Molecular mapping of the rust resistance gene in sunflower germplasm line PH 3

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Background

- Main causal organism: Fungus, *Puccinia helianthi* Schw.
- Lowest leaves usually become infected first. Infected leaves eventually wilt, dry up and die. Severe infections will cause defoliation.
- Loss of photosynthetic leaf area causes serious yield losses on susceptible hybrids. Yield loss with rust can be high :1400 lb/A to 200 lb/A in Mohall, ND in 2008
- Pustules can produce new spores after 10-14 days, which are spread by wind, and thus several cycles of rust infection can occur in the one cropping cycle.
- Control: Registered fungicides and resistant hybrids.

Sunflower rust

Rust problem in U.S.

- Increasing rust severity

  17% incidence in 2002

  60-77% incidence between 2005-2007

  Incidence in North Dakota very high

- Confections are generally more susceptible to rust, Limited resistance in commercial confection hybrids
- Genetic resistance very important

  Yield reduction of approx. 50% in Carrington hybrid performance trial, 2009 (Susceptible Confection Hybrids)

Screening for rust resistance in USDA breeding lines

- Race 336, the predominant race
- Race 777, the most virulent
- 104 lines were screened
  - 66 inbred lines
  - 14 interspecific lines and 24 foreign lines

* Gulya and Markell. 2009. Sunflower rust status-2008 race frequency across the Midwest and resistance among commercial hybrids.

Seven were selected as rust resistance

Lines	Type	Rust resistance		Origin of rust resistance
		Race 336	Race 777	
MC29		R	S	Wild *H. annuus* in Texas
HA-R2	OB	R	S	Argentina open pollinated varieties
HA-R3	OB	R	MR	Argentina open pollinated varieties
HA-R6	CB	R	R	A breeding line from France
HA-R8	OR	R	R	A landrace in Arizona, USA
RHA397	OR	R	R	South Africa
RHA464	OR	R	R	Wild *H. annuus* collected in California

OB: oil-B line OR: oil-R line CB: confection B line

All rust resistance genes in cultivated sunflower can be traced to wild species, primarily from wild *H. annuus*, *H. argophyllus* and *H.petiolaris*

