Development of SNP markers linked to the Downy Mildew Resistance Gene *Pl*$_8$ in Sunflower

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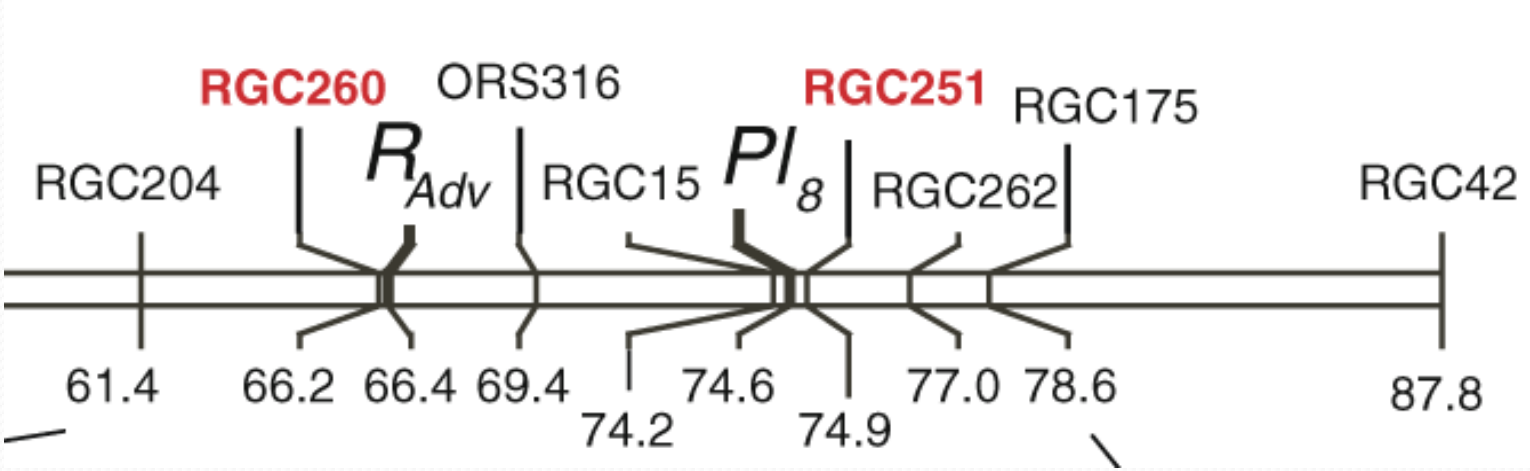
Background

- Downy mildew (DM) is the most destructive foliar disease in sunflower.
- The continuous use of sunflower hybrids with different resistance genes has led to a highly diverse pathogen population in the sunflower production regions.
- Prior to 1980, only two DM races were know, one each in Europe and North America.
- To date, at least 24 DM races were identified in Europe and 40 races in North America.

Background cont.

- RHA 340 was released in 1988 as an oilseed type male-fertility restorer line carrying the DM resistance gene *Pl_8*. This gene still confers resistance to most of the DM races.
- The DM resistance in RHA 340 was originated from *H. argophyllus* and previously mapped to the lower end of the linkage group (LG) 13 of the sunflower genome.

Background cont.



- SSCP (single-strand conformation polymorphism) markers, RGC15 and RGC251 are closely linked to PI_8.
- Genotyping of SSCP marker is time consuming and labor intensive.

Bachlava et al. 2011

Background cont.

- In recent years, single nucleotide polymorphism (SNP) markers have become widely used in plant breeding and research.
- SNPs are the most abundant and ubiquitous genetic variations in the eukaryotic genome and amenable to high-throughput, low-cost genotyping.
- The objective of this study is to develop SNP markers linked to Pl_8 for marker-assisted selection (MAS) in sunflower breeding programs.

