Ascorbate, also known as vitamin C, plays fundamental roles in biotic and abiotic stress resistance in plants. In green tissues, ascorbate is mainly synthesized through the Smirnoff-Wheeler pathway, whose regulation is not fully understood. Thus, it is necessary a thorough understanding of the regulation of biosynthetic pathway of ascorbate. It is known that VTC2 (GDP-L-Galactose Phosphorylase) is the bottleneck of the pathway, but little information is available on the regulation of the different biosynthetic enzymes at the biochemical and cellular level. Results were presented regarding protein regulation, localization and interaction among different biosynthetic components in Arabidopsis and Nicotiana plants.