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A Site-Directed Mutagenesis Interrogation of the Allosteric Region of Threonine Dehydratase/Deaminase (TD)

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TD is the key regulatory enzyme of the metabolic pathway leading to isoleucine biosynthesis in bacteria, yeasts, fungi and plants. When levels of isoleucine build up in a cell, isoleucine binds to TD changing its conformation and thus slowing down the production of more isoleucine (negative feedback control). In the small plant *Arabidopsis thaliana*, a mutant was isolated that overproduced isoleucine by 20-fold (Mourad and King, 1995). The mutation mapped to a new gene *omr1.1* which contained two point mutations of the base substitution type that leads to two amino acid substitutions at residues #499 and #544 of TD (Mourad et al., 1998; 2000). With the objective of maximizing the overproduction of isoleucine in plants for producing better food, we introduced 6 independent novel mutations in the wild type gene encoding TD of *Arabidopsis*. Each of the 6 new mutant alleles was spliced in front of the strong constitutive promoter CaMV 35S of the plant binary vector pBI121.1. Each of the 6 new mutant alleles was independently engineered into wild type *Arabidopsis thaliana* plants with the aid of *Agrobacterium tumefaciens* and the vacuum-infiltration method. All of the 6 different genetically engineered plant lines were able to germinate and grow in the presence of the toxic analog L-O-methylthreonine, an indication of their ability to overproduce isoleucine. We will further characterize these lines by assaying the sensitivity of their TD to isoleucine inhibition and by quantifying the amount of free isoleucine they produce using HPLC analysis.

Mourad G., King J. (1995) L-O-methylthreonine-resistant mutant of *Arabidopsis* defective in isoleucine feedback regulation. *Plant Physiology* 107:43-52.

Mourad G., Emerick R., Marion A., Smith A. (1998) Cloning and sequencing of a cDNA encoding threonine dehydratase/deaminase of *Arabidopsis thaliana* (Accession No. AF096281). *Plant Physiology* 118: 1534.

Mourad G., Emerick R., Smith A. (2000) Molecular cloning and sequencing of a cDNA encoding an isoleucine feedback insensitive threonine dehydratase/deaminase of *Arabidopsis thaliana* (Accession No. AF177212). *Plant Physiology* 122: 619.