Title Differential expression and ethylene regulation of β-galactosidase genes and isozymes isolated from avocado (*Persea americana* Mill.) fruit

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Abstract

β-Galactosidases (EC 3.2.1.23; β-Gals) consist of several isoforms which have different activity levels against native and synthetic substrates and play an important role in cell wall metabolism during fruit growth and ripening. In this study, we isolated three new β-Gal cDNA clones, *PaGAL2*, *PaGAL3* and *PaGAL4*, from the fruit of ripening avocado in addition to the *AV-GAL1* clone previously obtained. The expression patterns of these genes during fruit ripening were quite different. The *AV-GAL1* transcript, which was solely found in the fruit, accumulated with fruit ripening. *PaGAL2* transcript, which was detected in leaves, shoots, roots and fruit, showed a constant level throughout fruit ripening. The level of *PaGAL3* transcript in control fruit, which was not detected in root but only in other tissues, increased markedly at 2 days after treatment (DAT) (air treatment) and dropped quickly at 4 DAT in fruit. The transcript was not detectable at 6 DAT and thereafter. The *PaGAL4* transcript was detected in all tissues except for the fruit. In order to investigate the role of ethylene, on the regulation of β-Gal expression, pre-ripe fruit were treated with either ethylene or its inhibitor 1-methylcyclopropene (1-MCP). Exogenous ethylene promoted *AV-GAL1* expression but severely suppressed *PaGAL3* expression. Ethylene also affected the activities of fractionated β-Gal isozymes in a differential manner. Among the three isozymes, the increase in AV-GAL III activity with fruit softening were promoted by exogenous ethylene and delayed by 1-MCP. However, no apparent changes in the activities were observed in the other two isozymes. Based on the results obtained, it seems that *AV-GAL1*, which may encode AV-GAL III, is important for postharvest fruit softening while *PaGAL2*, *PaGAL3* and *PaGAL4* may be involved in galactose metabolism of cells or cell walls during development and ripening.