AMINOCICLOPRANO CARBOXILATO OXIDASA DE *Psidium guajava* L. "MEDIA CHINA": SECUENCIACIÓN PARCIAL DE ADNc[1]

[AMINOCYCLOPROPANE CARBOXYLATE OXYDASE FROM *Psidium guajava* L. "MEDIA CHINA": PARTIAL SEQUENCE FROM cDNA]

Silvia Flores-Benítez[1], Aleyna De la Luz-Monroy[2], Héctor Silos-Espino[1], Karla Yuritzi Amador-Rodríguez[1], Moisés Alberto Cortes-Cruz[3], Catarino Perales-Segovia[1], Luis Lorenzo Valera-Montero[1§]

[1]Tecnológico Nacional de México/I.T. El Llano (ITEL), Km 18, Carretera Aguascalientes-San Luis Potosí, El Llano, Aguascalientes, CP 20330. Aguascalientes, México. [2]Estudiante de Maestría en Ciencias en Biotecnología Agropecuaria (ITEL). [3]Centro Nacional de Recursos Genéticos-INIFAP, Boulevard de la Biodiversidad 400, CP 47600, Tepatitlán de Morelos Jal., México. [§]Autor para correspondencia: (luis.vm@llano.tecnm.mx).

RESUMEN

La guayaba mexicana denominada comúnmente como "Media China", procedente principalmente de los estados de Michoacán, Aguascalientes y Zacatecas, es uno de los tipos de guayaba con mayor demanda por su calidad. El periodo de vida del fruto es corto debido a su condición climatérica, que induce la maduración acelerada por la generación endógena de etileno. Esta hormona es producida por la enzima aminociclopropano carboxilato oxidasa o ACC oxidasa (ACO) y su presencia se traduce en una vida de anaquel corta y en una baja calidad de frutos no aptos para el consumo humano. En un esfuerzo por estudiar este fenómeno, se obtuvo una secuencia parcial de ADNc del gen ACO de guayaba (Pg_ACO, 786 nt de longitud) a partir de frutos maduros e inmaduros de guayaba "Media China" recolectados en Calvillo, Aguascalientes. Dicha secuencia se alineó mediante BLASTn y BLASTx en NCBI y los resultados mostraron una gran similitud con las secuencias reportadas para *Psidium guajava* y *Eucalyptus grandis* en ambos casos. La secuencia predicha de aminoácidos de Pg_ACO permitió modelar el ACO en 3D como un monómero. Esta secuencia fue alineada con las secuencias predichas de guayabas y eucaliptos reportados para construir un árbol filogenético que mostró todas las accesiones de guayaba en un solo grupo.

Palabras clave. ACC oxidasa, árbol filogenético, etileno, guayaba, modelo 3D.

ABSTRACT

The Mexican guava "Media China", coming mainly from the states of Michoacán, Aguascalientes and Zacatecas, behaves as a climacteric fruit whose ripening is accelerated by the endogenous generation of ethylene. This hormone is produced by the enzyme ACC oxidase (ACO) and its presence induces a short shelf life and a low quality of fruits not suitable for human consumption. In an effort to study this phenomenon, a partial cDNA sequence of the guava ACO gene (Pg_ACO, 786 nt in length) was obtained from mature and immature fruits of the "Media China" collected in Calvillo, Aguascalientes. That sequence was aligned by BLASTn and BLASTx in NCBI and the results showed great similarity with the sequences reported for *Psidium guajava* and *Eucalyptus grandis* in both cases. The predicted amino acid sequence of the Pg_ACO sequence

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allowed ACO to be 3D modeled as a monomer. This sequence was aligned with the reported guava and eucalyptus predicted sequences to construct a phylogenetic tree that showed all guava accessions in a single group.

Index words: ACC oxidase, phylogenetic tree, ethylene, guava, 3D-model.

INTRODUCTION

Guava (*Psidium guajava*) is a well-known fruit worldwide because of its flavor and nutritional properties (Kumari *et al*., 2017; Naseer *et al*., 2018; Omayio *et al*., 2019). It is cultivated in three Mexican states: Michoacán, Zacatecas and Aguascalientes, and these states account for the 93.6% of the national production. In México, during 2019, 22.4 thousand hectares (around 55.4 thousand acres) were cultivated with this species, producing 307,598 t worth $1827.2 million Mexican pesos, equivalent to $84.7 million USD (SIAP, 2020). The main problem for this fruit is its highly perishable condition with short shelf life that leads to economic losses and reduces the opportunity for guava export. This is due to its climacteric nature of some cultivars, since injuries from handling increase respiration producing ethylene, and this gas in turn, triggers ripening faster than normal condition (Jain *et al.,* 2003). Furthermore, some other publications mentioned that the classification of guava fruit as climacteric or non-climacteric varies with cultivars due to the fact that maximum respiratory activity as well as ethylene production was observed when the fruits were already ripe (Paul *et al*., 2012).

