2013 Progress for Molecular Mapping of the Downy Mildew Resistance Genes in Sunflower

Lili Qi[1], Yunming Long[2], Chao-Chien Jan[1],
Michael Foley[1], Thomas Gulya[1], Xiwen Cai[2]
[1]USDA-ARS, Northern Crop Science Laboratory
[2]NDSU, Dept. of Plant Sciences,

Project Objectives (2012-2014)

1. Phenotype $F_{2:3}$ population of the cross HA 89 × RHA 464 with 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 PI494573
3. Identify SSR markers linked to DM resistance genes in RHA 428, HA 458, and 803-1, and to a putative new gene from *H. argophyllus* accession PI 494573
4. Use SNP markers to saturate the regions where the new DM resistance genes reside

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. Mapping new DM R-gene derived from *H. argophyllus* accession PI 494573

3. Complete phenotyping of the two F_3 populations of RHA 428/HA 234 and HA 89/803-1

Mapping DM resistance gene in HA 458

Background

- HA 458 is an oilseed maintainer line resistant to all known DM races. The source of resistance for HA 458 is derived from wild *H. annuus* collected from Idaho

- An F_2 population was developed from the cross of HA 458 with HA 274

DM phenotyping

- A total of 188 F_2-derived F_3 families (30 seedlings per family) were screened with DM race 734
  - 47 homozygous susceptible
  - 90 heterozygous resistant
  - 51 homozygous resistant
- The DM resistance in HA 458 is controlled by a single dominant gene named as Pl_{17}

Marker development

- SSR marker screening of parents
  Among 860 SSR markers screened, 361 (42%) showed polymorphism between two parents

- Bulk segregant analysis was conducted with polymorphic SSRs, and *Pl_{17}* was located on LG4, which is the first DM gene mapped to LG4

- Genotyping of the 188 F_2 individuals with 13 polymorphic SSR markers from LG4 revealed two SSRs flanking *Pl_{17}* at 2.8 and 0.9 cM, respectively

Develop SNP markers linked to Pl_{17}

NSA SNP map	Public SNP map
16 SNPs, 13.9 cM covering Pl_{17}	25 SNPs, 9.2 cM covering Pl_{17}

- A total of 41 SNPs were screened between the two parents
- 15 polymorphic SNPs were genotyped in the F_2 population and integrated with the SSR map
- Two SNP markers flanking Pl_{17} at 2.1 and 1.8 cM, respectively

Mapping a new DM R-gene from *H. argophyllus*

Background

- The accession of *H. argophyllus* PI 494573 was identified to be resistant to downy mildew hot races. The cross was made between HA 89 and *H. argophyllus* PI 494573 in 2009

- The BC_1F_2 and BC_1F_3 populations were developed from this cross

DM phenotyping

- A total of 142 BC_1F_2-derived F_3 families (30 seedlings per family) were screened with DM race 734
  - 42 homozygous susceptible
  - 66 heterozygous resistant
  - 34 homozygous resistant

- The DM resistance derived from *H. argophyllus* is controlled by a single dominant gene named as Pl_{18}

Marker development

- SSR marker screening of parents
  Among 849 SSR markers screened, 427 (50%) showed polymorphism between the two parents

- Bulk segregant analysis was conducted with polymorphic SSRs, and *Pl_{18}* was located on LG2, which is the first DM gene mapped to LG2

- Genotyping of the 142 F_2 individuals with 19 polymorphic SSR markers from LG4 revealed two SSRs flanking *Pl_{18}* at 1.1 and 0.4 cM, respectively

Germplasm development

Table 1. Selection of homozygous resistant lines of BC_2F_3 from the cross of HA 89 and *H. argophyllus* PI 494573

		No. seed	No.	DM score	
Plant No.	Materials	germinated	planted	S	R
	HA335	40	25	25	
	HA 89	40	30	30	
11-261-4	HA89//(NMS HA89 X *H. argophyllus* PI 494573)BC_2F_3	50	30		30
11-261-8		50	21		21
11-261-14		40	20		20
11-261-15		50	24		24
11-261-16		50	30		30
11-264-1		50	30		30
11-264-2		50	30		30
11-264-4		50	25		25

Table 2. Downy mildew tests of the resistant lines using DM hot races collected from 2012 fields

	DM	DM Races		DM Score		DM Races		DM Score	
Lines	R-genes	Races	ID#	S	R	Races	ID#	S	R
Car 272 (S-check)	-	730^+	16	12		734^+	120	10	
RHA 340 (R-check)	Pl_8				16			2	21
HA 89 (S-parent)	-			9				12	
11-261-16/BC_2F_3	**Pl_{18}**				**20**				**26**
Car 272 (S-check)	-	734^+	117	9		770^+	58	12	
RHA 340 (R-check)	Pl_8				22				17
HA 89 (S-parent)	-			10				8	
11-261-16/BC_2F_3	**Pl_{18}**				**20**				**10**
Car 272 (S-check)	-	774^+	41	11		774^+	46	5	
RHA 340 (R-check)	Pl_8				19				33
HA 89 (S-parent)	-			9				9	
11-261-16/BC_2F_3	**Pl_{18}**				**22**				**36**
Car 272 (S-check)	-	774^+	47	8		774^+	130	12	
RHA 340 (R-check)	Pl_8				25			1	21
HA 89 (S-parent)	-			12				11	
11-261-16/BC_2F_3	**Pl_{18}**				**20**				**13**

S: susceptible; R: resistant

Completion of downy mildew phenotyping of two populations

RHA 428

- RHA 428 is an oilseed male-fertility restorer line resistant to downy mildew. The source of resistance for RHA 428 is derived from wild *H. annuus* collected from New Mexico

- An F_2 population was developed from the cross of RHA 428 with HA 274

- A total of 200 F_3 families (~6,000 individuals) were phenotyped with DM hot race 734

803-1

- The line 803-1 has been used as one of the nine standard differential lines to identify DM races. The DM resistance in 803-1 originated from *H. tuberosus*

- An F_2 population was developed from the cross of HA 89 with 803-1

- A total of 170 F_3 families (~5,100 individuals) were phenotyped with DM hot race 734

The Goals for 2014

1. Develop SNP markers linked to the gene Pl_{18}

2. Complete mapping of DM resistance genes in RHA 428 and 803-1, respectively

Acknowledgment

Angelia Hogness
Cheryl Huckle
Cullen Walser
Emily Wentzel

National Sunflower Association for financial support

Thank you