Title Evaluation of the role of the endo-β-(1,4)-glucanase gene *FaEG3* in strawberry fruit softening

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Abstract

In strawberry, *Fragaria* × *ananassa* Duch., fruit, two different endo-β-1,4-glucanase genes, *FaEG1* and *FaEG3*, also named *Cel1* and *Cel2*, are expressed during the softening process that occurs during fruit ripening. It has also been suggested that *FaEG3*, which contains a putative cellulose-binding domain, could play a key role in fruit development, since previous attempts to down-regulate this gene through transgenesis have been unsuccessful. In this investigation, we obtained transgenic strawberry plants containing an antisense sequence of the *FaEG3* gene under the control of the 35S promoter. Ripened fruit from transgenic lines (Acel lines) showed large variation in *FaEG3* silencing, but fruit firmness was similar to control fruit in all the lines. Two Acel lines showing almost 95% reductions in *FaEG3* mRNA levels were selected for further study. In these lines, *FaEG3* down-regulation was high, from 78 to 95%, at all fruit developmental stages, whereas *FaEG1* was only slightly suppressed. In spite of the high *FaEG3* silencing achieved, EGase activity was not modified in ripe fruit. At the cell wall level, walls from transgenic ripe fruit contained a significantly higher amount of the 4 M KOH fraction, which is enriched in hemicellulosic polymers. The analysis of this fraction by size exclusion chromatography showed that transgenic cell walls contained a smaller amount of higher molecular mass polymers than controls. Altogether, these results indicate that *FaEG3* does not play a key role either in fruit development or fruit softening. However, its silencing affects the amount and, in a minor way, the size of hemicellulosic polymers.