Simple Sequence Repeat Markers for *Puccinia Graminis* F. Sp. *Lolii* from the Willamette Valley

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Stem rust disease caused by *Puccinia graminis* f. sp. *lolii* (*Pgl*) can cause complete crop failure in grasses grown for seed. The Willamette Valley of Oregon is the primary seed growing region for perennial ryegrass, tall fescue, and fine fescues. Stem rust, while effectively controlled with fungicides, consistently occurs in these grasses. Genetically diverse plant pathogens have a greater chance of overcoming control measures and may require multi-tactic disease control approaches. The tools do not exist to study diversity of *Pgl* in Western Oregon and little is known about the diversity of the pathogen in this region. The pathogen is a biotroph and multiplication of individual genotypes for DNA extraction is difficult. Alternatively, isolated single infection pustules can be readily identified and sampled from plant leaves. These types of samples often yield limited amounts of DNA for diversity studies, making simple sequence repeat (SSR) markers useful since SSRs often require less DNA than other types of markers. 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. One hundred and twenty-two single infection pustules were collected from seven field sites throughout the Willamette Valley and DNA extracted. The samples were taken from perennial ryegrass, tall fescue, chewings fescue and creeping red fescue. One hundred seventy one SSR markers reported from studies of Puccinia graminis collected from wheat and oat (Berlin et al, 2013; Gnacato et al. 2018) were tested for amplification of DNA from eight *Pgl* isolates by PCR according to methods of Schuelk (2000). Reactions products were run on an AB 3730 capillary DNA sequencer to size amplification fragments and alleles were scored manually using Geneious Prime software v. 2020.2.4. Based on the amount of polymorphism, 36 markers were further tested using eight additional *Pgl* isolates. From these experiments, 23 markers with higher polymorphism and clearly identifiable alleles were then designed into six multi-plex pools of up to 4 markers each using the software multi-plex manager 1.2. The multi-plex reactions were used to evaluate 122 *Pgl* isolates. From these reactions, the 23 markers generated four to nine alleles each, resulting in 42 multi-locus genotypes. The Simpson's index and Nei's gene diversity index both ranged from 0.59 to 0.80. Nine of the markers amplified fragments in the size range previously reported for *Puccinia graminis*. For the remaining 14 markers no size information was available and one product from a pcr reaction from each marker was sequenced; the sequence in all cases was consistent with the expected short randem repeat. These results demonstrate that these markers are useful to study *Pgl* diversity.

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