Identification of markers associated with race-specific resistance to Aphanomyces root rot in alfalfa

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INTRODUCTION

Aphanomyces root rot (ARR), caused by *Aphanomyces euteiches*, is one of the most important diseases of alfalfa in the United States resulting in poor seedling stands and root rot of adult plants. Two races of the pathogen are currently recognized based on host resistance. Molecular markers are needed to facilitate breeding for resistance and to clarify race/resistance gene structure. Resistant gene mapping and identification in alfalfa is hampered by autotetraploid outcrossing genetics and lack of a genome sequence. The goals of this project are to:

- Determine the mechanisms of resistance to the pathogen.
- Ascertain if more than two races of the pathogen exist in nature.
- Identify QTLs for disease resistance.

METHODS

Race-specific resistance
- Hypocotyls of alfalfa seedlings were inoculated with 400 zoospores in a 5 µl drop.
- Cross sections were made at 48 h after inoculation, stained with wheat germ agglutinin-FITC, and visualized under UV illumination.

Isolation and race typing of *A. euteiches* strains from soil samples
- Seeds of differential cultivars were sown in field soil collected from alfalfa fields in MN and NY.
- After plant emergence, soil was saturated with water for 5 days. Plants were scored at 21 days after planting.
- Surface sterilized seedlings were placed on water agar. Hyphal tips of *A. euteiches* emerging from seedlings were excised and cultured on corn meal agar.
- Zoospores (400 spores/plant) of isolated strains were used to inoculate seedlings of differential cultivars.

Development and phenotyping of F1 populations
- Seedlings of differential cultivars were inoculated with race 1 and race 2 strains.
- Resistant and susceptible plants were rescued.
- Biparental crosses were made between resistant and susceptible plants.
- F1 populations were tested for resistance to race 1 and race 2 strains.

Genotyping-by-sequencing (GBS)
- Vegetative cuttings of 370 F1 progeny were phenotyped for resistance to race 1 and race 2 strains.
- DNA from each plant was used for genotyping using GBS followed by genotype calling using FreeBayes pipelines.

Cultivar	Race 1	Race 2
Saranac	C	C
WAPH-1	I	C
WAPH-5	I	I
53V52	I	I

Differential cultivars for ARR. C=compatible interaction (susceptible plant). I=incompatible interaction (resistant plant).

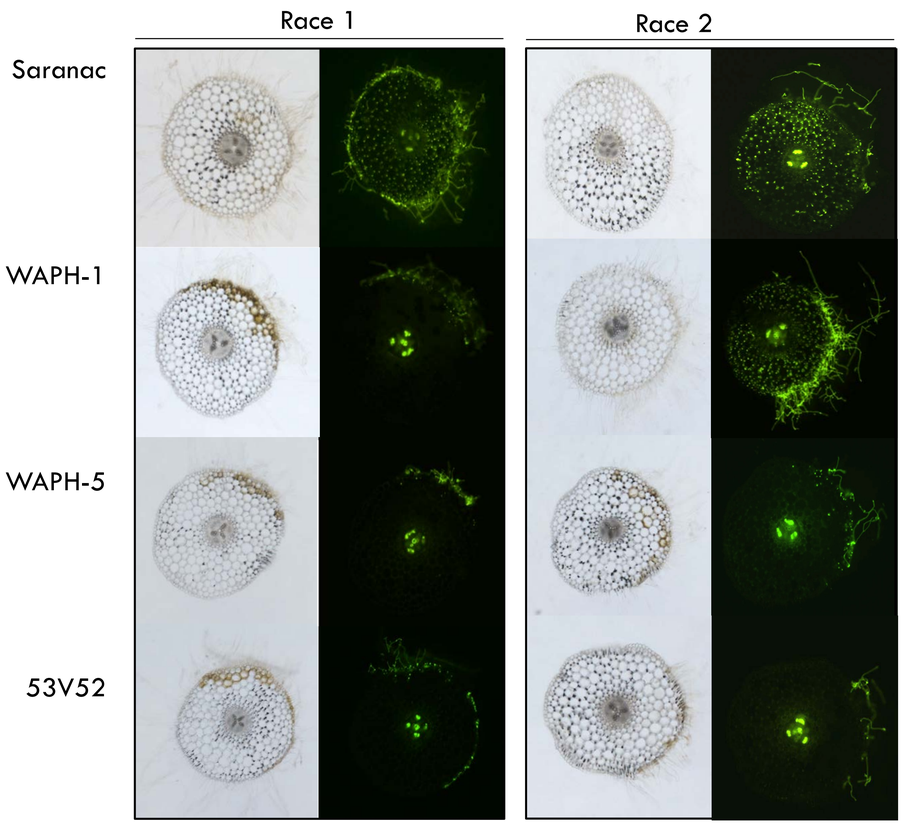
53 soil samples from MN and 40 from NY were evaluated for ARR. Seedlings of WAPH-5 were highly diseased in 21% of MN and 5% of NY soil samples, suggesting that novel race types were present. *A. euteiches* was isolated from these soils and phenotyped with the differential cultivars.

State	Soil	WAPH-1 resistant plants (%)	WAPH-5 resistant plants (%)	Predominant race
MN	GRE	6	15	Undetermined
MN	JIM	0	5	Undetermined
MN	WAS	14	28	Undetermined
NY	CAR	13	32	Undetermined
NY	ELK	32	87	2
NY	MER	0	40	2
NY	ROY	0	21	Undetermined
NY	STE	59	66	None
NY	WIL	78	78	None

RESULTS

Resistance to ARR was associated with a hypersensitive response

At 48 h after inoculation, susceptible plants were fully colonized by *A. euteiches*. The pathogen was limited to the epidermis and the first layer of cortical cells in resistant plants. Colonized cells of resistant plants showed necrosis and accumulation of phenolic compounds consistent with a hypersensitive response.



