2018 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
  - **Fine mapping of DM *R*-genes *Pl*$_{17}$ *and Pl*$_{19}$**
  - **Development of diagnostic markers for *Pl*$_{17}$ and *Pl*$_{19}$**
  - **Analysis of candidate gene of *Pl*$_{17}$**
- Future work
- Acknowledgements

Downy mildew

(Photo by Markell and Gong)

- *Plasmopara halstedii*
- Incidence: 16% of 2015 and 9% of 2017 (NSA surveys)
- Development of resistant hybrids is most effective management tool (economic & environmental)

DM threats sunflower production

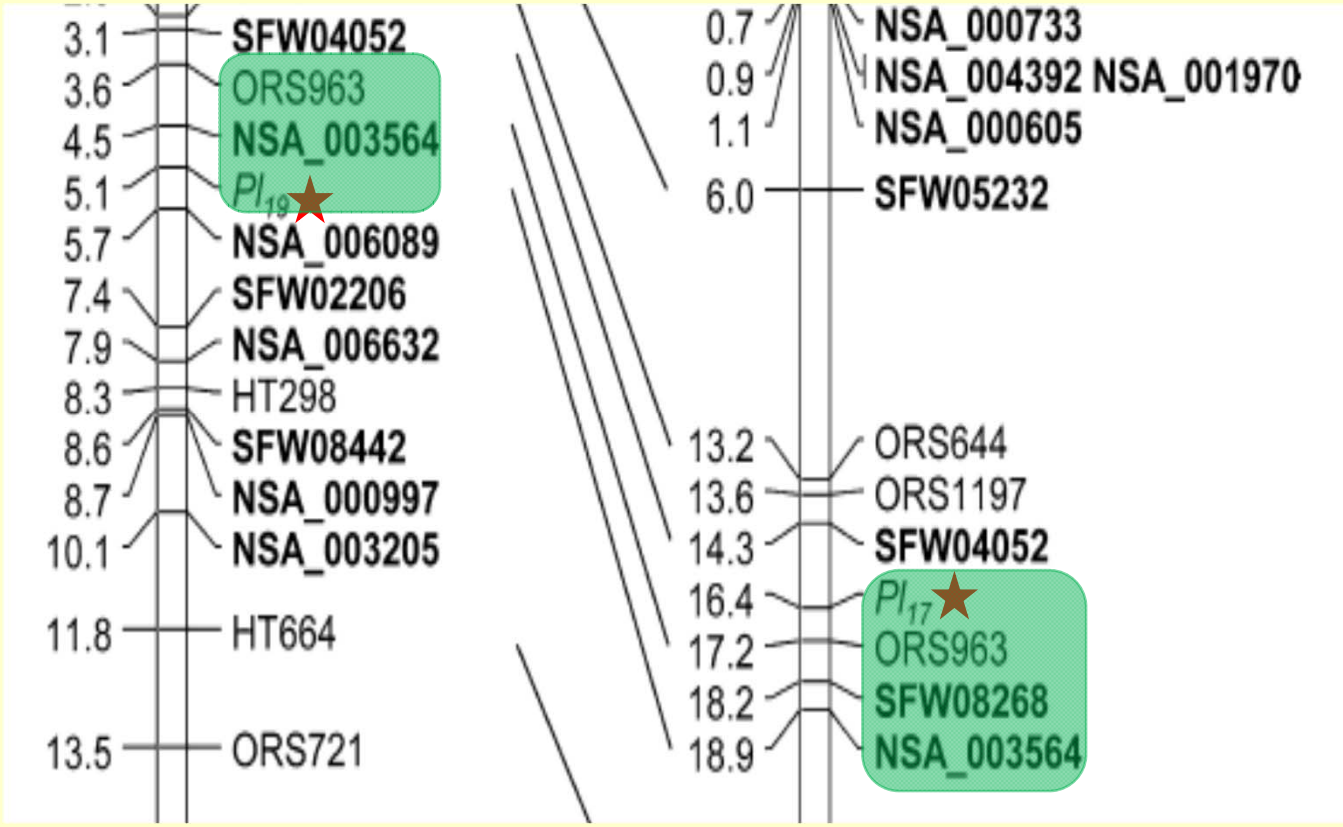
- New DM races are emerging, making current DM *R*-genes ineffective
- Potential threat to sunflower production
  - Gilley (2014-2015) identified only five *R*-genes [Pl_{Arg}, Pl_{15}, Pl_{17}, Pl_{18}, and TX16R (Pl_{33}, recently published)] were resistant to 185 DM isolates collected in ND, SD, MN, NE
- New DM *R*-gene is continuously needed (Pl_{19}, Pl_{20}, and more)

