Abstract

Fruit softening is a result of weakening of the cell wall and degradation of the middle lamella as a result of coordinated activity of various cell wall modifying enzymes. Pertinent to apple (*Malus pumila* Mill.) fruit physiology, activities of three cell wall modifying enzymes, polygalacturonase, β-galactosidase, and α-L-arabinofuranosidase were studied in eight apple genotypes during storage. Fruit were stored up to eight weeks and evaluated at two week intervals, when enzyme activities and fruit texture were measured. Some of the genotypes did not soften at all during the eight weeks of storage and others softened at a variable rate. Variation in softening was related to patterns of enzyme activities. In another experiment, a suppression subtractive hybridization experiment identified 15 cDNAs differentially expressed in fresh or three-months-stored 'Macoun' fruit. Transcript levels of these 15 cDNAs were further quantified with quantitative real-time polymerase chain reaction (qRT-PCR) in fresh and three-months-stored fruit from Macoun, a genotype that softens during storage and Honeycrisp, a genotype that does not soften during storage. Three cDNAs, potentially important for postharvest changes in apple fruit were identified based on their differential expression patterns in fresh and three-months-stored 'Macoun' and 'Honeycrisp' fruit. Expression analyses of fruit ripening related genes for four cell wall modifying enzymes, expansin (*MdEXPA2*) polygalacturonase (*MdPG*), β-galactosidase (*pABG1*), and α-L-arabinofuranosidase (*MdAFase1*), suggested that the combination of a late increase in *MdEXPA2* and decreased levels of *MdPG* and *MdAFase1* transcript levels in 'Honeycrisp' fruit during storage may lead to its non-softening phenotype. Additionally we identified two alleles of the fruit softening specific expansin gene *MdEXPA2* that differ in their 3' untranslated region (UTR). We examined the presence of these alleles in 15 different apple genotypes differing in postharvest softening. One of the alleles appeared to be associated with all 7 non-softening phenotypes, while the other allele was associated with 6 out of 8 softening phenotypes. The 3'-UTR does not appear to alter translation of expansin protein in either phenotype.