

ISOLATION AND CHARACTERIZATION OF PISANG TONGKAT LANGIT (*Musa troglodytarum* L.) *ACSI* AND *ACO1* GENE EXPRESSION DURING FRUIT RIPENING PROCESS

FENNY MARTHA DWIVANY^{1,2,3*}, KRISTI LENCI PATTY^{1,4} and CINDY NOVIANTI¹

¹School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, Indonesia

²Bioscience and Biotechnology Research Center, Institut Teknologi Bandung, Bandung, Indonesia

³Bali International Research Center for Banana, Indonesia

⁴Department of Agrotechnology, Faculty of Engineering and Agriculture, Universitas Nani Bili Nusantara, Sorong, West Papua, Indonesia

*E-mail: fenny@sith.itb.ac.id

Accepted 25 April 2020, Published online 6 July 2020

ABSTRACT

Pisang tongkat langit (*Musa troglodytarum* L.) is a banana cultivar originated from Eastern Indonesia. Previous studies showed that *ACSI* and *ACO1* are genes that play an important role in banana ethylene biosynthesis. However, characterization and analysis of *M. troglodytarum ACSI* and *ACO1* genes have not been reported. This study aims to isolate and characterize the *ACSI* and *ACO1* gene expression profile during fruit ripening process. Gene fragment isolation was performed using PCR based method. Results showed that the isolated genomic fragments of *ACSI* was 400 bp and *ACO1* was 500 bp. cDNA fragments of *ACSI* was 200 bp and *ACO1* was 300 bp. Analysis of genomic fragments using BLAST NCBI showed that *ACSI* had a high homology with 1818 bp *ACSI* gene of *Musa acuminata* AAA Group (GenBank: GQ396304.1), while *ACO1* gene had the highest homology with 1593 bp *ACO1* gene of *M. acuminata* AAA Group (GenBank: AJ223232.1). *ACSI* genomic fragments of *M. troglodytarum* had one intron and two exons, while *ACO1* had two introns and three exons. Homology analysis showed that gene fragment of *ACSI* and *ACO1* of *M. troglodytarum* had conserved region similar to *M. acuminata*.

Key words: Pisang tongkat langit, *Musa troglodytarum*, *ACSI*, *ACO1*, fruit ripening

INTRODUCTION

Bananas are the world's number four food commodity after rice, wheat, and corn. In 2013, world banana productivity reached 105.96 million tons, however Indonesia ranked sixth banana-producing countries of total world production (FAOSTAT, 2016). One of local banana cultivars in Indonesia that has economic potential is pisang tongkat langit (*Musa troglodytarum* L.). Pisang tongkat langit grows naturally only in eastern Indonesia, namely in Maluku to the territory of French Polynesia (Lim, 2012). Pisang tongkat langit has unique characteristics compared to bananas in general, i.e. has bunches that grow upwards, purple sap, and are generally resistant to pests and diseases. People in Maluku have developed pisang tongkat langit as an alternative food and used them as a raw

material for medicine (Cheesman, 1949; Satuhi & Supriyadi, 2005; Ploetz *et al.*, 2007).

Pisang tongkat langit is an interspecific hybrid, which is included in Australimusa banana group (Ploetz *et al.*, 2007). However, the genetic data on this banana group is still very limited. Meanwhile, molecular studies on the ripening process of pisang tongkat langit has not been reported.

Bananas are included in climacteric fruit, where its ripening stages are associated with an increased rate of respiration and ethylene production. Ripening process of climacteric fruit is regulated by three consecutive enzymes i.e. SAM synthetase (S-adenosylmethionine synthetase), ACC synthase (ACS) and ACC oxidase (ACO). SAM synthetase catalyzes methionine to SAM (S-adenosylmethionine). SAM becomes MTA (5-methylthioadenine), which enters into methionine cycle and is catalyzed by ACC synthase (ACS) to become a sulfur atom and 1-aminocyclopropane-1-

* To whom correspondence should be addressed.

carboxylic acid (ACC)). Furthermore, in the presence of oxygen, ACC oxidase (ACO) will catalyze ACC into ethylene and CO₂ (Yang & Hoffmann, 1984). ACC synthase enzyme is encoded by *ACS* gene while ACC oxidase is encoded by *ACO* gene. *ACS* and *ACO* genes are gene families, whose expression is regulated by many different signals such as environment, fruit development, and number of plant hormones (Karmawan *et al.*, 2009; Dwivany *et al.*, 2016). Recently, it has been known that *ACS1* and *ACO1* genes have an important role in fruit ripening. Research on both genes is widely performed in *Musa acuminata* AAA Group but has never been done in pisang tongkat langit (*Musa troglodytarum* L.). Therefore, this research is focused on the characterization and analysis of pisang tongkat langit *ACS1* and *ACO1* genes as well as to determine whether the process of fruit ripening in pisang tongkat langit is correlated with genes that play a role in fruit ripening. The results from this study can be used for future postharvest management of pisang tongkat langit.

MATERIALS AND METHODS

Materials

Pisang tongkat langit (*Musa troglodytarum* L.) used for this study were obtained from Ullath Village, Saparua Island, Central Maluku, Maluku Province, Indonesia. Pisang tongkat langit were transported carefully to avoid any physical damage.

Isolation and analysis of *ACS1* and *ACO1* genes

Isolation of DNA fragments from fruit pulp was done using the method by Doyle and Doyle (1990) which has been modified by Lodhi *et al.* (1994). Primers used in PCR analysis were *ACS1*, *ACO1*, and *GAPDH* (housekeeping gene) of *Musa acuminata* AAA Group which has been optimized by Karmawan *et al.* (2009) and Handayani and Dwivany (2014) (Table 1). The DNA fragments were amplified using GoTaq® Green Master Mix reagents from Promega (Catalog No. M7122). DNA fragments were sequenced using sequencing services from MACROGEN and the results were identified by BLASTN (www.ncbi.nlm.nih.gov/BLAST).

