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Characterization of CsTSI in the Biosynthesis of Theanine in Tea Plants (*Camellia sinensis*)

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Title

Characterization of CsTSI in the Biosynthesis of Theanine in Tea Plants (*Camellia sinensis*)

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Abstract

Theanine is a unique major amino acid in tea plants responsible for umami taste and mental health benefits of tea. However, theanine biosynthesis and physiological role in tea plants are not fully understood. Here, we demonstrate that tea plant theanine synthetase is encoded by a glutamine synthetase gene CsTSI. The expression pattern of CsTSI is closely correlated with theanine and glutamine levels in various tissues. CsTSI transcripts were accumulated in root tip epidermal cells, pericycle and procambial cells, where CsTSI presents as a cytosolic protein. Ectopic expression of the gene in *Arabidopsis* led to greater glutamine and theanine production than controls when fed with ethylamine (EA). RNAi knockdown or overexpression of CsTSI in tea plant hairy roots reduced or enhanced theanine and glutamine contents, respectively, compared with controls. The CsTSI recombinant enzymes used glutamate as an acceptor and ammonium or EA as a donor to synthesize glutamine and theanine, respectively. CsTSI expression in tea roots responded to nitrogen supply and deprivation and was correlated with theanine contents. This study provides fresh insights into the molecular basis for the biosynthesis of theanine, which may facilitate the breeding of high-theanine tea plants for improving the nutritional benefit of tea.

