

Plant metabolites in response to pathogen and gene mutations

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Abstract: In response to environmental stresses, plants induce complex signal transduction cascades that can lead to dramatic changes in profiles of gene expression and metabolites. In some instances, plants respond to pathogen stress by increasing production of defense chemicals such as polyphenols. Normal cellular functions of polyphenols depend on methylation of their hydroxyl groups at specific positions via reactions catalyzed by *O*-methyltransferases (OMTs), some of which are induced in pathogen stress. OMTs belong to a group of enzymes involved in the methylation of hydroxyl groups from many compounds in both animals and plants. *O*-methylation catalyzed by OMTs involves the transfer of the methyl group from *S*-adenosyl-L-methionine (SAM) to the hydroxyl group of an acceptor molecule, with the formation of its methylated derivative and *S*-adenosyl-L-homocysteine (SAH) as products. In this study, we summarized changes on plant secondary metabolites in response to pathogen stress and gene mutations, particularly to OMT mutants.

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1. Introduction

The objectives of this study are to explore the functions of *Arabidopsis* *O*-methyltransferase (OMT) genes in responses to stress. Polyphenols are some of the most abundant secondary metabolites in plants and their production indicates change in biochemical metabolism in plants under pathogen stress [1]. Normal cellular functions of polyphenols depend on methylation of their hydroxyl groups on specific positions, and these reactions are catalyzed by *O*-methyltransferases (OMTs). To study the functions of OMTs in *Arabidopsis thaliana*, giving insight into mechanisms of how polyphenols are involved in pathogen resistance, plants with mutated *OMT* genes are usually tested for changes of metabolite profiles compared with wild type plants both in control and pathogen infection. Loss of functional *OMT* genes is expected to disturb multiple metabolic pathways, particularly if specific polyphenol metabolites contribute to signaling. By studying the differences in metabolite profiles between wild type and *omt* mutants, it is possible to identify not only the substrates for OMTs, but also OMT-dependant metabolites and pathways critical for plant responses to stress.

2. Pathogen stress and secondary metabolites

Since plants are immobile, their successful resistance to pathogens depends on their ability to detect pathogens and generate biochemical responses. Pathogen detection by plants is followed by a complex system of signal transduction events that

ultimately result in dramatic changes in gene expression profiles. Plants also produce secondary metabolites and other natural products that can confer disease resistance [2]. However, the intermediates and end products of secondary metabolism are not well characterized, although their modulations are likely to be the major result of the numerous induced changes in transcriptional activity occurring in infected plant cells [3]. It is expected that levels of a diverse range of plant secondary metabolites will change dramatically during pathogen stress. Polyphenols are some of the most abundant secondary metabolites in plants and their production indicates change in biochemical metabolism in plants under pathogen stress [1]. Glucosinolates, oxylipins and indole conjugates are also thought to play an important role in plant pathogen responses [4]. Polyphenols are metabolites characterized by the presence of at least one phenolic hydroxyl group per molecule. They are generally subdivided into tannins and phenylpropanoids such as lignins and flavonoids derived from simple polyphenolic units produced by the shikimate pathway [5]. Polyphenols are important to the plant cell and act as phytoalexins, UV sunscreens, pigments, signaling molecules, and major structural components [6]. Polyphenols are also potent inhibitors of free radical oxidations; changes in polyphenol levels or reactivity have been postulated to influence the course of the oxidative burst response [7, 8]. Some polyphenols are reported to be antifeeding compounds that inhibit herbivory by insects and other animals; the roles of polyphenols in

microbial pathogen infection are not clear [9, 10]. It has been reported that the phenolic metabolite salicylic acid (SA) is the systemic signal for systemic acquired resistance (SAR) [11]. Enzymes in the phenylpropanoid pathway can transform phenylalanine into a variety of important secondary products, including lignin, sinapate esters, stilbenes, and flavonoids. Flavonoids are derived from the C15 flavonoid skeleton, which is synthesized via the condensation of *p*-coumaroyl-CoA and three molecules of malonyl-CoA [12]. Flavonoids occur

widely in plants and have diverse subgroups that can be divided into anthocyanins, flavonols, flavones, flavanols, flavanones, chalcones, dihydrochalcones and dihydroflavonols. [9, 13]. The induced formation of flavonoids after injury by pathogens or pests is a well-known phenomenon [14, 15]. The phenolic unit can often be modified through esterification, glycosylation, or methylation. Dimerization or further polymerization will create new classes of polyphenols.

