Title The role of prolyl 4-hydroxylases in tomato fruit ripening

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Abstract

Prolyl 4-hydroxylases (P4H) belong to the family of 2-oxoglutarate-dependent dioxygenases and catalyze the formation of 4-hydroxyproline, requiring 2-oxoglutarate and O_2 as cosubstrates, Fe2+ as cofactor and ascorbate for optimal activity. 4-Hydroxyproline-rich glycoproteins (HPRGs) comprise a superfamily of cell wall proteoglycans, serving as structural components of cell wall but also playing significant role in development, differentiation, signaling and plant defense. Members of HPRGs are: arabinogalactan proteins (AGPs), extensins, proline rich proteins (PRPs) and lectins in Solanaceae. Our approach is to reveal their function by regulating the action of P4Hs, which through hydroxylation determine their glycosylation pattern. Investigating the role of P4Hs in fruit ripening, requires the use of a plant like tomato, which is the model plant for the study of fleshy fruit; development. Quantitative RT-PCR of 9 putative SIP4Hs showed that members of the gene family exhibit different expression profiles during fruit ripening. Six P4Hs had similar expression profile to climacteric ethylene production. Three were not expressed in immature fruit (SlP4Hl, SlP4H2, SlP4H7) and one had senescence related expression pattern (SlP4H6). Moreover transcript levels of SlP4H5 and SlP4H9 were not significantly altered during ripening. To further investigate the role of P4Hs in: ripening process of tomato, we treated MG and TU excised tomato pericarp discs with the P4H inhibitor pyridine 2, 4-dicarboxylic acid (2,4-PDCA) for 4 days. Treatment with 1-2-3 mM PDCA resulted in reduced ethylene production, higher firmness and inhibition of color development. The delay in ripening was also supported by the lower PG mRNA transcripts in· treated discs. The involvement of AGPs and their regulation by P4Hs during ripening is currently under investigation.