Environment Effect on Fruit Ripening Related Gene to Develop a New Post Harvest Technology

Article · February 2014

DOI: 10.1063/1.4868801

9 authors, including:

Fenny MARTHA Dwivany
Institut Teknologi Bandung-Indonesia, Bali Int…
33 PUBLICATIONS **33** CITATIONS
SEE PROFILE

Rizkita Esyanti
Bandung Institute of Technology
37 PUBLICATIONS **22** CITATIONS
SEE PROFILE

Jekson Robertlee
Osaka University
3 PUBLICATIONS **3** CITATIONS
SEE PROFILE

Dina Hermawaty
University of Western Australia
4 PUBLICATIONS **3** CITATIONS
SEE PROFILE

Environment effect on fruit ripening related gene to develop a new post harvest technology

Fenny Dwivany, Rizkita Rahmi Esyanti, Jekson Robertlee, Indra Chandra Paramaputra, Rinda Kania Permatadewi, Dina Hermawaty Tambun, Resnanti Utami Handayani, Aksarani 'Sa Pratiwi, and Herafi Zaskia

Citation: AIP Conference Proceedings **1589**, 285 (2014); doi: 10.1063/1.4868801
View online: http://dx.doi.org/10.1063/1.4868801
View Table of Contents: http://scitation.aip.org/content/aip/proceeding/aipcp/1589?ver=pdfcov
Published by the AIP Publishing

Environment Effect on Fruit Ripening Related Gene to Develop a New Post Harvest Technology

Fenny Dwivany[1*], Rizkita Rahmi Esyanti, Jekson Robertlee, Indra Chandra Paramaputra, Rinda Kania Permatadewi, Dina Hermawaty Tambun, Resnanti Utami Handayani, Aksarani 'Sa Pratiwi and Herafi Zaskia

School of Life Sciences and Technology-Institut Teknologi Bandung, Indonesia
** Email: fenny@sith.itb.ac.id*

Abstract. Ripening process of fruits is a very complex process, which involves ethylene production, causing alteration on molecular and physiology level. Environmental stress caused by biotic and abiotic stress conditions (such as pathogen, mechanical stress, physical and physiology stress) can stimulate ethylene production. High levels of ethylene in turn can also inhibit growth, cause premature ripening and induce the onset of senescence, which then potentially reduce plant productivity. The ACC Synthase (ACS) and ACC Oxidase (ACO) genes are genes that have role in the ethylene production. By regulating those genes, especially ethylene biosynthesis genes, we might improve the quality of fruit at post harvest condition. Therefore, in this research we studied fruit ripening related genes expression on banana such as *MaACS* family at different environment condition. The result of study can give contributions in developing of new plants with desired traits or new post harvest technologies.

Keywords: *fruit ripening, FSC, banana, MaACS5 and MaACS6*
PACS: 87.85.md

INTRODUCTION

Natural ethylene which is synthesized from *S*-adenosyl-methionine and 1-aminocyclopropane-1-carboxylic acid (ACC) precursors has important role in fruit ripening process. Ethylene biosynthesis is catalyzed by ACC synthase (ACS) and ACC oxidase (ACO) which are encoded by *MaACS* and *MaACO* gene family in banana [1-2]. Ethylene will then induce metabolic process and activate many genes that correlate to ripening and senescence. The induced metabolic process for example are changes in respiration and carbohydrate composition [3]. Delaying fruit ripening could be performed by reducing O_2 concentration or elevating CO_2 concentration, this approaches have been known as *controlled atmosphere* (CA) [4]. Many attempts to delay banana ripening process have also been reported, such as placed fruits at particular O_2 gas concentration composition [5-6]. The other methods were performed using ethylene absorbent, CO_2 scrubber or PVC film [7-8]. Several studies of environment effects on ethylene biosynthesis related genes during ripening process have been reported. However, there was a little information of O_2 and CO_2 effects on ethylene biosynthesis related genes. Our previous gene expression study on *MaACO* and *MaACS* genes showed that only three genes (*MaACO*, *MaACS1* and *MaACS5*) were highly expressed in fruit (pulp and peel) at normal open condition, while in vegetative tissues (roots and leaves) expression of these three genes were very low [9]. Our preliminary study showed that fruits placed at modified atmosphere condition (Fruit Storage Chamber or FSC) ripe slower than open normal condition [10]. Gene expression study showed that *MaACO* gene expression increased at normal open condition, whereas at FSC had reverse profile. On the other hand, *MaACS1* gene expression increased at both condition [10]. Based on those result, gene expression study of other potential ripening related gene, *MaACS5*, is important. In this study we showed different expression profile of *MaACS5* gene together with *MaACS6* gene when fruits were place at both normal open condition and FSC.

