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Shelly A. Deck

Indiana University - Purdue University Fort Wayne

Eric L. Garcia

Indiana University - Purdue University Fort Wayne

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A Trifluoroleucine-Resistant Mutant of *Arabidopsis thaliana*
Overproduces Free Levels of the Essential Amino Acid Leucine

Shelly A. Deck and Eric L. Garcia

George S. Mourad

Department of Biology

Indiana University-Purdue University Fort Wayne

Isopropylmalate Synthase (IPMS) is the first and key regulatory enzyme of the pathway for leucine biosynthesis in bacteria, yeast, fungi and plants. IPMS is subject to negative feedback control by the end product leucine. When leucine levels build up in the cell, it binds to IPMS at a regulatory site causing a conformational change that alters the catalytic site. This in turn results in inhibiting IPMS activity and slowing down leucine biosynthesis. A toxic analog of leucine, 5,5,5-trifluoro-D,L-leucine (TFL), inhibits cell growth by incorporating into cellular proteins during protein synthesis resulting in their destabilization leading to cell death. To isolate *Arabidopsis thaliana* mutants that overproduce leucine, we screened EMS-mutagenized populations of seed for germination and growth in the presence of wild type inhibiting levels of TFL. The strategy of the mutant selection is that a mutant plant could survive in the presence of TFL only if they contain a mutation in the gene encoding IPMS which would result in loss or reduction of leucine negative feedback control. Such TFL-resistant plants would overproduce leucine above normal levels, and this excess of free leucine outcompetes TFL for incorporation into cellular proteins. We have isolated two TFL-resistant mutant plants and have shown that TFL resistance is inherited to the progeny of such mutants. High Performance Liquid Chromatography on extracts from the two mutant lines revealed free leucine overproduction in one of the lines.