## Arbuscular Mycorrhizal Symbiosis in Trema orientalis: Effect of a Naturally-Occurring Symbiosis Receptor Kinase Mutant Allele

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Abstract: The Trema genus represents a group of fast-growing tropical tree species within the Cannabaceae. Interestingly, five species nested in this lineage -known as Parasponia- can establish rhizobium nitrogen-fixing root nodules, similar to those found in legumes. Parasponia and legumes use a conserved genetic network to control root nodule formation, among which are genes also essential for mycorrhizal symbiosis (the so-called common symbiotic pathway). However, Trema species lost several genes that function exclusively in nodulation, suggesting a loss-of the nodulation trait in Trema. Strikingly, in a Trema orientalis population found in Malaysian Borneo we identified a truncated SYMBIOSIS RECEPTOR KINASE (SYMRK) mutant allele lacking a large portion of the c-terminal kinase domain. In legumes this gene is essential for nodulation and mycorrhization. This raises the question whether Trema orientalis can still be mycorrhized. To answer this question, we established quantitative mycorrhization assay for Parasponia andersonii and Trema orientalis. Plants were grown in closed pots on half strength Hoagland medium containing 20 µM potassium phosphate in sterilized sand and inoculated with 125 spores of Rhizopagus irregularis (Agronutrion-DAOM197198). Mycorrhization efficiency was determined by analyzing the frequency of mycorrhiza (%F), the intensity of the mycorrhizal colonization (%M) and the arbuscule abundance (%A) in the root system. Trema orientalis RG33 can be mycorrhized, though with lower efficiency compared to Parasponia andersonii. From this we conclude that a functional SYMRK kinase domain is not essential for Trema orientalis mycorrhization. In ongoing experiments, we aim to investigate the role of SYMRK in Parasponia andersonii mycorrhization and nodulation. For this two Parasponia andersonii symrk CRISPR-Cas9 mutant alleles were created. One mimicking the TorSYMRKRG33 allele by deletion of exon 13-15, and a full Parasponia andersonii SYMRK knockout.

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