Isolation of RNA from banana fruit pulp on day 1 of fruit ripening was done using Cordeiro *et al.* (2008) method with modification, then the RNA fragments were used as templates for cDNA synthesis using an iScript™ cDNA Synthesis (Biorad, Catalog No. 170-8890). The cDNA was amplified using *ACS1*, *ACO1*, and *GAPDH* primers listed in Table 1. The cDNA fragments were sequenced using sequencing services from MACROGEN. Sequencing Results were identified by BLASTN (www.ncbi.nlm.nih.gov/BLAST), then aligned using Clustal Omega – Multiple Sequence Alignment (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) to compare both *ACS1* and *ACO1* gene structures.

In addition, BLASTX (www.ncbi.nlm.nih.gov/BLAST) analysis was used to analyze structures of *ACS1* and *ACO1* proteins. Gene alignment analysis was done using clustalW of BIOEDIT and the MEGA 5.2 was used to construct the phylogenetic tree.

RESULTS AND DISCUSSION

Gene isolation and characterization

Pisang tongkat langit DNA amplification was performed using *ACS1*, *ACO1* and *GAPDH* primers, which were designed from *Musa acuminata* AAA Group. Results showed that *GAPDH* gene fragment was 250 bp, *ACS1* was 400 bp and *ACO1* was 500 bp (Figure 1A). Identification of *ACS1* and *ACO1* genes using BLASTN showed that the *ACS1* gene had 93% similarity with *ACS1* of *Musa acuminata* AAA Group (access code GenBank: GQ396304.1), while *ACO1* had 96% similarity with *ACO1* of *Musa acuminata* AAA Group (access code Gen Bank: AJ223232. 1). Full-length *ACS1* gene of *Musa acuminata* AAA Group was 1,818 bp in size (Karmawan *et al.*, 2009), while full-length *ACO1* of *Musa acuminata* AAA Group was 1,593 bp in size (Chen *et al.*, 1999).

Pisang tongkat langit cDNA amplification was performed using same *ACS1*, *ACO1* and *GAPDH* primers for amplifying the pisang tongkat langit DNA. Results showed that *GAPDH* cDNA fragment was around 250 bp, *ACS1* was 200 bp, and *ACO1*

Table 1. Primer sequences used in PCR analysis

Primer	Oligonucleotide sequence (5' → 3')	Size (bp)	Reference
<i>MaGAPDH</i> RT-F	TCAACGACCCCTTCATCAC	250	Karmawan <i>et al.</i> , 2009
<i>MaGAPDH</i> RT-R	AGCAGCCTTGTCCTTGTC		
<i>MaACS1</i> RT-F	CCGAGACTGGATGAAGAAGAA	172	Karmawan <i>et al.</i> , 2009
<i>MaACS-1</i> RT-R	GTCTGGGTCAAATCTGGCTC		
<i>MaACO1</i> RT-F	CGAGATGCTTGCGAGAAATGG	240	Handayani & Dwivany, 2014
<i>MaACO1</i> RT-R	TGCAGCAAATTCCTTCATCGC		

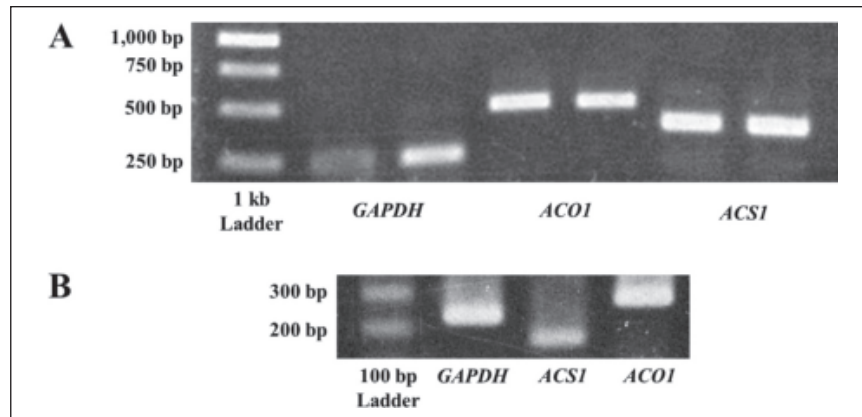


Fig. 1. Electropherogram of *ACSI*, *ACO1* and *GAPDH* gene fragments. (A) Gene fragments amplification using genomic DNA of pisang tongkat langit (*Musa troglodytarum* L.). (B) Gene fragments amplification using cDNA of pisang tongkat langit (*Musa troglodytarum* L.) pulp on day 1 of fruit ripening.

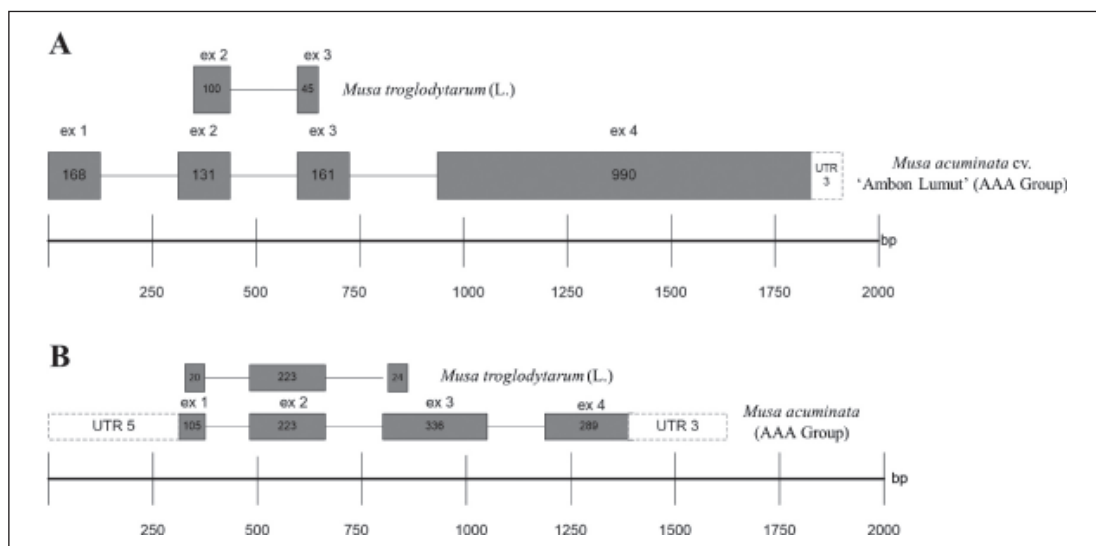


Fig. 2. Pisang tongkat langit (*Musa troglodytarum* L.) and *Musa acuminata* AAA Group *ACSI* (A) and *ACO1* (B) gene structure.

