Isolation and First Characterization of Two O-Methyltransferase Genes Involved in Phenylpropanoid Pathway in *Coffea canephora*

N. CHABRILLANGE, P. TALAMOND, C. MOREAU, L. LE GAL, M. BOURGEOIS, S. HAMON, M. NOIROT, A. DE KOCHKO AND C. CAMPA

IRD, Génomique et Qualité du Café, UMR 1097 DGPC, BP64501, 343094 Montpellier Cedex 5, France

SUMMARY

The phenylpropanoid pathway plays an important role in plant secondary metabolism as it leads to the synthesis of a great variety of phenolic compounds, such as flavonoids, coumarins, or lignin. This pathway plays a particular role in coffee, especially in *Coffea canephora* where high levels of cinnamic esters were found in green beans. These compounds are involved in the determination of the coffee cup quality. Several O-methyltransferases intervene in this pathway and participate to the lignin biosynthesis. Two full length cDNA clones corresponding to genes encoding S Adenosyl Methionine (SAM) dependent O-Methyltranferases were isolated from *C. canephora* cDNA libraries. The first cDNA contained an open reading frame encoding a 350 amino acid protein. A blastx search showed that this protein belongs to the Methyltransferase II family and more precisely, that it was 85 % identical to the Caffeic acid O-Methyltransferase (COMT) from *Catharanthus roseus*. The second cDNA's ORF encoded a 247 amino acid peptide belonging to the Methyltransferase III family. This protein presented 89 % identity with the Caffeoyl CoA O-Methyltransferase (CCoAOMT) from *Betula platyphylla*. These results showed that the *Coffea canephora* genome contains at least one gene for each of these two types of structurally and functionally different OMTs involved in the phenylpropanoid pathway by performing the methylation of caffeic acid or caffeoyl-CoA Interestingly, in two EST *C. canephora* libraries, from young leaves and fruits, representing over 5 000 unigenes, only 1 CCoAOMT cluster was found but not a single EST for COMT.

RÉSUMÉ

La voie des phénylpropanoïdes joue un rôle important dans le métabolisme secondaire des plantes conduisant à la synthèse d'une grande variété de composés phénoliques, tels que des flavonoïdes, des coumarines, ou de la lignine. Cette voie joue un rôle particulier chez les caféiers, particulièrement chez *Coffea canephora* où des niveaux élevés d'esters d'acide cinnamique ont été trouvés dans les grains verts. Ces composés sont impliqués dans la détermination de la qualité de tasse du café boisson. Plusieurs O-methyltransferases interviennent dans cette voie et participent à la biosynthèse de lignine. Deux ADNc pleine longueur correspondants aux gènes codant pour des O-Methyltranferases-S Adenosyl Méthionine (SAM) dépendantes ont été isolées dans banques d'ADNc de *C. canephora*. Le premier ADNc possède un cadre ouvert de lecture codant une protéine de 350 acides aminés. Une recherche par Blastx a montré que cette protéine appartenait à la famille des Méthyltransférases II et qu'elle était à 85% identique à la «Caffeic acid O-Methyltransferase» (COMT) de *Catharanthus roseus*. Le deuxième ADNc code pour un peptide de 247 acides aminés appartenant à la famille des Méthyltransférases III. Cette protéine a présenté 89%

d'identité avec la «Caffeoyl CoA O-Methyltransferase» (CCoAOMT) de *Betulla platyphylla*. Ces résultats ont montré que le génome de *Coffea canephora* contient au moins un gène de chacune des deux OMTs différemment impliquées dans la voie des phénylpropanoïdes, en méthylant soit l'acide caféique soit le caffeoyl-CoA. Il est intéressant de noter que dans les 2 banques EST de *C. canephora* réalisées à partir de jeunes feuilles et de fruits, représentants plus de 5 000 unigènes, seulement 1 EST a été trouvé pour la CCoAOMT et aucun pour la COMT.

INTRODUCTION

S-Adenosyl L-methionine (SAM) dependent O-methyltransferases (OMTs) are involved in phenylpropanoid pathway. These enzymes catalyse the transfer of a methyl group on hydroxycinnamates, leading to the synthesis of ferulate, sinapate or feruloyl-CoA. In plants, they are implicated in lignin synthesis, response to pathogen and secondary metabolism synthesis. A high content of chlorogenic acids (11% DW), hydroxycinnamoylquinic acids including feruloyl esters, has been found in *Coffea canephora* seeds. This accumulation led us look for genes encoding for OMTs and expressed in fruits or leaves of *C. canephora*.

