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

Lili Qi¹, Yunming Long², Chao-Chien Jan¹, Michael Foley¹, Thomas Gulya¹, Xiwen Cai²
¹USDA-ARS, Northern Crop Science Laboratory
²NDSU, 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 F₂ 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
16 SNPs, 13.9 cM covering $Pl_{17}$

Public SNP map
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$_1$F$_2$ and BC$_1$F$_3$ populations were developed from this cross.
DM phenotyping

● A total of 142 BC$_1$F$_2$-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 $P_{l18}$
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
## Table 1. Selection of homozygous resistant lines of BC$_2$F$_3$ from the cross of HA 89 and *H. argophyllus* PI 494573

<table>
<thead>
<tr>
<th>Plant No.</th>
<th>Materials</th>
<th>No. seed germinated</th>
<th>No. planted</th>
<th>DM score</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA335</td>
<td></td>
<td>40</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>HA 89</td>
<td></td>
<td>40</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>11-261-4</td>
<td>HA89//(NMS HA89 X <em>H. argophyllus</em> PI 494573)BC$_2$F$_3$</td>
<td>50</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>11-261-8</td>
<td></td>
<td>50</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>11-261-14</td>
<td></td>
<td>40</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>11-261-15</td>
<td></td>
<td>50</td>
<td>24</td>
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<td>11-261-16</td>
<td></td>
<td>50</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>11-264-1</td>
<td></td>
<td>50</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>11-264-2</td>
<td></td>
<td>50</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>11-264-4</td>
<td></td>
<td>50</td>
<td>25</td>
<td>25</td>
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</tbody>
</table>
Table 2. Downy mildew tests of the resistant lines using DM hot races collected from 2012 fields

<table>
<thead>
<tr>
<th>Lines</th>
<th>DM R-genes</th>
<th>DM Races</th>
<th>DM Score</th>
<th>DM Races</th>
<th>DM Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Car 272 (S-check)</td>
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<td>730+</td>
<td>16</td>
<td>12</td>
<td>734+</td>
</tr>
<tr>
<td>RHA 340 (R-check)</td>
<td>Pl8</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HA 89 (S-parent)</td>
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<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-261-16/BC2F3</td>
<td>Pl18</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Car 272 (S-check)</td>
<td>-</td>
<td>734+</td>
<td>117</td>
<td>9</td>
<td>770+</td>
</tr>
<tr>
<td>RHA 340 (R-check)</td>
<td>Pl8</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HA 89 (S-parent)</td>
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<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-261-16/BC2F3</td>
<td>Pl18</td>
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<tr>
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<td>41</td>
<td>11</td>
<td>774+</td>
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<tr>
<td>RHA 340 (R-check)</td>
<td>Pl8</td>
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<td>HA 89 (S-parent)</td>
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<tr>
<td>Car 272 (S-check)</td>
<td>-</td>
<td>774+</td>
<td>47</td>
<td>8</td>
<td>774+</td>
</tr>
<tr>
<td>RHA 340 (R-check)</td>
<td>Pl8</td>
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<td>12</td>
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<tr>
<td>11-261-16/BC2F3</td>
<td>Pl18</td>
<td></td>
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</tr>
</tbody>
</table>

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 F₂ population was developed from the cross of RHA 428 with HA 274

- A total of 200 F₃ families (~6,000 individuals) were phenotyped with DM hot race 734
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 \( P_{18} \)

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

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Thank you