1-Aminocyclopropane-1-Carboxylate Oxidase (ACO) is a member of a superfamily of non-heme iron-containing proteins which is known as 2-oxoglutarate-dependent dioxygenase (2OGD), which probably appeared on seed plants during the Devonian period, plays a significant role in angiosperms since this enzyme is responsible for the evolution of 1-aminocyclopropane-1-carboxlylic acid (ACC) into ethylene, which in influences seed germination, wound response, flower and leaf abscission, ripening of climacteric fruits and senescence (Iqbal *et al*., 2017: Dorling and McManus, 2019; Houben and Van de Poel, 2019). The first ACO gene was found in tomato using antisense expression of pTOM13, a clone of unknown function (Holdsworth *et al*., 1987; Alexander and Grierson, 2002;). Later on, other gene sequences of 1-Aminocyclopropane-1-Carboxylate Synthase (ACS) and ACO were found in peanut, papaya, coffee plants, Japanese morning glory, white clover, tomato and zucchini, among others (Sato and Theologis, 1989; López-Gómez *et al*., 2004; Pereira *et al*., 2005; Chen and McManus 2006; Jafari *et al*., 2013; Wilmowicz *et al*., 2014; Sun *et al*., 2015).

Signaling of stress and cell damage, were the early function for ethylene release on land plants but differences exist among species. Nowadays, angiosperms show a different biosynthetic pathway from algae and primitive land plants. Phylogenetic analysis of ACO, sorted sequences into polyphyletic clades of dicots, monocots, gymnosperms and mosses/lycophytes indicating separated diversification early in the evolution plants (Clouse and Carraro, 2014).

Knowledge about ethylene production by plants and sequences of involved genes have been used in transgenic plants expressing ACC deaminases (Jonathan *et al*., 2009) and antisense ACC synthase or ACC oxidase for delaying tomato fruit ripeness (Guittinan and Deikman, 1994) and other climacteric fruits. Guava is considered mostly climacteric with some exemptions (Parra-Coronado, 2014), but Mexican cv. “Media China” was reported as climacteric (Mercado-Silva *et*

al., 1998). This condition makes it a highly perishable fruit with short shelf life that soon become unfit for consumption in fresh (Rana *et al*., 2015). This way, determining the sequence of the ACO gene, specific to the local germplasm of orchards in production of the guava type "Media China", can help to delay maturation by means of antisense technology, as mentioned by Aguero *et al*. (2003). Therefore, the goal of this work was to obtain a partial cDNA of the ACC oxidase for guava native to the North-Central region of Mexico.

MATERIALS AND METHODS

Plant material and RNA extraction

"Media China" guava fruits were collected from an orchard located in Calvillo, Aguascalientes (Mexico). Fruit tissue was obtained from the exocarp and mesocarp from ripe (soft and yellow in color) and immature fruits (hard and bright green in color), which were placed in liquid nitrogen until required. After several trials with a commercial kit for plants and two more published customized protocols, extraction of good quality RNA was achieved adapting the methodology of López-Gómez and Gómez-Lim (1992). Briefly: Frozen fruits were ground and exposed at room temperature to the lysis buffer (2% SDS, 1% β-mercaptoethanol, 50 mM EDTA, and 150 mM Tris base with pH adjusted to 7.5 with 1 M H_3BO_3 acid; 2 to 3 $mL \cdot g^{-1}$ fresh tissue). The mixture was homogenized adding 0.25 v/v absolute ethanol and 0.11 v/v 5 M potassium acetate and vortexed for 1 minute. After this, extraction was performed with chloroform- Isoamyl alcohol (49:1) and centrifugation at 14,000 rpm for 10 minutes. The supernatant was collected on a fresh tube adding one volume of phenol-chloroform (1:1) and vortexed for 1 minute. The aqueous phase was collected and the chloroform-isoamyl alcohol step was repeated. The aqueous phase was recovered in a fresh tube; RNA was precipitated after 24 hours of exposure to 3 M LiCl at -20 C and centrifugation for 90 minutes. The pellet was resuspended in sterile water, and potassium acetate was added to a final concentration of 0.3 M. Then the RNA was precipitated adding two volumes of absolute ethanol and centrifugation for 10 minutes. The pellet was washed twice with 75% ethanol and resuspended in sterile water.

ACO cDNA obtained through PCR and its sequencing

Total RNA obtained was used to generate cDNA using the Kit Affinity Script Multiple Temperature cDNA Synthesis (Agilent®) following the procedures specified by the supplier. In order to obtain a ACO fragment amplification, PCR reaction was performed with the following reaction mix: 2 µl cDNA, 1 µM of oligonucleotides 5'-AAYTGGGGHTTYTTTGAG-3´ and 5´-GCGNAGYTTCATRTARTCYTC-3´ (Aguero *et al*., 2003) plus 25 µl of PCR Master mix (Go Taq® Green Master Mix, Promega®). The PCR program (Techne-512® thermocycler) included an initial denaturation at 94 ˚C for 5 min; 32 cycles (95 ˚C, 1 min; 50 ˚C, 1 min; 72 ˚C, 1 min) and a final extension at 72 ˚C for 7 minutes. PCR products were visualized on 1.5 % TBE agarose gels run at 120 V for 1 hour. Gels were stained with EtBr for 10 min before visualized under UV light. Sequencing was performed by an ADN 3500XL Genetic Analyzer (Applied Biosystems) at LANBAMA, IPICYT Research Center (Mexico).

Bioinformatics analyses

BLASTn and BLASTx alignments and virtual translation for the obtained sequences were requested to the NCBI. ACO Sequences having ≥90% similarity to the query were considered for further comparisons. BioEdit® software was used to check for consensus among the selected sequences. ClustalX2 was used to align these sequences and to construct a phylogenetic tree. Protein modelling was achieved with both Phyre2 (Protein Fold Recognition Server) and SWISS-MODEL from ExPASy.