RESULTS

Two coding sequences, corresponding to 2 different SAM-OMTs were isolated by screening *Coffea canephora* leaf and fruit cDNA libraries.

The first sequence was identified as a Caffeoyl CoA O-methyltransferase (CCoAOMT), which catalyses the methylation of caffeoyl CoA to form feruloyl CoA. The cDNA was 744 bp long. The deduced peptide (247 amino acids) shared the domains E, F, G and H, characteristic of plant OMT I. (Figure 1)

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                                                    ▸                   D
C.canephora          -----------MAQNGEG-KDSQNLRHQEVGHKSLLQSDALYQYILETSVYPREPEPMKE   48
M.sativa_U20736      -----------MATNEDQ-KQTESGRHQEVGHKSLLQSDALYQYILETSVFPREHEAMKE   48
N.tabacum_Q42945     -----------MAENGAA-QENQVTKHQEVGHKSLLQSDALYQYILETSVYPREPEPMKE   48
A.thaliana_NM_119566 MATTTTEATKTSSTNGEDQKQSQNLRHQEVGHKSLLQSDDLYQYILETSVYPREPESMKE   60
                                ▸                       E
C.canephora          LRELTAKHPWNIMTYSADEGQFLNMIIKLINAKKTMEIGVYTGYSLLATALALPEDGKIL   108
M.sativa_U20736      LREVTAKHPWNIMTYSADEGQFLSMLLKLINAKNTMEIGVYTGYSLLATALAIPEDGKIL   108
N.tabacum_Q42945     LRELTAKHPWNLMTYSADEGQFLSMLLKLIIAKNTMEIGVYTGYSLLATALALPDDGKIL   108
A.thaliana_NM_119566 LREVTAKHPWNIMTYSADEGQFLNMLIKLVNAKNTMEIGVYTGYSLLATALALPEDGKIL   120
                                     F                                  G
C.canephora          AMDINREWYELGLPVIEKAGVSHKIDFREGPALPVLDELIEDDKNHGSFDFIFVDADKDN   168
M.sativa_U20736      AMDINKEWYELGLPVIKKAGVDHKIDFREGPALPVLDEMIKDEKNHGSYDFIFVDADKDN   168
N.tabacum_Q42945     AMDINKEWYELGLPVIQKAGVAHKIDFREGPALPVLDLMIEDKNNHGTYDFIFVDADKDN   168
A.thaliana_NM_119566 AMDVNREWYELGLPIIEKAGVAHKIDFREGPALPVLDEIVADEKNHGTYDFIFVDADKDN   180
                            SAM binding A            SAM binding B        SAM binding C
                                          ▸                ▸ ▸  ▸
C.canephora          YLNYHKRIIELVKVGGMIGYDNTLWNGSVVAPPDAPMRKYVRYYRDFVLELNKALAADPR   228
M.sativa_U20736      YLNYHKRLIDLVKVGGVIGYDNTLWNGSVVAPPDAPLRKYVRYYRDFVLELNKALAVDPR   228
N.tabacum_Q42945     YINYHKRIIELVKVGGVIGYDNTLWNGSVVAPPDAPMRKYVRYYRDFVLELNKALAADPR   228
A.thaliana_NM_119566 YINYHKRLIDLVKIGGVIGYDNTLWNGSVVAPPDAPMRKYVRYYRDFVLELNKALAADPR   240
                                H
C.canephora          IEICMLPVGDGITLCRRVS   247
M.sativa_U20736      IEICMLPVGDGITICRRIK   247
N.tabacum_Q42945     IEICMLPVGDGITLCRRIS   247
A.thaliana_NM_119566 IEICMLPVGDGITICRRIS   259
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▸ : Active site substract binding / positioning residues
Bold : Conserved residues and motifs for SAM binding

Figure 1. Sequence alignment of 4 CCoAOMTs. Alignment was performed using ClustalW algorithm.

