

# 2020 Progress for enhancing rust resistance in confection sunflower production through next-generation technologies

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# Outline

- Background knowledge (rust,  $R$  genes, *etc.*)
- Overall research objectives
- Research progress
  1. Development of  $R$  genes stacks
  2. Saturation mapping of rust  $R$  genes  $R_{13a}/R_{13b}$
  3. Fine mapping of  $R_{13a}$
- Future work

# Rust

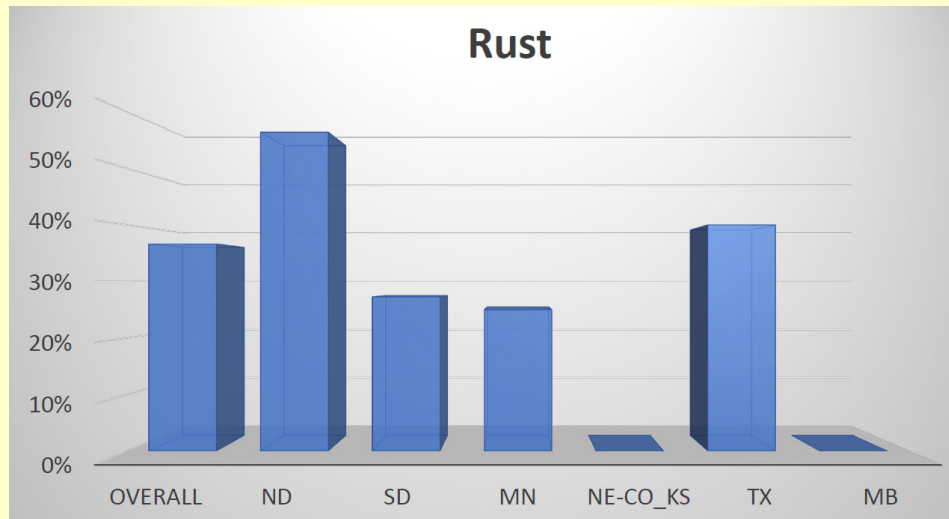


- *Puccinia helianthi* Schwein.
- Teliospores can overwinter

# Rust survey

	2013	2015	2017
Rust	65%	62%	38%

*Rust incidence in U.S.*



*Geographic trends*

(Gulya et al., 2017 NSA survey)

- Highest prevalence during the entire survey period among five diseases surveyed (Gulya et al. 2018)
- Development of resistant hybrids is most effective management tool (economic & environmental)

# Rust threatens sunflower production

- Occur in cultivated sunflower, ornamentals, and sunflower wild relatives in the *Helianthus* genus
- Important disease in North America, Australia, Argentina, South Africa, Russia, India, and China
- Appearance and spread of new *P. helianthi* races may lead to epidemics when weather favors

# Genetics of rust resistance in sunflower

- Dominant resistant ( $R$ ) genes in sunflower control rust resistance
- New rust races make current  $R$  genes ineffective
  - 7 ( $R_{11}$ ,  $R_{12}$ ,  $R_{13a}$ ,  $R_{13b}$ , and  $R_{14}$ - $R_{16}$ ) out of 15 reported rust  $R$  genes remain effective
- New rust  $R$  genes continuously need to be discovered and utilized ( $R_{17}$  &  $R_{18}$  are on the way)

# Research objectives 2020-2022

- Fine mapping of new rust resistance genes  $R_{13a}$ ,  $R_{12}$ , and  $R_{15}$
- Develop diagnostic molecular markers for  $R_{13a}$ ,  $R_{12}$ , and  $R_{15}$
- Identify candidate genes associated with rust resistance in the sunflower genome
- Stack effective rust  $R$  genes to generate lines with improved rust resistance

# Rust $R$ genes $R_{13a}$ & $R_{13b}$

	$R_{13a}$	$R_{13b}$
$R$ donor	HA-R6 (confection)	RHA 397 (oil)
$R$ origin	PI 650362, France	RO-20-10-3-3-2, South Africa
Chrom. location	Linkage Group 13	
Resistance	All races identified in U.S.	
Released germplasm	<b>Confection:</b> HA-R12 ( $R_2$ & $R_{13a}$ ); HA-R13 ( $R_5$ & $R_{13a}$ )	<b>Oilseed:</b> HA-R16 ( $R_{12}$ , $R_{13b}$ & $Pl_{Arg}$ ); HA-R17 ( $R_{15}$ & $R_{13b}$ )

- $R_{13a}$  shows broad-spectrum resistance
- Develop breeder-friendly markers for the gene  $R_{13a}$ 
  - Closer markers (best option is  $R$  gene itself)
  - Unique markers