RESULTS AND DISCUSSION

ACO cDNA PCR products

Both samples obtained from mature and unripe fruits were good source of RNA. Nevertheless, only RNA obtained from mature fruits yielded cDNA 786 bp that showed a good coincidence with that of 783 bp previously obtained by Aguero *et al*. (2003). Other amplicons higher than 1 kb were obtained from unripe fruits and one of the samples of mature fruit appearing as a faint band (Figure 1); these were discarded. Only the control (a sample of tomato leaf) gave no bands at all probably due to handling and the state of development of the tissue, since only young leaves were collected.

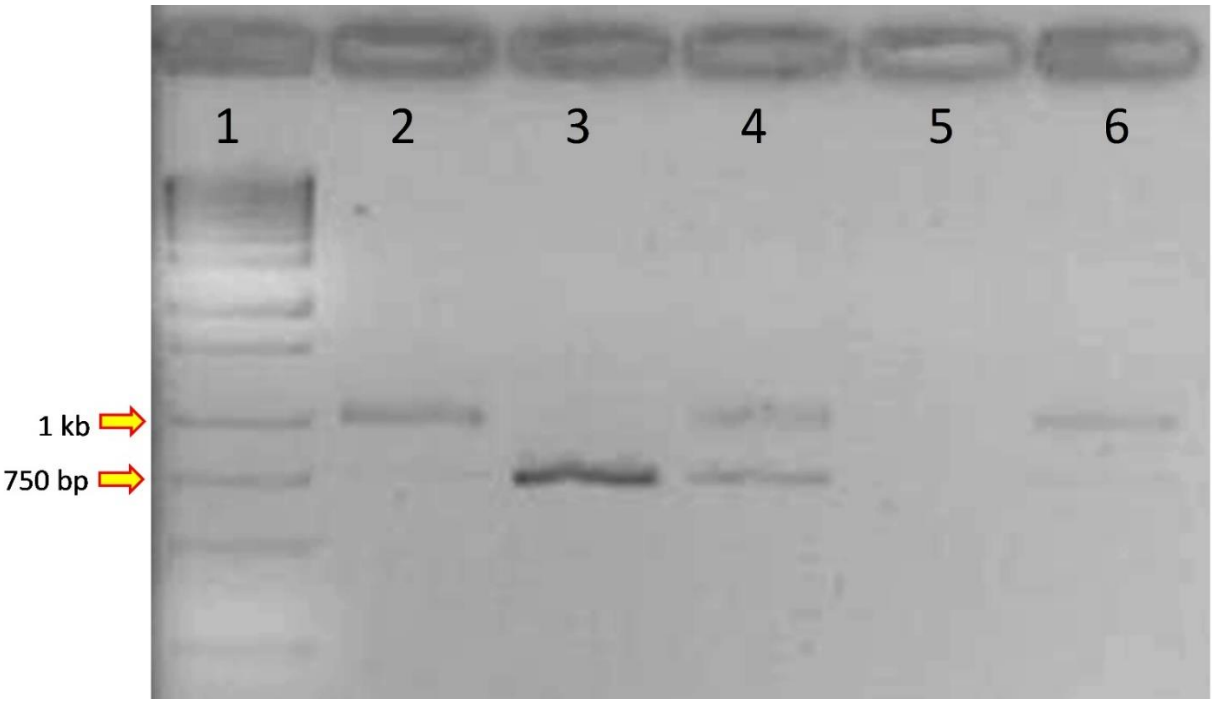

Figure 1. Partial cDNA amplification of guava ACO through PCR. Lane 1: 1 kb Molecular Marker, Lanes 2 and 6: Unripe fruits, Lanes 3 and 4: Mature fruits, Lane 5: Tomato leaf (control).

Figura 1. Amplificación parcial de ADNc del gen ACO de guayaba mediante PCR. Línea 1: Marcador de Peso Molecular 1kb, Líneas 2 y 6: Frutos inmaduros, Líneas 3 y 4: Frutos maduros, Línea 5: Hojas de tomate (control).

Analyses of the ACO sequence through BLASTn and BLASTx

One out of three obtained cDNA sequences was chosen (Pg_ACO) for bioinformatics analyses after checking the respective electropherograms (Figure 2). BLASTn results, restricted to similarity alignments of >90 %, yielded three accessions from *Psidium guajava*: AY123201.1 (98%) from Cuba, HM124457.1 (98%) and HM124456.1 (91%), both from Taiwan (Liu and Wu, 2010). Similarities within the range 80-90% belong to eight accessions of *Eucalyptus grandis* from the NCBI. Other accessions with lower similarity are significantly more distant; while high similarity between guava and eucalyptus is not surprising since these belong to the same family. As matter of comparison, full length ACO cDNA from *Guzmania ruiz* showed 94% similarity to that of pineapple; although, some other homologues were significant such as rice, moso bamboo, Malay banana (seeded), banana ABB (*Musa* × *paradisiaca*), sugar cane and barley (Liu *et al*., 2017).

