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High resolution melting (HRM) analysis in sugar beet: identification of SNP markers associated to Fusarium resistance

ABSTRACT

Fusarium spp. cause severe damage in many agricultural crops including sugar beet. Sugar beet needs to be protected from these soil borne pathogens to guarantee an optimal sugar yield in the field. The genetic control is the key to overcoming this disease. Identification of single nucleotide polymorphism (SNPs) markers linked to the resistance can be a powerful tool for the introgression of valuable genes needed to develop Fusarium-resistant varieties. The use of molecular markers to breed for Fusarium resistance is a cost effective tool to enhance conventional selection. An association study is in progress at the University of Padova (Italy) to identify SNP markers linked to Fusarium resistance. DNA was isolated from 12 sugar beet lines, each using 4 resistant individuals and 4 susceptible individuals to *Fusarium oxysporum*, for a total of 96 samples. Twenty resistant analogue genes (RGAs) were screened by means of HRM analysis, and results were validated by Sanger sequencing. The difference in genotype distribution between resistant and susceptible samples was assessed by the Chi-square test. This study identified a mutation in one of the screened resistance candidate genes, which was significantly associated (p<0.01) with Fusarium resistance. This mutation can be accurately identified in the HRM difference plot curves. Further validation by Sanger sequencing indicated the allelic status of resistant individuals. The frequency of the resistant allele was 83% in individuals with the resistant phenotype. Our results demonstrate that HRM analysis is a rapid, feasible and reliable method for detecting SNP mutations linked to disease resistance traits in sugar beet. The next step will be to validate the identified SNPs in a wider germplasm base segregating for Fusarium resistance.