



Two Combined Mutations That Introduce The Second Entry Pathway To Synthesized Lignin From Tyrosine In Plants

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Inventors: Hiroshi Maeda, Yuri Takeda-Kimura, Bethany Moore

The Invention

UW-Madison researchers have identified two amino acids in the active site of phenylalanine tyrosine ammonia-lyase (PTAL) critical for the activity of the enzyme in catalyzing the synthesis of p-coumarate from phenylalanine and tyrosine in grasses. They used this knowledge to engineer phenylalanine ammonia-lyase (PAL) to convert that enzyme from only using phenylalanine as a substrate in the formation of p-coumarate into an enzyme that uses both phenylalanine and tyrosine as substrates. Creating transgenic plants that can use both amino acids as substrates should enhance the levels of p-coumarate formed from atmospheric carbon, improving the carbon sequestration in plants and increasing the value of the plant material by increasing the amount of aromatic carbon molecules in the plant. His140 has been previously proposed as a critical residue for tyrosine ammonia-lyase (TAL) activity; however, site-directed mutagenesis of Phe140 to His in a *J. ascendans* PAL (JaPAL) enzyme only partially increased TAL activity, which was still much weaker than those of native PTAL enzymes. Phylogeny-guided biochemical characterization, sequence comparison, and repeated site-directed mutagenesis further identified a novel residue Ile112 responsible for the transition from PAL to PTAL in this lineage. Furthermore, introduction of the S112I mutation together with F140H converted monofunctional PAL enzymes of *J. ascendans* as well as distantly-related *Arabidopsis* into bifunctional PTAL enzymes.

Additional Information

For More Information About the Inventors

- [Hiroshi Maeda](#)

Tech Fields

- [Animals, Agriculture & Food : Plant biotech](#)
- [Clean Technology : Other clean technologies](#)

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