>Pg_ACO
AACCTGGGGGCTTCTTTGAGCTGGTGAATCATGGGATTCCTCCCGAGATG
ATGGACACAATCGAGAGAATGACAAAGGGACACTACAAGAAGTGCATGGA
GCAGAGGTTCAAAGAGCTAGTGGTGAGCAAGGGGCTCGAGTATGTCCAAA
AAGAGGTCCATGACTTGGATTGGGAAAGCACCTTCCACTTGAAGCATCTT
CCTGAATCCAACATCTTTCAAATCTCTGACCTTGATGATGACTATCGAAA
AGTCATGAAGGAGTTTGCAGTGAAATTAGAGAAGCTGGCAGAGCAGCTAT
TGGACCTATTGTGTGAGAACTTGGGGCTAGAGAAACTGTACTTGAAAAAG
GCCTTTTATGGGTCCAACGGGCCGACTTTCGGCACCAAGGTTAGCAACTA
CCCGCCGTGCCCGAAGCCGGACCTAATCAAGGGTCTCCGGGCCCACACCG
ACGCCGGTGGCATCATCTTGCTCTTTCAAGATGACAAGGTTAGTGGCTTG
CAGCTTCTCAAGGACGGCCAATGGGTCGATGTGCCCCCGATGCACCACTC
CATCGTCGTCAACCTCGGCGACCAACTCGAGGTGATAACCAATGGGAAAT
ACAAGAGTGTGCTACATAGGGTGGTGGCCCAGACAGATGGGAATAGGATG
TCCATAGCTTCATTCTACAACCCAAGCACCGACGCTGTGATCTACCCAGC
ACCGGCACTCGTGGAGAGAGAAGAAGAGGAGGCTGGCAAAGGGACTTATC
CAAAATTTGTGTTTGAGGACTACATGAAACTACGCA

Figure 2. Partial cDNA sequence (786 nt) of ACO obtained from ripe guava fruit from Calvillo, Aguascalientes, Mexico.

Figura 2. Secuencia parcial de ADNc (786 nt) de ACO obtenido de fruto maduro de guayaba de Calvillo, Aguascalientes, México.

On the other hand, from the predicted translated sequence BLASTx revealed two putative conserved overlapped domains. The first domain, Oxidoreductase 20G-Fe(II) from the oxygenase superfamily (20G-FeII_Oxy), related to the iron/ascorbate-dependent oxidoreductase family and was ubiquitously present, as mentioned by Clouse and Carraro (2014). The family also includes lysyl hydrolases, isopenicillin synthases. The second one was a domain for Isopenicillin N synthase (PcbC) related to the dioxygenase superfamily, also present from bacteria to eukaryotes

(Figure 3). The presence of both conserved domains were confirmed with the help of PRODOM tool from ExPASy.

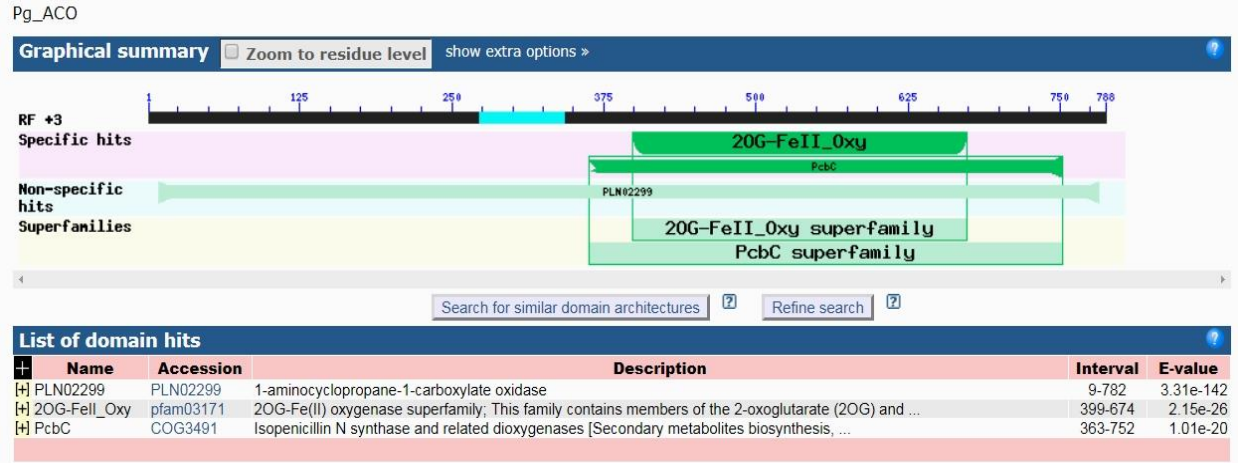

Figure 3. Putative conserved domains found on the Pg_ACO cDNA from *Psidium guajava* cv. "Media China".

Figura 3. Dominios putativos conservados en un ADNc de Pg_ACO de *Psidium guajava* cv. "Media China".

