Simple sequence repeat markers for *Puccinia graminis* f. sp. *lolii* from the Willamette Valley.

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Introduction:

- Oregon produces the majority of the world's temperate grass seed, with 300,000+ acres of perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinacea*) and fine fescue grown for seed annually.
- Stem rust disease in these crops caused by *Puccinia graminis* f. sp. *lolii* (*Pgl)* can result in crop failure (**Fig. 1A**).
- Host resistance to the disease is known, but most cultivars do not have adequate resistance to eliminate fungicide use.
- Little is known about the genetic variability of *Pgl* in the Pacific Northwest and therefore its ability to evolve.
- The presence of Oregon grape (*Mahonia*) and barberry (*Berberis*) as alternate hosts (**Fig. 1B**) potentially provides a source of pathogen genetic diversity through sexual recombination.
- The pathogen can also overwinter and clonally propagate using urediniospores produced on grass plants.

Objectives:

- The objective of this study was to develop simple sequence repeat (SSR) markers that can be used to assess *Pgl* genetic diversity in the Willamette Valley of Oregon.

Fig. 1 A) Perennial ryegrass plants that are resistant (top) and suscptible (bottom) to stem rust. B) Oregon grape is a known alternate host enabling sexual recombination of cereal isolates of *Puccinia graminis* (Upadhaya et al, 2022). Oregon grape grows wild and as an ornamental in Western Oregon alongside perennial grasses grown for seed. C) Stem rust pustules (p) on perennial ryegrass caused by *Pgl (Photo by Cynthia M. Ocamb, 2001, https://pnwhandbooks.org/plantdisease/host-disease/grass-seed-stem-rustI*.) Pustules result from infection of a single urediniospore

Methods:

- 122 single pustule *Pgl* isolates (**Fig. 1C**) were collected from tall fescue, perennial ryegrass and fine fescue from seven fields throughout the Willamette Valley **(Fig. 2**).
- DNA was extracted from single pustule isolates using a KingFisher Flex extraction system.
- 171 SSR markers previously used to study stem rust from wheat and oat (Szabo, 2007; Berlin et al., 201; Gnocato et al., 2018) were tested for amplification of *Pgl* DNA by PCR.
- Reactions products were run on an AB 3730 capillary DNA sequencer to size fragments and alleles were scored manually using Geneious Prime software v. 2020.2.4.

Fig. 2 * indicate the locations of *Pgl* collections in the mid and North Willamette Valley. Cities Portland, Salem, Eugene and Corvallis are shown for reference.

- Data was analyzed using Poppr (Kamvar et al. 2014).
- Index of association to measure sexual recombination and clustering analysis were conducted on a subpopulation of 89 isolates of tall fescue, perennial ryegrass and fine fescue using poppr.

Results:

- 23 markers with higher polymorphism and clearly identifiable alleles were designed into six multi-plex pools of up to 4 markers each.
- Test of these markers on 122 *Pgl* isolates result in 42 multi-locus genotypes.
- Nei's gene diversity index for each marker ranged from 0.59 to 0.80.
- Sexual reproduction in the subpopulation is negligible to absent and the population has extensive clonality (**Fig. 3)**.
- Clustering in the subpopulation grouped *Pgl* isolates by host suggesting host specialization with *Pgl* (**Fig. 4)**.

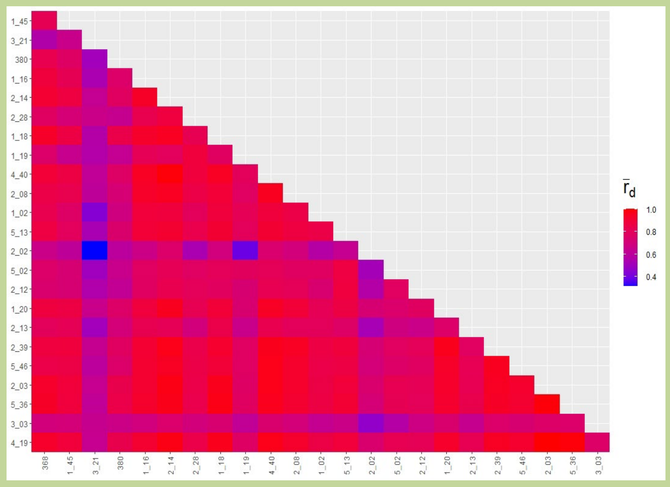

Fig. 3: Heat map of index of association (r_d) for 23 simple sequence repeat markers on 31 tall fescue, 48 perennial ryegrass and 10 fine fescue single pustule isolates of *Pgl.* Higher values indicate higher linkage disequilibrium. The overall r_d for this data is 0.78 indicating limited to non-existent sexual recombination.

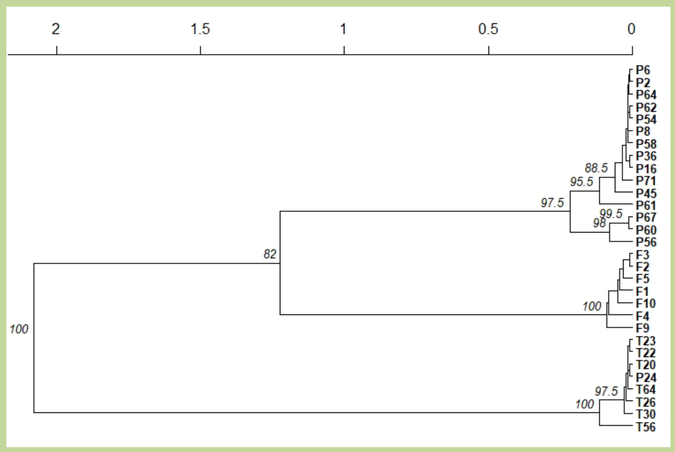

Fig. 4: Nei's Distance was used to make the dendrogram, with 200 replications and bootstrap support of 80 or greater is indicated on the tree. Hosts are indicated by P (perennial ryegrass), F (fine fescue), and T (tall fescue). Marker data was from 23 simple sequence repeat markers on 31 tall fescue, 48 perennial ryegrass and 10 fine fescue isolates of *Pgl.*

Impact & Future Work:

- Knowledge of *Pgl* genetic diversity informs seed production management decisions
  - The 23 SSR markers are useful for studying *Pgl* population genetics.
  - *Pgl* is genetically diverse in seed production regions, but the tested *Pgl* collections do not support sexual. recombination in the Willamette Valley
  - The subpopulation appears to maintain itself as a set of clones that propagate through urediniospores.
- Future research with larger samples will explore population structure across PNW seed production locations, the extent of variation within fields, sexual recombination, and host adaptation.

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