2017 Progress for applying genomic tools to accelerate breeding for disease resistance in confection sunflower

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Outline

- Background knowledge (DM, *Pl*$_{17}$, *Pl*$_{19}$, *etc*.)
- Research objectives
- Research progress
  - **Allelic test of *Pl*$_{17}$ and *Pl*$_{19}$**
  - **Fine Mapping of DM *R*-gene *Pl*$_{17}$**
  - **Saturation mapping of DM *R*-gene *Pl*$_{19}$**
- Future work
- Acknowledgements

Downy mildew

(Photo by Markell and Gong)

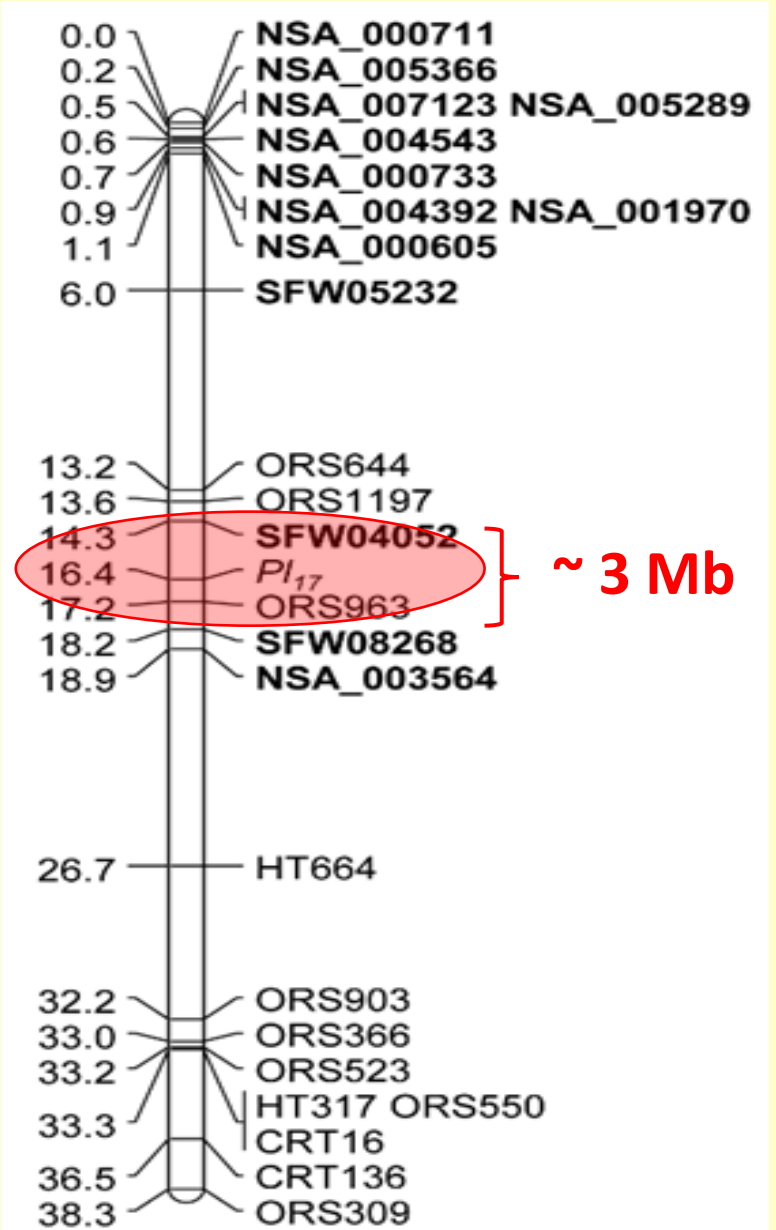
- *Plasmopara halstedii*
- >15% infection in US (2013 and 2015 NSA surveys)
- Development of resistant hybrids is most effective management tool (economic & environmental)

DM threats sunflower production

- New DM races are emerging, making current DM *R*-genes ineffective
- Potential threat to sunflower production
  - Pl_6 was overcome by six new DM races in France 2000-2008
  - Pl_6 was first reported to be overcome by race 734 in U.S. in 2009
  - Pl_6 and Pl_7 were overcome by five DM races in U.S. by 2010
  - Pl_{15} was overcome in Argentina in 2013
  - Gilley (2014-2015) identified only five *R*-genes [Pl_{Arg}, Pl_{15}, Pl_{17}, Pl_{18}, and TX16R (unknown)] were resistant to 185 DM isolates collected in ND, SD, MN, NE
- New DM *R*-gene is continuously needed (Pl_{19}, Pl_{20}, and more)