The blast of predicted protein showed higher number of accessions having similarities >90 %. Still, the highest values were for guava (AAM74522.1, AEC32838.1 and AEC32837.1) and eucalyptus accessions (XP_010035506.1, XP_010035507.1, XP_010035510.1, KCW46927.1, KCW46928.1 and XP_010035513.1). Alignment with the BioEdit software of these sequences showed remarkable consensus, especially where the putative domains are located (Figure 4). Furthermore, guava ACOs cluster together since they were very similar, and that cluster lies apart from eucalyptus ACOs (Figure 5). In spite of the similarity observed between the guava sequences, it is important to highlight that our sequence presents at least twelve amino acid residues that differ from those previously reported for guava (AAM74522.1, AEC32838.1 and AEC32837.1, Figure 4), which makes it a new contribution to the knowledge of this enzyme. Reyes Silva *et al*., (2003) reported the partial sequence of this gene (PgAC01, accession JQ639887.1) of 320 bp that encodes a partial protein of 106 aa. This sequence was not included in the alignment of our sequence due to the reduced number of residues compared to those that were used in the average had 300 aa. These are good results considering that for sugar cane ACO, the highest similarity, reached about 86% (amino acid sequence) for close monocot species such as bamboo and rice. Lower percentages were found when compared to dicots such as banana, tomato, potato, melon and carnation (Wang *et al*., 2003). Furthermore, BLASTp of ACO deduced amino acid from *Guzmania ruiz* showed the maximum similarity (76%) to ACO of Malay banana (seeded); on the other hand, kiwifruit, moso bamboo and others showed lower values (Liu *et al*., 2017).

3D Protein model

The predicted protein sequence was sent to Phyre[2] (Protein Fold Recognition Server) which was used to model the 3D monomer of ACO of guava cv. "Media China" (Figure 6). Among others, two template molecules were used by the server with a 100% confidence: the crystal structure of

Arabidopsis thaliana ACCo2 and a Penicillin synthase-like, having 73% and 80% identity, respectively. This model originally was shown as a tetramer when using ExPASy software. Zhang *et al*. (2004) described a model for ACO from *Petunia hybrida* as a tetramer and a monomer with similar shape to that obtained for this work.

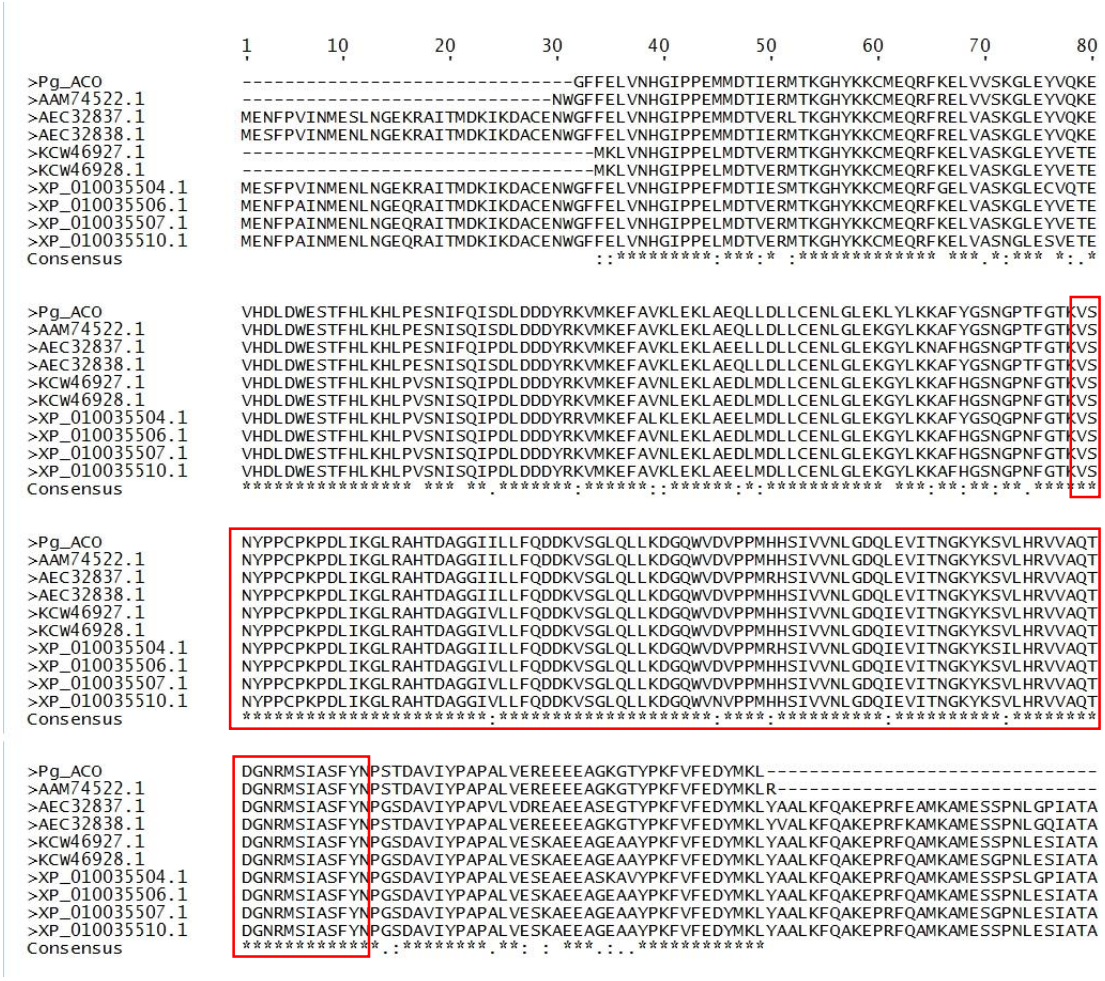

Figure 4. Alignment of the translated cDNA sequence Pg_ACO from guava and those from accessions whose sequences are annotated at the NCBI as guava (AAM and AEC accessions) and eucalyptus (XP and KCW accessions). Linking the three boxes together include the placement of the putative domains of 20G-FeII_Oxy superfamily and PcbC superfamily.