DM *R*-genes Pl_{17} *and* Pl_{19}

	Pl_{17}	Pl_{19}
Publication	Qi et al., 2015	Zhang et al., 2017
Resistant line	HA 458	HA-DM5
Chro. location	LG4	LG4
Resistance to all U.S. races	Yes	Yes
Confectionary	Yes (HA-DM3)	Yes (HA-DM5)

- Broad-spectrum and new resistance
- 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

Positions of Pl_{17} and Pl_{19}



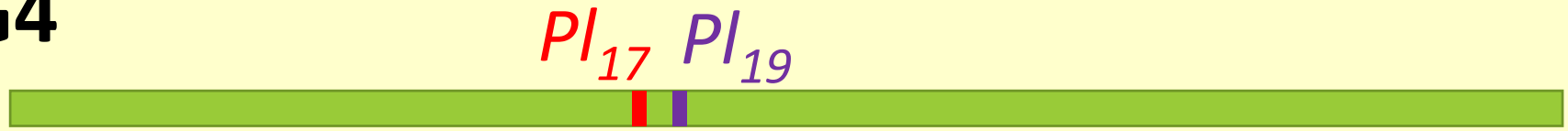
Common markers

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

~ 3.2 Mb

Achievement by 2017

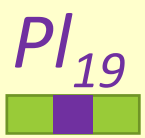
LG4



3.2 Mb

SSR marker development from reference seq; 2017

150 kb ***(4.7%)***



69 kb ***(2.2%)***

Whole genome sequencing; 2018

?

?

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

Research objectives 2018

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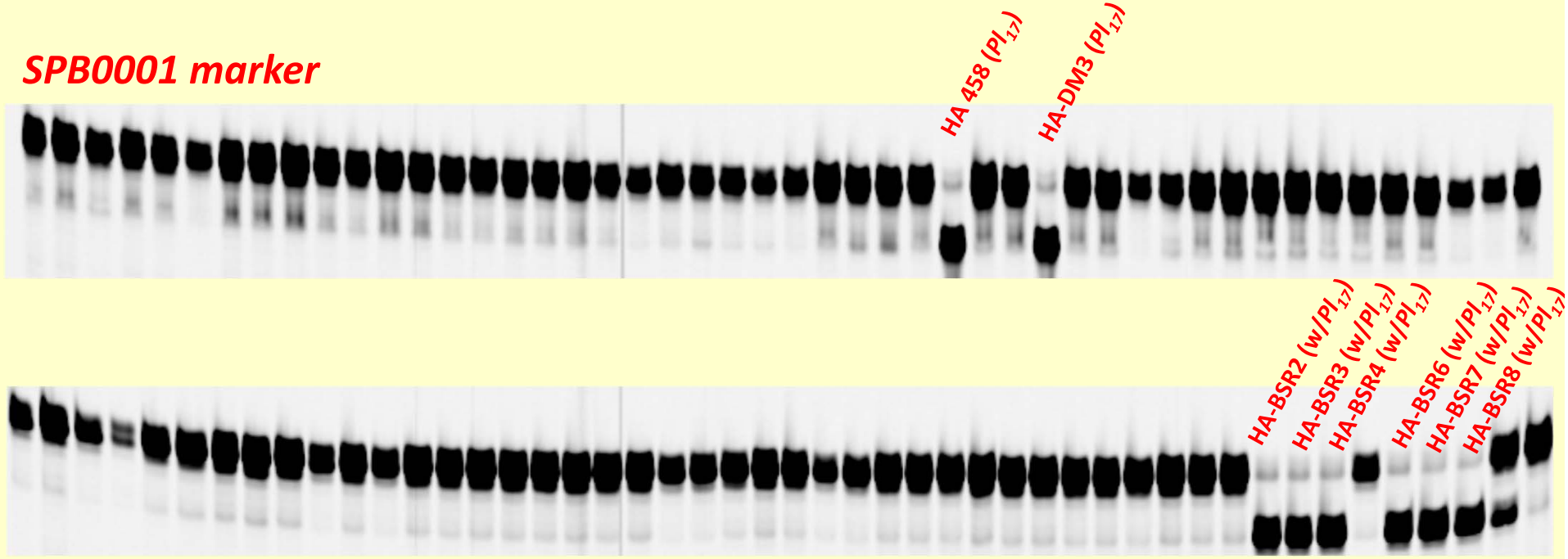
Fine mapping of Pl_{17}

