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CAPE peptides are involved in root architecture and nodulation in *Medicago truncatula*.

Nitrogen (N) is one of the major limiting nutrients in plant productivity. Nitrogen availability determines root architecture. Under nitrogen-deficient conditions, plants develop an exploratory root system by elongating their primary root length and reducing the number of lateral roots for N-foraging in lower soil layers. Studying mechanisms underlying nitrogen acquisition is vital to understanding their role in plant growth and development. In addition to changes in root architecture, to augment the nitrogen supply, plants belonging to the Fabaceae family uniquely establish symbiotic relationships with nitrogen-fixing bacteria called rhizobia and form specialized structures known as root nodules. Root growth and symbiotic nodule formation are developmental processes regulated by Small Signaling Peptides (SSPs). However, to date, only 6 of 47 families of SSPs have been studied for their roles during nodulation. We investigated the role of hitherto unstudied CAP-derived peptides (CAPEs) in the model legume *Medicago truncatula*. Using publicly available data, we screened the gene expression profile of 21 *M. truncatula* CAPEs under N-deficiency. The gene encoding *MtCAPE16*, in particular, was identified to be transcriptionally responsive to both nitrogen deficiency and nodulation. Spatial expression analysis using the 3kb upstream region of *MtCAPE16* with GUS fusion revealed MtCAPE16 to be expressed in lateral roots as well as meristematic and infection zones of root nodules. To understand *MtCAPE16* function *in planta* we generated transgenic lines that overexpress *MtCAPE16* and knockout CRISPR mutant lines. Our data show that *MtCAPE16* overexpression lines had 30-40% reduced production of root nodules 21 days post inoculation with *Sinorhizobium meliloti* as well as a reduction of 30-45% lateral roots under Full N availability. To develop CRISPR mutant lines, two guide RNAs each targeting *MtCAPE16* and its homolog *MtCAPE17* were utilized. We identified five different mutant lines with frameshift deletions creating functional knockout mutation lines. These lines will be further evaluated for changes in root architecture, nodule number, and nitrogen use efficiency. This study sheds light on the role of CAPE peptides in root nodule symbiosis and reveals a function in lateral root formation under N-deficient conditions.