Figura 4. Alineamiento de la secuencia ADNc traducido de Pg_ACO con accesiones disponibles en el NCBI de guayaba (AAM y AEC) y de eucalipto (XP y KCW). Los recuadros incluyen los dominios putativos de las superfamilias 20G-FeII_Oxy y PcbC.

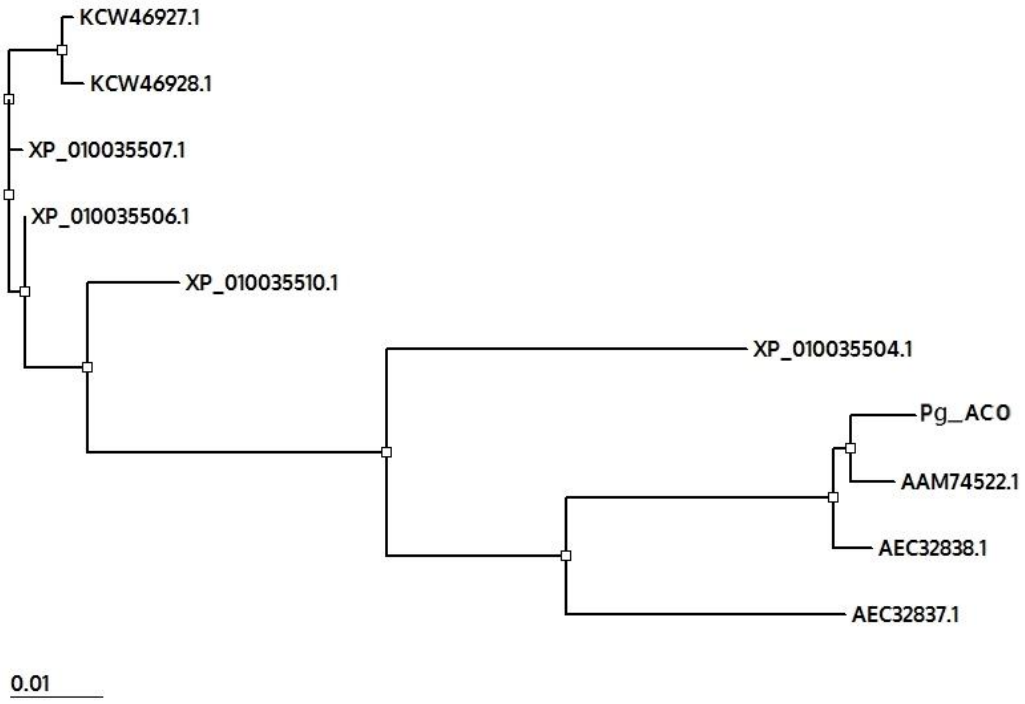

Figure 5. Phylogenetic tree constructed with the Pg_ACO predicted amino acid sequence and nine ACO sequences annotated in the NCBI Data Bank. These sequences have >90 % similarity and were obtained from guava (AAM and AEC accessions) and eucalyptus (XP and KCW accessions).

Figura 5. Árbol filogenético construido con la secuencia traducida de Pg_ACO y nueve secuencias del Banco de Datos del NCBI. Estas secuencias tienen >90 % de similitud y fueron obtenidas de accesiones de guayaba (AAM y AEC) de eucalipto (XP y KCW).

CONCLUSIONS

This work reports a partial fragment (786 nucleotides) of cDNA from ACO, showing the conserved domains present in most of the species related. BLASTn of "Media China" ACO cDNA showed the characteristic domains of Oxidoreductase 20G-Fe(II) and Isopenicillin N synthase (PcbC). Translated ACO sequence obtained from Mexican guava cv. "Media China" is very similar to that published from a Cuban guava by Aguero *et al*. (2003). Also, these two sequences cluster together with their guava Asian counterparts (Liu and Wu, 2010). Furthermore, modelling this sequence into a 3D image showed the characteristic shape of the ACO monomer. Our results confirm the isolation of an ACO sequence involved in the ripening of guava fruits of the "Media China" type from orchards in Calvillo, Aguascalientes. Unlike the previous reports of ACO in guava, in this study native plants were taken as the source of plant material. This is of great importance for the future improvement of this germplasm, since this type of creole guava known as "Media China", having great economic importance, it is also considered the genetic base of the different cultivars recently registered by INIFAP (Mexico). We hope these results will be a contribution to the knowledge of the sequence of ACO gen in guava and its expression during the ripening of the fruit.

