New sunflower rust projects in the USDA Sunflower Research Unit

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USDA-ARS Sunflower Unit

Rust is becoming a serious threat to the sunflower industry in North America

- Sunflower rust appeared earlier than normal in the field
- Rust incidence has continued to increase from 17% in 2002 to over 70% of fields affected in the last two years
- New rust races have arisen to which current hybrids have little resistance

Ongoing rust projects

- Evaluation of rust resistance to virulent races of USDA-released breeding lines
- Transfer of rust resistance genes from oil sunflower to confection sunflower
- Molecular mapping of rust resistance genes

Evaluation of resistance to virulent races of USDA released breeding lines

A total of 106 lines that previously reported resistance to one or more pathotypes of sunflower rust were selected

- 66 inbred lines
  - 9 oil-B lines
  - 26 oil-R lines
  - 28 confection-B lines
  - 3 confection-R lines
- 14 introgression lines
- 26 introduced lines from Canada, Australia, and Argentina

Selected rust races

- Race 336 - more prevalent race
- Race 777 – more virulent race

Sunflower lines resistant to race 336 or 777

Sunflower line	Type	Reaction to		Putative rust gene (s)
		race 336	race 777	
Inbred lines (6/66)				
Susc. check		S	S	
HA-R2	OB	R	S	R_5
HA-R3	OB	R	MR	R_4
HA-R6	CB	R	R	
HA-R8	CR	R	R	
RHA 397	OR	R	R	
RHA 464	OR	R	R	
Introgression lines (6/14)				
Ph3		R	R	R_{4m}
Ph4*		R	R	
Ph5*		R	R	
Ph6*		R	S	R_{4o}
TX16R*		R	R	
Rf ANN-1742*		R	R	
Introduced lines (7/26)				
CM29		R	S	$R_2 + R_{10}$
Suncross 53 self		R	MR	R_{4u}
Hysun 36 self		R	R	
Hysun 37 self		R	R	
Hysun 47self		R	R	
PI497938		R	S	
PI650362		R	R	

OB: oil-B line; OR: oil-R line; CB: confection-B line; CR: confection-R line. *Resistance segregation

Transfer of rust resistance genes from oil sunflower to confection sunflower

Selected recurrent confection parents

- CONFSCLB1, a B-line released in 2006
- CONFSCLR5, a R-line released in 2006

Selected rust resistance genes

- R_2 gene in CM29, resistant to 90% of isolates
- R_4 gene in HA-R3, resistant to 86% of isolates
- R_5 gene in HA-R2, resistant to 88% of isolates

Traditional backcross-based gene introgression

Recurrent parent X R-gene donor
(RP)

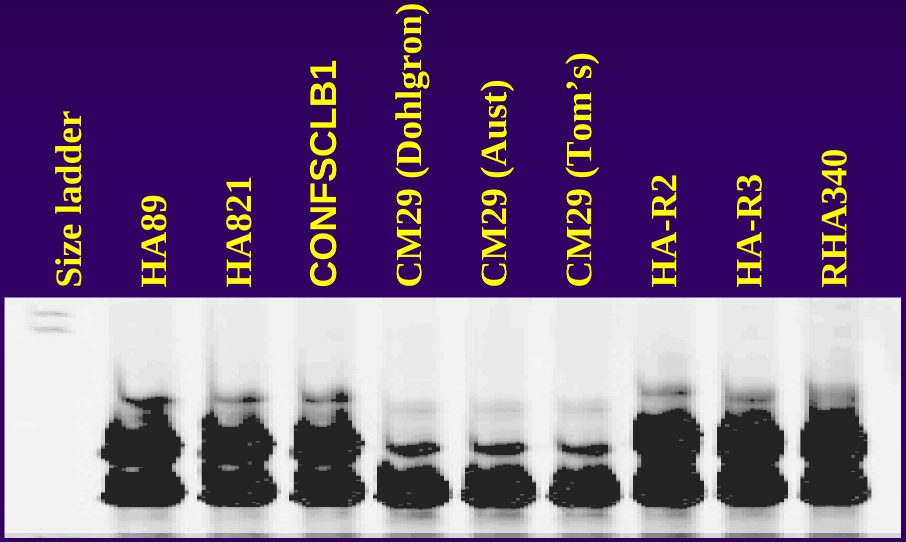
↓	
RP X F_1	50% RP genome
↓	
RP X BC_1F_1	75% RP genome
↓	
RP X BC_2F_1	88% RP genome
↓	
RP X BC_3F_1	94% RP genome
↓	
BC_4F_1	97% RP genome
↓	
BC_4F_2	
↓	
$BC_4F_{2:3}$	Select homozygous R-gene in RP background