- 22 recombinants were detected from large F_2 pop (3,008 plants) with two flanking markers
- F_3 phenotyping
- HA 458 whole genome resequencing data (40x coverage) *vs*. references XRQ and HA 412-HO
- Single nucleotide polymorphism (SNP) identification and primer design
  - **Outcome**: 19 (out of 88) SNP markers were mapped around Pl_{17}, and Pl_{17} was placed in 11 kb interval (0.34% of initial 3.2 Mb)

Development of PI_{17} diagnostic markers

- 7 SNP markers are unique to PI_{17}, and different from PI_{19}
- 6 markers could differentiate PI_{17} from 96 selected sunflower lines, will be useful in MAS



Candidate Pl_{17} gene identification

- Pl_{17} candidate gene: HanXRQChr04g0095641 in XRQ genome
- Belongs to TIR-NBS-LRR (typical for *R* gene) class with 32.329 kb
- 16 polymorphic markers fall in the region
- Working on gene sequence retrieval and correction, and functional analysis

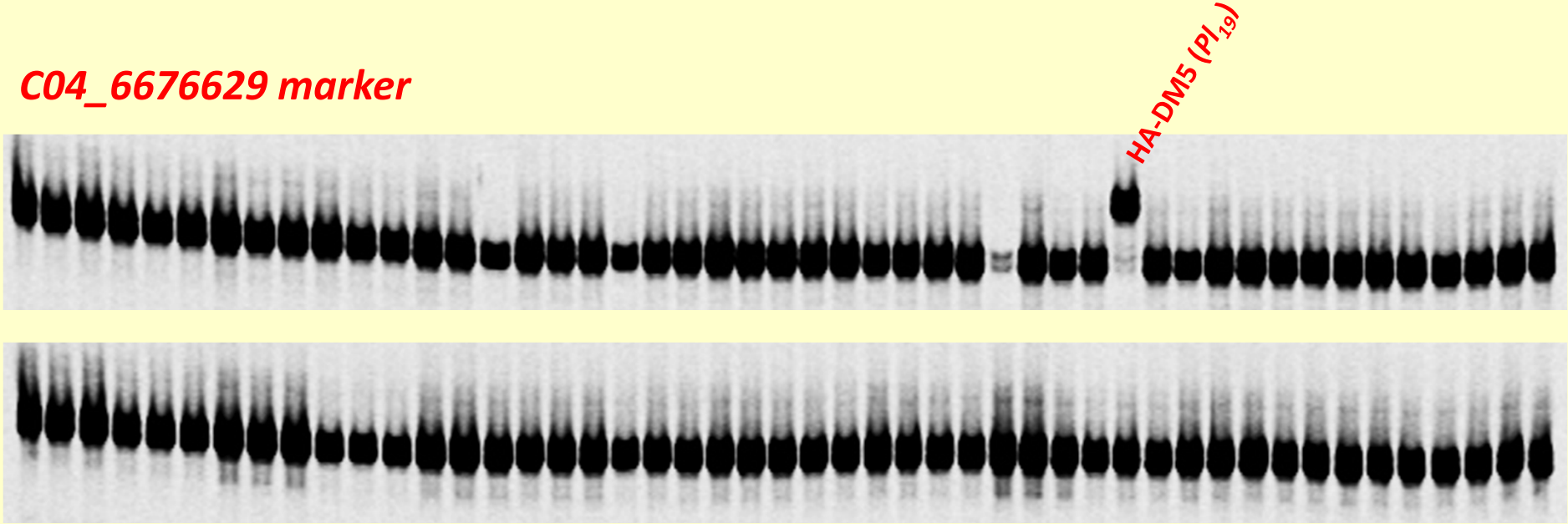
Fine mapping of *Pl*$_{19}$

- 23 recombinants were detected from large F_2 pop (2,256 plants) with two flanking markers
- F_3 phenotyping
- HA-DM5 whole genome resequencing data (40x coverage) *vs*. references XRQ and HA 412-HO
- Single nucleotide polymorphism (SNP) identification and primer design
  - **Outcome**: 52 SNP markers were mapped around *Pl*$_{19}$, and *Pl*$_{19}$ was placed in 36 kb interval of XRQ assembly (1.12% of initial 3.2 Mb)