The second sequence (1153 nucleotides) was identified as a Caffeic acid O-methyltransferase (COMT), which catalyses the conversion of caffeic acid to ferulic acid. The deduced peptide sequence (350 amino acids) was analysed by alignment with other COMTs. It shared the domains I, J, K, L that characterize the plant OMTs II. (Figure 2)

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                                                                  ● ●
C. canephora          ------------MAEEEACLFAMSLASASVLPMVLKSAIELDLLELIAKAGPGAYVSPS   47
M. sativa_AAB46623    MGSTGETQITPTHISDEEANLFAMQLASASVLPMILKSALELDLLEIIAKAGPGAQISPI   60
N. tabacum_CAA52461   MGSTSESQSNSLTHTEDEAFLFAMQLCSASVLPMVLKSAVELDLLELMAKAGPGAAISPS   60
A. thaliana_NM_124796 MGSTAETQLTPVQVTDDEAALFAMQLASASVLPMALKSALELDLLEIMAKNG--SPMSPT   58
                                                  /
C. canephora          ELAAQLPTHNPEAPIMLDRILRLLATYSVLDCKLNNLADGGVERLYGLAPVCKFLTKNAD  107
M. sativa_AAB46623    EIASQLPTTNPDAPVMLDRMLRLLACYIILTCSVRTQQDGKVQRLYGLATVAKYLVKNED  120
N. tabacum_CAA52461   ELAAQLSTQNPEAPVMLDRMLRLLASYSVLNCTLRTLPDSSVERLYSLAPVCKYLTKNAD  120
A. thaliana_NM_124796 EIASKLPTKNPEAPVMLDRILRLLTSYSVLTCSNRKLSGDGVERIYGLGPVCKYLTKNED  118

C. canephora          GVSMAPLLLMNQDKVLMESWYHLKDAVLDGGIPFNKAYGMTAFEYHGTDPRFNKVFNQGM  167
M. sativa_AAB46623    GVSISALNLMNQDKVLMESWYHLKDAVLDGGIPFNKAYGMTAFEYHGTDPRFNKVFNKGM  180
N. tabacum_CAA52461   GVSVAPLLLMNQDKVLMESWYHLKDAVLDGGIPFNKAYGMTAFEYHGTDPRFNKVFNRGM  180
A. thaliana_NM_124796 GVSIAALCLMNQDKVLMESWYHLKDAILDGGIPFNKAYGMSAFEYHGTDPRFNKVFNNGM  178
                        ▸                    SAM binding A                    J
C. canephora          SNHSTITMKKILEVYRGFEGLKTVVDVGGGTGATLNMIISKYPTIKGINFELPHVVEDAP  227
M. sativa_AAB46623    SDHSTITMKKILETYTGFEGLKSLVDVGGGTGAVINTIVSKYPTIKGINFDLPHVIEDAP  240
N. tabacum_CAA52461   SDHSTMSMKKILEDYKGFEGLNSIVDVGGGTGATVNMIVSKYPSIKGINFDLPHVIGDAP  240
A. thaliana_NM_124796 SNHSTITMKKILETYKGFEGLTSLVDVGGGIGATLKMIVSKYPNLKGINFDLPHVIEDAP  238

                              K          SAM binding B                    SAM binding C
C. canephora          SHPGVEHVGGDMFVSVPKGDAIFMKWICHDWSDDHCRKLLRNCYQALPDNGKVILAECVL  287
M. sativa_AAB46623    SYPGVEHVGGDMFVSIPKADAVFMKWICHDWSDEHCLKFLKNCYEALPDNGKVIVAECIL  300
N. tabacum_CAA52461   TYPGVEHVGGDMFASVPKADAIFMKWICHDWSDEHCLKFLKNCYEALPANGKVIIAECIL  300
A. thaliana_NM_124796 SHPGIEHVGGDMFVSVPKGDAIFMKWICHDWSDEHCVKFLKNCYESLPEDGKVILAECIL  298
                                        ▸  ▸▸   ▸       L
C. canephora          PEAPDTSLATQNVVHVDVVMLAHNPGGKERTEKEFEALAKGAGFKEFRKVCSAVNTWIME  347
M. sativa_AAB46623    PVAPDSSLATKGVVHIDVIMLAHNPGGKERTQKEFEDLAKGAGFQGFKVHCNAFNTYIME  360
N. tabacum_CAA52461   PEAPDTSLATKNTVHVDIVMLAHNPGGKERTEKEFEALAKGAGFTGFARLVALTTLGSWN  360
A. thaliana_NM_124796 PETPDSSLSTKQVVHVDCIMLAHNPGGKERTEKEFEALAKASGFKGIKVVCDAFGVNLIE  358

C. canephora          LCK--   350
M. sativa_AAB46623    FLKK-   364
N. tabacum_CAA52461   STSN-   364
A. thaliana_NM_124796 LLKKL   363
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● : Active site dimer
▸ : Active site substract binding / positioning residues
Bold : Conserved residues and motifs for SAM binding

Figure 2. Sequence alignment of 4 COMTs. Alignment was performed using ClustalW algorithm.

