'Raja Bulu' banana MaACS1 and MaACO1 gene expression during postharvest storage

Article *in* Acta horticulturae · July 2016

DOI: 10.17660/ActaHortic.2016.1120.16

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'Raja Bulu' banana *MaACS1* and *MaACO1* gene expression during postharvest storage

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Abstract

Indonesia is one of world's biggest banana producers, however only a small portion of bananas are exported. Mechanical injuries during postharvest handling can increase respiration which leads to ethylene production. Ethylene accelerates fruit ripening process which leads to quality deterioration and waste of fruit. In order to maintain good quality environment condition, distribution process and storage condition need to be optimized. This study examined the effect of fungicide treatment and temperature during storage of banana ripening process, on the Indonesian native banana, 'Raja Bulu'. Expression of banana ripening-related genes, *MaACS1* and *MaACO1*, were analyzed. The results showed that the longest shelf-life was obtained on bananas stored at 20°C which were immersed in fungicide prior to storage. Quantitative PCR (qPCR) analysis showed that there was a difference in the expression of ripening-related genes between control group and treated banana (stored at best storage condition).

Keywords: *Musa paradisiaca*, quantitative PCR, ethylene, ripening, fungicide

INTRODUCTION

Banana is a climacteric fruit. Ripening of climacteric fruit is associated with increase of respiration rate and ethylene production. Two important enzymes, ACC synthase (which is encoded by ACS gene) and ACC oxidase (which is encoded by ACO gene) catalyse the conversion from S-adenosyl-L-methionine (*S*-AdoMet/SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) which becomes ethylene (Yang and Hoffman, 1984).

'Raja Bulu' (*Musa paradisiaca* L. 'Raja Bulu') is an indigenous banana to Indonesia and is usually consumed as a dessert or as a raw material for baking. Large volumes of 'Raja Bulu' are produced by small farmers in Indonesia's rural areas where there are not enough storage facilities to keep the fruit in good condition. Our previous study examined the simple cool room storage chamber and natural fungicide to delay fruit ripening. In this study, ethylene biosynthesis gene expression were examined in control fruit (stored at room temperature and treated or not with fungicide) and treated fruit (stored at 20°C and treated or not with fungicide). Analysis of *MaACS1* and *MaACO1* gene expression were performed by qPCR.

MATERIALS AND METHODS

Fruit treatment

Fruit was stored at 27°C (room temperature) and treated or not with fungicide as control. This study compared the control fruit with fruit which had treated or not with fungicide at 20°C. Fungicide made from natural ingredients was supplied by local Fruit Supplier (PT. SRA, Bandung, Indonesia). Physical assessment and RNA isolation were conducted every two days in fourteen days after harvesting. Physical assessment such as peel color and pulp to peel weigh ratio were conducted according to banana routine post-harvest screening method (Dadzie and Orchad, 1997).

RNA isolation and qPCR analysis

RNA was isolated using method described by Zhuang et al. (2006). RNA was then

converted into cDNA. Total cDNA was synthesized using iScript cDNA Synthesis Kit from BioRad and used as material for qPCR analysis. The MaACS1, MaACO1 specific primers and housekeeping gene were used in analysis (Karmawan et al., 2009; Dwivany et al., 2014; Handayani and Dwivany, 2014). Analysis of qPCR data was performed using relative quantification.

RESULTS AND DISCUSSION

Based on physical assessment analysis, fruit which were stored in control conditions tended to have a shorter shelf life than fruit stored at 20°C room and treated or not with fungicide (Figure 1). Fruit which were stored in the control conditions lasted for eight days whereas fruit stored at 20°C and treated or not with fungicide lasted for 14 days. Physical assessment showed that peel color was gradually changed from green to yellow with brown spots in ripening banana. In the control treatment, peel turned into yellow at seven days whereas those treated or not with fungicide at 20°C turned to yellow after eight days. There were no significant differences between bananas that treated or not with fungicide. Bananas which were stored at 20°C and treated or not with fungicide had lower sucrose contents compared to those in the control. The ratio of pulp to peel was also measured and showed that treatment at 20°C with or without fungicide had lower ratio (data not shown; Hermawaty, 2014).

