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

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

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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 O₂ concentration or elevating CO₂ 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 O₂ gas concentration composition [5-6]. The other methods were performed using ethylene absorbent, CO₂ 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 O₂ and CO₂ 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:O₂:CO₂ = 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°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
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% O₂ enzymes activities were suppressed, appearance of yellow color as well as sugar accumulation were also delayed [6]. Although low O₂ concentration can delay ripening process, O₂ below 2% will give anaerobic condition for fruits, while upper limit for CO₂ 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 (N₂:CO₂:O₂ = 90%:5%:5%).
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.

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