Isolation and characterisation of an ACC oxidase gene in cassava

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Introduction

Ethylene is involved in a wide range of physiological processes in plants including germination, abscission, senescence, flowering and fruit ripening. Ethylene production is also induced by stresses such as wounding, drought, chilling and pathogen attack. During cassava postharvest physiological deterioration (PPD), ethylene production in the roots was found to increase after a lag of 6 to 16 hours after harvesting (Plumbley *et al.*, 1981). 1-Aminocyclopropane-1-carboxylate (ACC oxidase or ACO) catalyses the last step of ethylene biosynthesis in plants. A number of ACC oxidase genes have been cloned, mainly from ripening fruits or flowers and some from vegetative tissues of different plant systems (Lasserre *et al.*, 1996). In most plant systems studied, ACC oxidase was encoded by a multigene family. An ACC oxidase cDNA clone, designated as MeACO1, has been isolated and characterized from a PPD-related cDNA library (Han, 2000). Here we report the isolation and characterization of an ACC oxidase genomic clone.

Material and Methods

About 2.3×10^6 plaques of a genomic library of cassava cultivar MBRA 534 DNA in λEMBL3, a generous gift of Professor M A Hughes, University of Newcastle, were screened with the cDNA MeACO1 probe (Han, 2000) and one positive clone was isolated. PCR was carried out using the lysate of the positive plaque and primers designed from cMeACO1. The sequence of PCR product showed high homology to ACC oxidase genes from other plants. However, when compared with the cDNA clone, MeACO1, the similarity was 90% over a 300 bp length. Therefore, the isolated clone was an ACC oxidase clone but not the one corresponding to cDNA MeACO1. This genomic clone was designated gMeACO2.

The λ DNA of gMeACO2 clone was extracted, purified and digested respectively with *Hind*III and *Kpn*I, and the resulted two fragments (6 kb and 2 kb) were cloned into the pUC19 vector and sequenced. Putative exons, introns and promoter regions were identified by Genefeature at http://vega.igh.cnrs.fr.bin.align-guess.cgi, and by comparing the gene with other ACC oxidase genes.

Results and Discussion

The determined sequence of the ACC oxidase gene, gMeACO2, was 6349 bp, including 4928 bp of 5´flanking region and four putative exons interrupted by three introns. The deduced amino acid sequence was 312 amino acids.

Motifs or putative regulatory elements similar to those in ACO promoters of other plant gene promoters were identified in the 5' flanking regions of the gMeACO2. The TCA motif (TCATCT-TCTT) and near matches, which were present in over 30 stress-induced genes (Goldsbrough *et al.*, 1993), was identified in gMeACO2 (at –723 to –733). Ethylene-inducible elements ATT-TCAAA or AATTCAAA were initially identified in the E4 gene in tomato and glutathione-S-transferase (GST1) gene in carnation (Montgomery *et al.*, 1993; Itzaki *et al.*, 1994), and also present in ethylene-inducible ACO genes in tomato and mung bean (Blume *et al.*, 1997). There were two such motifs in the cassava gMeACO2 (–471 to –465, –2127 to –2121 and –2431 to –2424).

ACC oxidase genes from different plant species are of high similarity both in their nucleic acid and deduced amino acid sequences. Cassava gMeACO2 had 85% similarity to the ACC oxidase gene from *Prunus persica* in the coding region (Tang *et al.*, 1993). The isolated cassava ACO genes, cDNA MeACO1 and gMeACO2, shared very high amino acid sequence identity with a number of ACO genes from other plants such as *Carica papaya* (84% identity and 88% similarity, gene access number: U68215), *Prunus persica* (81% and 89%, AF129074), petunia (81% and 86%, Q08507) and *Nicotiana glutinosa* (79% and 88%, U54565). To demonstrate the high similarity, the deduced amino acid sequence of the two cassava genes were compared with four tomato ACO genes, a well-characterized ACO gene family (Figure 1). As can be seen, the deduced amino acid sequences of these ACO genes were highly conserved from the N terminal to the C terminal except for two short regions near the C terminal. The identities between gMeACO2 and tomato ACO genes, LeACO1, LeACO2, LeACO3 and LeACO3, were 83%, 79%, 82% and 81%, respectively. This means that gMeACO2 was much more similar to tomato LeACO1 and LeACO3 than to MeACO1 (81% identity with gMeACO2).

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MeACO2          MET-FPVIDLSKLSGEERKPTMEMIQDACENWGFFELVNHGISHELMDTVERLTKEHYKK
MeACO1          ME--FPVINLEKLNGEERAATMAKIKDACENWGFFELLNHGIEPEFLDRVESMTKGHYRK
LeACO1          MEN-FPIINLEKLNGDERANTMEMIKDACENWGFFELVNHGIPHEVMDTVEKMTKGHYKK
LeACO2          MEN-FPIINLEKLNGAERVATMEKINDACENWGFFELVNHGIPHEVMDTVEKLTKGHYKK
LeACO3          MEN-FPIINLENLNGDERAKTMEMIKDACENWGFFELVNHGIPHEVMDTVEKLTKGHYKK
LeACO4          MESNFPVVDMGLLQTEKRPEAMDKIKDACENWGFFELVNHGISHELLDAVENLTKGHYKK
                **  **:::: *.  :*  :*  *:***********:****  *.:* ** :** **:*

