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OC - 32

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The GDP-D-mannose epimerase (GME, EC 5.1.3.18), which converts GDP-D-mannose to GDP-L-galactose, is generally considered as a central enzyme of the major ascorbate biosynthesis pathway in higher plants but experimental evidence for its role in planta is lacking. By using transgenic tomato lines RNAi-silenced for the two GME genes, we could decipher the respective function of GME1 and GME2 proteins during tomato development. Both GME1 and GME2 participate to AsA biosynthesis pathway confirming that GMEs indeed play a key role in the regulation of ascorbate biosynthesis in tomato plants. Regarding the role of the GME activity in the cell wall biogenesis, our data suggest the existence of a specific cell wall-related activity of SlGME1 and SlGME2 according to the considered plant tissue and its developmental stage. On the basis of the gene expression patterns, SlGME2 displayed predominance during the vegetative growth phase, since only RNAi-GME2 transgenic lines exhibited growth delay. On the other hand, GME1 seems to be the major player during the early phase of development of reproductive tomato organs, namely flowers and fruits, as shown by the smaller fruits produced by RNAi-SlGME1 lines. When considered together, these findings confirm the intimate linkage of ascorbate and cell wall biogenesis in tomato plants, and they also reveal the possible existence of specificity of the GME activities related to cell wall that depends on the organ type and its stage of development.