was 300 bp (Figure 1B). Identification of *ACSI* and *ACO1* cDNA using BLASTN showed that the *ACSI* cDNA fragment had 97% similarity with *ACSI* of *Musa acuminata* AAA Group mRNA (access code GenBank: Y15739.1), while *ACO1* cDNA fragment had 92% similarity with *ACO1* of *Musa acuminata* AAA Group mRNA (access code Gen Bank: EU131109.1). According to Choudhury *et al.* (2008), *M. acuminata* (AAA Group, Subgroup Cavendish) has 1,477 bp of full-length *ACSI* cDNA sequence and 957 bp of full-length *ACO1* cDNA sequence. Moreover, Dwivany *et al.* (2016) reported that both genes were also expressed during fruit ripening in “Raja Bulu” banana while other *MaACS* gene (*MaACS2*) was expressed in leaf and root of fungi-infected banana tissue culture (Handayani & Dwivany, 2014).

Schematic structure of the *ACSI* and *ACO1* gene fragments of pisang tongkat langit (*Musa*

troglodytarum L.) compared with *Musa acuminata* AAA Group can be seen in Figure 2 (the alignment results could be seen in Supplemental Data 1 and 2). The exon and intron of *ACSI* and *ACO1* were found using alignment tool of Clustal Omega, the results of which can be seen in Supplemental Data 3 and 4.

Sequence alignment and phylogenetic analysis of *ACSI* and *ACO1* genes

Protein sequence alignment analysis of *ACSI* (Figure 3) showed amino acid sequence differences between *Musa acuminata* AAA Group and pisang tongkat langit (*Musa troglodytarum* L.). Results of BLASTX (Figure 3) showed that protein sequences obtained from both bananas have conserved sequences ‘FQDYHGLP’ as mentioned by Karmawan *et al.* (2009). However, *ACSI* protein sequences of pisang tongkat langit have three

different amino acid residues ('FQDYSRPP'). ACS1 protein in bananas forms homodimers and forms a complex with the cofactor PLP (pyridoxal 5'phosphate), amino acid residues Ser-Leu-Ser-Lys-Ser-Lys-Asp-Leu-Val-Gly-Pro-Gly-Phe-Arg-Val-Gly (sequence no. 276-289) were mentioned as a site to bind to aminotransferase class-1 pyridoxal-phosphate (Choudhury *et al.*, 2009). However, further study to isolate full-length sequence of ACS1 protein is needed.

Phylogenetic analysis showed four main groups based on branching: Group 1 consisted of

Musa acuminata AAA Group and their cultivars. Group 2 consisted of pisang tongkat langit (*Musa troglodytarum* L.), *Musa acuminata* AAA Group, *Musa acuminata* AAA Group cv. 'Ambon Lumut', *Musa acuminata* AAA Group clone GMACS12 and GMACS clone-9. Group 3 consisted of *Arabidopsis thaliana* derived from dicotyledonous plant group and group 4 consisted *Saccharum officinarum* representing the group of monocots plants and *Musa acuminata* AAA Group GMACS clone-1 (Figure 4).

Protein alignment of both pisang tongkat langit and *Musa acuminata* AAA Group (GenBank

Score	Expect	Method	Identities	Positives	Gaps	Frame
86.3 bits(212)	2e-21	Compositional matrix adjust.	42/48(88%)	44/48(91%)	0/48(0%)	+3
Query 3	KNPQASICT	EGVSEFEAIANFQDYSRPP	TFRKAI	QFM	KVRGGRAR	146
Sbjct 6	KNPQASICT	EGVSEFEAIANFQDYSRPP	TFRKAI	QFM	KVRGGRAR	53
Score	Expect	Method	Identities	Positives	Gaps	Frame
92.4 bits(228)	8e-21	Composition-based stats.	44/48(92%)	44/48(91%)	0/48(0%)	+3
Query 3	KNPQASICT	EGVSEFEAIANFQDYSRPP	TFRKAI	QFM	KVRGGRAR	146
Sbjct 71	KNPQASICT	EGVSEFEAIANFQDYSRPP	TFRKAI	QFM	KVRGGRAR	118

Fig. 3. The ACS1 protein alignment. Black box shows sequence differences. Grey box shows conserved area for ACS. Query: pisang tongkat langit (*Musa Troglodytarum* L.) ACS1, Subject: *Musa acuminata* AAA Group ACS1 (access code GenBank: ACV30004.1; CAA75749.1).