DM phenotyping

- An F_2 population was developed from the cross of HA 434 (S-parent) with RHA 340 (R-parent).
- A total of 130 F_3 families (30 seedlings per family) were phenotyped with DM hot race 734.
- The segregation of DM resistance in 130 $F_{2:3}$ families fits the 1:2:1 segregation ratio, confirming that the DM resistance derived from RHA 340 is controlled by a single dominant gene *Pl_8*.

Development of SNP markers linked to *Pl*$_8$

- The parents were screened with 41 SNPs (12 NSA and 29 SFW SNPs) selected from the lower end of LG13 covering the *Pl*$_8$ region.
- Nine SNPs that showed polymorphism between the parents were genotyped in the F_2 population.
- The SNP markers delimited *Pl*$_8$ at an interval of 1.7 cM.

Genetic linkage map of Pl_8

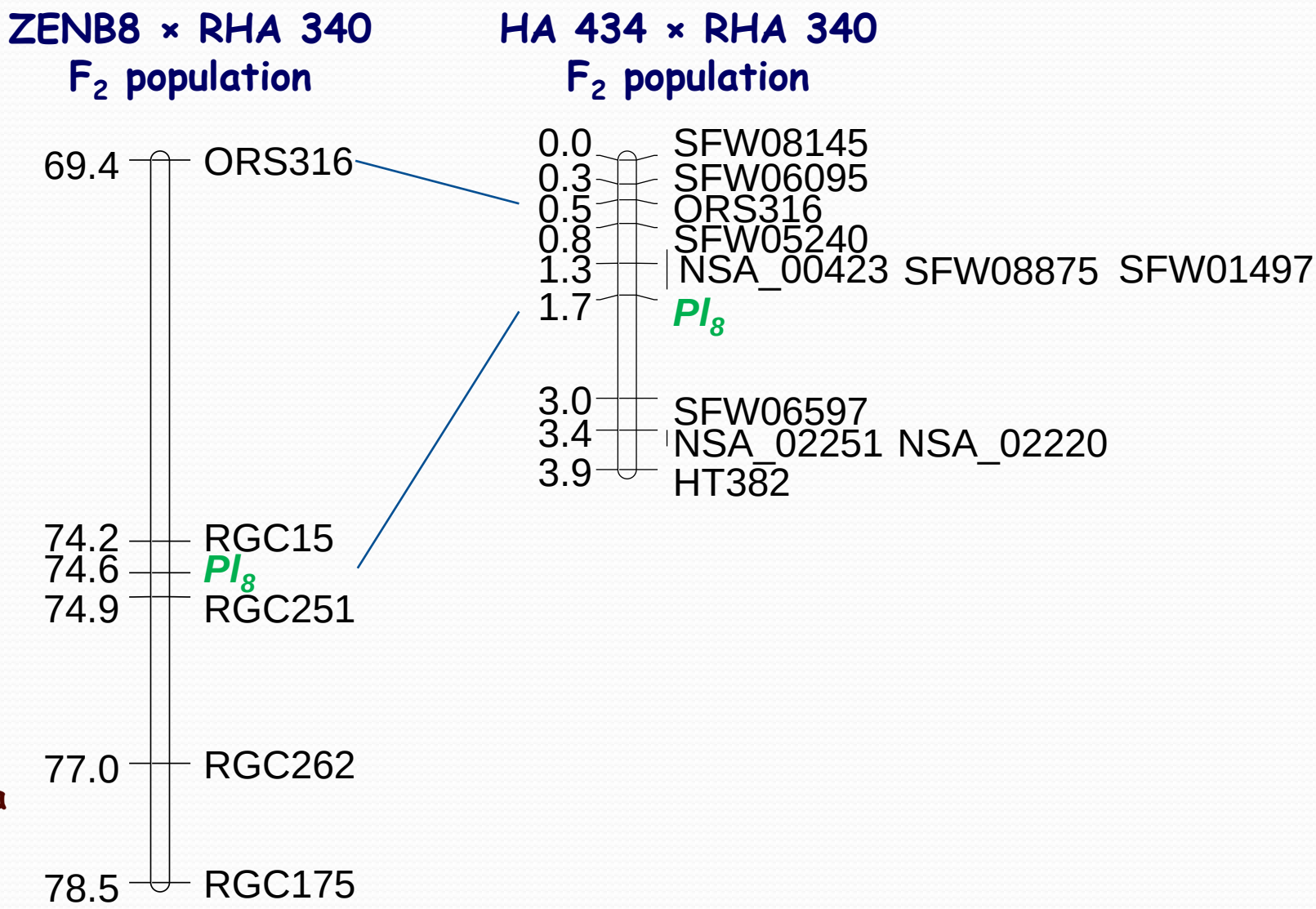


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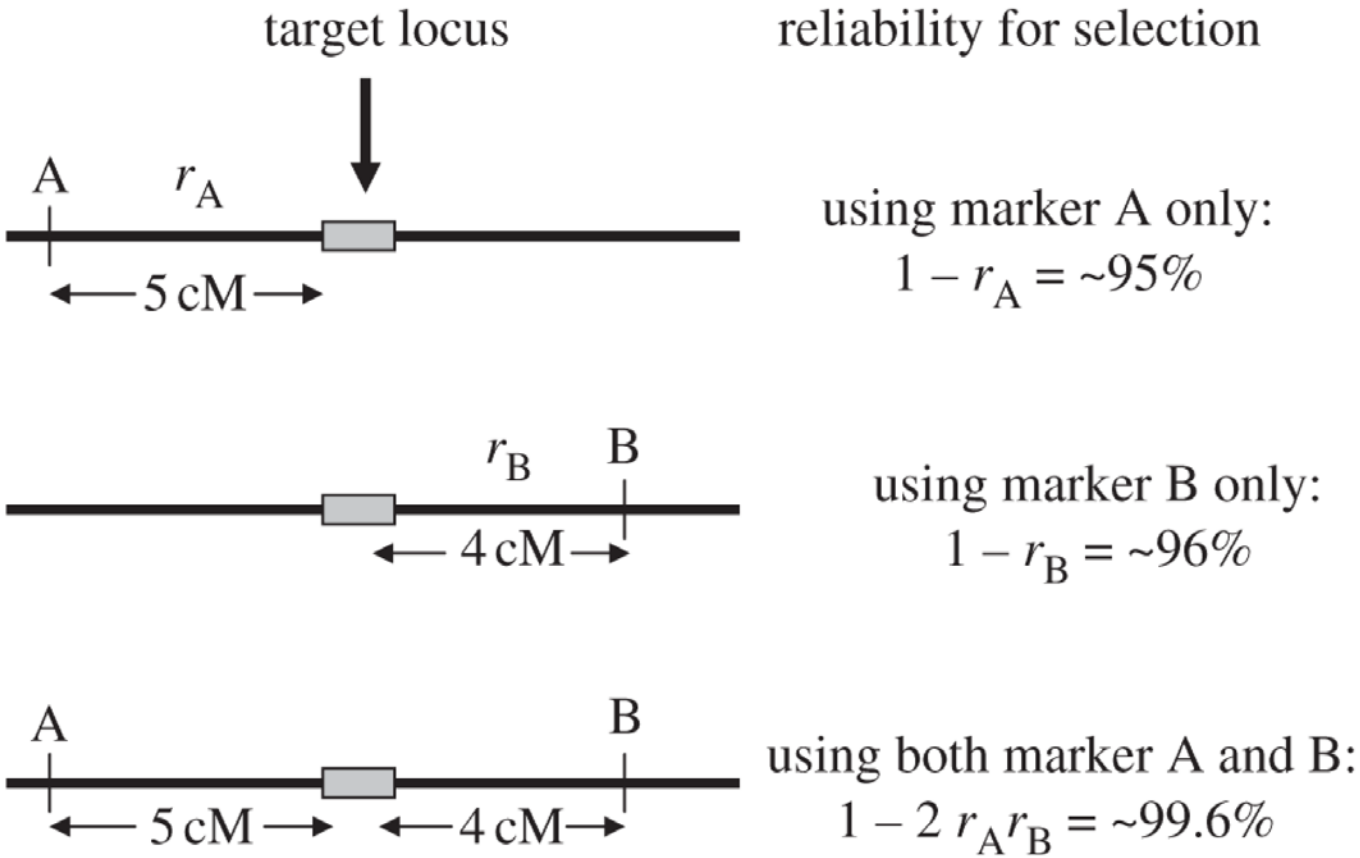
Table 1 Distribution of RHA 340 allele in the 548 sunflower lines tested with three NSA SNP markers linked to *Pl*$_8$