Marker-assisted background selection (MABS)-based gene introgression

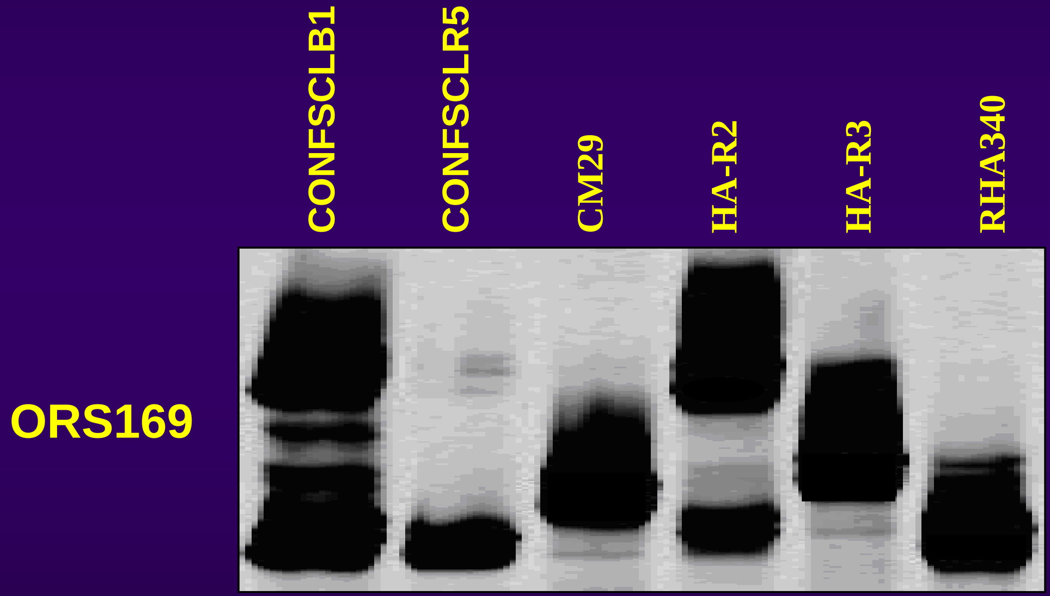
- ►Molecular markers linked to known R-gene will be used to target rust resistance gene in progeny

Resistance source	Resistance gene	Marker	Linkage (cM)	Linkage group	Reference
CM29	R_2	ORS333	0.0	9	Jan, personal communication
HA-R2	R_5	ORS630	0.0	13	Sendall et al. 2006
HA-R3	R_4	-	-	-	

SSR marker ORS333 linked to R_2 gene in CM29



- Selection of molecular markers for RP genome
  - Screen polymorphism between recurrent parent and R-gene donor parent with SSR markers



  - Polymorphic markers for RP genome will be used for marker-assisted background selection

Scheme of MABS-based gene introgression

Recurrent parent X R-gene donor parent
(RP)

RP X F_1 1st Backcross

- Screening of 500-1,000 BC_1F_1 for rust resistance.
- The selected resistant plants will be genotyped with the R-gene carrier chromosome markers.
- Plants with most RP background in the carrier chromosome will be used to make BC_2F_1