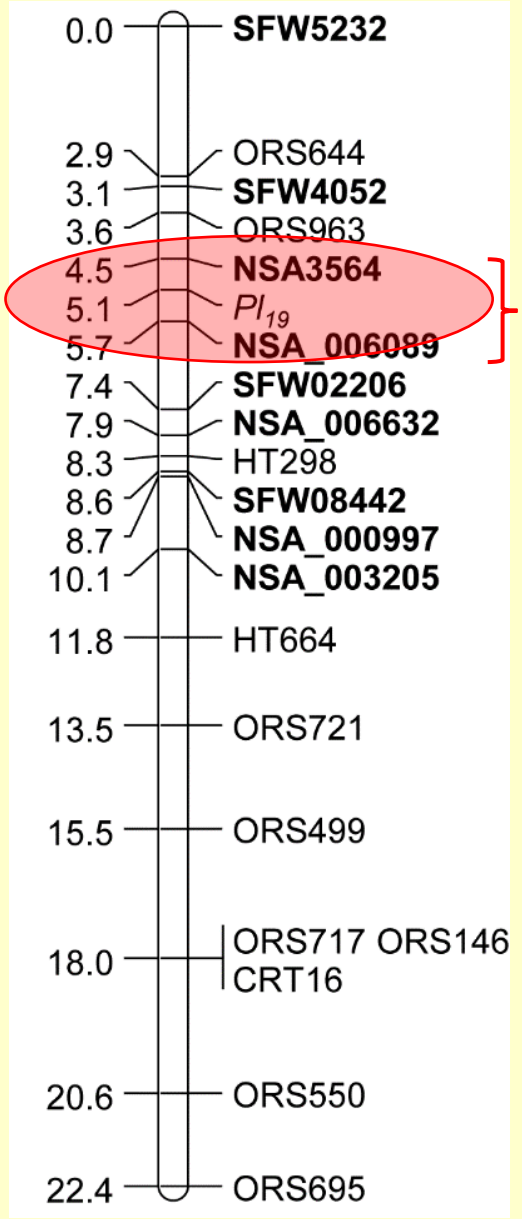
DM *R*-gene PI_{17}

- Qi et al., Theor. Appl. Genet. (2015) 128:757-767
- Identified from HA 458 (USDA inbred line)
- Mapped to LG4 of the sunflower genome (2.9 cM & 3 Mb interval)
- Resistant to all *P. halstedii* races in U.S.
- Transferred to confection sunflower – HA-DM3 (rust and DM resistance)

