA 89 and RHA 464 with DM race 734

b. Integrating phenotype data with SNP genotype data to identify SNP markers linked to *Pl_{ARG}*



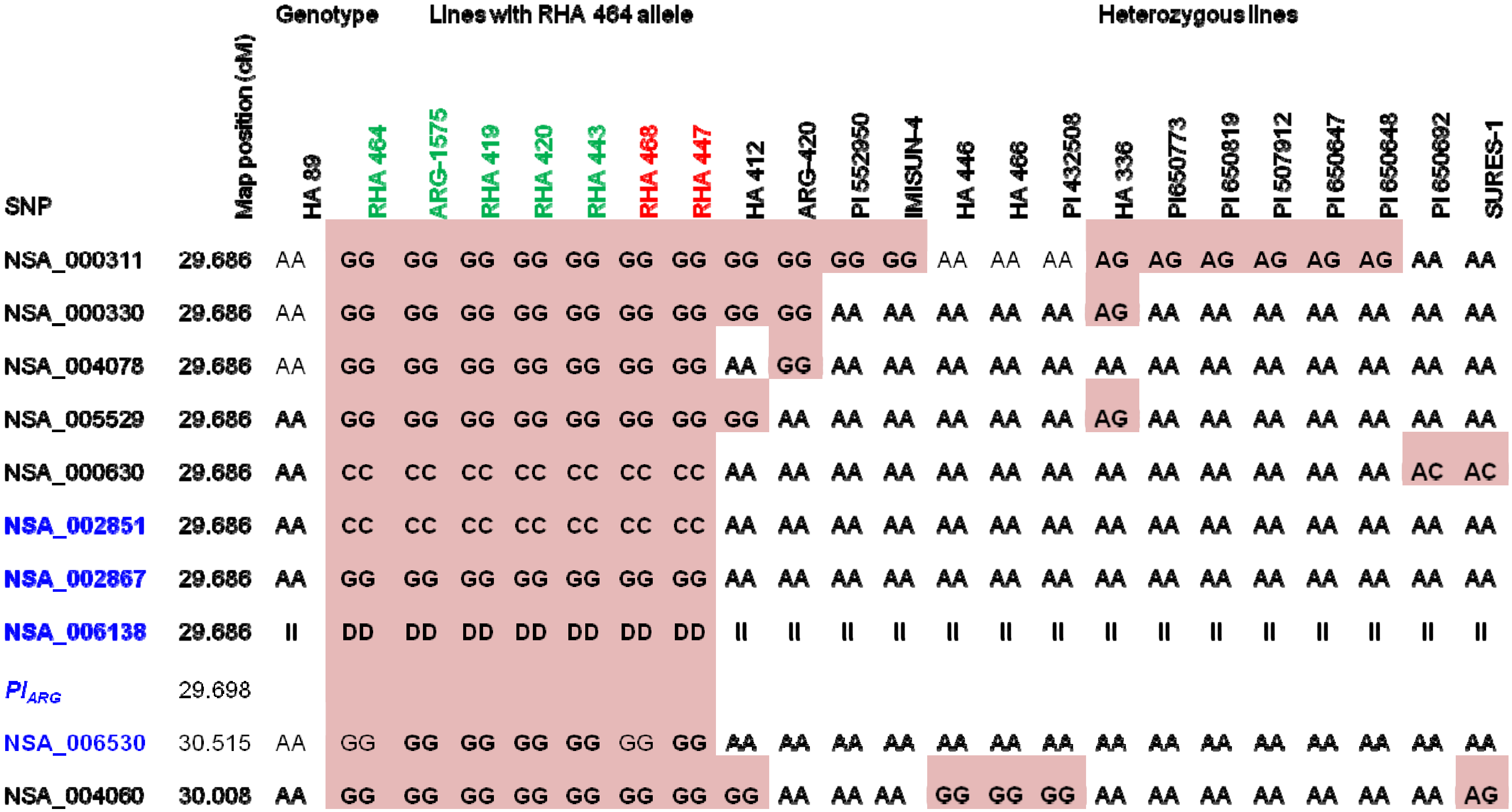
87 SNPs linked to *Pl_{ARG}* within 0.8 cM interval

c. Analysis of genotype data of 550 sunflower lines with 87 SNP markers around Pl_{ARG} to identify SNPs closely linked to Pl_{ARG}