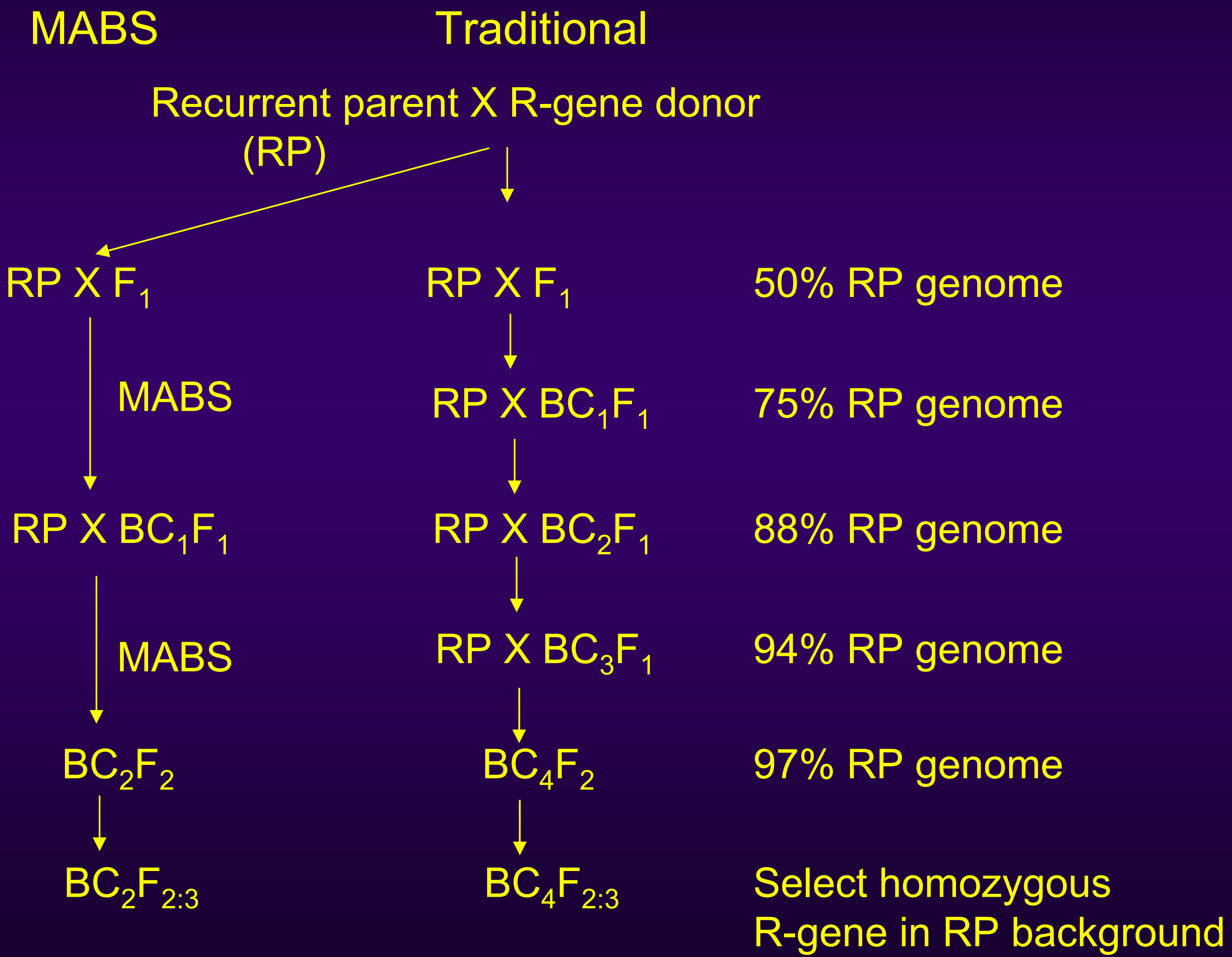
RP X BC_1F_1 2nd Backcross

- Screening of 500-1,000 BC_2F_1 for rust resistance.
- The selected resistant plants will be genotyped with the R-gene carrier chromosome markers.
- Selected plants will be genotyped with RP makers.
- Plants with most PR loci will be selected and selfed.