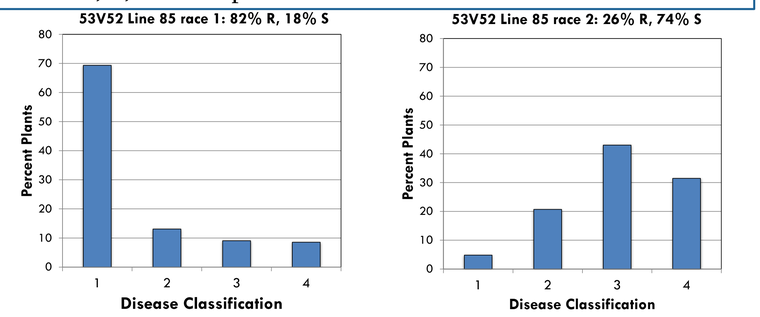
Strains of *A. euteiches* were classified as race 1 or race 2

Individual strains of *A. euteiches* recovered from infected plants could be phenotyped as either race 1 or race 2 with the differential cultivars. WAPH-5 was resistant to all strains tested.

Strain	WAPH-1 resistant plants (%)	WAPH-5 resistant plants (%)	Race
GRE1	24	86	2
GRE3	0	67	2
GRE4	3	86	2
GRE5	0	81	2
GRE6	100	96	1
GRE7	14	86	2
GRE8	0	98	2
JIM1	0	66	2
JIM2	0	50	2
JIM3	71	100	1
JIM4	0	77	2
JIM5	0	67	2
WAS4	74	97	1
WAS5	45	88	1
WAS W5-1	69	97	1
WAS6	100	100	1
CAR1	60	78	1
CAR2	58	85	1
CAR3	5	94	2
CAR W5-1	0	92	2
ELK6	67	91	1
ELK7	63	55	1
ELK8	0	88	2
MER4	0	71	2
MER5	0	87	2
MER6	6	76	2
ROY1	0	79	2
ROY3	0	91	2
ROY4	0	76	2
ROY5	0	71	2
ROY6	13	94	2
ROY8	0	86	2
ROY11	0	70	2
STE1	0	44	2
STE2	0	58	2
STE3	0	48	2
STE4	0	62	2
STE5	0	79	2
STE6	7	66	2
STE11	8	82	2
STE14	100	100	1
STE17	8	67	2
STE19	0	90	2
WIL W5-1	0	74	2

F1 populations segregated for disease phenotype

F1 plants resulting from a cross between a plant resistant to both races and a plant susceptible to both races from 53V52 were predominantly resistant to race 1 and susceptible to race 2. Similar results were obtained with seedlings and vegetative cuttings. 1, 2=resistant; 3, 4=susceptible.



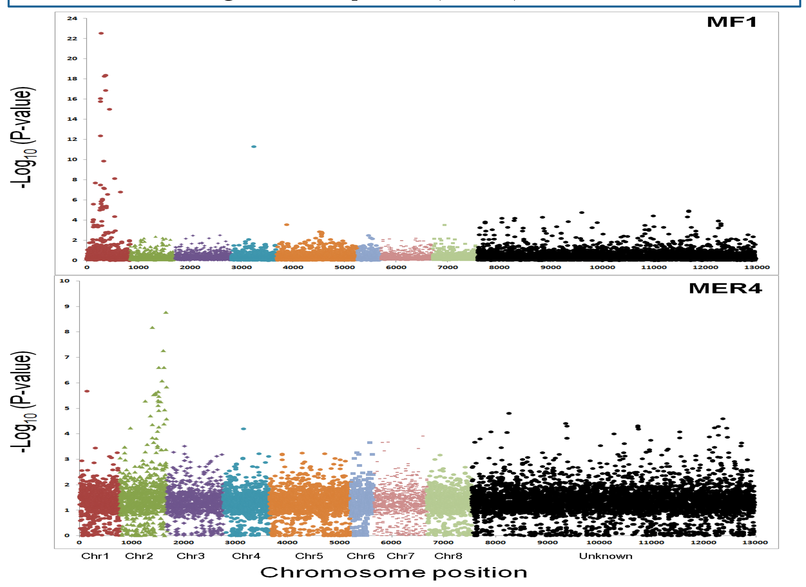
Segregation for resistance in individual F1 plants

74 F1 plants from 53V52 line 85 were tested for reaction to five strains of *A. euteiches* that were phenotyped as race 2 using the differential cultivars. Plants segregating for resistance to single strains were identified. These results provide evidence for multiple races and the presence of a multiple resistance genes in 53V52.

MER4	GRE5	ROY8	STE2	JIM1	Number of plants
R	R	R	R	R	2
S	S	S	S	S	16
R	S	S	S	S	6
S	R	S	S	S	2
S	S	R	S	S	5
S	S	S	R	S	2
S	S	S	S	R	3

GWAS identifies markers associated with resistance to race 1 (MF1) and race 2 (MER4)

Manhattan plots of genome-wide association mapping showing significant SNPs associated with resistance to ARR. The positions of SNPs were based on the alignment of sequence tags of alfalfa to the *M. truncatula* genome sequence (v 4.01).



CONCLUSIONS

- Resistance to ARR was mediated by a hypersensitive response.
- Segregation for resistance in F1 plants to individual *A. euteiches* strains suggests that multiple races of are present in U.S. soils and resistant differentials have multiple resistance genes.
- Markers associated with resistance to a race 1 strain (MF1) was identified on chromosome 1 and resistance to a race 2 strain (MER4) on chromosome 2. Several candidate genes involved in the hypersensitive response were identified using the flanking sequences of the resistance loci. These markers may be useful in marker-assisted selection for breeding alfalfa.