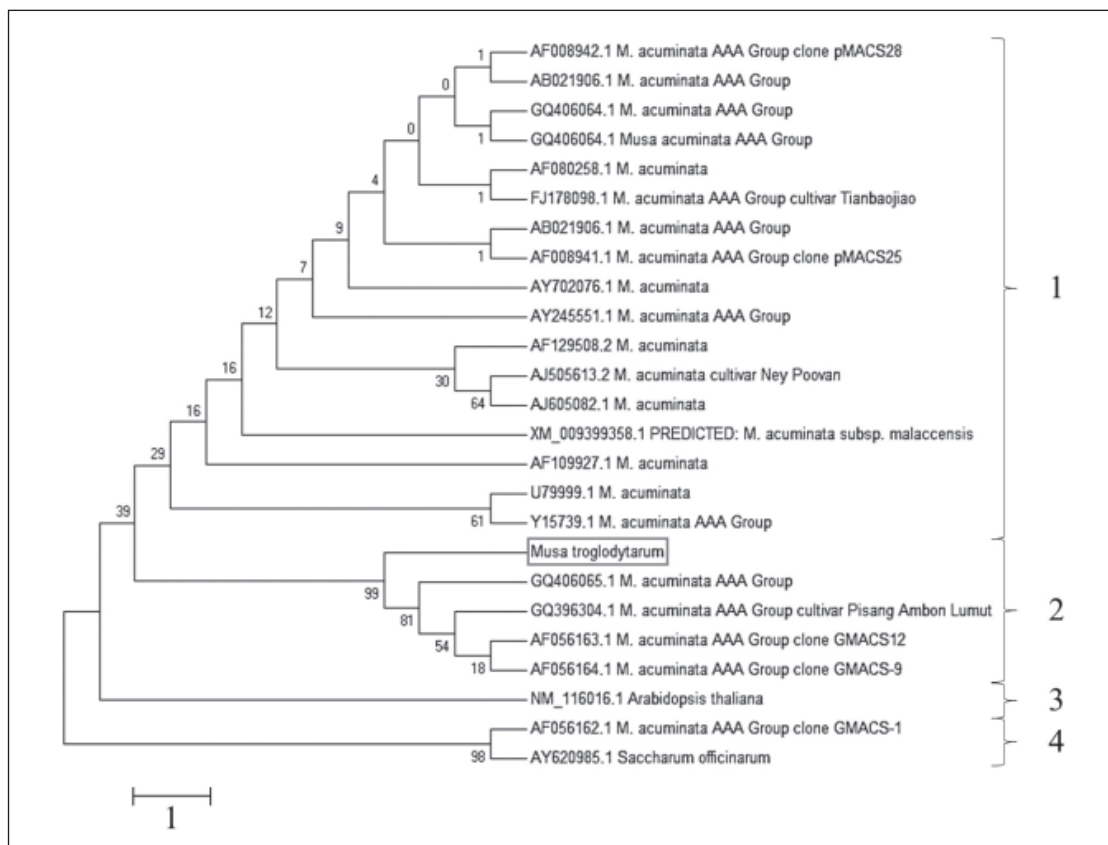


Fig. 4. Phylogenetic tree based on nucleotide sequence alignment of ACS1 from *Musa acuminata* AAA Group, *Musa troglodytarum*, *Arabidopsis thaliana* and *Saccharum officinarum* using bootstrap consensus neighbor-joining.

access code: CAA11200.1) ACO1 are shown in Figure 5. There are only three amino acid sequence differences between *Musa acuminata* AAA Group and pisang tongkat langit on sequence 42, 58, and 60. It has been reported previously that ACO enzyme family is highly conserved (Hubert & Mbeguie-A-Mbeguie, 2012). ACC oxidase (ACO) is a member of superfamily group of non-heme-iron that utilizes Fe (II) as a co-factor and 2-oxoglutarat (2OG) as co-substrat (John, 1991; Bidonde *et al.*, 1998).

Phylogenetic analysis showed the presence of two main groups based on branching: Group 1 consisted of *Musa acuminata* AAA Group, their

cultivars and pisang tongkat langit (*Musa troglodytarum* L.), Group 2 consisted of *Musa* ABB Group, *M. indica* which represents dicot plant and *Oryza sativa* representing the monocot plant (Figure 6).

Results from this study showed that *ACSI* and *ACO1* gene fragments were successfully isolated from pisang tongkat langit (*Musa troglodytarum* L.), which also having high similarity to *Musa acuminata*. Both genes were also expressed during fruit ripening process, which was indicated by cDNA amplification result (Figure 1B).

Score	Expect	Method	Identities	Positives	Gaps	Frame
152 bits(384)	4e-42	Compositional matrix adjust.	72/75(96%)	74/75(98%)	0/75(0%)	+3
Query	108	ILNHGITHDLMDEVEKVNKEQYDKGREQKFN	FANKALENADSEIDHLDWESTFFLRHLP	287		
Sbjct	36	ILNHGITHDLMDEVEKVNKEQYDKGREQKFN	FANKALENADSEIDHLDWESTFFLRHLP	95		
Query	288	VSNISEIPDLDDQYR	332			
Sbjct	96	VSNISEIPDLDDQYR	110			

Fig. 5. The ACO1 protein alignment. Black box shows sequences differences. Query: pisang tongkat langit (*Musa troglodytarum* L.) ACO1, Subject: *Musa acuminata* AAA Group ACO1 (GenBank access code: CAA11200.1).

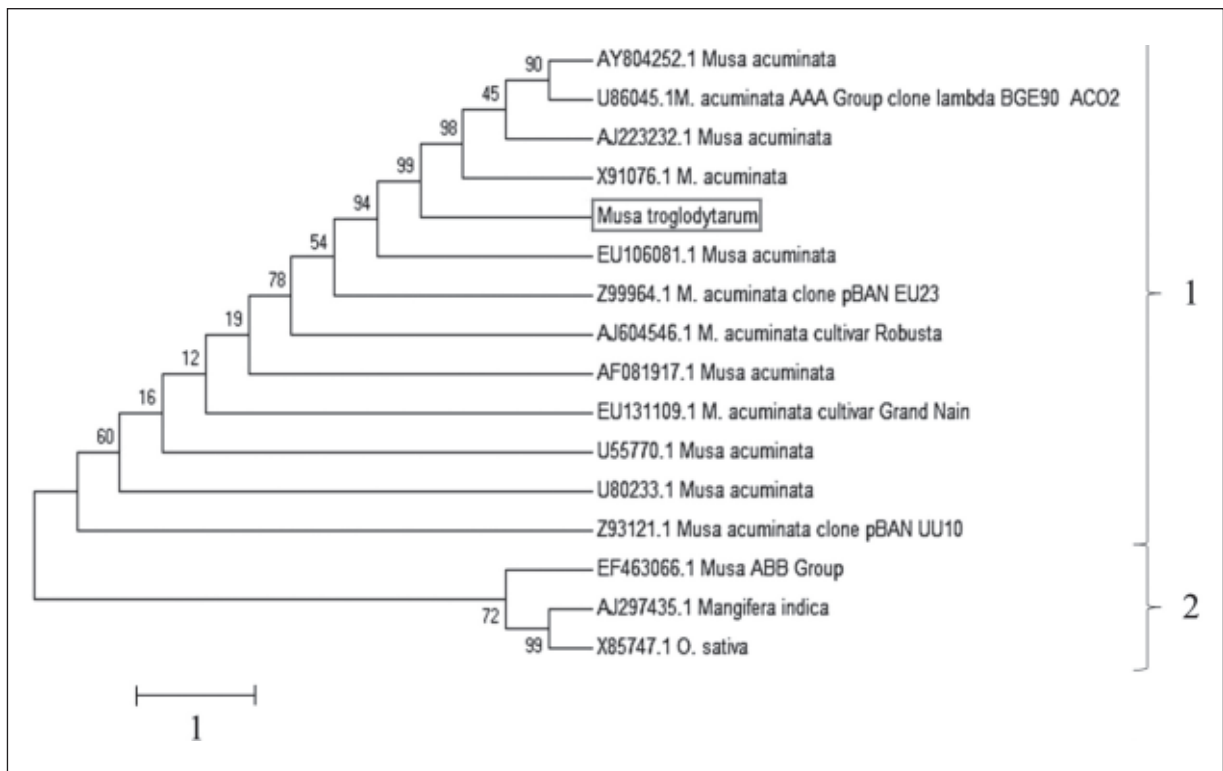


Fig. 6. Phylogenetic tree based on nucleotide sequence alignment of *ACO1* from *Musa acuminata* AAA Group, pisang tongkat langit (*Musa troglodytarum* L.), *M. indica*, *Musa* ABB Group and *Oryza sativa* using Bootstrap consensus neighbor-joining.

