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Molecular characterization and stress signaling of the MsCOMT gene by transient assay

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Objectives

Lignin is most commonly found as a polymeric macromolecule in plant cell walls, so it plays multiple roles in providing mechanical support for plant tissues and protects the plant from pathogen invasion. Most Caffeic acid O-methyltransferase (COMT) possess a broad substrate allowance and can potentially participate in various branches of monolignol biosynthetic processing Gene transient expression analysis is a powerful method to describe plant functions. we describe the function of a gene encoding COMT that was isolated from *Miscanthus sinensis* (Ms).

Materials and Methods

- Construction of the cDNA library.
- cDNA Sequencing and Sequence Analysis.
- Plant material and chemical treatments
  (control buffer, 400mM NaCl, 400mM mannitol, 50 μM methylviologen(MV), 100 μM ABA)
- Analysis of the MsCOMT gene sequence.
- Isolation of RNA and the RT reaction.
- Antisense construction using a binary vector and the *Agrobacterium-mediated* transient assay.

Results

The transcript level of the MsCOMT gene responded only to abscisic acid signaling among environmental stressors such as mannitol, NaCl, and methyl viologen related to drought, salt, and reactive oxygen species. However, MsCOMT expression decreased following the NaCl and methyl viologen treatments.The PAL transcript level decreased when the antisense-MsCOMT gene was transiently overexpressed. PR group genes led to a decrease in MsCOMT-AS gene expression in leaves. The NbAPX expression level decreased following a decrease in NbPAL and PR group gene expression. PAL is related to lignin biosynthesis and secondary metabolism. The results showed that MsCOMT has functional specialization in environmental stress signaling and lignin biosynthesis.

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Fig. 1. Representations for functional annotation and classification of unigenes. (A) Functional annotation of unigenes with BLASTX program in NCBI. (B) Distribution of ESTs using functional catagories.

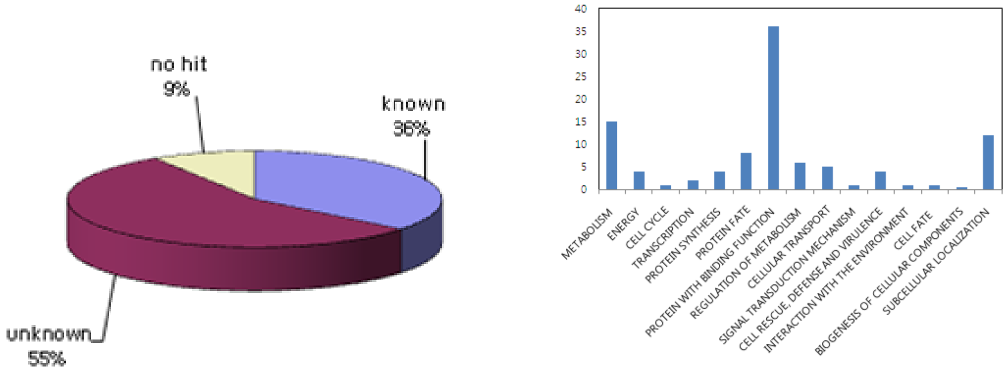
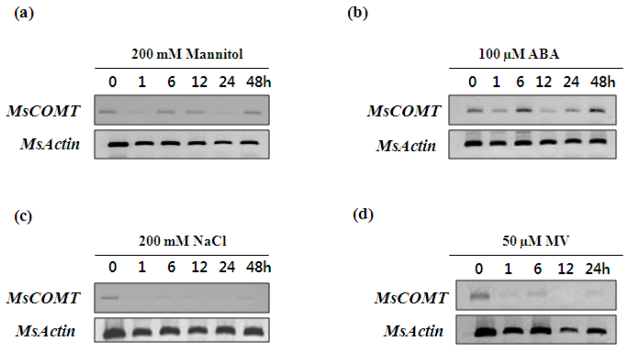


Fig. 3. RT-PCR analysis of *MsCOMT* gene in leaves of Miscanthus treated with chemicals related to environmental stresses.



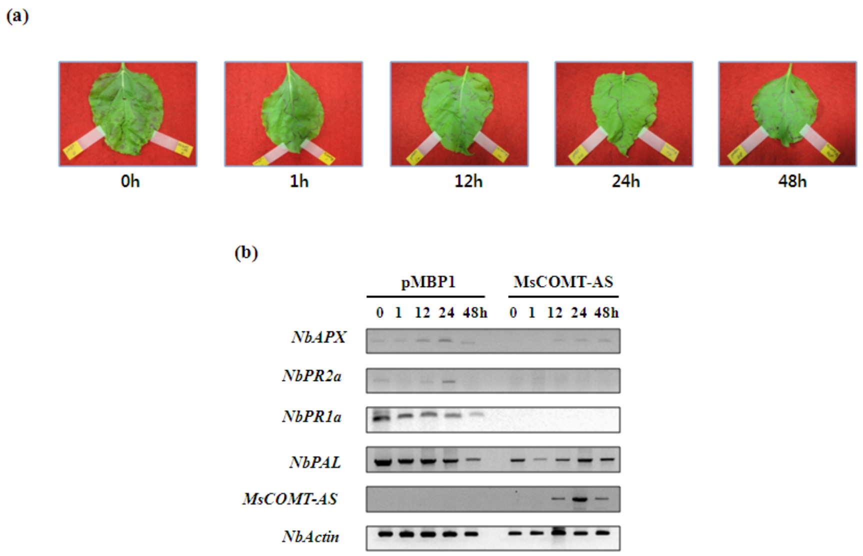


Fig. 4. Molecular characterization of MsCOMT by transient overexpression in leaves of *N. benthamiana*.