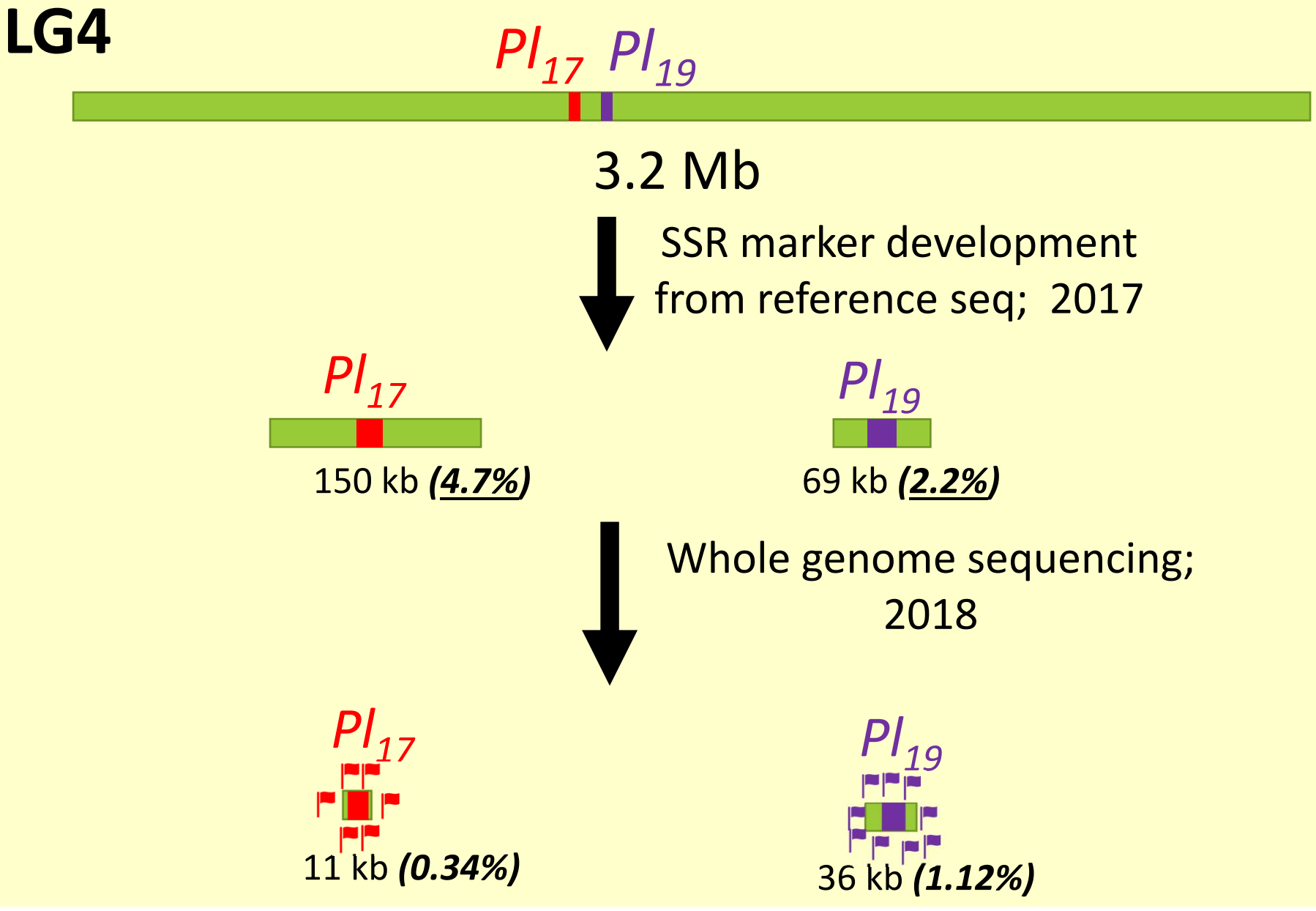
	From XRQ	From HA 412-HO	Total
Designed markers	**168**	**104**	**272**
Polymorphic markers	**47**	**5**	**52**

Development of PI_{19} diagnostic markers

- 19 SNP markers are unique to PI_{19}, and different from PI_{17}
- 9 markers could differentiate PI_{19} from 96 selected sunflower lines, will be useful in MAS



Summary of 2018 achievement



Future work (2019)

- *PI_{17}* and *PI_{19}* fine mapping are complete, and will work on manuscript writing
- Candidate gene of *PI_{19}* identification
- HA-DM1 (*PI_{18}*) whole genome has been sequenced, and currently working on the fine mapping and diagnostic marker development
- Candidate *PI_{18}* gene identification

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