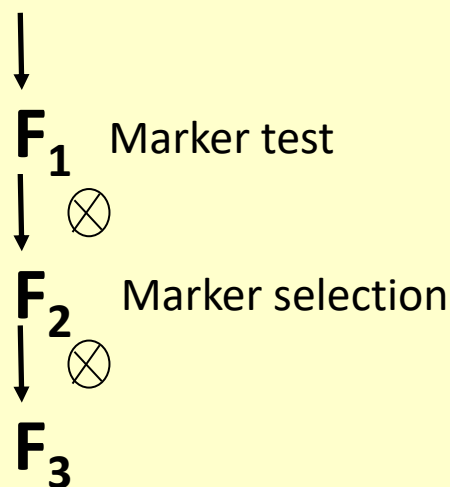


# Positions of $R_{13a}$ & $R_{13b}$

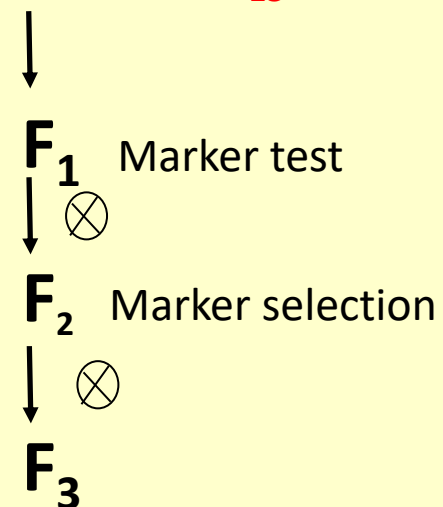
SNP/gene	Genetic position (cM)		Physical position in XRQ assembly		
	$R_{13a}$ map	$R_{13b}$ map	Start	End	
SFW08188	3.4	5.9	190,945,091	190,945,210	
SFW01499	3.4	5.9	190,716,677	190,716,796	
SFW06095	3.4	5.9	190,803,806	190,803,688	
SFW05240	3.4	5.9	190,827,363	190,827,244	
SFW08283	3.4	5.9	190,750,047	190,749,927	
SFW05630	3.4	5.9	190,719,258	190,719,163	
SFW08875	3.4	5.9	193,131,235	193,131,123	
SFW05453	3.4	5.9	193,062,603	193,062,484	
SFW01497	3.4	5.9	193,089,467	193,089,349	
$R_{13a}$	3.8	-			<b>3.4 Mb</b>
$R_{13b}$	-	6.8			
SFW04317	-	9.2	196,474,077	196,473,983	
SFW05743	4.0	9.2	196,521,145	196,521,026	

# R genes stacks

HA-DM2 ( $R_{12} + PI_{Arg}$ ) × HA-R8 ( $R_{15}$ )



HA-DM3 ( $R_{13a} + PI_{17}$ ) × HA-R8 ( $R_{15}$ )



No. of F <sub>2</sub> tested	No. of triple homozygous F <sub>2</sub>	Genotype of triple homozygous F <sub>2</sub>
188	9	$R_{12}R_{12}/R_{15}R_{15}/PI_{Arg}PI_{Arg}$
188	4	$R_{13a}R_{13a}/R_{15}R_{15}/PI_{17}PI_{17}$

# Saturation mapping of $R_{13a}$ & $R_{13b}$

- ***Sequence-based SNP markers development***
  - HA-R6 ( $R_{13a}$ ) was whole-genome re-sequenced at 40× coverage
  - Whole genome sequence was aligned with two reference genomes, and variants (SNPs & InDels) were called
  - 432 SNPs/InDels potentially around  $R_{13a}$  were chosen and primers were designed (316 from XRQ and 116 from HA412-HO)

# Saturation mapping of $R_{13a}$ & $R_{13b}$

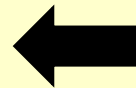
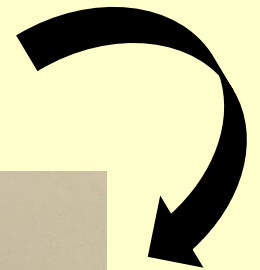
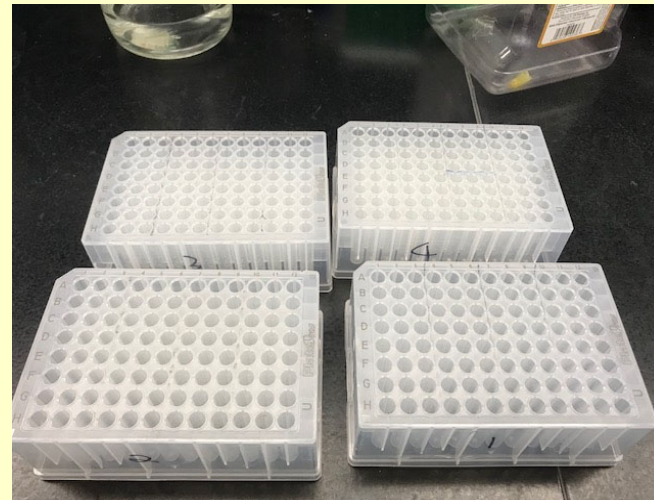
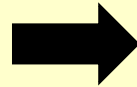
- ***Saturation mapping with the developed SNP markers***
  - Polymorphism screening of the developed SNP markers between HA 89/HA-R6 ( $R_{13a}$ ) and HA 89/RHA 397 ( $R_{13b}$ )
  - Identified 12 ( $R_{13a}$ ) and 7 ( $R_{13b}$ ) polymorphic markers
  - Genotyped on  $R_{13a}$  and  $R_{13b}$   $F_2$  pops with polymorphic SNP markers, respectively
- ❖ **Outcome: 5 and 4 SNP markers were mapped** around  $R_{13a}/R_{13b}$ , respectively, reducing gene interval from **3.4 Mb** to **0.93 Mb** in the XRQ reference genome