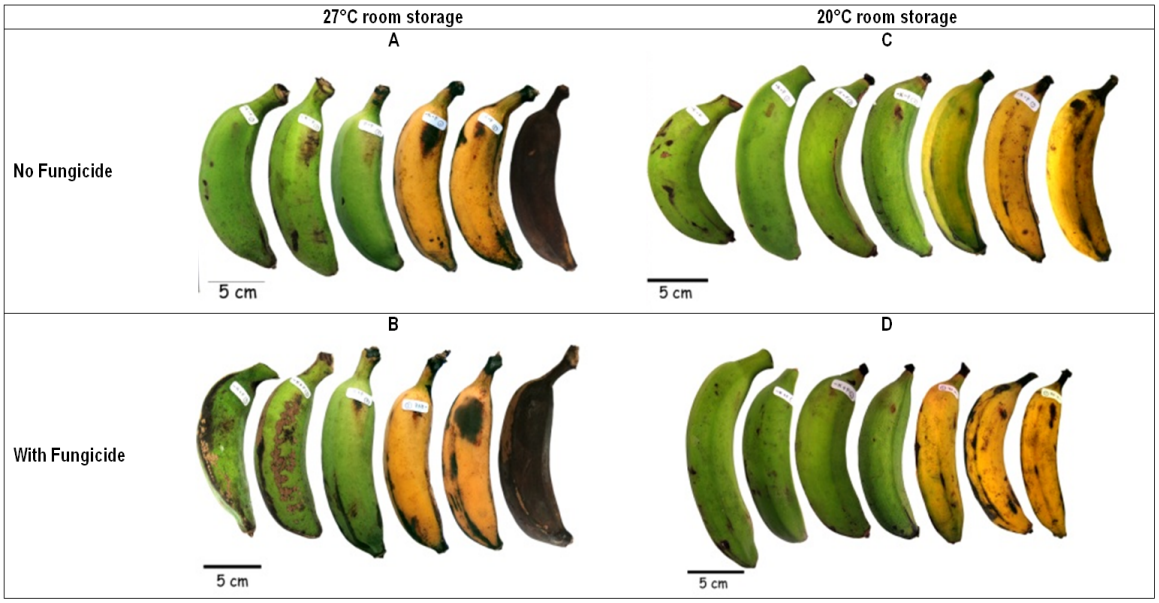

Figure 1. Physical assessment of banana ripening process on day 0, 1, 5, 7, 8, 11, and 13 respectively. A-B. Control samples, C-D. Treated samples.

Banana samples that were stored at 27°C with no fungicide treatment were considered as controls and those stored fruit stored at 20°C with fungicide were considered as the treated samples in the gene expression analysis. Both MaACS1 and MaACO1 gene expression profiles were analysed using qPCR method (Figure 2). MaACS1 gene expression in the control sample increased at day 5 while in treated sample it started to increase at day 7. Gene expression level was also lower in treated sample compare to that in the control fruit. On the other hand, *MaACO1* gene expression of treated sample slightly increased at day 5 and decreased at day 7, while expression level during ripening in the control sample was almost similar from day 1 to 7.

In this study, the lower temperature (20°C) and fungicide treatment resulted in 'Raja Bulu' having longer shelf life. Fungicide treatment suppressed fungal growth in the treated samples (data not shown; Ardea, 2013). However, the shelf life of fruit was almost similar at both control and 20°C temperature with or without fungicide treatment. This indicates that

the temperature affected fruit shelf life more than fungicide. MaACS1 gene expression was also affected, as shown in decreasing of its expression to 2-10 fold and changed its expression profile.