Molecular marker-assisted *R*-gene selection

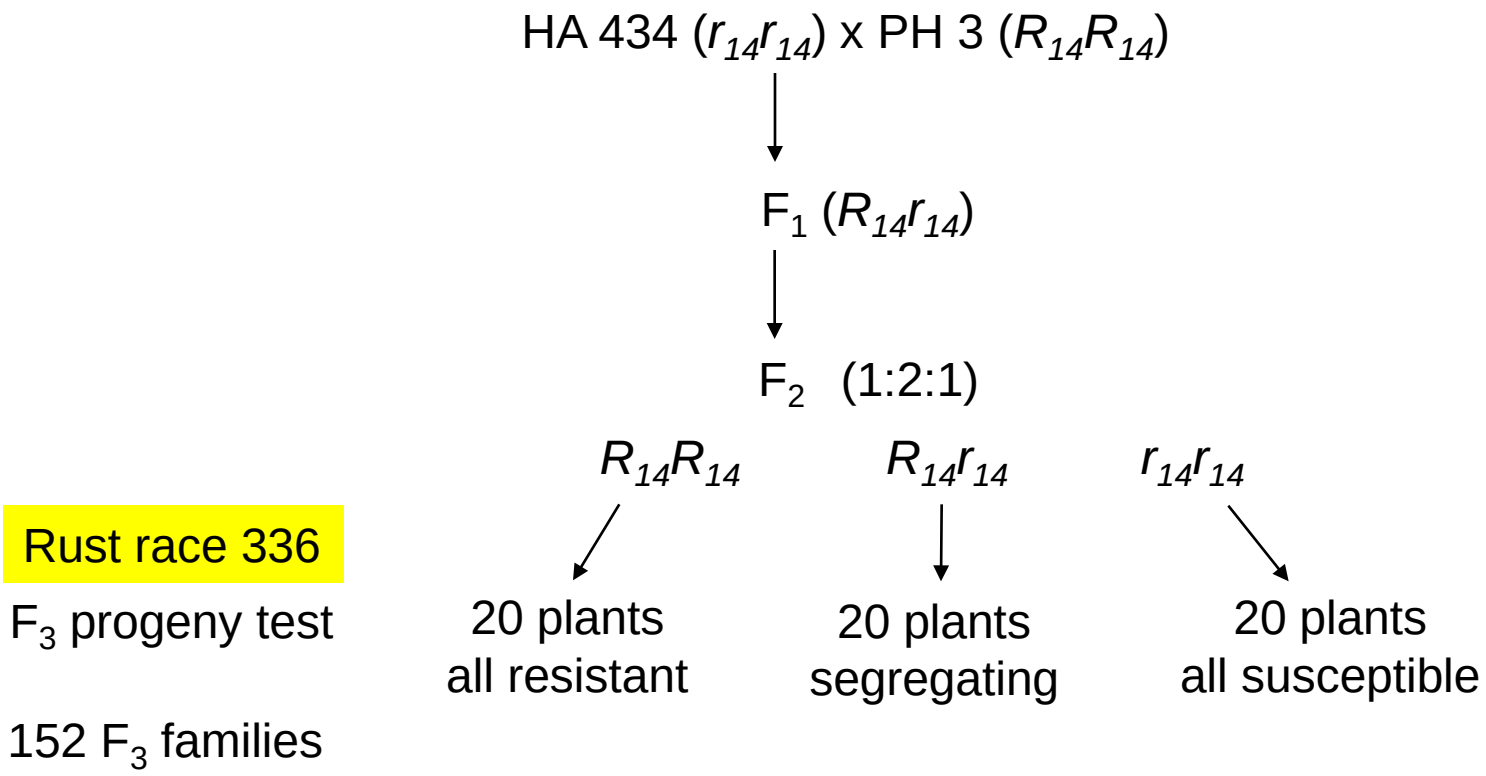
Molecular markers linked to new R-genes

R-gene donors	Genes	Linked markers	Linkage (cM)	LG	References
RHA 279	R_1	$SCT06_{950}$	0.0	8	Lawson et al. 1998
MC29	R_2	ORS 333	0.0	9	Lawson et al., 2010
HA-R3	R_4	ZVG61 ORS581	2.1 0.8	13	Qi et al., 2011
HA-R2	R_5	ORS1197-2 ORS653a	3.3 1.8	2	Qi et al., 2012
Rf ANN-1742	R_{11}	ORS45 ORS728	1.0 0.3	13	Qi et al., 2012
RHA464	R_{12}	CRT275	1.0	11	Gong et al., 2013
HA-R6 RHA397	R_{13a} R_{13b}	RGC15/16	0.3 0.1	13	Gong et al., 2013

PH 3, a rust resistant germplasm

- PH 3 was registered in 2004, carrying a dominant rust resistance to the prevailing North American races 1 to 4 (Jan et al. 2004), tentatively designated R_{14} gene
- Derived from PI 413038, a South Dakota wild *H. annuus*
- Pedigree: HA 89//PI 413038/HA 89*2, BC_2F_4
- Resistant to 10 rust races tested so far (races 300, 304, 334, 336, 337, 376, 377, 734, 736, 776, and 777) (Gong et al. 2013)
- PH 3 has purple hypocotyl color

