Development of Super Confection Sunflower Effectively Resistant to Downy Mildew and Rust

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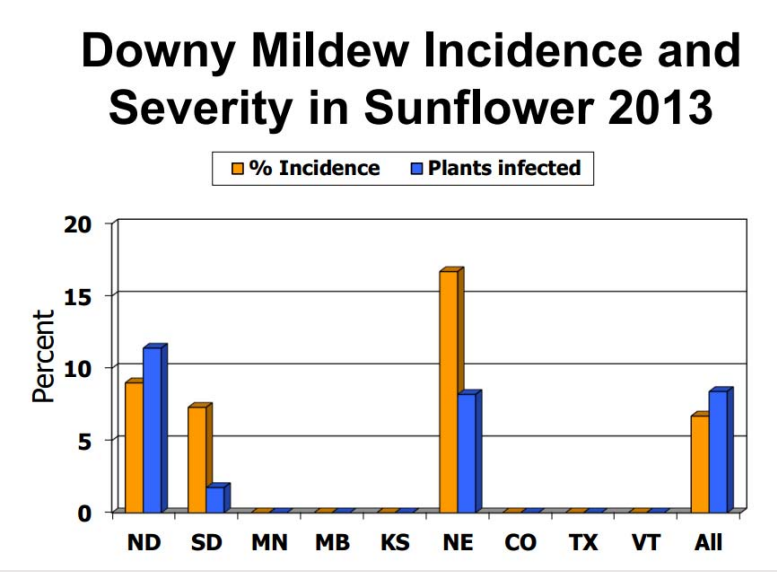
Outline

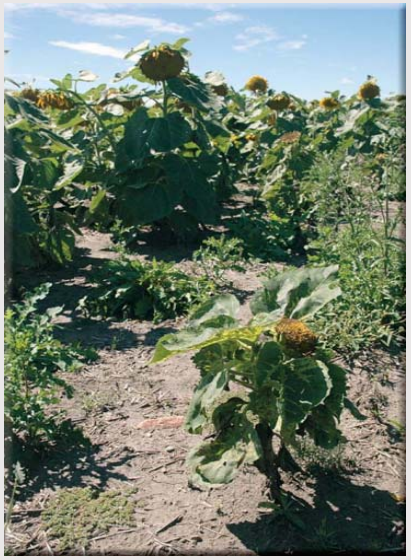
- Background knowledge
- Research objectives
- Research progress of 2014
- Mapping of RHA 468 DM resistance gene
- Future work
- Acknowledgements

Background Knowledge

- Sunflower downy mildew (DM), caused by *Plasmopara halstedii* (Farl.) Berl. et de Toni, one of the serious sunflower diseases in some regions of the world
- Significant yield- and quality-limiting factor
- *P. halstedii* survives for up to 10 years in soil as sexual, thick-walled oospores



(Kandel, 2014 NSA forum)



(Photo by Markell)

Background Knowledge, *cont*.

- Management includes crop rotation, fungicide treatment (seed treatment), control of weed hosts, DM resistant hybrids, *etc.*
- Development of DM resistant hybrids is top choice for disease management
- More than 20 DM resistance genes (Pl_1-Pl_{18}, Pl_{21}, Pl_v, Pl_w, $Pl_{x\text{-}z}$, Pl_{ARG}, *etc*.) have been identified in sunflower and wild species
- None of them in confection sunflower

Progress of Molecular Mapping of DM R-Genes in Sunflower

Gene	Linkage Group	Source line	R-gene origin	Reference
Pl_{13}	1	HA-R5		Mulpuri *et al*. 2009
Pl_{14}	1	HA-R4		Bachlava *et al*. 2011
Pl_{16}	1	HA-R4		Liu *et al*. 2012
Pl_{ARG}	1	RHA 419	*H. argophyllus*	Dußle *et al*. 2004
Pl_{18}	2	-	*H. argophyllus*	Qi *et al*. 2013
Pl_{17}	4	HA 458, PI 468435	Wild *H. annuus*	Qi *et al*. 2013
Pl_{1}	8	RHA 266, RHA 274	Wild *H. annuus*	Mouzeyar *et al*. 1995
Pl_{2}	8	AMES 3235, PI 497250, RHA 274	Wild *H. annuus*	Vear *et al*. 1997
Pl_{6}	8	HA 335, HA 336	Wild *H. annuus*	Roeckel-Drevet *et al*. 1996
Pl_{7}	8	HA 337, HA 338, HA 339	*H. praecox*	Bert *et al*. 2001
Pl_{15}	8	RNID		De Romano *et al*. 2010
Pl_{5}	13	INRA inbred line XRQ, Progress	*H. tuberrosus*	Bert *et al*. 2001
Pl_{8}	13	RHA 340	*H. argophyllus*	Radwan *et al*. 2003, 2004
Pl_{21}	13	HA 61		Vicourt *et al*. 2012

DM Resistance Testing in Sunflower Seedlings

(Photos by Liu)

Research Objectives (2014 – 2017)

- Transfer DM resistance genes from oil-sunflower into confection sunflower
- Create superior confection sunflower germplasms with both DM and rust resistance
- Develop DNA markers linked to disease resistance genes to facilitate marker-assisted selection

Research Strategy

Special crosses

- ✓ CONFSCLR5 × RHA 464 (PI_{ARG} + R_{12})
- ✓ HA-R6 (R_{13a}) × HA 458 (PI_{17})
- ✓ HA-R6 (R_{13a}) × new DM R-line (PI_{18}, from *H. argophyllus* PI 494573)