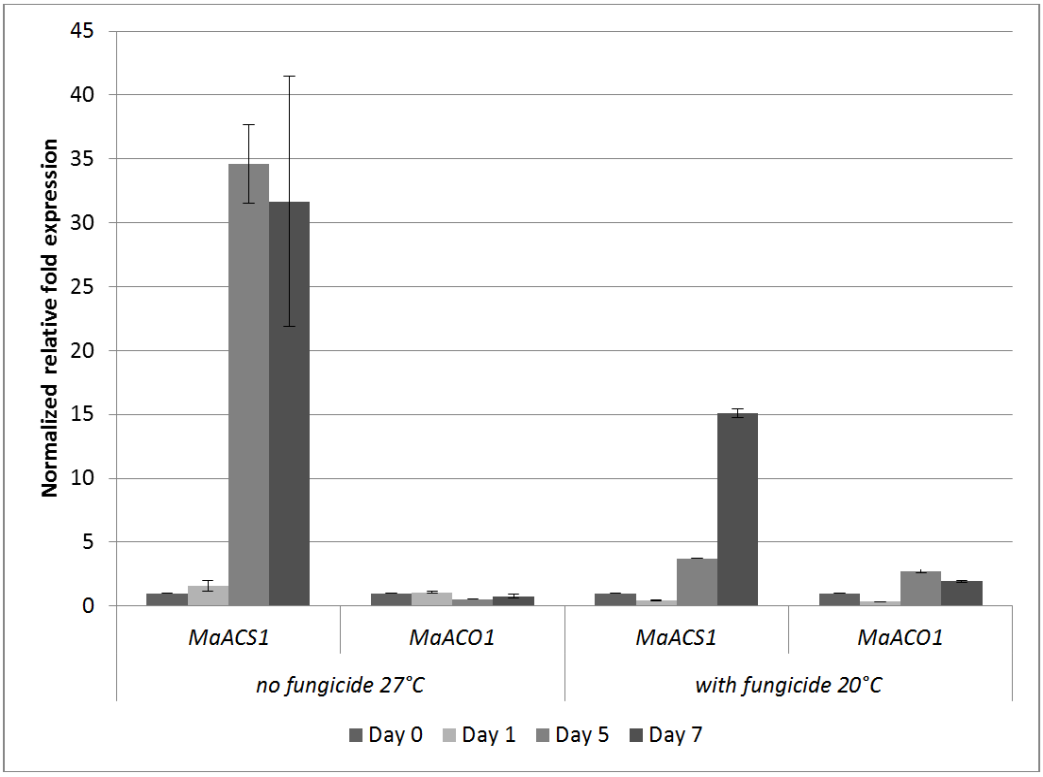

Figure 2. Relative quantification analysis of *MaACS1* and *MaACO1* gene expression from banana pulp during ripening process in two storage condition: with no fungicide at 27°C (as control sample) and with fungicide at 20°C (as treated sample).

Several studies have reported that under normal storage conditions, *MaACS1* gene expression level increases significantly at the onset of ripening and followed by decrease of gene expression (Liu et al., 1999; Inaba et al., 2007; Karmawan et al., 2009). In this study, its expression profile changed with temperature treatment. At room temperature, the peak of expression was at day 5, whereas treatment at 20°C made expression of *MaACS1* increased until day 7. Since *MaACS1* is a member of gene family that encodes an important enzyme in ethylene biosynthesis, its lower expression may have influence on ACC production and result in lower ethylene production. Although *MaACO1* was slightly increased at 20°C, it appeared that this higher expression did not accelerate ripening process. A previous study from our group on the effects of modified atmosphere (CO_2 and O_2) on *MaACS1* and *MaACO1* gene expression showed that modified atmosphere resulted in longer banana fruit shelf life and affected both *MaACS1* and *MaACO1* gene expression profiles (Robertlee, 2012). Other study also showed changes in *MaACS1* and *MaACO1* gene expression level due to various storage conditions, such as the use of nitric oxide (Cheng et al., 2009).

CONCLUSIONS

Treatment of 'Raja Bulu' at 20°C and fungicide gave longer shelf life and affected both *MaACS1* and *MaACO1* gene expression. Lower expression of *MaACS1* of treated sample might influence ethylene production and resulted in longer shelf life. However, further study to measure enzyme activity that involve in ethylene production, such as ACS and ACO, and measurement of production of ethylene will be very useful.

ACKNOWLEDGEMENTS

This study was partly founded by ITB Innovation Research Grant and MP3EI Grant from Indonesian Higher Education. Authors would like to thank the banana group-ITB for research collaboration during this study.

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