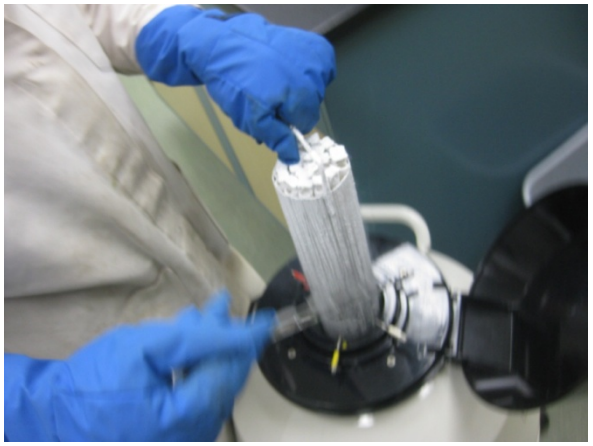
Materials and methods



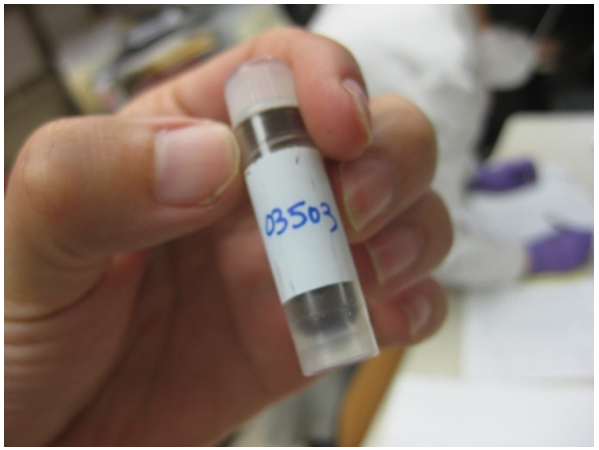
► **Progeny test of 152 $F_{2:3}$ families (20 plants for each family, a total of 3,040F_3 individuals) was performed to confirm the phenotype and assign the genotype of the F_2 plants.**

Rust inoculation and sporulation

14-day-old seedlings for inoculation



Rust spores in liquid nitrogen



Heat shock before inoculation

The next day after inoculation

12-14 days after inoculation

RUST SCORE

Score	Symptom	Pustule coverage
0	Immune	
0;	As necrotic flecks	0-0.5%
1	Necrosis with low sporulation	0-0.5%
2	Necrosis with medium sporulation	0-0.5%
3	Necrosis with medium to high sporulation	>0.5%
4	Necrosis with full sporulation	>0.5%

Qi et al. 2012

Results

Phenotypes in the F_2 population derived from HA 434 X PH 3

HA 434 PH 3 F_1

F_3 individuals

0 1 2 3 4

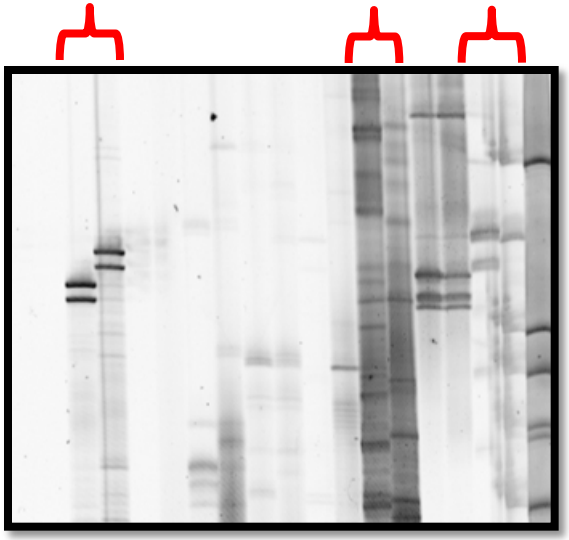
Phenotypes in the F_2 population derived from HA 434 X PH 3

Phenotype	Rust resistance	Hypocotyl color
A	35	37
H	94	87
B	23	28

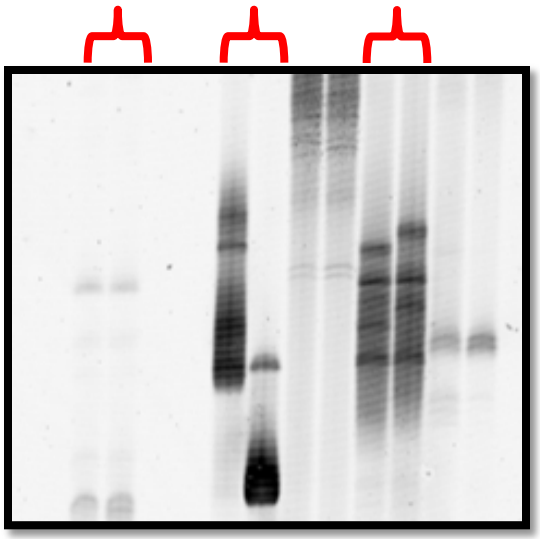
Bulks:
Bulk 1: Homozygous resistant to rust race 336, and homozygous purple hypocotyl color
Bulk 2: Homozygous susceptible to rust race 336, and homozygous green hypocotyl color

Marker polymorphism screening between parents and bulks

	Primers No.	No. of Polymorphic SSRs	Percentage (%)
Between parents	515	330	64.08
Between bulks	330	61	18.49