ACKNOWLEDGEMENTS

Author would like to thank Institut Teknologi Bandung, Indonesia and Ministry of Research, Technology and Higher Education, Indonesia for supporting the research.

REFERENCES

- Bidonde, S., Ferrer, M.A. & Zegzouti, H. 1998. Expression and characterization of three tomato 1-aminocyclopropane-1-carboxylate oxidase cDNAs in yeast. *European Journal of Biochemistry*, **253**(1): 20-26. [doi:10.1046/j.1432-1327.1998.2530020.x]
- Cheesman, E.E. 1949. Classification of the bananas: Critical notes on species: *Musa fehi*. *Kew Bulletin*, **4**(4): 445-449. [doi:10.2307/4109051]
- Chen, Y.M., Lu, B.W., Hagenbeek, D., Li, N. & Yang, S.F. 1999. A nucleotide sequence of *Musa acuminata* cv. Cavendish Grand Nain (AAA) encoding an ethylene inducible 1-Aminocyclopropane-1-Carboxylate Synthase (accession no. AJ223232) (PGR 99-062). *Plant Physiology*, **120**: 340.
- Choudhury, S.R., Roy, S. & Sengupta, D.N. 2008. Characterization of transcriptional profiles of *MA-ACS1* and *MA-ACO1* genes in response to ethylene, auxin, wounding, cold and different photoperiods during ripening in banana fruit. *Journal of Plant Physiology*, **165**: 1865-1878. [doi:10.1016/j.jplph.2008.04.012]
- Choudhury, S.R., Roy, S. & Sengupta, D.N. 2009. *MA-ACS1*: A key operator in ethylene biosynthesis in banana – its role and regulation during fruit ripening. *ISHS Acta Horticulturae*, **897**. [doi: 10.177660/ActaHortic.2011.897.20]
- Cordeiro, M.C.R., Silva, M.S., Oliveira-Filho, E.C., de Miranda, Z.J.G., Aquino, F.G., Fragoso, R.R., Almeida, J. & Andrade, L.R.M. 2008. Optimization of a method of total RNA extraction from Brazilian native plants rich in polyphenols and polysaccharides. In: *IX Simposio Nacional Cerrado*. October 12-17, 2008, ParlaMundi, Brazil.
- Doyle, J.J. & Doyle, J.L. 1990. Isolation of plant DNA from fresh tissue. *Focus*, **12**: 13-15.
- Dwivany, F.M., Hermawaty, D. & Esyanti, R.R. 2016. 'Raja Bulu' banana *MaACS1* and *MaACO1* gene expression during postharvest storage. *Acta Hortic*, **1120**: 111-114. [doi: 10.17660/ActaHortic.2016.1120.16]
- FAOSTAT. *Production and Trade* (2016). FAO, <http://faostat.fao.org/>. Accessed on January 2016.
- Handayani, R.U. & Dwivany, F.M. 2014. Analysis of *MaACS2*, a stress-inducible ACC Synthase Gene in *Musa acuminata* AAA Group Cultivar Pisang Ambon. *Journal of Mathematical and Fundamental Sciences*, **46**(2): 194-203. [doi: 10.5614/j.math.fund.sci.2014.46.2.8]
- Hubert, O. & Mbéguié-A-Mbéguié, D. 2012. Expression Patterns of Ethylene Biosynthesis Genes from Bananas During Fruit Ripening and in Relationship with Finger Drop. *AoB Plants*, **pls041**. [doi:10.1093/aobpla/pls041]
- John, P. 1991. How plant molecular biologists revealed a surprising relationship between two enzymes, which took an enzyme out of a membrane, where it was not located and put it into the soluble phase where it could be studied. *Plant Molecular Biology Reporter*, **9**(3): 192-194. [doi:10.1007/BF02672067]
- Karmawan, L.U., Suhandono, S. & Dwivany, F.M. 2009. Isolation of *MA-ACS* gene family and expression study of *MA-ACS1* gene in *Musa acuminata* Cultivar Pisang Ambon Lumut. *Hayati Journal of Biosciences*, **16**(1): 35-39. [doi: 10.4308/hjb.16.1.35]
- Lim, K.T. 2012. *Edible medicinal and non-medicinal plants. Vol. 3: Fruits*. Springer Science + Business Media B.V, Dordrecht.
- Lodhi, M.A., Guang-Ning Ye, Norman, F.W. & Bruce, I.R. 1994. A simple and efficient method for DNA extraction from grapevine cultivars, vitis species and Ampelopsis. *Plant Molecular Biology Reporter*, **12**(1): 6-13. [https://doi.org/10.1007/BF02668658]
- Ploetz, R.C., Kepler, A.K., Daniells, J. & Nelson, S.C. 2007. Banana and Plantain-an overview with emphasis on Pasific Island Cultivars. Species Profiles for Pasific Island. *Agroforestry*, **1**: 1-27.
- Satuhu, S. & Supriyadi, A. 2005. *Pisang: Budi daya, pengolahan, dan prospek dasar*. Penebar Swadaya, Jakarta. (Bahasa Indonesia)
- Yang, S.F. & Hoffman, N.E. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology*, **35**: 155-189. [https://doi.org/10.1146/annurev.pp.35.060184.001103]