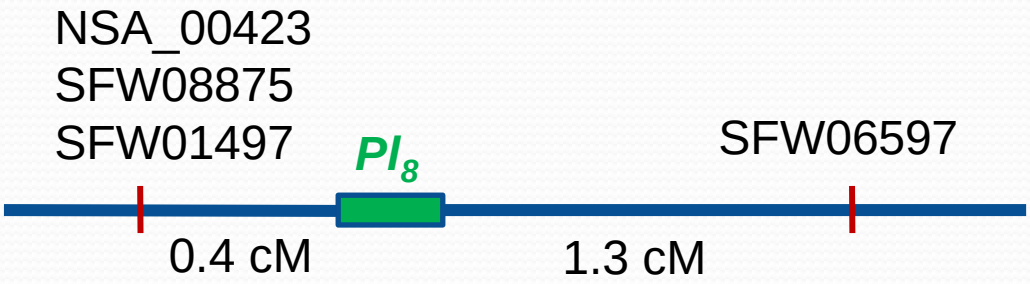
SNP ID	Map position (cM)	HA 434	RHA 340	Summary			
				Lines with data	Lines with HA 434 allele	Line with RHA 340 allele	Lines with heterozygous
NSA_000423	1.3	AA	GG	546	471	63 (11.5%)	12 (2.2%)
Pl$_8$	1.7						
NSA_002220	3.4	GG	AA	548	472	63 (11.5%)	13 (2.4%)
NSA_002251	3.4	AA	GG	547	348	130 (23.8%)	37 (6.8%)

Reliability of marker selection



Bertrand et al. (2008)

Reliability of marker selection cont.



Reliability of MAS selection for PI_8

Using flanking markers:

1- (2 x 0.004 x 0.013) = 99.986%

Factors affecting accuracy of SNP genotyping

SNP genotyping methods

- Multiplexed chip-based technology: large-scale studies requiring genotypic data for individual samples with thousands of SNPs.
- Single-plex PCR-based SNP genotyping: small or moderate numbers of SNPs for a large number of samples, such as KASP (KBioscience Competitive Allele-Specific PCR).

- **Allele-specific primers designed for length polymorphism (the method was developed in my lab).**

Genotyping of SNP NSA_01570 linked to the rust R-gene R_{12}

Accuracy of SNP genotyping cont.

- DNA quality: we recommend to use DNeasy Kits (Qiagen) for high quality sunflower DNA
- Population structure

Accuracy of SNP genotyping cont.

- Genetic distance vs physical distance

Table 2 Genetic and physical positions of four SNP markers currently used for marker selection of Pl_{Arg} in LG1 of the sunflower genome

SNP or Gene	Genetic position (cM)	Physical position (bp)
NSA_002851	29.682	124006083-124006420
NSA_002867	29.682	129149732-129150065
Pl_{Arg}	29.693	
NSA_004060	30.511	143164483-143164830
NSA_006530	30.511	143859036-143859455

Genetic distance between NSA_002867 and NSA_004060: 0.829 cM

Physical distance between NSA_002867 and NSA_004060: 14,0 Mbp

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Thank you