Backcrosses

Recurrent Parent × R-Parent

F_1 × Recurrent Parent

BC_1 × Recurrent Parent

BC_2 × Recurrent Parent

BC_3 × Recurrent Parent

BC_4

⊗

BC_4F_2

MAS of Double Resistant Homozygotes

BC_4F_4

Field testing & release

Final products (BC_4F_4)

Confection line 1: PI_{ARG} + R_{12}

Confection line 2: PI_{17} + R_{13a}

Confection line 3: PI_{18} + R_{13a}

Available DNA Markers for Selection of Double Resistant Homozygotes in BC_4F_2

Gene	Available SSR marker	Available SNP marker	Reference
Pl_{ARG}	Yes	Yes	Wieckhorst *et al*. 2010; Qi *et al*. 2012
Pl_{17}	Yes	Yes	Qi *et al*. 2013
Pl_{18}	Yes	Yes	Qi *et al*. 2013, 2014
R_{13a}	Yes	Under development	Gong *et al*. 2013

2014 Goals

- Create the first cross in greenhouse in January and backcross (BC_1) in the summer greenhouse and BC_2 generation in the winter greenhouse
- Test all generations in laboratory/greenhouse for resistance
- Begin the process of identifying molecular markers for the DM resistance gene derived from RHA 468

1st Year Research Progress

- Made crosses: CONFSCLR5 × RHA 464, HA-R6 × HA 458, and HA-R6 × new DM resistant line from *H. argophyllus* PI 494573
- DM resistance testing (race 734) of F_1 of HA-R6 × HA 458 and HA-R6 × new DM resistant line
- DM and rust (race 366) testing of F_1 of CONFSCLR5 × RHA 464
- Backcross of F_1 to recurrent parents to produce BC_1

1st Year Research Progress, *cont*.

- DM testing of BC_1 of HA-R6 × HA 458 and HA-R6 × new DM R-line
- DM and rust testing of BC_1 of CONFSCLR5 × RHA 464

BC_1	No. of seeds germinated	No. of seeds inoculated	No. of DM R-plants	No. of DM & rust double R-plants
CONFSCLR5 × RHA 464	405	243	14	5
HA-R6 × HA 458	100	32	8	8
HA-R6 × new DM R line	120	75	33	33

- Backcross of the selected BC_1 to recurrent parents to produce BC_2

1st Year Research Progress, *cont*.

Molecular mapping of DM-R gene in RHA 468

- Mapping populations

  F_2 and F_3 populations were developed from the cross of HA-R8/RHA 468

  - HA-R8: resistant to rust, but susceptible to downy mildew
  - RHA 468: resistant to downy mildew, but susceptible to rust

- Downy mildew evaluation in F_3 population

  - 183 $F_{2:3}$ families (30 seedlings each; 5,490 in total) were inoculated with DM race 734
    - 53 homozygous susceptible
    - 30 homozygous resistant
    - 100 heterozygous resistant
  - DM resistance in RHA 468 is controlled by a single dominant gene

1st Year Research Progress, *cont*.

Molecular mapping of DM-R gene in RHA 468

- Pedigree of RHA 468: RHA 428/RHA 426//RO 12-13/3/RHA 274/PRS 5
- RHA 428 was thought to be the DM R-gene donor for RHA 468. However, RHA 468 is resistant to race 774, whereas, RHA 428 is susceptible to race 774
- Difference of DM resistance specificity between RHA 468 and RHA 428

Line	No. seed germinated	No. inoculated	DM spore		DM score	
			Race	ID#	S	R
Car 272 (S-check)	20	11	774	41	11	
RHA 340 (R-check)	20	11				11
RHA 428	20	11			11	
RHA 468	20	12				12
Car 272 (S-check)	20	12	774	47	8	
RHA 340 (R-check)	20	13				13
RHA 428	20	14			10	4
RHA 468	20	12				12
Car 272 (S-check)	20	12	774	131	12	
RHA 340 (R-check)	20	14				14
RHA 428	20	12			11	1
RHA 468	20	13				9

1st Year Research Progress, *cont*.

Molecular mapping of DM R-gene in RHA 468

- No LG13 molecular markers associated with DM resistance in RHA 468, but LG1 markers
  - Hypothesis: RHA 468 DM gene is derived from RHA 428 (LG13)
  - Bulked Segregant Analysis (BSA) was conducted in RHA 468 F_2 population with LG13 markers (64 SSRs and 47 SNPs), and no marker-trait association was found
  - However, LG1 SSR markers showed association with R-bulk

- The DM R-gene in RHA 468 is located on LG1

- Genotyping of F_2 population with LG1 SSR and SNP markers is underway

Future Work (2015)

- Create BC_3 generation in spring greenhouse and BC_4 generation in winter greenhouse
- Continue lab/greenhouse test of all generations for resistance
- Complete molecular mapping of the resistance gene in the line RHA 468
- Begin the process of identifying molecular markers for the DM resistance gene derived from *H. argophyllus* PI 494578

Acknowledgements

- Angelia Hogness
- Dr. Yunming Long
- Dr. Zahirul Talukder
- Chris Misar
- Cullen Walser
- Emily Wentzel

- National Sunflower Association
- Specialty Crop Block Grant, USDA-AMS
- ND Department of Agriculture

Thank you