SUPPLEMENTAL DATA

Supplemental Data 1. Alignment result of *Musa acuminata* cv. 'Ambon Lumut' AAA Group and pisang tongkat langit (*Musa troglodytarum* L.) *ACS1* gene sequence. Starred sequences shows similar sequences (considered as conserved sequences).

```

ACS1_M.acuminata      TACGGCGAGGAGCACCCAAATCAGCAGATCCTCTCTCGGATCGCGACCAACGACGGCCAT 60
ACS1_M.troglodytarum ----- 0

ACS1_M.acuminata      GCGGAGAACTCCTCCTACTTCGATGGGTGGAAGGCCCTACGAGAAGGATCCTTTCCACCTC 120
ACS1_M.troglodytarum ----- 0

ACS1_M.acuminata      ACCGACAACCCACGGGGTTCATCAAATGGGACTCGCAGAAAACCAGGTTAGAGTTCCT 180
ACS1_M.troglodytarum ----- 0

ACS1_M.acuminata      TCATGGTGATGATTAATCGCACATGCCTTCGGTCAATTGCCACTCCCTGCGGTTGCTAAT 240
ACS1_M.troglodytarum ----- 0

ACS1_M.acuminata      CTAATCTGTATGTGGGTTTTGGGTCTTTCTTCTCAGCTTTCCTCGACTTGATCCGAG 300
ACS1_M.troglodytarum ----- 0

ACS1_M.acuminata      ACTGGATGAAGAAGAACCACAGGCTTCGATCTGCACCGAAGAAGGGGTCTCAGAGTTCA 360
ACS1_M.troglodytarum -----AGAAGAACCCACAGGCTTCGATCTGCACCGGGGAAGGGGTCTCAGAGTTCG 51
                        *****

ACS1_M.acuminata      AAGCAATGCCAACTTTCAGGACTATCATGGCCTCCCAGCCTTCCGAAAGGTAATGATTT 420
ACS1_M.troglodytarum AAGCAATGCCAACTTTCAGGACTATTCACGGCCTCCCACCTTCCGAAAGGTAAGTACTGATTT 111
                        *****

ACS1_M.acuminata      CAACCCAAAACGCAGCGCTGCAGCTGCTTGTCTCACTGTCCAAGTAGCTACATACGTCC 480
ACS1_M.troglodytarum CAACCCGAAACGCAGAGCTGCAGCTGCTTGTCTCACTGTCCAAGTAGCTACATACGTCC 171
                        *****

ACS1_M.acuminata      AATATGATAAAGCTGGGACTGACAGCCACTTACGGCCCGAGCCCTGCCTGCTCACCCCTGG 540
ACS1_M.troglodytarum AATACGATAAAGCTGGGACTGACAGCTACCTACGGCCTGAGACCTACCTGCTCAGCTGG 231
                        ****

ACS1_M.acuminata      ATAAGGGATAAGCTAATGATGGTGTGATTTGCTGACACGCGCAGGCCATCGCCCAGTTCA 600
ACS1_M.troglodytarum AT--GGGATAAGCTAATGATGATGTGATTTGCCGACACGCGCAGGCCATCGCCCAGTTCA 289
                        **

ACS1_M.acuminata      TGGAGAAGGTGAGAGGGGACGAGCCAGATTTGACCCAGACCGCATCGTGATGAGCGGTG 660
ACS1_M.troglodytarum TGGAGAAGGTGAGAGGAGGCCGAGCCAGAT----- 319
                        *****

ACS1_M.acuminata      GAGCCACCGGCGCTCAGGAAACCATCGCCTTTTGCTGGCTGATCCTGGCGAGGCCTTCT 720
ACS1_M.troglodytarum ----- 319

ACS1_M.acuminata      TGATTCCAACGCCATATTATCCGGGTAAGTATTTAGGTGTACTAATCTACCGAGTTCTT 780
ACS1_M.troglodytarum ----- 319

ACS1_M.acuminata      TATCCGGCAGAGGATCTAATGGCATCTGCATGGTTTCCAGATTCGATCGAGACTTCAGGT 840
ACS1_M.troglodytarum ----- 319

ACS1_M.acuminata      GGAGGACAGGAGTTCAGCTCCTCCCATTCACTGCCACAGTTCCAACAAGTTCAAGATCA 900
ACS1_M.troglodytarum ----- 319

```

ACS1_M.acuminata	CCCAAGCCGCACTGGAGACTGCTTACAGGAAGGCTCGAAACTCACACATTAGAGTCAAAG	960
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	GAATACTGGTGACCAACCCATCGAACCCCTCTGGGCACAACCATGGACAGAGAGACGCTGA	1020
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	GAACCCTAGTCAGCTTCGTCAACGAGAAAAGGATGCACTTGGTGTGCGACGAGATCTTCT	1080
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	CCGGAACCGTCTTCGACAAGCCGAGTTACGTGAGCGTCTCCGAGGTGATCGAAGACGAGC	1140
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	CCTACTGCGACAGGGATCTGATTCACATCGCCTACAGCCTCTCCAAGGACCTGGGCGTCC	1200
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	CTGGCTTCGCGTTCGGCGTCATATACTCCTACAACGACGCCGTGGTTCAGCTGCGCGAGGA	1260
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	AGATGTCGAGCTTTGGACTGGTCTCGTCGACAGCAGCACCTGCTCGCTTCCATGTTGG	1320
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	GAGACGAGGAGTTCACCACGAGTTCTTAGCGACGAGCCGGACGAGGTTGTGCGGGCGGC	1380
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	GAGACGAGGAGTTCACCACGAGTTCTTAGCGACGAGCCGGACGAGGTTGTGCGGGCGGC	1380
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	GCAGGGTCTTTACGGACGGCCTCAAGCGAGTCGGGATTCATTGCTTGGACGGCAACGCGG	1440
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	GGCTGTTCTGCTGGATGGACTTGAGGCCGTTGCTGAAGGAAGCGACGGTGGAGGCGGAGC	1500
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	TCCGGCTGTGGCGGGTGATCATCAACGACGTGAAGCTCAACATCTCGCCGGGGTCTGTCCT	1560
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	TCCACTGCTCGGAGCCGGGGTGGTTCAGGGTGTGCTTCGCCAACATGGACGACACGGCCA	1620
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	TGAAGATAGCGCTGAGGAGATCGAGAGTTTCGTGTACCGGGAGAACGACCCGCTGTGC	1680
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	AGCGAAGAACAAAGAGGAGGTGGGACGAAGCGCTGCGGCTGAGCTTGCTCGTGGAGGT	1740
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	TCGAGGATCCGACCATCATGACACCACATCTGATGTCTCCCCACTCGCCTCTCGTTCAAG	1800
ACS1_M.troglodytarum	-----	319
ACS1_M.acuminata	CCGCCACCTGAAACATCG	1818
ACS1_M.troglodytarum	-----	319