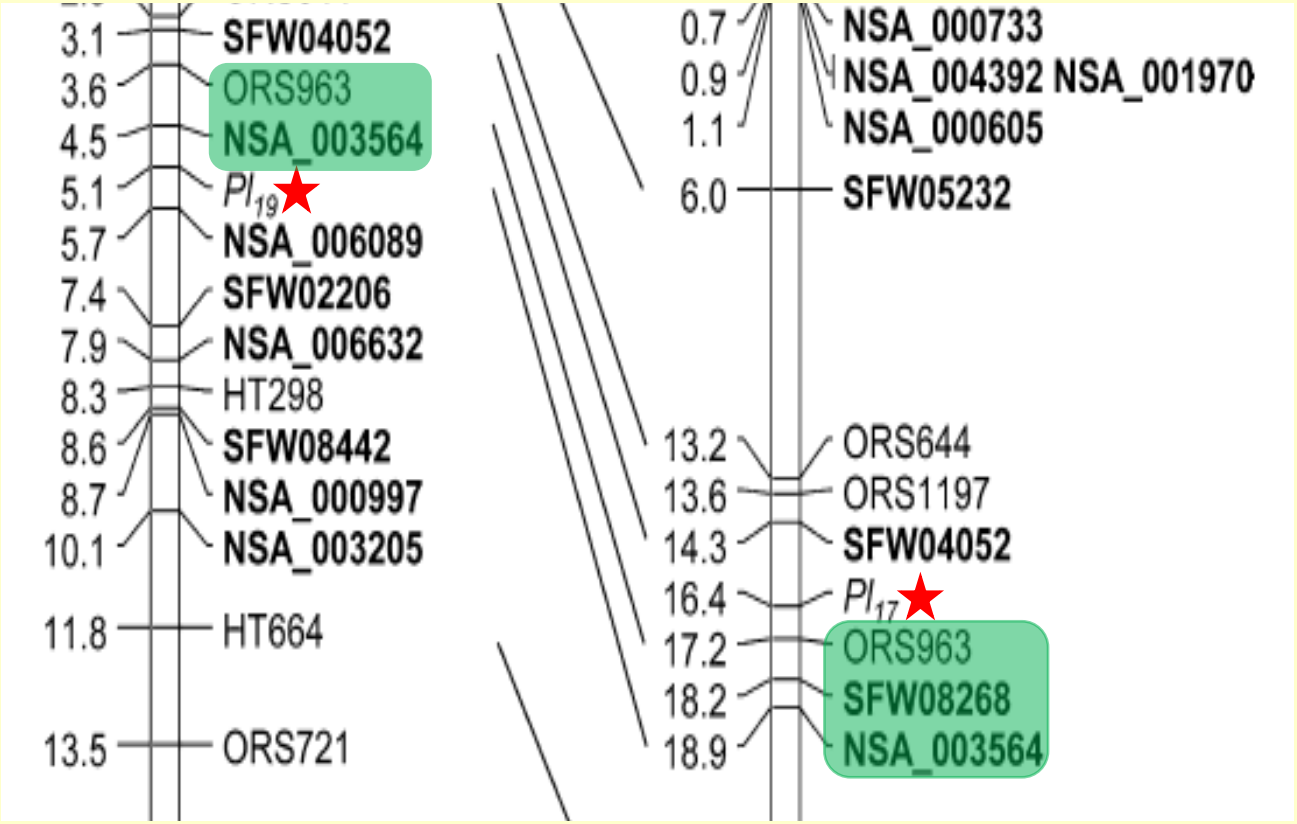


DM *R*-gene *Pl*$_{19}$

- Zhang et al., Theor. Appl. Genet. (2017) 130:29-39
- Identified from wild *Helianthus annuus* PI 435414
- Mapped to LG4 of the sunflower genome (1.2 cM interval)
- Resistant to all *P. halstedii* races in N.A.
- Introduced to confection sunflower
  - HA-DM5



Positions of PI_{17} and PI_{19}



Common markers

SFW05232
ORS644
SFW04052
PI_{17}
ORS963
NSA_003564
PI_{19}
HT664
CRT16
ORS550

~ 3.2 Mb

Why fine mapping of PI_{17} and PI_{19}

- Broad-spectrum DM resistance
- Not used in commercial scale
- Long-term mission: making the *R*-genes easier to use (breeder-friendly markers)
  - ❖ Closer markers (best would be in *R*-gene itself)
  - ❖ More unique markers

Research objectives 2017-2019

- Analyze allelic relationships of the two new DM *R*-genes, Pl_{17} and Pl_{19}
- Conduct high-resolution genetic and physical mapping of Pl_{17}, Pl_{19}, and Pl_{18} (LG2)
- Identify candidate genes of DM resistance
- Develop user-friendly markers of these DM *R*-genes

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Allelic test of *Pl_{17} and Pl_{19}*

HA 458 (*Pl_{17}*) × HA-DM5 (*Pl_{19}*)

↓

F_1 Marker Test

↓ ⊗

F_2 DM Test (large F_2 pop; 5043 plants)

❖ Outcome: No susceptible F_2 was detected, meaning there was no recombination event happened in this F_2 population

Saturation mapping of Pl_{17}

- ***STEP 1-sequence-based SSR marker development***
  - Pl_{17} and Pl_{19} was delimited to a 3.2 Mb region on LG4
  - The sequence was extracted from reference genome of HA 412
  - 94 pairs of primers (55 SSR & 39 STS) were designed from the sequence
  - Polymorphism screening between HA 234/HA 458 (Pl_{17})
  - Genotyped 186 F_2 individuals with 8 polymorphic markers

❖Outcome: 3.2 Mb interval reduced to 150 kb

(95.3% reduction)