Table 3 The most unique SNP markers linked to Pl_{ARG}

SNP	Map position (cM)	No. lines genotyped	No. lines with data	No. HA 89 allele	No. RHA 464 allele	No. heterozygous
NSA_000311	29.686	550	539	532	11	6
NSA_000330	29.686	550	550	540	9	1
NSA_004078	29.686	550	549	541	8	0
NSA_005529	29.686	550	550	541	8	1
NSA_000630	29.686	550	549	540	7	2
NSA_002851	29.686	550	543	536	7	0
NSA_002867	29.686	550	550	543	7	0
NSA_006138	29.686	550	550	543	7	0
Pl_{ARG}	29.698					
NSA_006530	30.515	550	549	542	7	0
NSA_004060	30.008	550	549	537	11	1

Table 4 Genotype data of the sunflower lines with RHA 464 allele among ten most unique SNPs



		Genotype	Lines with RHA 464 allele														Heterozygous lines							
SNP	**Map position (cM)**	**HA 89**	**RHA 464**	**ARG-1575**	**RHA 419**	**RHA 420**	**RHA 443**	**RHA 468**	**RHA 447**	**HA 412**	**ARG-420**	**PI 552950**	**IMISUN-4**	**HA 446**	**HA 466**	**PI 432508**	**HA 336**	**PI650773**	**PI 650819**	**PI 507912**	**PI 650647**	**PI 650648**	**PI 650692**	**SURES-1**
NSA_000311	29.686	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	AA	AA	AA	AG	AG	AG	AG	AG	AG	AA	AA
NSA_000330	29.686	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AA	AA	AA
NSA_004078	29.686	AA	GG	GG	GG	GG	GG	GG	GG	AA	GG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
NSA_005529	29.686	AA	GG	GG	GG	GG	GG	GG	GG	GG	AA	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AA	AA	AA
NSA_000630	29.686	AA	CC	CC	CC	CC	CC	CC	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AC	AC
NSA_002851	29.686	AA	CC	CC	CC	CC	CC	CC	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
NSA_002867	29.686	AA	GG	GG	GG	GG	GG	GG	GG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
NSA_006138	29.686	II	DD	DD	DD	DD	DD	DD	DD	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II
PI_{ARG}	29.698																							
NSA_006530	30.515	AA	GG	GG	GG	GG	GG	GG	GG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
NSA_004060	30.008	AA	GG	GG	GG	GG	GG	GG	GG	GG	AA	AA	AA	GG	GG	GG	AA	AA	AA	AA	AA	AA	AA	AG

- ♦ PI_{ARG} originates from ARG-1575 (*H. argophyllus*). RHA 464, RHA 419, RHA 420, and RHA 443 all carry the gene PI_{ARG}
- ♦ Four SNP markers, NSA_002851, NSA_002867, NSA_006138, and NSA_006530, were the most unique markers linked to PI_{ARG}

II. Advance of F_2 populations to F_3 generations

HA 458 × HA 234
↓
240 F_2 individuals
↓
211 F_3 families

RHA 428 × HA 234
↓
240 F_2 individuals
↓
151 F_3 families

HA 89 × 803-1
↓
200 F_2 individuals
↓
171 F_3 families

HA 89 × *H. argophyllus* PI 494573
↓
240 BC_1F_2 individuals
↓
169 BC_1F_3 families

III. Screening of polymorphism between each pair of parents, susceptible and resistant, with SSRs

Table 5 Summary of polymorphism between susceptible and resistant parents with SSR markers

Combinations	No. SSR tested	No. polymorphic SSR
HA 89 × *H. argophyllus* PI 494573	860	518 (60%)
HA 89 × 803-1	860	283 (33%)
HA 458 × HA 234	860	361 (42%)
RHA 428 × HA 234	860	342 (40%)

IV. Location of DM *R*-gene in HA 458 to linkage group 4 of sunflower

a. Phenotyping of 188 F_3 families (~5, 600 individuals) derived from the cross of HA 458 and HA 234 with DM race 734

b. Bulked segregant analysis

- ♦ S-bulk: ten homozygous susceptible F_2 plants
- ♦ R-bulk: ten homozygous resistant F_2 plants
- ♦ Screening of 369 polymorphic SSRs between S- and R-bulks
- ♦ Four SSRs from linkage group 4 were found to be associated with DM resistance in HA 458