Supplemental Data 2. Alignment result of *Musa acuminata* AAA Group and pisang tongkat langit (*Musa troglodytarum* L.) *ACO1* gene sequence. Starred sequences shows similar sequences (considered as conserved sequences).

```

ACO1_M.acuminata      ACGCGAGAGGTGGAAGCAAGGAGGGTGGAGAACCAGGCCAAAGTGGTGGGGCTGAGAGA 60
ACO1_M.troglodytarum  ----- 0

ACO1_M.acuminata      TGGCCAACTGGGTCAACCTATGGAATCGGCTCCGTTACGTCTTCCACTGCTGTTGCTCTC 120
ACO1_M.troglodytarum  ----- 0

ACO1_M.acuminata      GTCGATAGATCCTTCTCCAACCTTGCTTCCTCATTCAATTCGTCCTCGACGTCAAGAAC 180
ACO1_M.troglodytarum  ----- 0

ACO1_M.acuminata      GCCTATAAATTGCCTGGTAATCAGCAGCACCTAGCACACTCCAGATAGAAAGCACAAAGTG 240
ACO1_M.troglodytarum  ----- 0

ACO1_M.acuminata      CAATCAGGAAGAAAGAGCGTGTGATGGATTCCCTTCCGGTTATCGACATGGAGAAGCTT 300
ACO1_M.troglodytarum  ----- 0

ACO1_M.acuminata      TTGGGAAGGGAGAGAGGAGCAGCCATGGAGATCCTCCGAGATGCTTGCGAGAAATGGGGC 360
ACO1_M.troglodytarum  -----GGAGAAATGGGGC 13
                        *****

ACO1_M.acuminata      TTCTTTGAGGTGCTGAAGCATACATAACTGGTTTTGCTTCTTTGAACTATATATATGCT 420
ACO1_M.troglodytarum  TCTTTGAGGTCCTGAAGCATACATAACTGGTTTTGCTTCTTTGAACTATACACTGCT 73
                        *****

ACO1_M.acuminata      AAAAATGTAATTTGCGCATGCAATCTGTGTGATAGATTTTAAACCATGGCATCTCACAT 480
ACO1_M.troglodytarum  --AAATGTAATTTGCACATGCAATCTGTGCGTAGATTTTAAACCATGGCATCACACAT 131
                        *****

ACO1_M.acuminata      ACCTCATGGATGAAGTGGAGAAGGTGAACAAAGAACAGTACAACAAATGCAGGGAGCAA 540
ACO1_M.troglodytarum  ACCTCATGGATGAAGTGGAGAAGGTGAACAAAGAGCAGTACGATAAAGGCAGGGAGCAA 191
                        *****

ACO1_M.acuminata      AAGTTCAACGAGTTCGCCAACAAAGCACTGGAAAACGCCGACTCAGAAATCGACCACCTC 600
ACO1_M.troglodytarum  AAGTTCAACGAGTTCGCCAACAAAGCACTGGAAAACGCCGACTCAGAAATCGACCACCTC 251
                        *****

ACO1_M.acuminata      GACTGGGAAGACACCTTTTTCTGCGTCATCTCCCGTCTCCAACATTTCTGAGATCCCC 660
ACO1_M.troglodytarum  GACTGGGAAGACACCTTTTTCTGCGTCATCTCCCGTCTCCAACATTTCTGAGATCCCC 311
                        *****

ACO1_M.acuminata      GATCTTGATGACCAGTATAGGTTGCACGATCTGATCATGATGTCATCTTCTAGCCTTGTC 720
ACO1_M.troglodytarum  GATCTTGATGACCAGTATAGGTTGCACGATCTGATCATGATGTCATCTTCTAGCCTGGCC 371
                        *****

ACO1_M.acuminata      TTTTCACCTTGCTCATCGTTTCGTTTCTTGGGACGATGACTGCGTGCAGGAAGGCGATGA 780
ACO1_M.troglodytarum  TTTTCACCTTGCTCATCGTTTCGTTTCTTGGGACGATGACTGCGTGCAGAAAGGCGATGA 431
                        *****

ACO1_M.acuminata      AGGAATTTGCTGCAGCGATAGAGAAGCTGGCAGAGCGGCTGCTCGACTTGCTGGGTGAGA 840
ACO1_M.troglodytarum  AGGAATTTGCTGCAAGGCCGGAGACGCCAGGGGACGCCACCCGCCGAAA----- 481
                        *****

ACO1_M.acuminata      ACCTGGAGCTGGAGAAGGGGTACCTGAAGAAAGCCTTCTCTAATGGATCCAAGGGGCCAA 900
ACO1_M.troglodytarum  ----- 481

ACO1_M.acuminata      CCTTTGGGACCAAGGTCAGCAGCTACCCACCATGCCACGCCCGGACCTGGTGAAGGGCC 960
ACO1_M.troglodytarum  ----- 481