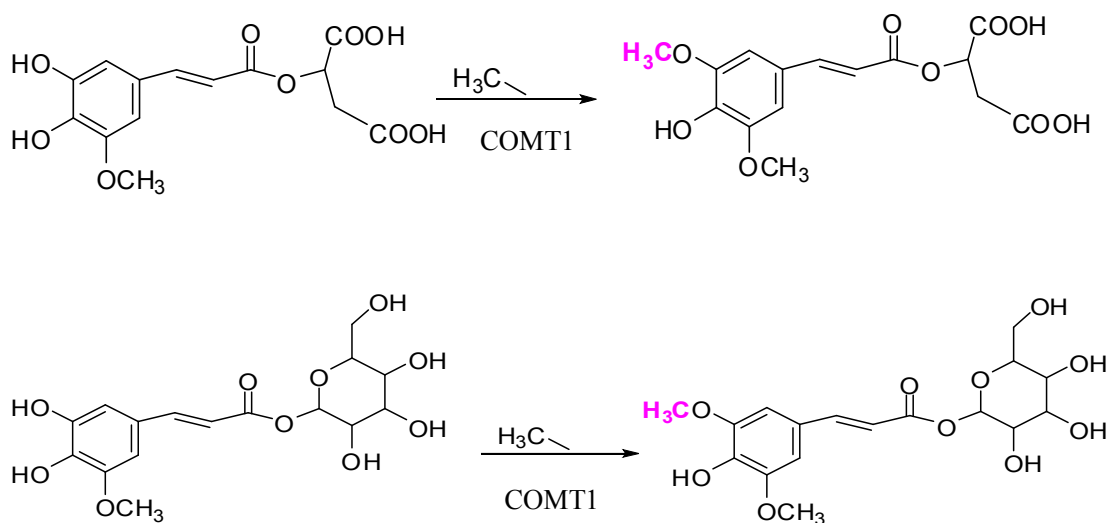


Fig. 1. Putative *O*-methylations catalyzed by COMT1 from 5-hydroxyferuloyl malate (5HFM) to sinapoyl malate (SM) (top panel) and from 5-hydroxyferuloyl glucose (5HFG) to sinapoyl glucose (SG) (lower panel).

In addition to polyphenols, other metabolites are expected to be induced in plant responses to pathogen stress. Glucosinolates (GS) are well-characterized antimicrobial and anti-insect compounds [16, 17]. The 120 different GS identified to date share a common core structure of a β -D-thioglucose group linked to a sulfonated aldoxime moiety and a variable aglycone side chains [18]. In *A. thaliana*, the source of the side chain defines three major classes of GS: aliphatic GS are derived principally from methionine, indolyl GS are from tryptophan, and aromatic GS are from phenylalanine. Among oxylipins, jasmonic acid (JA), methyl JA (MeJA) and 12-oxophytodienoic acid (OPDA) are important mediators of signal transduction [19]. A growing research effort focuses on the roles of oxylipin conjugates in signal transduction [20]. Accumulation of soluble and wall-bound indolic metabolites in *Arabidopsis* leaves has also been

found with the infection of *Pseudomonas syringae* [21]. Most prominent among the accumulating soluble substances were tryptophan, -D-glucopyranosyl indole-3-carboxylic acid, 6-hydroxyindole-3-carboxylic acid 6-*O*- β -D-glucopyranoside, and the indolic phytoalexin camalexin.

3. *O*-methyltransferase (OMT)

The phenolic unit in polyphenols can often be methylated by OMTs, some of which are induced upon pathogen stress. Methylation of polyphenols is critical for some biological functions. OMTs belong to a group of enzymes involved in the methylation of hydroxyl groups from many compounds in both animals and plants. *O*-methylation catalyzed by OMTs [EC 2.1.1.6.x] involves the transfer of the methyl group from *S*-adenosyl-L-methionine (SAM) to the hydroxyl group of an acceptor molecule, with

the formation of its methylated derivative and *S*-adenosyl-L-homocysteine (SAH) as products [22]. Lignin, one of the polymeric products of the general phenylpropanoid pathway, is an abundant biopolymer that strengthens plant tissues and is thought to be an important component of some disease resistance mechanisms. Two classes of “lignin-specific” OMTs have been proposed [23, 24]. Class I consists of low molecular weight (23–27 kDa subunits), Mg^{2+} -dependent OMTs. Class I enzymes were initially identified as caffeoyl-CoA OMTs (CCoAOMTs), and

these catalyze methylations that are involved in biosynthesis of the guaiacyl moiety of phenolic CoA esters in lignin biosynthesis. Larger OMTs (38–43 kDa subunits) methylating caffeic acid or caffeoyl aldehyde and caffeoyl alcohol (COMTs) independent of Mg^{2+} were designated class II, and were considered to methylate primarily 5-hydroxyconiferaldehyde residues during the lignin biosynthesis [23, 24]. COMT-deficient plants exhibit altered lignin structures [25, 26].

