Mapping and pyramiding adult plant resistance loci to manage oat crown rust caused by *Puccinia coronata* f. sp. *avenae*

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Abstract

Oat crown rust, caused by the fungus *Puccinia coronata* f. sp. *avenae*, is one of the most devastating cereal diseases in the world. Breeding for genetic resistance is viewed as a sustainable and cost-effective strategy to manage this disease. While race-specific resistance breaks down within a few years of deployment, adult plant resistance (APR) or partial resistance has been shown to provide durable protection against the pathogen. In this study, we are mapping APR loci that are linked to crown rust resistance using nine recombinant inbred mapping populations. These populations have been evaluated in the Buckthorn Nursery in St. Paul, MN, where a natural sexual population of crown rust exists. Plants with extreme phenotypes were subjected to selective genotyping using the 6K Oat SNP Chip. After SNP calling and QTL mapping based on allele frequencies, eleven loci linked to APR were detected from the nine populations, of which four have already been validated. Using marker-assisted selection (MAS), we plan to combine these APR loci and release the pyramided lines as breeding germplasm.

Introduction

- Losses due to crown rust usually vary from 10-40%. In 2014, Minnesota and South Dakota suffered 35-50% yield losses due to the disease.
- Vertical resistance is short-lived in the field, lasting only for a few years, due to high variability in the pathogen.
- Pyramiding *R* genes is a viable approach to achieve durable resistance.
- The macrocyclic and heteroecious nature of *P. coronata* f. sp. *avenae* likely contributes to its genetic variability (Figure 1).
- Mutations and somatic hybridization may occur during the asexual phase while meiosis takes place in its alternate host, buckthorn (*Rhamnus* sp.), and leads to re-assortment of virulence factors.
- To date, no *R* genes nor effector genes have been cloned and the molecular interaction of oat-*P. coronata* f. sp. *avenae* remains unclear.

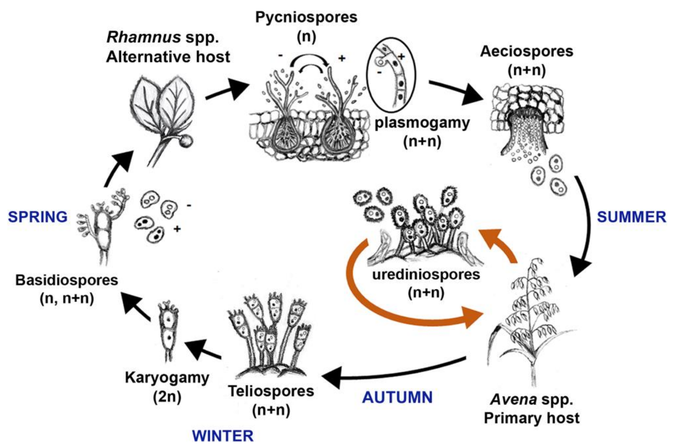


Figure 1. Life cycle of *P. coronata* f. sp. *avenae* (Nazareno, et al., 2017).

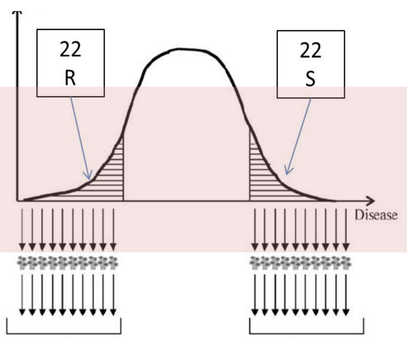
Materials and Methods

- Nine mapping populations were developed in 2010 to find new sources of APR (Table 1), which were evaluated in the Buckthorn Nursery in St. Paul, MN for three seasons (Figure 2).
- Crown rust was assessed by obtaining disease severity and reaction class per entry using the modified Cobb's scale. Disease severity was multiplied to numerically-converted reaction class to obtain the coefficient of infection. Data were combined using rank analysis to determine individuals that consistently exhibit extreme phenotypes.

Table 1. Mapping populations for adult plant resistance.

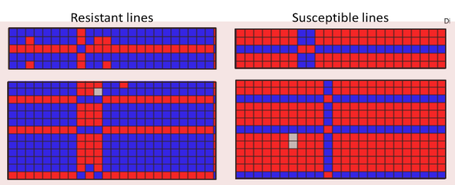
	Population	Lines	Generation
1	OtanaA x CI7035-1	172	F8
2	OtanaA x CI4706-2	130	F8
3	Otana x PI189733	185	F9
4	OtanaA x CI8000-4	227	F9
5	OtanaA x CI9416-1	195	F9
6	OtanaA x PI266887-1	154	F11
7	OtanaD x PI260616-1	191	F11
8	OtanaI x PI263412-1	173	F11
9	OtanaA x CI1712-5	195	F7

Phenotyping at Buckthorn Nursery, St. Paul, MN

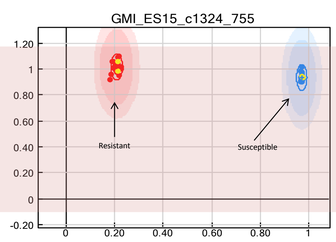


Selective sampling
(Collard et al. 2008)

6K SNP Chip genotyping (Illumina)