On both sequences, alignment with *Medicago sativa* OMTs allowed to identify amino acids implicated in substrate and cofactor binding. (Figures 1 and 2).

A putative transit peptide (20 amino acids) was only identified in the COMT sequence. The addressing of this peptide to the secretory path suggests that phenylpropanoid compound methylation occurs in different cell compartments.

Interestingly, in two EST *C. canephora* libraries, from young leaves and fruits, representing over 5 000 unigenes, only 1 CCoAOMT cluster was found but not a single EST for COMT.

Alignment of the two OMTs of *C. canephora* was performed with homologues from species where the two kinds of peptide were simultaneously found. (Figures 3 and 4).

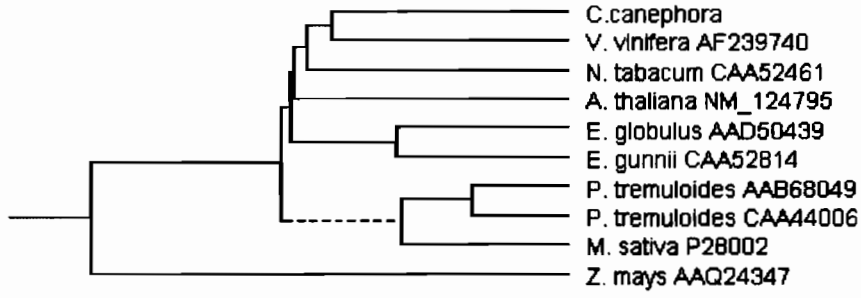

Figure 3. Phylogenic tree of COMT proteins. Multialignment was performed using ClustalW algorithm.

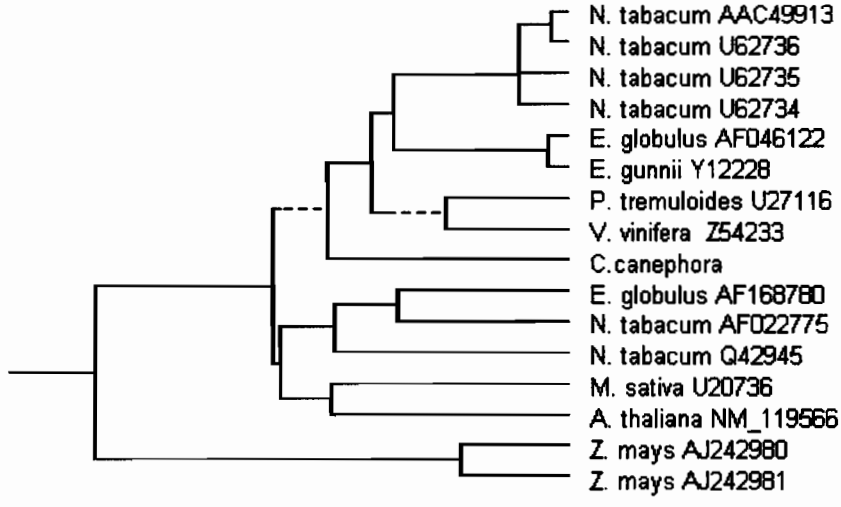

Figure 4. Phylogenic tree of CCoAOMT proteins. Multialignment was performed using ClustalW algorithm.

The two *C. canephora* OMTs gather together with dicotyledonous OMTS, suggesting the implication of these OMTs in lignin synthesis and response to pathogens.

CONCLUSION

This preliminary work is ongoing with the identification of homologous OMT genes and the functional characterization of the encoded proteins in coffee plants. The recent isolation of a second CCoAOMT sequence led us to understand the implication of each isozyme in chlorogenic acids biosynthesis.

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