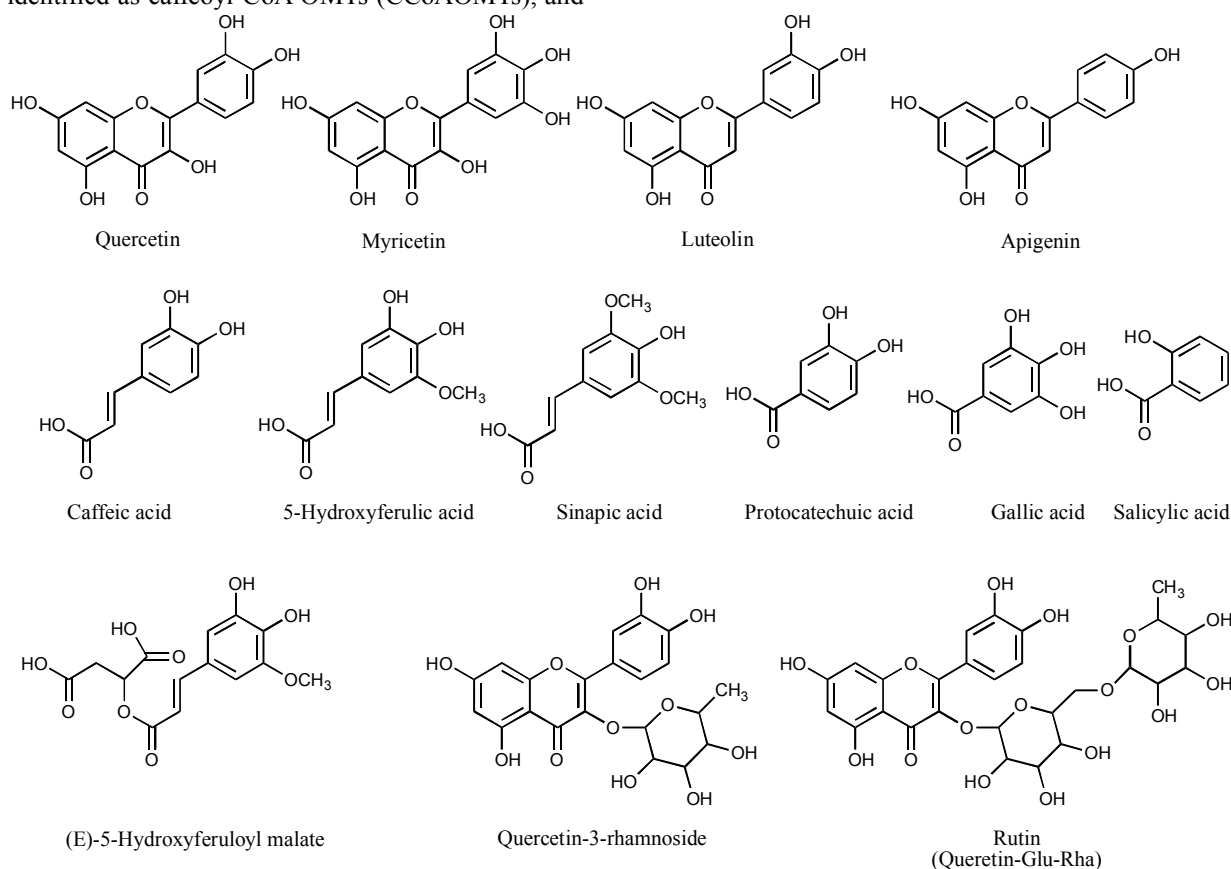


Fig. 2. Structures of putative substrates for the COMT1 activity.

The *Arabidopsis thaliana* genome contains 29 loci that encode members of the OMT family [27]. Only a few of these OMT genes have been studied so far [25, 28–31]. Caffeic acid *O*-methyltransferase 1 (COMT1) (At5g54160) is one of the most studied OMTs in *Arabidopsis*. COMT1 was originally thought to be a bifunctional enzyme that methylates caffeic acid and 5-hydroxyferulic acid [30], based on high similarity and identity to COMT in other species. Transgenic studies revealed that another predominant role of COMT is the methylation of 5-hydroxyconiferaldehyde and 5-hydroxyconiferyl alcohol to sinapaldehyde and sinapyl alcohol,

respectively, which are also metabolites in the lignin synthesis pathway (Fig. 1 and 2) [25, 32]. In 2000, COMT1 was cloned and reported to use the flavanol quercetin as the preferred substrate, but neither of the hydroxycinnamic acids, caffeic or 5-hydroxyferulic, to any significant extent [31]. More recently, it was reported that stem extracts of the COMT1 T-DNA knockout mutant exhibited only about 7% of activity toward caffeic acid and 5-hydroxyferulic acid relative to that of wild type, and an overexpressing line gave a 5-fold increase in activity toward these two substrates relative to wild type [25]. Since the finding from T-DNA mutant is inconsistent with the

biochemical observations regarding COMT1 activities, a more complete assessment of the kinetic properties of COMT1 is needed to resolve these differences. In addition, Tohge *et al.* [33] recently showed that COMT in *Arabidopsis* root extracts is also involved in the methylation of flavonol precursors of isorhamnetin. In conclusion, previous studies on substrate specificities of COMT1 gave conflicting results. This enzyme may have additional activities that may be revealed by further characterization.

OMTs are involved in many different pathways with relatively minor sequence modifications resulting in significant modifications in substrate affinity [34, 35]. Thus, comparisons of sequence similarity/identity are not sufficient to determine the function of *OMT* gene family members. To fill in the gap from gene annotations to gene functions, metabolite profiles of leaves from wild-type plants and T-DNA insertion mutants may provide evidence for the metabolic role of different *OMT* genes.

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