A Novel Tobacco Gene Encodes a Pistil-Specific Methyltransferase Capable to Produce Methyljasmonate, Methylbenzoate and Methylsalicylate

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A pistil-specific gene was identified by differential screening of a tobacco stigma/style cDNA library. After sequence analysis using the BlastX program, the identified clone showed high similarity to a benzoic/salicylic acid methyltransferase from *Nicotiana suaveolens*. These methyltransferases (MT) catalyze the transfer of methyl groups from S-adenosyl-L-methionine to different substrates as salicylic, benzoic or jasmonic acids. The esters methylsalicylate, methylbenzoate and methyljasmonate are involved in important plant process such as pollinator attraction and plant development. Additional experiments showed the existence of several pistil-specific methyltransferase transcripts, possibly produced by alternative splicing, from a gene containing 8 exons. RT-PCR experiments allowed us to identify and clone the expected full-length cDNA (MT1). The MT1 was subcloned in the expression vector pDEST17 and the recombinant protein was expressed in BL21(DE3) Rosetta *E. coli* strain. The rMT1 protein was assayed *in vitro* in the presence of each of the three putative substrates. After GC-MS analyses, the results showed that the rMT1 protein efficiently produced methyljasmonate. Methylbenzoate was also produced but with a lower efficiency, while methylsalicylate was hardly detected. This is the first MT described to methylate the three compounds. The total protein extracts from leaves and pistils were assayed in an ELISA experiment, using a polyclonal antibody produced in Balb/C mice, against the rMT1 protein. This antibody was capable to recognize the MT1 native protein efficiently in the stigma/style tissues with a high specificity. Additional experiments are in progress to understand the role of the different methyltransferase transcripts in the pistil of *N. tabacum* flowers.

Financial Support: FAPESP, CAPES and CNPq

Key words: Methyltransferase, Pistil, *Nicotiana tabacum*, Polyclonal antibody, Methyljasmonate

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