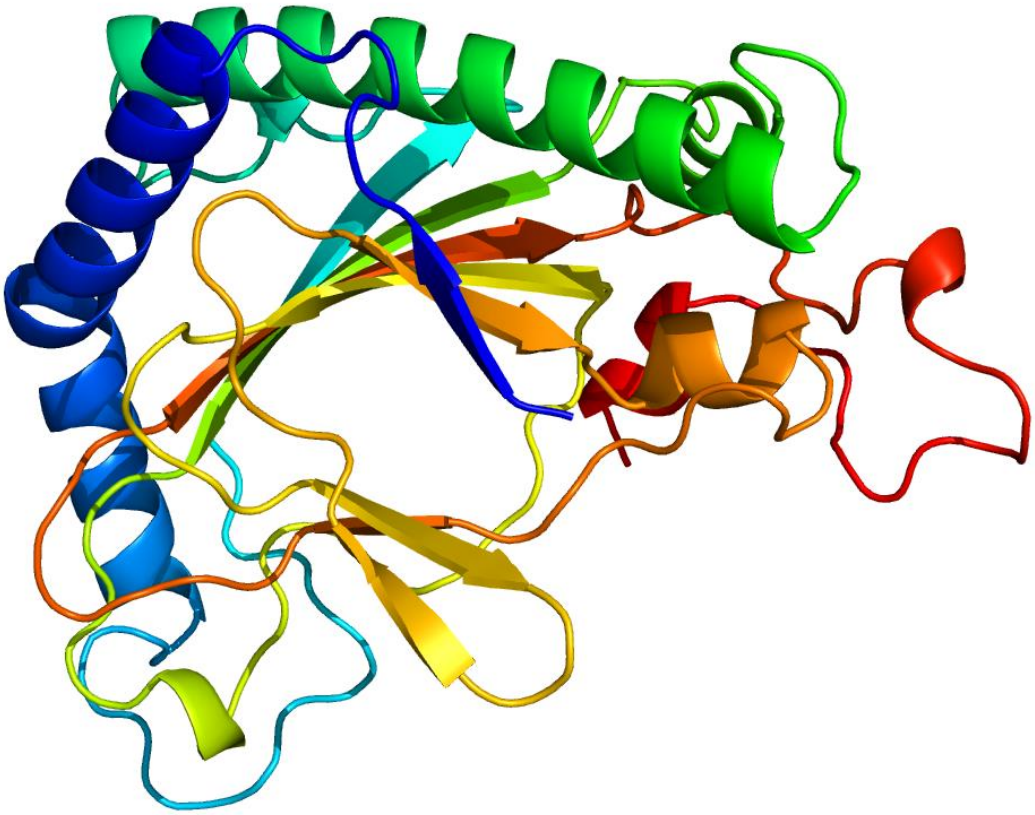

Figure 6. Monomer of a 3D model of "Media China" guava ACO built using Phyre[2] (Protein Fold Recognition Server).

Figura 6. Monómero de un modelo 3D de ACO de guayaba "Media China" generado con Phyre[2] (Protein Fold Recognition Server).

LITERATURE CITED

Aguero, G., E. Rodríguez, L. Díaz, N. Bacallao y E. Jiménez. 2003. Aislamiento de un fragmento del ADNc ACC oxidasa expresado durante la maduración del fruto de Guayaba Enana. Biotecnología Vegetal 2:187-189.

Alexander, L. and D. Grierson. 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. Journal of Experimental Botany, Fruit Development and Ripening Special Issue 53:2039–2055.

Chen, B.C.M. and M.T. McManus. 2006. Expression of 1-aminocyclopropane-1-carboxylate (ACC) oxidase genes during the development of vegetative tissues in white clover (*Trifolium repens* L.) is regulated by ontological cues. Plant Molecular Biology 60:451–467. DOI 10.1007/s11103-005-4813-3.

Clouse, R.M. and N. Carraro. 2014. A novel phylogeny and morphological reconstruction of the PIN genes and first phylogeny of the ACC-oxidases (ACOs). Frontiers in Plant Science 5:1-15.

Dorling, S. J. and M.T. McManus. 2012. The fate of ACC in higher plants. *In*: McManus MT (ed) Annual Plant Reviews: the plant hormone ethylene, vol 44. Wiley, Chichester. pp 83–115.

Guittinan, M.J. and J. Deikman. 1994. Molecular and genetic approaches to the study of plant hormone action. *In*: Horticultural Reviews, J. Janick (Ed.), Wiley & Sons, Inc., N.J. pp: 1-32.

Holdsworth, M.J., C.R. Bird, J. Ray, W. Schuch and D. Grierson. 1987. Structure and expression of an ethylene-related mRNA from tomato. Nucleic Acids Research 15:731–739.

Houben, M. and B. Van de Poel. 2019. 1-Aminocyclopropane-1-Carboxylic Acid Oxidase (ACO): The enzyme that makes the plant hormone ethylene. Frontiers in Plant Science 10 :695, 15 pages. https://doi.org/10.3389/fpls.2019.00695.

Iqbal, N., A. K. Nafees, A. Ferrante, A. Trivellini, A. Francini and M. I. R. Khan. 2017. Ethylene role in plant growth, development and senescence: Interaction with other phytohormones. Frontiers in Plant Science 8: 19 pages. https://doi.org/10.3389/fpls.2017.00475.

Jafari, Z., R. Haddad, R. Hosseini and G. Garoosi. 2013. Cloning, identification and expression analysis of ACC oxidase gene involved in ethylene production pathway. Molecular Biology Reports 40:1341–1350. DOI 10.1007/s11033-012-2178-7.

Jain N., K. Dhawan, S. Malhotra and R. Singh. 2003. Biochemistry of fruit ripening of guava *(Psidium guajava* L.): compositional and enzymatic changes. Plant Foods for Human Nutrition 58: 309-315.

Jonathan, M., L. McDonnell and S. Regan. 2009. Plant encoded 1-aminocyclopropane-1-carboxylic acid deaminase activity implicated in different aspects of plant development. Plant Signaling & Behavior 4:1186-1189.

