Caffeic acid O-methyltransferase (*COMT*) gene of the phenyl propanoid pathway involved in resistance to Corynespora leaf disease in rubber (*Hevea brasiliensis*)

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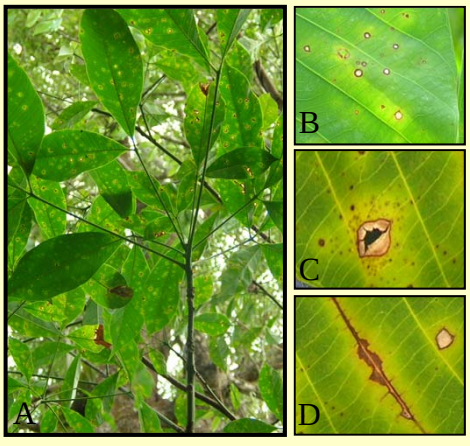
Corynespora leaf disease of rubber



Severely infected branch (A), various symptoms of the disease viz., spots (B), papery lesions (C) and fishbone or railway track symptoms along the veins (D).

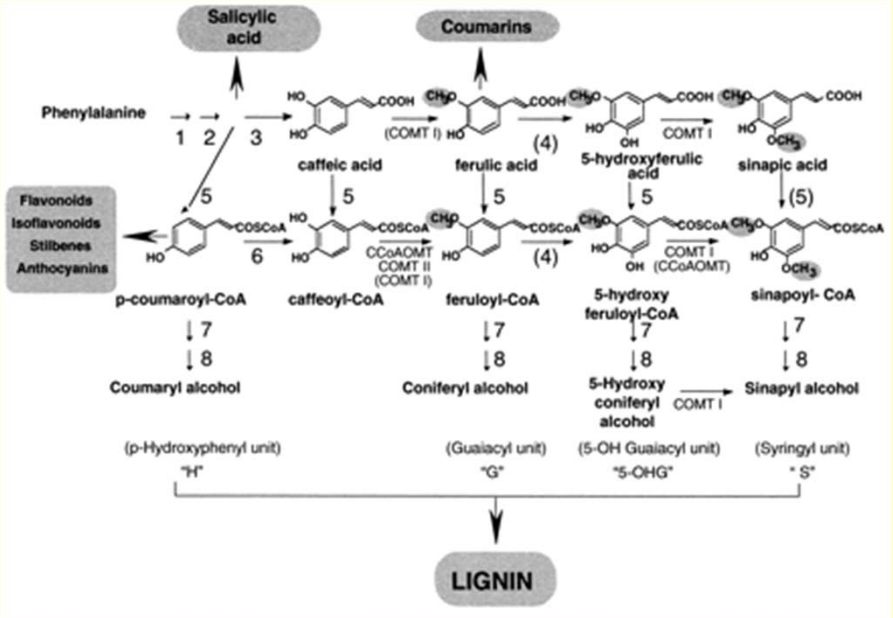
Why this study?

Increase in lignification is often observed in response to pathogen attack. Since lignin is a non-degradable mechanical barrier for most microorganisms, it increases the tolerance of the host by blocking pathogen invasion.

Caffeic acid O-methyltransferase (COMT) catalyzes the multistep methylation reactions of hydroxylated monomeric lignin precursors and is believed to occupy a pivotal position in the lignin biosynthetic pathway.

Differential patterns of expression of the two COMT classes in different plant tissues have suggested a specific role for class I enzyme in lignin biosynthesis and the involvement of class II COMT in the production of defense-related compounds.

Therefore, an effort was made to understand the role of COMT in response to pathogen infection.