QTL mapping and validation using KASP (qPCR) assay

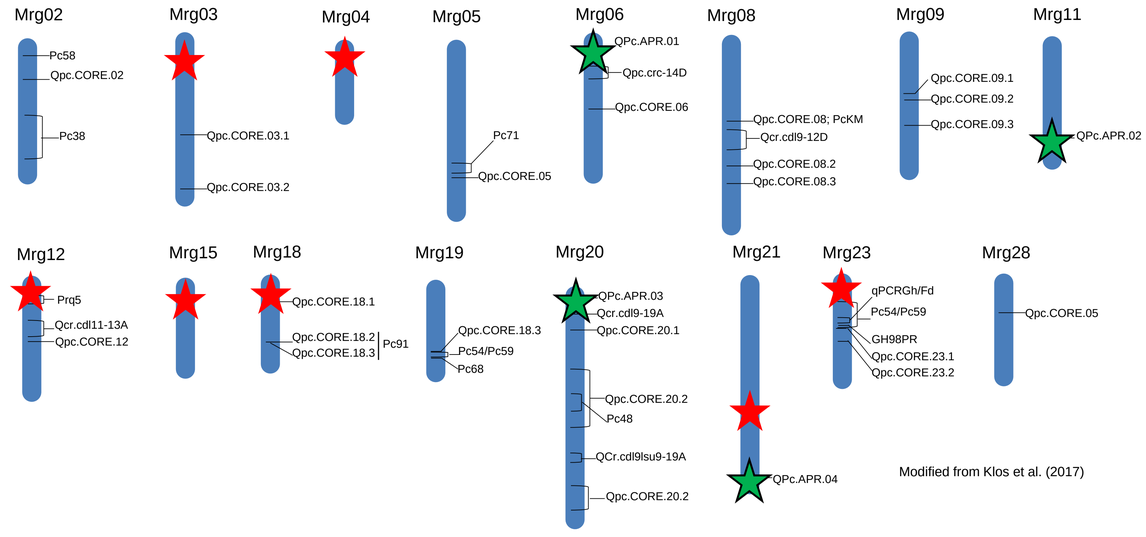


SNP calling in GenomeStudio2

Figure 2. Workflow for allele frequency-based mapping of APR loci using selective genotyping approach.

Results and Discussion

- SNP filtering in GenomeStudio2 yielded 1200-1900 markers per population, which were used in the mapping.
- A total of eleven APR loci in ten linkage groups were detected from the nine mapping populations (Figure 3): CI8000-4 and PI260616-1 (Mrg21), CI1712-5 (Mrg06, Mrg11), PI266887-1 (Mrg04, Mrg15), CI9416-2 (Mrg03, Mrg21), CI7035-1 (Mrg15, Mrg23), CI4706-2 (Mrg18, Mrg20), PI263412-1 (Mrg06, Mrg12), and PI189733 (Mrg21).
- Five of these loci are in regions where genes or QTL for crown rust have been reported previously while six are potentially new based on the most recent oat consensus map.
- Four of the eleven loci (Figure 3) have been validated by developing Kompetitive Allele-Specific PCR (KASP) markers and genotyping the whole population. SNP markers were converted to KASP (M2 and M9), which were used to genotype the whole populations of CI8000-4 and PI260616-1 (Figure 4). M2 and M9 markers have r^2 of 0.30-0.51.



Modified from Klos et al. (2017)

Figure 3. The eleven APR loci identified in this study; four were validated using KASP (green stars) while seven are still for validation (red stars).

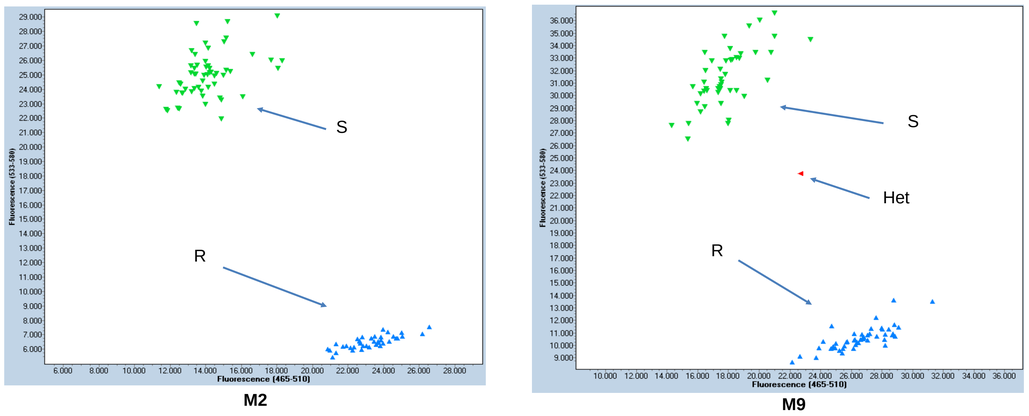


Figure 4. Segregation of marker genotypes (R- resistant, S- susceptible, Het- Heterozygous) in the KASP assay for the PI260616-1 population.

Conclusions and Future Work

- We identified eleven APR loci from nine mapping populations.
- Selective genotyping reduces cost and the KASP assay is effective in marker validation.
- The KASP assay works in differentiating the marker genotypes and will be used in the future for marker-assisted selection (Figure 5); pyramided lines will be released as germplasm donors for crown rust resistance.

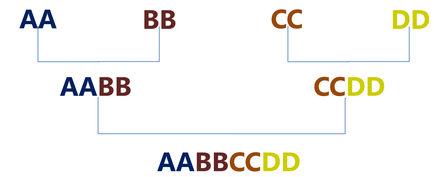


Figure 5. APR loci pyramiding approach using MAS.

Acknowledgements

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