Kumari, K. U., G. Rishitha, K. Rajendra and P.S. Kumar. 2017. Value added products of guava. Agriculture Update 12: 2171-2177. doi:10.15740/HAS/AU/12.TECHSEAR(8)2017/2270-2276.

Liu, J., H. Ding, W. Wang, H. Liu and D. Tian. 2017. Molecular characterisation and expression analysis of ACC oxidase gene from *Guzmania* Ruiz & Pav. Pakistan Journal of Botany 49:147-154.

Liu, T.C. and C.T. Wu. 2010. Cloning and analysis of the cDNA clones encoding ACC oxidase in guava. Direct submission to NCBI. Retrieved on 11/22/2017 from https://www.ncbi.nlm.nih.gov/protein/AEC32838.1

López-Gómez, R. and M. Gómez-Lim. 1992. A method for extracting intact RNA from fruits rich in polysaccharides using ripe mango mesocarp. Journal of Horticultural Science 27: 440-442.

López-Gómez, R., F. Morales-Domínguez, O. Mendoza-Alcázar, and M.A. Gómez-Lim. 2004. Identification of a genomic clone to ACC Oxidase from papaya (*Carica papaya* L.) and Expression Studies. Journal of Agricultural and Food Chemistry 52:794−800.

Mercado-Silva, E., P.B. Bautista and M.A. Garcia-Velasco. 1998. Fruit development, harvest index ripening changes of guavas produced in Central Mexico. Postharvest Biology and Technology 13: 143-150.

Naseer, S., Hussain, S., Naeem, N., Pervaiz M. and Rahman, M. 2018. The phytochemistry and medicinal value of *Psidium guajava* (guava). Clinical Phytoscience 4: 32. https://doi.org/10.1186/s40816-018-0093-8.

Omayio, D. G, G.O. Abong, M. W. Okoth, C. K. Gachuiri and A. W. Mwang'ombe. 2019. Current status of guava (*Psidium Guajava* L) Production, Utilization, Processing and Preservation in Kenya: A Review. Current Agriculture Research 7(3). doi: http://dx.doi.org/10.12944/CARJ.7.3.07

Paul, V., R. Pandey and G.C. Srivastava. 2012. The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene—An overview. J. Food Sci. Technol. 49:1–21.

Parra-Coronado, A. 2014. Ripening and postharvest behavior in guava (*Psidium guajava* L.): A review. Revista Colombiana de Ciencias Hortícolas 8: 314-327.

Pereira, L.F.P., R.M. Galvão, A.K. Kobayashi, S.M.B. Cação and L.G. Esteves-Vieira. 2005. Ethylene production and ACC oxidase gene expression during fruit ripening of *Coffea arabica* L. Braz. J. Plant Physiol. 17: 282-289.

Rana, S., S. Siddiqui and A. Goyal. 2015. Extension of the shelf life of guava by individual packaging with cling and shrink films. Journal of Food Science and Technology 52: 8148–8155.

Reyes Silva, A. I., H. G. Núñez Palenius, G. Hernández Guzmán, A. G. Alpuche Solís, C. Garcidueñas Piña, y J. F. Morales Domínguez. 2013. ADNc relacionados con la maduración del fruto de guayaba (*Psidium guajava* L.): Caracterización y análisis de expresión. Revista Fitotecnia Mexicana 36: 117-125.

Sato, T. and A. Theologis. 1989. Cloning mRNA encoding 1-aminocyclopropane-1-carboxylate synthase, the key enzyme for ethylene biosynthesis in plants. Proceedings of the National Academy of Sciences 86: 6621-6625.

Sistema de Información Agroalimentaria y Pesquera (SIAP). 2020. SIACON-NG. Sistema de Información Agroalimentaria de Consulta. Servicio de Información Agroalimentaria y Pesquera. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. México, D.F. (Consultado: 07/09/2020). Disponible en: https://www.gob.mx/siap/acciones-y-programas/produccion-agricola-33119.

Sun, Q., X. Wang, Y. Tang, Q. Wu, Y. Wang, Q. Zhang, G. Cao, S. Meng and C. Wang. 2015. Cloning and sequence analysis of a novel ACC Oxidase gene from peanut (*Arachis hypogaea* L.). *In*: T.C. Zhang & M. Nakajima (eds.), Advances in Applied Biotechnology, Lecture Notes in Electrical Engineering 333. DOI 10.1007/978-3-662-46318-5_10.

Wang, Z.Z., Y.R. Li, S.Z. Zhang, J.F. Lin and L.Q. Guo. 2003. Cloning and sequencing of ACC oxidase gene from sugarcane [Article in Chinese]. Yi Chuan Xue Bao 30: 62-69.

Wilmowicz, E., K. Frankowski, A. Kućko, J. Kęsy, and J. Kopcewicz. 2014. Involvement of the IAA-regulated ACC oxidase gene *PnACO*$_3$ in *Pharbitis nil* flower inhibition. Acta Biologica Cracoviensia. Series Botanica 56: 90–96. DOI: 10.2478/abcsb-2014-0013.

Zhang, Z., J.-S. Ren, I.J. Clifton and C.J. Schofield. 2004. Crystal structure and mechanistic implications of 1-aminocyclopropane-1-carboxylic acid oxidase-The ethylene-forming enzyme. Chemistry & Biology 11:1383–1394.