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SUGAR BEET DHLS PRODUCTION: ESTIMATION OF DNA PLOIDY LEVELS AND GAMETES ORIGIN CONFIRMATION

ABSTRACT

Doubled haploidy technology is a fundamental tool in plant breeding as it provides the fastest way to generate populations of meiotic recombinants in a genetically fixed state. Diploid gynogenic plantlets should be investigated for their source of origin before using in breeding programs to avoid mistakes in assessing the homo/heterozygosity of progeny. Rapid screening techniques are needed to validate that the regenerated in vitro putative DHLs are indeed homozygous. Enzymatic mismatch cleavage (EMC) techniques commonly used for TILLING (Targeting Induced Local Lesions IN Genomes) were adapted for the evaluation of heterozygosity in parental F1 and putative DH plants. 28 amplicons were tested. Experiments were performed using self-extracted single-strand-specific nuclease (CEL1) and standard native agarose gels. Some primer pairs did not produce a PCR product, while others produced a high yield of PCR product, but weak or no detectible enzymatic cleavage. Seven primer pairs produced a high yield of PCR product and high yield of banding. In addition, cleavage products in synthetic mixtures of genomic DNA of the two parents prior to PCR were observed indicating homozygous polymorphisms between the parents. This resulted in the validation of 7/28 primer pairs suitable for DH screening. Ultimately this technique allows to replace the widely used isozymes or SSR methods to identifying of the maternal origin in spontaneous occurred putative Doubled Haploid plants.