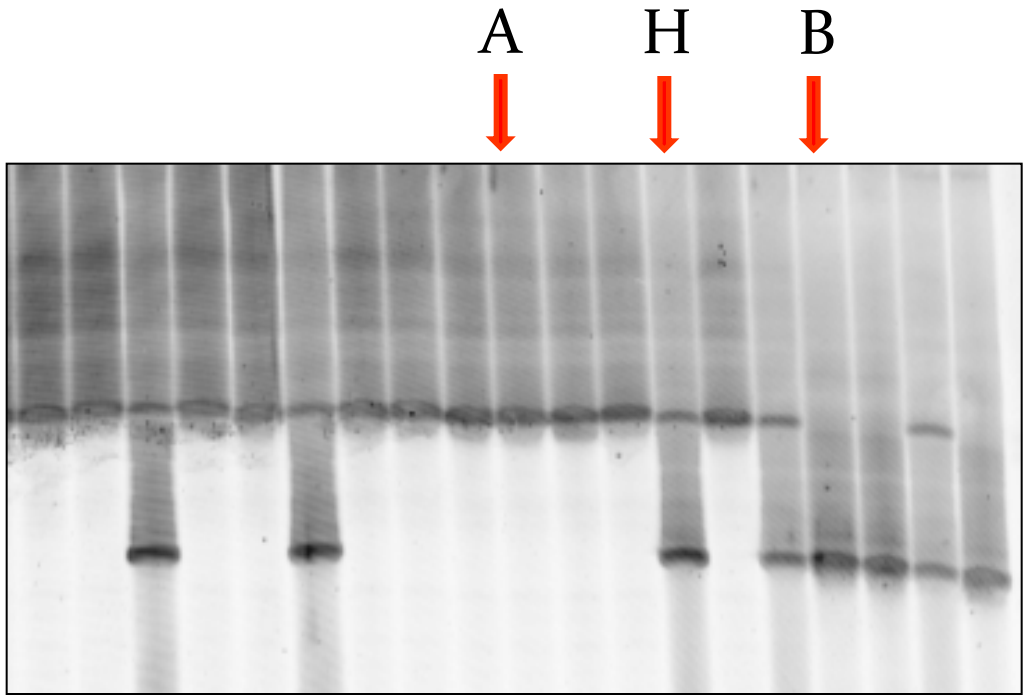


◆Polymorphism between parents



◆Polymorphism between bulks

F_2 population for polymorphic markers between bulks



A: $r_{14}r_{14}$; H: $R_{14}r_{14}$; B: $R_{14}R_{14}$

Segregation of the rust resistance (R_{14}) gene, purple hypocotyl color gene (*PHC*), molecular markers linked to the genes in the F_2 population of the cross HA 434 x PH 3

Traits or markers	Number of F_2 plants[a]	Observed number[b]					Ratio tested	χ^2	P	
		A	H	B	C	D				
R_{14} gene	152	35	94	23			1:2:1	10.42	0.005	←
PHC gene	152	37	87	28			1:2:1	4.25	0.119	
ORS 542	152	33			119		1:3	0.88	0.349	
ORS 261	152	36	94	22			1:2:1	11.11	0.004	←
ORS 956	152	37	91	24			1:2:1	8.14	0.017	←
ORS 748	152			29		123	1:3	2.82	0.092	
ORS 990	152	37	90	25			1:2:1	7.05	0.029	←
ORS 697	152			25		127	1:3	5.93	0.015	←
ORS 326	152	38	87	27			1:2:1	4.78	0.092	
ORS 1227	151			24		127	1:3	6.68	0.010	←
ORS 1146	152	38	88	26			1:2:1	5.68	0.058	
ORS 5	152	37	89	26			1:2:1	6.04	0.049	←
ZVG 53	150	40			110		1:3	0.22	0.637	

→ Segregation distortion (P< 0.05)

Summary

- The segregation distortion of the rust resistance gene R_{14} and purple hypocotyl color gene *PHC* was observed for the F_2 population of the cross HA 434 x PH 3
- The rust resistance gene was mapped to LG 11, with dominant marker ORS 542 as the closest marker at about 1.7 cM
- This gene is also linked with a purple hypocotyl color gene, at about 10.5 cM
- The purple hypocotyl color gene could be used as a marker for selecting homozygous rust resistant materials derived from PH 3

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Thanks for your attention!
ABORATORY
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