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Czech J. Genet. Plant Breed.

Gawłowska M., Święcicki W.:

The application of high resolution melting in the analysis of simple sequence repeat and single nucleotide polymorphism markers in a pea (*Pisum sativum* L.) population

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The aim of this study was to verify the high resolution melting (HRM) method in the analysis of single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers in pea (*Pisum sativum* L.). A recombinant inbred line population, Carneval × MP1401, was tested for three SNP and 103 SSR markers. HRM analysis was conducted

on a LightScanner 96 instrument with LC Green dye. The melting curve shape permitted two polymorphic genotypes to be distinguished. The results were confirmed by gel electrophoresis. Three SSR markers were sequenced and analysed by the melting prediction software. The results confirmed the presence of one polymerase chain reaction (PCR) product with two melting domains. Sequence tagged site (STS) markers produced specific products: Psat_EST_00189_01_1 (300 bp), Pis_GEN_18_2_1 (400 bp), Pis_GEN_7_1-2_1 (600 bp). Amplicons contained one, four and seven single nucleotide polymorphisms, respectively. Melting curve differences enabled the population genotyping except for Psat_EST_00189_01_1 where resolution was too low. Primers for Psat_EST_00189_01_1 were redesigned to obtain a shorter (100 bp) PCR product which increased the resolution. The number of SNPs and amplicon length are crucial for HRM resolution. The HRM method is fast and has a high throughput. The melting analysis of 96 samples takes less than 10 min. Agarose gel analysis confirmed the reliability of HRM, which

eliminates laborious post-PCR analysis.

Keywords:

genotyping; high resolution melting; molecular markers; *Pisum sativum* L.

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