BC_2F_2 Selfing

$BC_2F_{2:3}$ Select homozygous
R-gene in RP background

Comparison of MABS and traditional gene introgression



MABS

Traditional

Recurrent parent X R-gene donor
(RP)

MABS		Traditional	
RP X F_1		RP X F_1	50% RP genome
	MABS	RP X BC_1F_1	75% RP genome
RP X BC_1F_1		RP X BC_2F_1	88% RP genome
	MABS	RP X BC_3F_1	94% RP genome
BC_2F_2		BC_4F_2	97% RP genome
$BC_2F_{2:3}$		$BC_4F_{2:3}$	Select homozygous R-gene in RP background

Progress of the project

Crosses were made

- (CONFSCLB1 X CM29) F_1
- (CONFSCLB1 X HA-R2) F_1
- (CONFSCLR5 X HA-R3) F_1

F_1 hybrids were tested with the rust race 336 and backcross is underway

Materials	No. plants	Race 336	
		IT	Severity (%)
Susc. check 7350	6	4	20
CONFSCLB1	6	4	20
CONFSCLR5	6	4	20
CM29	8	1	0
HA-R2	8	1	0.1
HA-R3	8	1	0.1
(CONFCLB1 X CM29) F_1	12	2	0.5
(CONFCLB1 X HA-R2) F_1	12	3	2-5
(CONFCLR5 X HA-R3) F_1	16	3	1-2

IT: infection type; 0-2, resistance, 3-4 susceptible.
Severity: the percent leaf area affected by rust; 0-0.5%, resistance, >1%, susceptible.

Molecular mapping of rust resistance genes

♦ Pyramiding of two or more effective resistance genes into one cultivar may provide a more durable resistance.

♦ The selection of genotypes with gene combinations is often not possible using resistance tests.

♦ Marker-assisted selection is a powerful approach for pyramiding non-allelic rust resistance genes in sunflower.

♦ Only a few molecular markers are available for sunflower rust resistance genes.