Saturation mapping of PI_{17}

- ***STEP 2-sequence-based SNP marker development***

  - HA 458 pan-genome sequence (10x coverage) was aligned with another reference genome XRQ around PI_{17} region

  - Single nucleotide polymorphism (SNP) was analyzed and 27 sets of primers (SPB0001-SPB0027) were designed

  - ❖ Outcome: Eight SNP markers were mapped adjacent to and below PI_{17} within the 150 kb region

Fine mapping of *Pl*$_{17}$

- ***STEP 3-fine mapping***

  - A total of 3,008 F_2 plants were screened with two flanking markers, and 22 recombinants were detected

  - The recombinants were advanced to F_3 generation for further DM test

  - Genotyping of the new markers in the recombinant lines is underway, leading to increase of map resolution

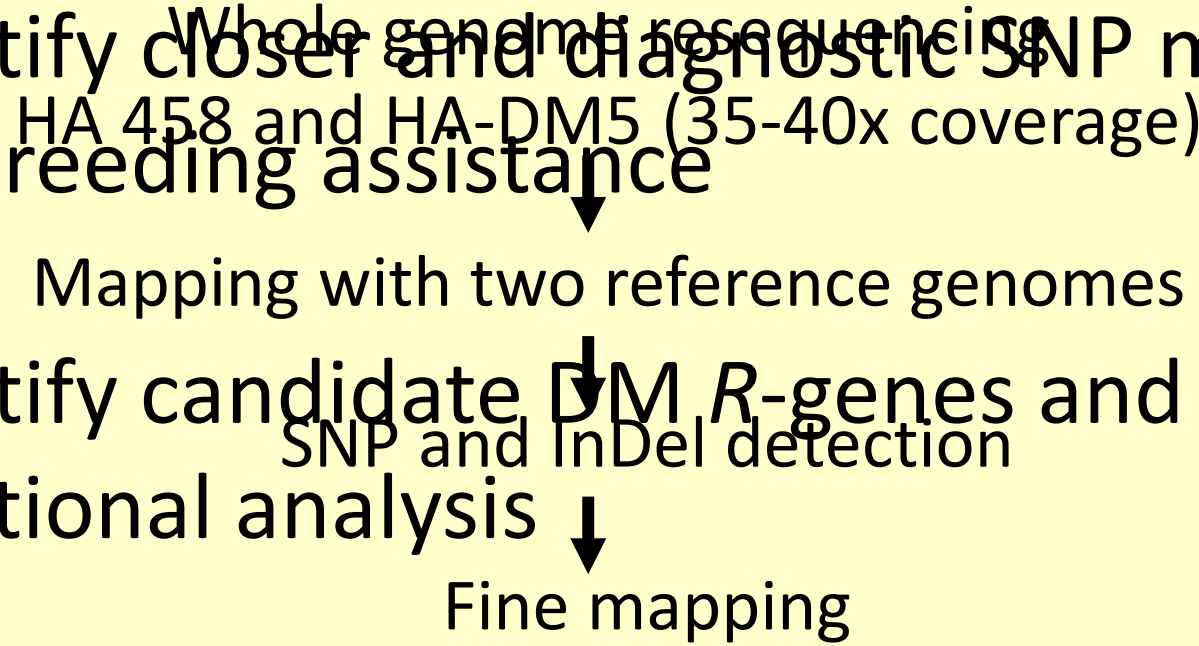
Saturation mapping of *Pl*$_{19}$

- 150 pairs of primers (111 SSR & 39 STS) were designed from the 3.2 Mb sequences from the reference genome of both HA 412 and XRQ
- Polymorphism screening between CONFSCLB1/HA-DM5 (*Pl*$_{19}$)
- Genotyped 140 F_2 individuals with 24 polymorphic markers
- 13 markers were mapped upstream of the *Pl*$_{19}$ gene and one marker downstream of the *Pl*$_{19}$ gene

❖Outcome: 3.2 Mb interval reduced to
258 kb (*91.9% reduction*) in HA 412
69 kb (*97.8% reduction*) in XRQ

Future Work (2018)

- Further narrow down the interval of Pl_{17} and Pl_{19} region
- Identify closer and diagnostic SNP markers for breeding assistance
- Identify candidate DM *R*-genes and functional analysis



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