Title Cloning, characterisation and expression analyses of cDNA clones encoding cell wall-modifying enzymes isolated from ripe apples

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Abstract

Fruit softening is accompanied by modifications of the cell wall pectic and hemicellulosic fractions, as the result of the combined action of several cell wall-modifying enzymes. The objective of this work was to clone specific cDNAs that encode isoforms of cell wall-modifying enzymes, which are expressed during the final stages of apple softening, and to establish a temporal sequence of their accumulation. A cDNA library enriched with mRNA isolated from over-ripe fruit was constructed and screened. A pectin methylesterase (*Md*PME1), a pectate lyase (*Md*PL1), an α-l-arabinofuranosidase (*Md*AF1) an endo-1,4-β-glucanase (*Md*EG1), two xyloglucan endotransglucosylase/hydrolases (*Md*-XTH1 and *Md*-XTH2), and an alpha-expansin (*Md*EXPA3) specific cDNAs were identified by homology-based cloning, and their mRNA accumulation was examined during fruit growth and ripening. The expression of an apple β-galactosidase (β-Gal; pABG1) and a polygalacturonase (PG; pGDPG-1) mRNA previously reported was also investigated using the same biological material. Transcripts of all enzymes, except *Md*PME1, could be unambiguously detected by semi-quantitative RT-PCR in fruit during ripening. However, transcripts of *Md*EG1 were more abundant at fruit set and *Md*PL1 exhibited higher expression before commercial maturity. The strongest RT-PCR signals in over-ripe fruit were observed for PG, β-Gal and *Md*-XTH1 clones. Two XTHs were detected in over-ripe fruit. While *Md*-XTH1 acts constitutively during fruit development, *Md*-XTH2 showed a ripening-related pattern. The *Md*-XTH2-encoded protein was heterologously expressed in *Saccharomyces cerevisiae* and showed both transglycosylase and hydrolase activities. Expression analyses conducted in flowers, peduncles, young and expanded leaves, and petioles of senescent leaves revealed that none of the cloned cDNAs is fruit specific.