Aphanomyces and Phytophthora Root Rots; development of molecular markers associated with tolerance in alfalfa

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Aphanomyces euteiches and *Phytophthora medicaginis* are major causes of decline of established stands of alfalfa in northern production areas particularly in wet, saturated soil conditions on poorly drained soils or on clay loam soils during extended periods of wet weather. Marker-assisted selection could help accelerate the introgression of resistance genes in germplasm of high agronomic value. In this study, two alfalfa cultivars, Gilbratar and Manitou were used for marker selection linked to Aphanomyces root rot (ARR) superior tolerance. For each cultivar, 1500 seedlings were challenged with a mixture of four pathogenic isolates of *Aphanomyces euteiches* (from eastern and western Canada). The best 100 ARR-tolerant phenotypes were selected and DNA was extracted from each plant individually (first cycle of the recurrent selection; ARR-R1 Gibraltar and ARR-R1 Manitou). Pooled DNA samples for each population were generated and used for a bulk segregant analysis of DNA polymorphisms using the SRAP technique. Polymorphic fragments associated with tolerance to ARR were identified within both Gibraltar AAR-1 and Manitou AAR-R1. The genotypic frequency of these specific fragments will be determined. A second cycle of recurrent selection is in progress with seeds resulting from crosses of the best 100 genotypes of the first round of selection.

In the last three years, we used a PRR-sensitive Apica background population initially improved for superior freezing-tolerance (Apica-TF3) to develop PRR-tolerance by recurrent selection. In each cycle, 1500 seedlings were challenged with a blend of four isolates of *P. medicaginis* and the 100 best PRR-tolerant phenotypes were selected and intercrossed to generate three Apica-derived populations (Apica PRR-R1, R2 and R3). DNA polymorphisms associated with PRR tolerance were subsequently uncovered with sequence related amplified polymorphism (SRAP) amplification of bulked DNA within each population. Among the 280 SRAP primer pairs assessed, an amplicon obtained with the F11-R9 primer pair was closely associated with PRR-tolerance. After three cycles of recurrent selection, we improved the initial Apica background from sensitive (S, 0-5% resistant plants) to highly resistant (HR, >50% resistant plants) to PRR.