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TESTING HIGH-RESOLUTION MELTING FOR SNP DISCOVERY AND GENOTYPING IN DIPLOID AND POLYPLOID CEREALS

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High Resolution Melting (HRM) is emerging as a novel technique for single-nucleotide polymorphism (SNPs) discovery and genotyping. With HRM, allelic differences are detected based on differences in melting temperature using a DNA intercalating dye. This makes HRM a fast, simple and relatively cheap close-tube technique which does not require expensive chemistry for probe labelling and any processing after PCR.

In order to explore the potential of HRM technique in both diploid and polyploid species, a composite study was performed in barley (*Hordeum vulgare* L.) and durum wheat (*Triticum turgidum* L.*)*. We first addressed the sensitivity of HRM for SNP discovery by screening several amplicons containing known SNPs of all types (AT, AG, etc). In barley, the sensitivity reached at least one mutated copy out of 20 W.T., equivalent to 1 heterozygous plant in 10-plant bulks. Such sensitivity makes HRM fully suitable for TILLING (or EcoTILLING)-like mutation discovery approaches. In durum wheat, an HRM-based protocol was successfully tested for SNP discovery at target loci, in conditions where both homoeologous target sequences were amplified. We also tested the HRM suitability for genotyping of known SNPs and indel markers in durum wheat. When experimental biparental populations were utilized, HRM provided fully accurate genotyping results, including heterozygous SNPs in the presence of a second homoeolog amplicon. However, HRM profiles were difficult to interpret when applied to a population segregating for multiple alleles.

Based on our results, HRM confirmed to be a potentially interesting technique for molecular marker genotyping for mapping, marker-assisted selection and/or TILLING.