About lignin

- A complex polymer
- Abundant organic compound
- Provides mechanical strength to the cell wall
- Structural integrity of the plant cell
- Hydrophobicity to conducting vessel
- Defense against pathogen invasion
- Negative impact on biomass utilization

Methodology adopted

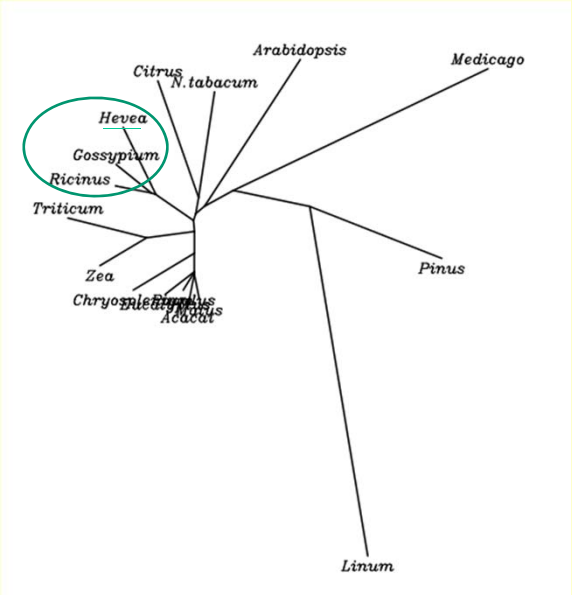
- Adopted RT-PCR technique to amplify partial gene sequence from the bark specific RNA pool using degenerated primer-pair:
- Cloning of cDNA ends of COMT gene from rubber was carried out using RACE technique and finally full-length gene sequence was deduced.
- Primers were designed based on the sequence of the RACE product to amplify full-length cDNA of OMT gene.
- Southern analysis was performed for understanding its genomic organization.
- Prediction of 3D structure of the conceptual resistance protein was made through homology modeling using Phyre2 Server.

Sequence of the full-length *HbCOMT* cDNA revealed 1104 bp long ORF. 21 bp 5'UTR and 204 bp 3'UTR

Three dimensional structure of conceptual translation of HbCOMT2 was predicted through homology modelling.

351 aminoacid residues of HbCMT2 have been modelled with 100% confidence by the single highest scoring template.

Phylogenetic analysis of *HbCOMT2*



Comparative gene expression profiling following *Corynespora* callange inoculation

Challenge inoculation was performed by spraying spore suspension of *Corynespora cassiicola* on immature leaf of RRII 105 and GT1.

Leaf samples were collected at different time intervals (0, 4, 12 and 24 h) following challenge inoculation and preserved at -86ºC.

RNA was isolated and converted to cDNA for template preparation.

Real-time quantification was performed using Roche LightCycler 480 system. Primer-pairs were designed at the 3' end of COMT for its specificity. *Hevea* actin was used as an internal control.

Differential expression of the gene *HCOMT* was noticed between these two clones, even in unchallenged condition and expression level was more in RRII 105 over the tolerant clone GT 1.

However, at 4 hours following infection, the expression levels shot up in both the cases. At 12 hours, expression level was found to reduce significantly only in case of RRII 105.

Even though an increasing trend of gene expression level was noticed in RRII 105 at 24 h of infection, it couldn't reach up to the control level.

Whereas in GT 1, expression shot up significantly after 12 h and eventually at 24 h it had increased by 62 fold compared to the control indicating that this form of OMT might participate in the hypersensitive reaction in GT 1 in response to *Corynespora cassiicola* infection.

Conclusion

- Full-length cloning and characterization of *HbCOMT* gene belonging to class-2 were performed.
- Maximum sequence homology was noticed with *Ricinus communis* COMT.
- Three dimensional protein structure of the conceptual translation of *HbCOMT* gene was predicted through homology modeling, which can help in identifying putative active sites, binding pockets and ligands.
- The involvement of *HbCOMT* gene in disease tolerance was assessed through real-time quantification of gene expression after challenging tolerant (GT 1) and susceptible (RRII 105) clones of rubber with *Corynespora cassiicola*.
- It appears that gradual increment in expression of *HbCOMT* in GT 1 after the infection in initial hours followed by a sudden boost during the period from 12 to 24 h when the pathogen establishes on the host could be one of the reasons for the tolerance observed in GT 1.
- *HbCOMT* could be used as a potential marker for disease tolerance, as a linear relationship is established between gene expression and its tolerance to *Corynespora* infection.