A central role for bifunctional aspartate/prephenate aminotransferase in the biosynthesis of amino acids in plant plastids.

Fernando de la Torre, Jorge El-Azaz, Concepción Ávila, Francisco M. Cánovas

Departamento de Biología Molecular y Bioquímica. Universidad de Málaga.

Bifunctional aspartate/prephenate aminotransferases (AAT/PAT) are plastid-located enzymes encoded by a single locus in all reported plants, which develop two different enzymatic activities: aspartate aminotransferase (AAT), the reversible combination of L-Asp and α-ketoglutarate to render L-Glu and oxaloacetate, and prephenate aminotransferase (PAT), the reversible combination of L-Asp and prephenate to render oxaloacetate and arogenate (1), (2), (3). Interestingly, L-Asp and prephenate are direct precursors for the biosynthesis of six different amino acids within plant plastids: L-Phe, L-Tyr, L-Met, L-Thr, L-Ile and L-Lys.

The genetic analysis of AAT/PAT-AT function in plants has remained elusive because its absence is lethal during embryogenesis as revealed by the study of *Arabidopsis* transposon mutants defective in female gametogenesis and embryo development (4). The observed lethal phenotype is consistent with the suppression of an essential enzyme for amino acid biosynthesis. Our attempts to obtain *Arabidopsis* transgenic plants suppressed for the corresponding At2g22250 gene were unsuccessful. In order to overcome this problem we chose to implement a different strategy using Virus Induced Gene Silencing in *Nicotiana benthamiana,* a useful technique to study the mature phenotype corresponding to the disruption of a gene described as essential during embryo development. *N. benthamiana* plants were successfully silenced for the endogenous *NbAAT/PAT* gene and exhibited severe reduction in growth, strong symptoms of chlorosis, and altered fresh weight-to-dry weight ratio in comparison with control plants (5). Metabolic analysis revealed that the silencing of *NbAAT/PAT* results in altered profiles of amino acids, chlorophylls, carbohydrates and very interestingly, affects lignin deposition in vascular bundles. Our results also demonstrates that both AAT and PAT activities, housed by *NbAAT/PAT,* are functional in plants and develops critical roles in the biosynthesis of amino acids in platids. Our biochemical and molecular data also provide consistent evidences with an alternative route for the biosynthesis of phenylalanine in plants similar to that described for various groups of microorganisms (5). Parallel research in petunia petals is also consistent with this hypothesis (6).

(1)de la Torre et al. (2006) *Plant Journal* 46(3):414-425.
(2)Maeda et al. (2011) Nat Chem Biol. 7(1):19-21.
(3)de la Torre et al. (2009). *Plant Physiology* 149(4):1648-1660
(4)Pagnussat et al. (2005) *Development* 132:603-614.
(5)de la Torre et al. (2014) *Plant Physiology* 164(1):92-104.
(6)Yoo et al. (2013) Nat Commun. 25(4):2833.