

DOWNREGULATION OF NAC TRANSCRIPTION FACTORS MODIFIES CELL WALL COMPOSITION AND INCREASE STRAWBERRY FRUIT FIRMNESS

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The strawberry is a soft fruit with a very short post-harvest shelf life. The changes in texture during fruit ripening are mainly due to the dissolution of the middle lamellae, reducing cell-to-cell adhesion, and the weakening of parenchymal cell walls as result of the action of cell wall modifying enzymes. At present, no master regulator of this process has been discovered yet. NAC transcription factors have been involved in numerous physiological processes, including fruit ripening. In strawberry, the NAC family comprises more than 110 genes, and at least 6 of them are expressed during fruit development. In this research, we performed a functional analysis of two ripening-related NAC genes, *FaNAC2* and *FaNAC3*, in *Fragaria x ananassa* Duch. cv. Chandler. Several RNAi transgenic lines showing low *FaNAC2* or *FaNAC3* mRNA levels in fruit were obtained through *Agrobacterium*-mediated transformation. These lines produced fruits significantly firmer than control at the ripe stage, being the increase in firmness higher in *FaNAC2* silenced plants. Cell walls were extracted from ripe transgenic fruits and characterized by ELISA and Epitope Detection Chromatography (EDC), using monoclonal antibodies against different polysaccharide epitopes. *FaNAC2* transgenic lines showed more extensive changes than *FaNAC3*; these modifications involved increased amounts of demethylated pectins (LM19) in water and CDTA fractions and an alteration of the lateral branches of RG-I, decreasing the amount of arabinan epitopes and increasing galactan epitopes detected by LM6 and LM5, respectively. The amount of arabinogalactan proteins recognized by the JIM13 antibody was also affected, decreasing in the Na₂CO₃ fraction and increasing in the 4M KOH and cellulase fraction of the transgenic lines. The results obtained indicate that NAC genes could be involved in the regulation of cell wall disassembly associated to strawberry fruit softening. The transcriptomes of transgenic fruits are currently being analyzed by RNAseq to determine genes encoding cell wall modifying proteins whose expression could be modulated by NAC transcription factors.

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