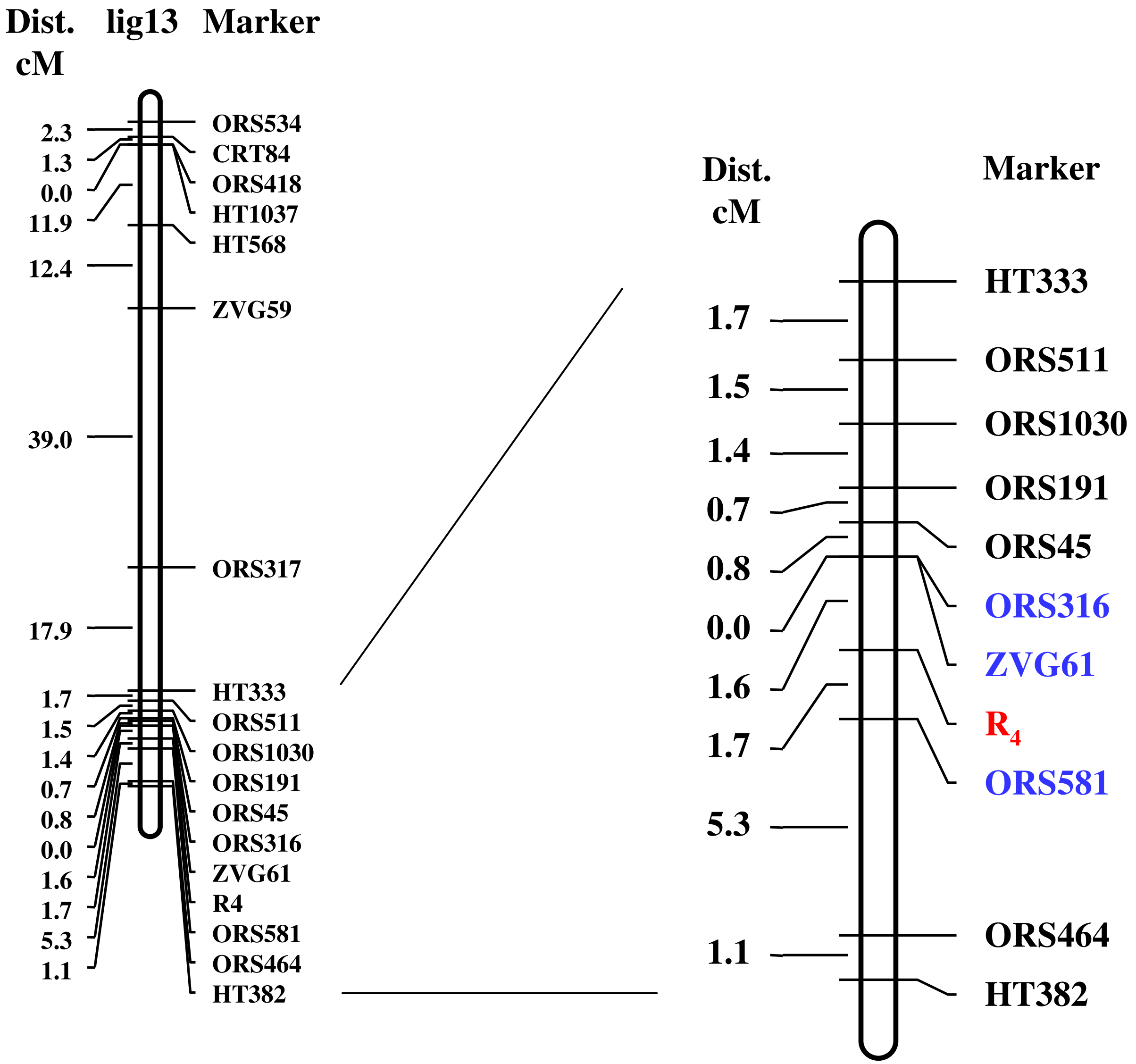
Mapping of rust gene R_4 in HA-R3

- Molecular marker source
  - 1,700 SSR primers are available
  - 870 have known positions in linkage map
  - 830 unmapped

- Screened 544 SSR primers between HA89 and HA-R3 and about 40% of the primers amplified polymorphic fragments in two parents.

- Twenty-two polymorphic SSR markers mapped to linkage 13 were first used to screen 94 plants of a F_2 population from the cross HA89 with HA-R3.

- 120 F_2 and 1900 F_3 plants from 94 $F_{2:3}$ families were tested with rust race 336.


Dist. cM
lig13
Marker
2.3
1.3
0.0
11.9
12.4
39.0
17.9
1.7
1.5
1.4
0.7
0.8
0.0
1.6
1.7
5.3
1.1
ORS534
CRT84
ORS418
HT1037
HT568
ZVG59
ORS317
HT333
ORS511
ORS1030
ORS191
ORS45
ORS316
ZVG61
R4
ORS581
ORS464
HT382
Dist. cM
Marker
1.7
1.5
1.4
0.7
0.8
0.0
1.6
1.7
5.3
1.1
HT333
ORS511
ORS1030
ORS191
ORS45
ORS316
ZVG61
R4
ORS581
ORS464
HT382

Future plan

Mapping of new rust resistance genes in RHA397 and RHA464

- RHA397 and RHA464 resist both rust races 336 and 777. RHA464 also resists downy mildew new race 734.

- The rust resistance in RHA397 may originate from a germplasm originating in South Africa

- The rust resistance in RHA464 was derived from a wild *H. annuus* accession PI 413047.

Acknowledgment

Angelia Hogness
Megan Townsend
Amanda Schoch
Hannah Worral