MATERIALS AND METHODS

Materials

Cavendish banana fruits were obtained from local fruit supplier then stored at normal open condition and FSC ($N:O_2:CO_2$= 90%:5%:5%) [10]. Starch analysis and fruit pulp sampling were performed at day 0, 1, 4, 5 and 7. All samples were then placed at -80^{o}C for RNA isolation.

RNA Isolation and cDNA Synthesis

RNA was isolated using modified Zhuang *et al.* method [11]. RNA was treated using DNAse from Fermentas (cat. no. #EN0521) and cDNA was then

4th International Conference on Mathematics and Natural Sciences (ICMNS 2012)
AIP Conf. Proc. 1589, 285-287 (2014); doi: 10.1063/1.4868801

synthesized using iScript *cDNA synthesis* from BioRad (cat. no 170-8890).

Quantitative Polymerase Chain Reaction (qPCR)
Gene expression analysis was conducted using Bio-Rad iCycler® CFX 96™ Thermal Cycler which was connected to iQ™5 *Real-Time* PCR Detection System. SsoFast™ EvaGreen® Supermix (No. catalog #172-5200) was used for reaction. Preparation of 20 µl qPCR mix was performed using *Filter tips* Xcluda™ in *Microseal 96-Well Skirted PCR Plates Low-Profile*™ plate. Three replications were performed for each treatment and negative control (*no template control* / NTC and water) was also used. qPCR cycle was performed 39 times at 95°C and 48°C for 10 second using gene specific primers of *MaACS5* and *MaACS6*. Quantitative analysis was then conducted using Livak *et al*. [12] method and *MaGAPDH* gene was used as a control.

RESULT AND DISCUSSION

Our study showed that FSC can be used to delay banana fruit ripening (Figure 1.). In addition to a longer greener color and delay in starch conversion to sugar, the respiration process also slower at FSC (data not shown). Other report showed that using 2.5% O_2, enzymes activities were suppressed, appearance of yellow color as well as sugar accumulation were also delayed [6]. Although low O_2 concentration can delay ripening process, O_2 below 2% will give anaerobic condition for fruits, while upper limit for CO_2 concentration is varies between fruits [4].

In this study, *MaACS5* gene expression increased at normal open condition, while it decreased drastically at FSC (Figure 2a). On the other hand, *MaACS6* gene expression was increased at both conditions (Figure 2b.). Our previous study also showed that *MaACO* gene expression has correlation with ripening process, its expression increased at normal open condition and decreased when fruit ripening was delayed at FSC [10]. It is interesting, since both *MaACS5* and *MaACO* genes expressed higher in fruit tissue compared to other genes at normal condition, but not in vegetative tissue [9]. Based on these results, both of genes may have important role in delaying of fruit ripening process and became limiting factors in ethylene production at FSC. The role may through reduction of ACC synthase and ACC oxidase enzymes. On the other hand, similar to *MaACS1* from previous study, *MaACS6* expression quite similar at both conditions and may have no correlation with delaying ripening at FSC.

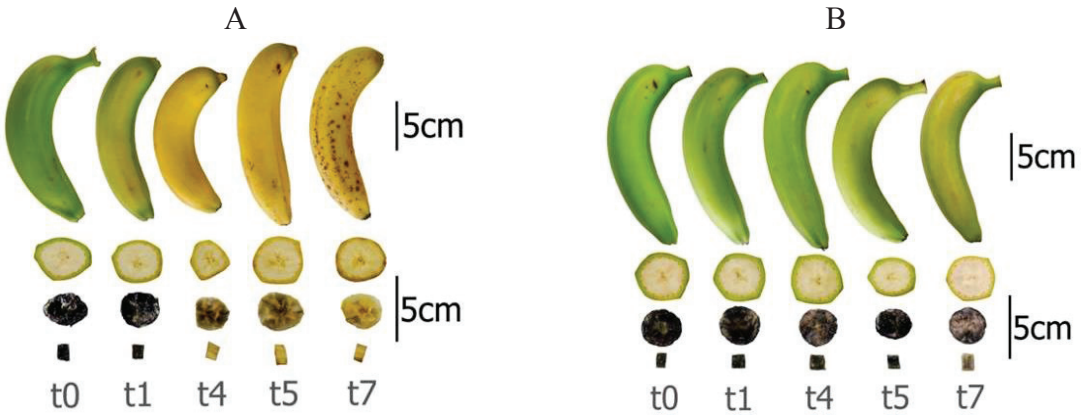

FIGURE 1. Ripening process of banana from day 0-7 and starch content. A. Normal open condition, B. Fruit Storage Chamber (N_2:CO_2:O_2 = 90%:5%:5%).