MeACO2          CMEQRFKEMVASKGLEAVQSEISDLDWESTFFLRHLPVSNMAEIPDLDEEYRKTMKEFAE
MeACO1          CMEQRFKEMVANKGLDAVQTEIKDMDWESTFFIRHLPDSNLAQLPDLDDEHRAVMKEFAA
LeACO1          CMEQRFKELVASKGLEAVQAEVTDLDWESTFFLRHLPTSNISQVPDLDEEYREVMRDFAK
LeACO2          CMEQRFKELVAKKGLEGVEVEVTDMDWESTFFLRHLPSSNISQLPDLDDVYREVMRDFRK
LeACO3          CMEQRFKELVASKGLEAVQAEVTDLDWESTFFLRHLPTSNISQVPDLDEEYREVMRDFAK
LeACO4          CMEQRFKEMVASKGLEAVQTEIDDLDWESTFFLKHLPVSNVYEVPDLDDEYRKVMKDFAL
                ********:**.***:.*: *: *:*******::*** **: ::****: :* .*::*

MeACO2          ELEKLAEQLLEVLCENLGLEKGYLKKAFYGSKGPTFGTKVSNYPPCPKPDLIKGLRAHTD
MeACO1          KLEKLAEDLLDLLCENLGLEKGYLKKAFYGSRGPTFGTKVSNYPPCPKPDLIKGLRAHTD
LeACO1          RLEKLAEELLDLLCENLGLEKGYLKNAFYGSKGPNFGTKVSNYPPCPKPDLIKGLRAHTD
LeACO2          RLEKLAEELLDLLCENLGLEKSYLKNTFYGSKGPNFGTKVSNYPPCPKPDLIKGLRAHTD
LeACO3          RLEKLAEELLDLLCENLGLEKGYLKNAFYGSKGPNFGTKVSNYPPCPKPDLIKGLRAHTD
LeACO4          KLEKLAENLLDLLCENLGLEKGYLKKAFYGSKGPTFGTKVSNYPPCPKPDLIKGLRAHTD
                .******:**::*********.***::****:**.************************
MeACO2          AGGIILLFQDDKVSGLQLLKDGQWFDVPPMKHSIVINIGDQLEVITNGKYKSVMHRVIAQ
MeACO1          AGGIILLFQDDRVSGLQLLKDGQWIDVPPMRHSIVVNLGDQLEVITNGKYKSVEHRVVAQ
LeACO1          AGGIILLFQDDKVSGLQLLKDEQWIDVPPMRHSIVVNLGDQLEVITNGKYKSVLHRVIAQ
LeACO2          AGGIILLFQDDKVSGLQLLKDGRWIDVPPMRHSIVVNLGDQLEVITNGKYKSVMHRVIAQ
LeACO3          AGGIILLFQDDKVSGLQLLKDEQWIDVPPMRHSIVVNLGDQLEVITNGKYKSVMHRVIAQ
LeACO4          AGGIILLFQDDKVSGLQLLKDGNWIDVPPMKHSIVINLGDQLEVITNGRYKSIEHRVIAQ
                ***********:********* .*:*****:****:*:**********:***: ***:**
MeACO2          TDGTRMSLASFYNPGSDAVIYPAPALVEK--EAEKSQVYPKFVFEDYMKLYAGLKFQAKE
MeACO1          TDGTRMSLASFYNPGSDAVIYPAPALVEKE-AEEKKQVYPKFVFEDYMKLYVGLKFQAKE
LeACO1          TDGTRMSLASFYNPGSDAVIYPAKTLVEKEAEES-TQVYPKFVFDDYMKLYAGLKFQAKE
LeACO2          KDGTRMSLASFYNPGNDALIYPAPALVDKEAEEHNKQVYPKFMFDDYMKLYANLKFQAKE
LeACO3          TDGTRMSLASFYNPGNDAVIYPAPSLIE----ES-KQVYPKFVFDDYMKLYAGLKFQPKE
LeACO4          QDGTRMSIASFYNPGSDAVIFPAPELIEKT-EEDIKLKYPKFVFEDYMKLYAGLKFQAKE
                 ******:*******.**:*:**  *::       .  ****:*:******..****.**
MeACO2          PRFEAMKA-------MGP-----IATA-
MeACO1          PRFEAMKAVENNVN-LGPNCYCLIINYY
LeACO1          PRFEAMKAMES-----DP-----IASA-
LeACO2          PRFEAMKAMES-----DP-----IAIA-
LeACO3          PRFEAMKAMEANVELVDQ-----IASA-
LeACO4          PRFEAMKAVETTVN-LGP-----IETV-
                ********        .      *
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Figure 1. Comparison of the deduced amino acid sequences of MeACO1 and MeACO2 with those of four tomato ACC oxidase genes. Access number for tomato ACO genes in GeneBank, LeACO1: X58273, LeACO2: Y00478, LeACO3: Z54199 and LeACO4: AB013101. * – single, fully conserved residue; : – conservation of strong groups;. – conservation of weak groups; – no consensus

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