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Another Route for Amino Acid Production?: Reverse Genetic Probing for a Functional Cytosolic Shikimate Pathway in Plants

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ABSTRACT

The shikimate pathway is a metabolic pathway that produces the three aromatic amino acids—phenylalanine, tryptophan, and tyrosine—which are essential to human diets and necessary for many plant functions. Consequently, the shikimate pathway is commonly targeted for antibiotic and herbicide strategies as well as genetic engineering in several fields. This pathway is known to be localized in the plastids, or double membrane-bound organelles, of plant cells; however, there is enzymatic evidence of another shikimate pathway in the cell fluid, or cytosol. To determine whether a complete cytosolic shikimate pathway exists, we used a modified gene for the first enzyme of the shikimate pathway, 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase, that will be produced exclusively in the cytosol. We inserted this gene into both *Petunia hybrida* cv. multiflora and *Petunia hybrida* cv. Mitchell, which produce high levels of phenylalanine-derived volatile organic compounds (VOCs). We will determine the gene's effect by measuring the change in the rate of production of the VOCs, which corresponds to overall activity of the shikimate pathway. Therefore, if there is a fully-functional, cytosolic shikimate pathway, this rate will increase compared to wild-type petunias. We are also investigating putative cytosolic, shikimate pathway enzymes 3-dehydroquinate synthase and 3-dehydroquinate dehydratase/shikimate dehydrogenase. We will determine their subcellular localization by performing confocal microscopy on green fluorescent protein variants and their function by interfering RNA suppression. While further investigation is required, this cytosolic function could provide a new, innovative target for antibiotics, herbicides, and genetic engineering.

KEYWORDS

shikimate pathway, amino acid biosynthesis, DAHP synthase, DHQS, DHD-SHD, lignin, metabolism