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CRISPR/Cas9 editing of pectinase genes to reduce strawberry fruit softening

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Strawberry is a soft fruit characterized by undergoing extensive softening during its ripening. The disassembly of the parenchyma cell walls, the loss of cell-to-cell adhesion due to the dissolution of the middle lamella and, of minor importance, the loss of cell turgor are the main determinant factors in strawberry fruit softening. Cell wall disassembly and the loss of middle lamella take place by the induction of ripening-related genes encoding enzymes or proteins acting on different cell wall components. In a previous study, *Fragaria x ananassa*, cv. Chandler, knock-out plants for a polygalacturonase gene, FaPG1, were obtained (López-Casado et al., 2023). These plants produced fruits significantly firmer than wild type with an extended shelf life. De-methyl esterified homogalacturonan pectins are the target of polygacturonases. This pectin domain is also degraded by pectate lyases. In strawberry, this activity is mainly encoded by three ripening-induced genes that are expressed at similar levels in ripe fruits. The key role of pectate lyase in strawberry softening was demonstrated by antisense silencing of one of these genes (Jiménez-Bermúdez et al., 2002). In this research, CRISPR-Cas9 mutant strawberry plants for both polygalacturonase and pectate lyase genes were obtained. A FaPG1 mutant line previously obtained and characterized by López-Casado et al. (2023) was introduced in vitro and re-transformed via *A. tumefaciens* with the plasmid pDe-CAS9-D10A containing a sgRNA guide specific for the pectate lyase genes FxaC_5g17410 and FxaC_6g23780. Nine kanamycin-resistant transgenic lines were obtained. Genomic DNA was isolated from leaves and a 443 bp of FxaC_5g17410 and FxaC_6g23780 genes was amplified by PCR and sequenced by Sanger method. The editing rate was determined by ICE software (Conant *et al*., 2022). Six out of the 9 lines obtained were successfully edited, ranging the editing efficiency from 60 to 95%. The most frequent editing events corresponded to the deletion of 1 or 2 bases, and the insertion of a single base. All the editing events induced frame-shifting and premature termination of protein transduction due to the introduction of stop codons. Transgenic plants were acclimated and transferred to the greenhouse for phenotypical evaluation. No significant alterations in the growth pattern of edited plants were observed. Experiments are currently underway to determine the effect of stacking mutations on polygalacturonase and pectate lyase genes on fruit quality and postharvest shelf life.

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