```

ACO1_M.acuminata	TGAGGGCGCACGCCGACGCCGGAGGCATCATCTTGCTCTTCCAGGACGACCAGGTCAGCG	1020
ACO1_M.troglodytarum	-----	481
ACO1_M.acuminata	GCCTGCAGTTCCTCAAGGACGGCGAGTGGCTGGACGTGCCCCCATGCGCCATGCCATCG	1080
ACO1_M.troglodytarum	-----	481
ACO1_M.acuminata	TCGTCAACCTCGGCGACCAGCTCGAGGTTGGGTCTCTTTGCTCTCGTTCCGCTGCC	1140
ACO1_M.troglodytarum	-----	481
ACO1_M.acuminata	GTCGTCTGTGATGTTGAATGCAACGAGGCTGCAGGTAATCACCAATGGCAAGTACAAGA	1200
ACO1_M.troglodytarum	-----	481
ACO1_M.acuminata	GCGTGGTGCACCGCGTGGTGGCTCAGACTGATGGCAACAGGATGTCGATTGCCTCCTTCT	1260
ACO1_M.troglodytarum	-----	481
ACO1_M.acuminata	ACAACCCCGGGAGCGACGCTGTGATCTTCCCGCCCCGCTCTTGTGGAGAAGGAAGCGG	1320
ACO1_M.troglodytarum	-----	481
ACO1_M.acuminata	AGGAGAAGAAGGAGGCTATCCGAGGTCGTGTTTCGAGGATTACATGAAGCTCTACGTCG	1380
ACO1_M.troglodytarum	-----	481
ACO1_M.acuminata	GGCATAAGTTCCAGGCCAAGGAGCCAAGATTGGAAGCCATGAAAGCCATGGAAGCAGTTG	1440
ACO1_M.troglodytarum	-----	481
ACO1_M.acuminata	CCACCCACCCAATCGCTACCTCTTAAGTGACAGCCCCCAAGTTAGTGCATGTCGCTGTAC	1500
ACO1_M.troglodytarum	-----	481
ACO1_M.acuminata	TTCGCGTTAGGAAGCTGTCGTCTATGTCTATGTAACCCGATGGAAGCGTGGTATGTACGT	1560
ACO1_M.troglodytarum	-----	481
ACO1_M.acuminata	GTTTGAGCCTTTTCTAATGAAGCAAGTCATATA	1593
ACO1_M.troglodytarum	-----	481

Supplemental Data 3. Alignment result of DNA and cDNA sequence of pisang tongkat langit (*Musa troglodytarum* L.) *ACS1*. Black boxes are considered as exons.

```

DNA_ACS1      AGAAGAACCCACAGGCTTCGATCTGCACCGGGGAAGGGCTCTCAGAGTTCTGAAGCAATTG 60
cDNA_ACS1     -----TGTCTGGGTCA----- 11
                * * * * *

DNA_ACS1      CCAACTTTCAGGACTATTCACGGCCTCCACCTTCCGAAA---GGTACTGATTTCAACCC 117
cDNA_ACS1     -----AATCTGGCTCGGCCTCCTCACCCTTCTCCATGAAGTGGGCATGGCCTTTC 63
                * * * * *
                * * * * *
                * * * * *

DNA_ACS1      GAAACGCAGAGCTGCAGCTGCTTGTCTCTCACTGTCCAAGTAGCTACATACGTCCAATACG 177
cDNA_ACS1     GGAAGGCTGGGAGG-CCGTGATAGTCCTG----- 91
                * * * * *
                * * * * *

DNA_ACS1      ATAAAGCTGGGACTGACAGCTACCTACGGCCTGAGACCTACCTGCTCAGCTGGATGGGA 237
cDNA_ACS1     --AAAGTTGGCAAT-----TGCTTCGAACTCTGAGACCCCTTCCCCGG-TGCAGATCG 141
                * * * * *
                * * * * *
                * * * * *
                * * * * *

DNA_ACS1      TAAGCTAATGATGATGTGATTTGCCGACACGCGCAGGCCATCGCCCAGTTCATGGAGAAG 297
cDNA_ACS1     AAGCCTGTGGGT--TCTTCTTCATCCAGTCTCGGA----- 174
                * * * * *
                * * * * *

DNA_ACS1      GTGAGAGGAGGCCGAGCCAGAT 319
cDNA_ACS1     ----- 174

```

Supplemental Data 4. Alignment result of DNA and cDNA sequence of pisang tongkat langit (*Musa troglodytarum* L.) *ACO1*. Black boxes are considered as exons.

```

DNA_ACO1      GGAGAAATGGGGCTTCTTTGAGGTCCTGAAGCATACTAACTGGTTTTGCTTCTTTGAAC 60
cDNA_ACO1     ----- 0

DNA_ACO1      TATACATACTGCTAAATGTACTATTTGCACATGCAATCTGTGCGTAGATTTTAAACCATG 120
cDNA_ACO1     -----CCCTTTCTATACCTGG 16
                *** ** *

DNA_ACO1      GCATCACACATGACCTCATGGATGAAGTGGAGAAGGTGAACAAAGAGCAGTACGATAAAG 180
cDNA_ACO1     TCATCAAGATCC-----GGGGATTCTCAGAAAATGTTGGAGACGG-----GGAGATGACG 66
                *****          **** * * * * * * * * * *          **** *

DNA_ACO1      GCAGGGAGCAAAAGTTCAACGAGTTCCCAACAAAGCACTGGAAAACGCCGACTCAGAAA 240
cDNA_ACO1     CCAGGAAAAAAGGGTGC-----TTTC----- 87
                ***** ** * * * *          ***

DNA_ACO1      TCGACCACCTCGACTGGGAAAGCACCTTTTTCTGCGTCATCTCCCCGTCTCCAACATTT 300
cDNA_ACO1     ---CCCAGTTCGAGTGTGGTCC-GATTTCTGAGTCCGGCGTTTTCCAGTGCCCTTT--- 139
                *** * * * * *          * * * * * * * * * * * * * *

DNA_ACO1      CTGAGATCCCCGATCTTGATGACCAGTATAGGTTGCACGATCTGATCATGATGTCATCTT 360
cDNA_ACO1     ---GTTGGCGAACCTCGTTGAA-----CTTTTGCTCCCCTGCCTTTATCGTACTGCT 188
                * * * * * * * * * *          ** * * * * * * * * * *

DNA_ACO1      CTAGCCTGGCCTTTTCACCTTGCTCATCGTTTCGTTGCTTGGGACGATGACTCGGTGCAG 420
cDNA_ACO1     CTTTGTTACCTTCTCCACTTCATCCATGAGGTCATGTGTGATGCCAT-----GGTTTAA 243
                ** * * * * * * * * * * * * * * * * * * * *          ** *

DNA_ACO1      AAAGGCGATGAAGG-AATTTGCTGCAAGCCGGGAGACGCCAGGGGACGCCACCCGCCGA 479
cDNA_ACO1     AATCTCAAAGAAGCCCCATTTCTCGCAAGCATCTCGA----- 280
                ** * * * * * * * * * * * * * * * *

DNA_ACO1      AA 481
cDNA_ACO1     -- 280

```