

MINIER, DOUGLAS H.¹, LINDA E. HANSON*², MARINA L. RAMON³ and FRANK N. MARTIN³, ¹PSM, Michigan State University and ²USDA-ARS, 1066 Bogue St., East Lansing, MI 48824, ³USDA-ARS, 1636 E. Alisal St., Salinas CA 93905. **Identification and validation of microsatellite markers for SSR genotyping of *Rhizoctonia solani* AG2-2.**

Rhizoctonia solani (Kühn) AG2-2 is an important, soilborne pathogen of sugarbeet (*Beta vulgaris*) as well as a number of other crops. An improved understanding of the diversity and population structure of this pathogen could benefit management practices. Microsatellites have become an invaluable tool for these types of studies; so in order to develop a set of useful microsatellite markers, we utilized an *in-silico* approach to identify potential loci. One isolate from each of three distinct phylogenetic groups was sequenced on a HiSeq4000 and assembled using CLC Genomics Workbench. Loci that were at least trinucleotide and of a suitable repeat length were selected from isolate Rs850. These preliminary loci were compared to the other two isolate assemblies to determine if there were differences in repeat length, there were no indels in the flanking regions and conserved primers could be developed. We generated 33 potential marker loci that were tested on isolate Rs850 for PCR amplification using a single annealing temperature and MgCl₂ concentration. Those that amplified well under these conditions were then tested on eight additional isolates, which represented the three distinct phylogenetic groups. Sixteen primer pairs amplified all nine isolates and showed probable polymorphisms in fragment length. These primer sets were paired based on suitability for multiplexing and labeled with either Hex or 6-Fam fluorescent dyes for automatic size detection on an ABI 3730 sequencer. Those that appear to be suitable markers will be further screened on 22 additional isolates to confirm sufficient variation. We expect that the effort invested by sequencing multiple isolates and developing markers from those assemblies will increase the probability that preliminary SSR marker selections will be successful.