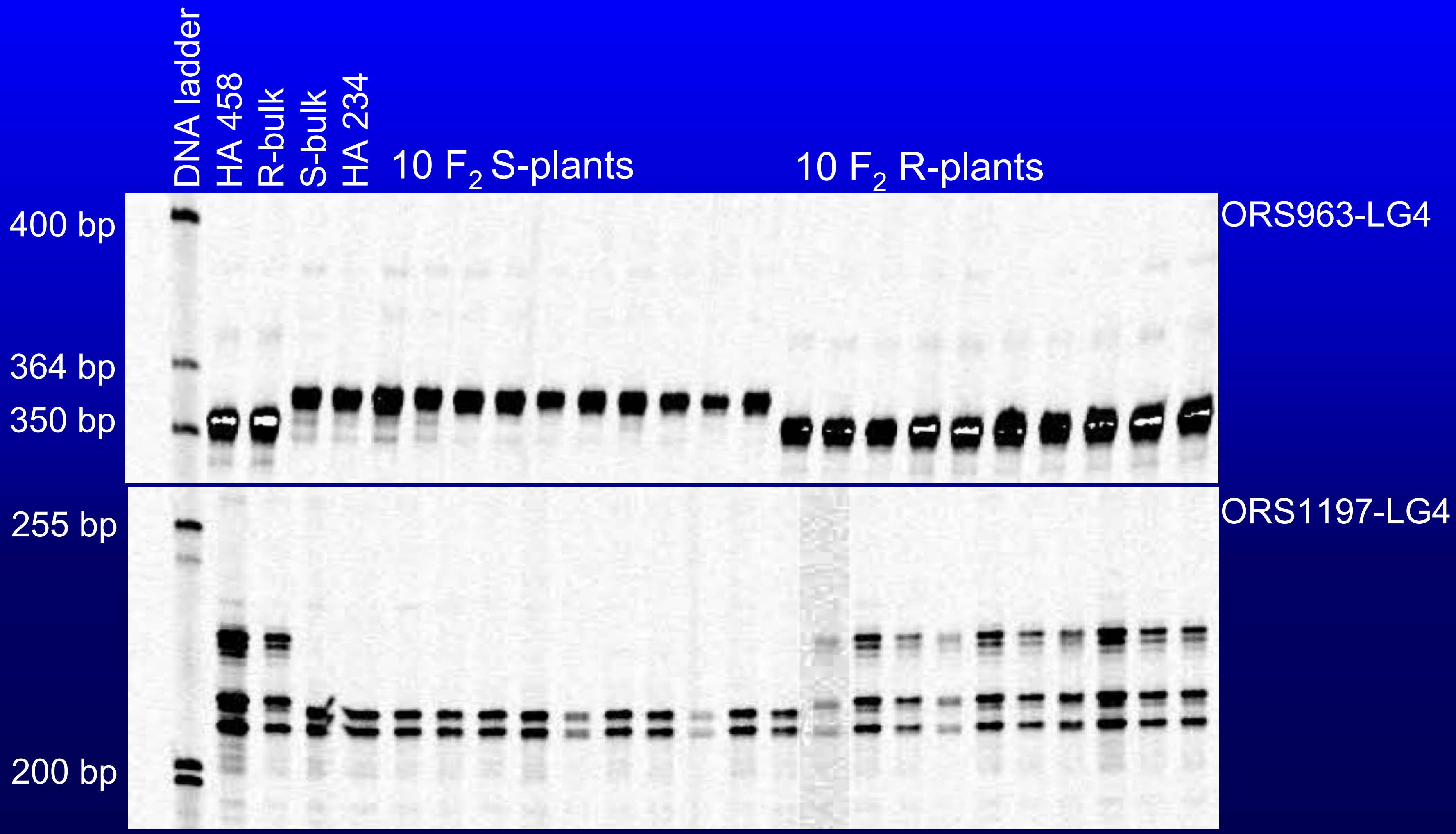


Fig. 1 Bulked segregant analysis. HA 458: resistant parent; HA 234: susceptible parent; R-bulk: 10 homozygous F_2 resistant plants; S-bulk: 10 homozygous F_2 susceptible plants

The Goals for 2013

1. Complete genotyping of 188 F_2 individuals and identify SSR markers linked to DM *R*-gene in HA 458

2. Complete phenotyping of the two F_3 populations of RHA 428/HA 234 and HA 89/PI 494573

3. Mapping DM R-gene in RHA 428

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Questions?