## Fine mapping of $R_{13a}$

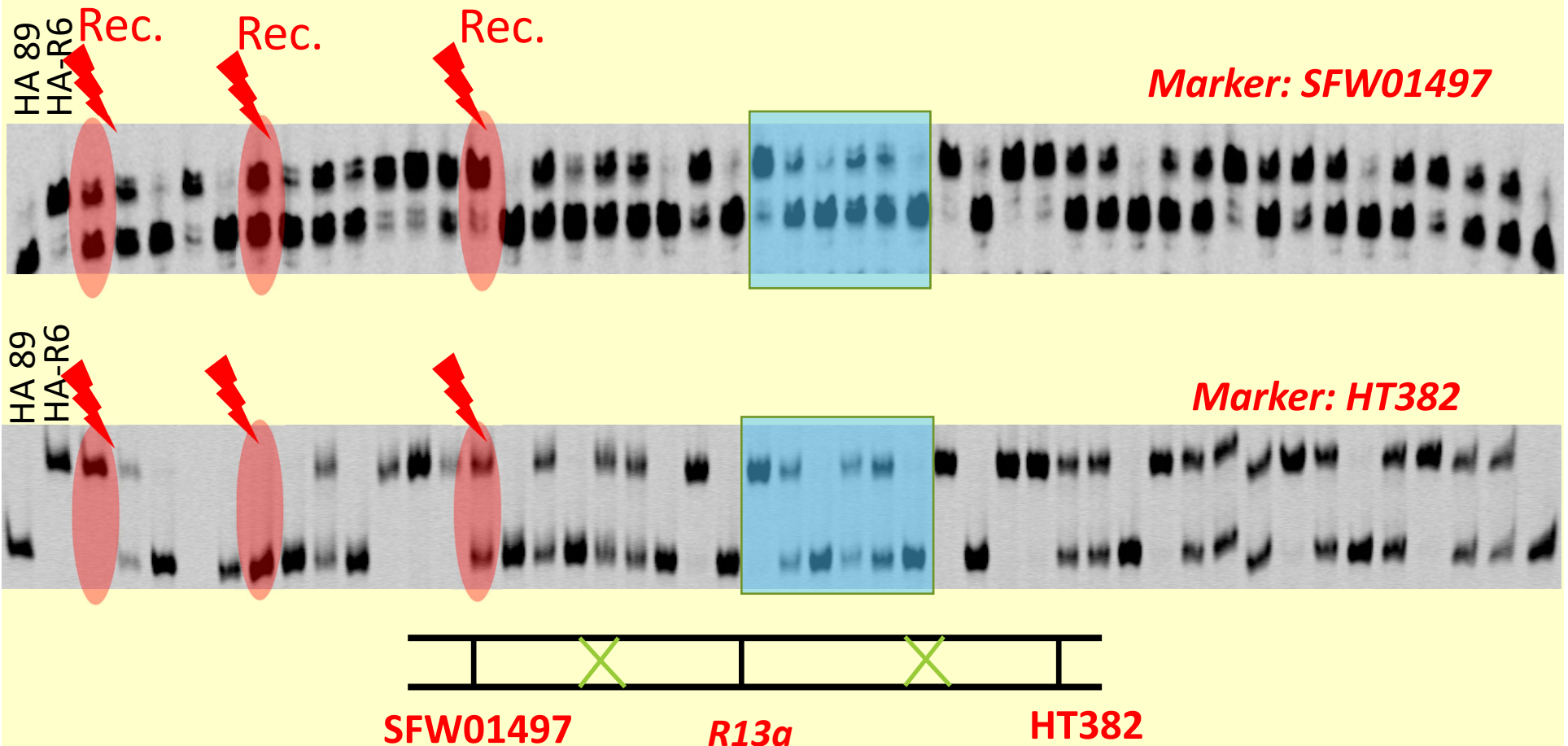
- Two flanking markers (SFW01497 & HT382) were used to test a large  $F_2$  pop (2,820 plants)
- 312 recombinants were identified and advanced to the next generation

# Recombinant selection of $R_{13a}$

- A total of 2,820  $F_2$  individuals were tested with flanking markers



# Recombinant selection of $R_{13a}$ , *cont'd*



- Recombinants were advanced to the next generation for further progeny tests

# Future work (2021)

- Complete fine mapping of  $R_{13a}/R_{13b}$
- Test and validate diagnostic markers for  $R_{13a}/R_{13b}$
- Identify candidate genes of  $R_{13a}/R_{13b}$
- Prepare manuscript
- Saturation and fine mapping of  $R_{12}$



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