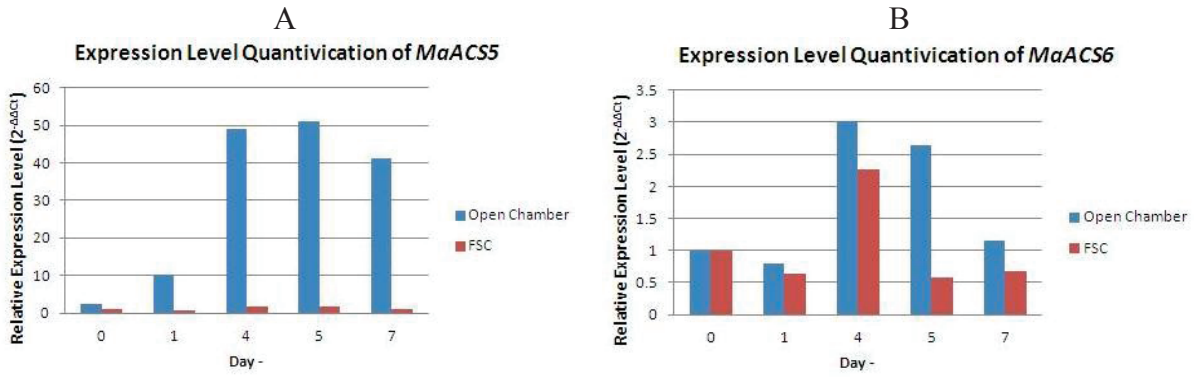

FIGURE 2. Expression profile of *MaACS5* (A) and *MaACS6* (B) genes during banana fruit ripening process day 0, 1, 4, 5 and 7.

CONCLUSION

The *MaACS5* gene may have correlation with delaying of fruit ripening process at our FSC. On the other hand, *MaACS6* gene may have no correlation with delaying fruit ripening at FSC since its expression profile almost similar in both normal open condition and FSC.

ACKNOWLEDGMENTS

We would like to thank Prof. Chunaeni Latif and Ari Ginaldi, S.Si. from LAPAN, Indonesia for their contribution in this study.

REFERENCES

1. X. S. Liu, A. Shiomi, Y. Nakatsuka, R. Kubo, Nakamura, and A. Inaba, *J. Plant Physiol.* **121**, 1257–1265 (1999).
2. A.Inaba, X. Liu, N. Yokotani, M. Yamane, W. J.Lu, R. Nakano, and Y. Kubo, *J Exp. Bot.* **58,** 1047-1057 (2007).
3. J. D. Bierhals, F.M. Lajolo, B.R. Cordenunsi, and J.R.O.D. Nascimento, *J.Agric. Food Chem*. **52,** 7412 – 7418 (2004).
4. A. A. Kader, *Food Tech*. **34**, 51- 54 (1980).
5. L.W. Mapson and J. E. Robinson, *J. Food Technol.* **1**, 215 – 225 (1966).
6. A.K. Kanellis, T. Solomos, and A.K. Mattoo, *J. Plant Physiol.* **90,** 251-258 (1989).
7. R. Choehom, S. Ketsaa, and W.G. Doornb, *J. Postharvest Bio.and Tech*. **31**, 167 – 175 (2004).
8. T.B.T. Nguyen, S. Ketsa, and W.G. Doorn, *Postharvest Bio. Tech.* **31,** 313 – 317 (2004).
9. R. U. Handayani, "Studi pola ekspresi keluarga multigen *MaACS* dan gen *MaACO* pada tahap perkembangan vegetative dan proses pematangan buah pisang Ambon (*Musa sp*. AAA group) menggunakan metode REALTIME PCR (qPCR)", Magister Thesis, ITB, 2011.
10. J. Robertlee, "Profil ekspresi gen MaACS1, *MaACS2* dan *MaACO* selama proses pematanagn buah pisang Cavendish (*Musa acuminata* AAA. Group dalam Fruit Storage Chamber". Bachelor Report, ITB, 2012.
11. J. Zhuang, S. Jing, C. Weixin, *Mol. Plant Breeding* **4**, 143 – 146 (2006).
12. K.J. Livak and T.D. Schmittgen, *Methods* **25,** 402–408 (2001).