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Abstract. The objective of the research was to determine the effect of microgravity simulation by 3D clinostat on Cavendish banana (*Musa acuminata* AAA group) ripening process. In this study, physical, physiological changes as well as genes expression were analysed. The result showed that in microgravity simulation condition ripening process in banana was delayed and the *MaACO1*, *MaACS1* and *MaACS5* gene expression were affected.

1. Introduction

According to several researches, microgravity conditions may lead to the limitation of air convection, resulted in a change in the composition of O_2 and CO_2 in the surface around the plant [1,2]. The condition can lead to a stagnant air layer that will thicken around plant surface, meanwhile the air from outside was difficult to diffuse, causing unbalanced in composition of CO_2 and O_2. CO_2 will increase as a result of respiration, while O_2 will decrease due to the limitations of air convection [2]. Changes in the composition of the air can change some metabolic processes in plants, for example ethylene biosynthesis.

Ethylene is a gaseous plant hormone that is naturally produced. One function of ethylene is a signal in the process of fruit ripening. In climacteric fruit such as banana, ethylene production will increase along with the increase of the rate of respiration. Increased ethylene does not occur in non-climacteric fruit. Along with increase of ethylene, the fruit will undergo maturity, changes in physiology, biochemistry, molecular and organoleptic characters [3].

Ethylene is synthesized from methionine and the reaction is catalyzed by two enzymes, ACC synthase and ACC oxidase [4]. Methionin is changed into S-adenosyl-methionin (SAM), and ACC synthase catalyze reaction of SAM into ACC (1-Aminocyclopropane-1-carboxylic acid). Finally, ACC oxydase catalyze ACC conversion into ethylene and this step really depends on O_2 and accelerated by fruit ripening [5]. It has been known that ACC synthase is encoded by multigene families ACS, whereas ACC oxidase is encoded by multigene families ACO. According to previous research, *MaACO1*, *MaACS1*, *MaACS5* were expressed higher in ripening bananas [6,7,8].

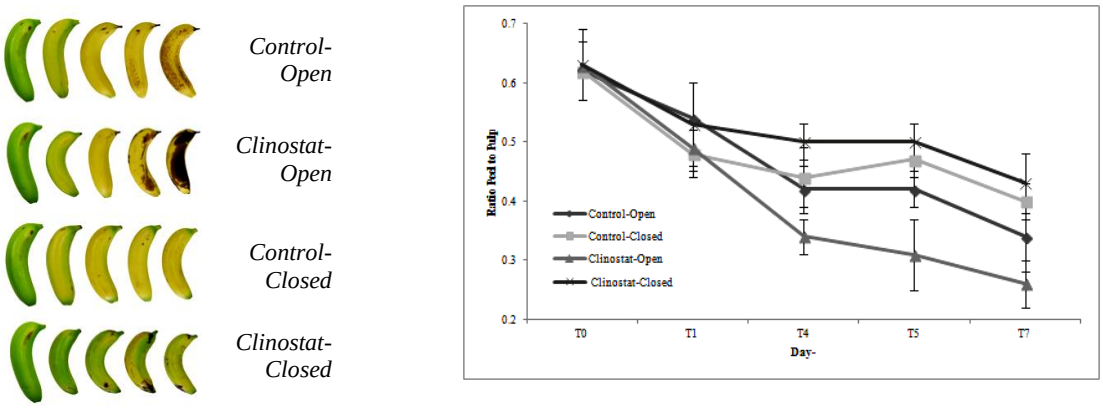
In this study, the effect of microgravity simulation performed by 3D clinostat and closed chamber condition on banana fruit ripening process were analysed based on physical, physiological and gene expression study. As control, banana was placed at open air condition as well closed chamber on ground and clinostat open condition. Until recently, report related to effect of microgravity on fruit ripening process was limited. Understanding the effect of space condition on plant response to various signals from the environment can also materialize to the idea of establishing a greenhouse/laboratory to minimize effect of microgravity for further space farming. In addition, understanding of fruit maturation and decay from this experiment can also be applied to design a better postharvest technology.

2. Methods

Mature green Cavendish Bananas, (*Musa acuminata* AAA Group), were used as samples after 24 hours treatment by ethylene. Banana samples were wrapped in paper tissues containing active charcoal to avoid physical injury and to absorb water from respiration process. Three replicates of samples were then placed into four groups. Experiment was conducted using 3D clinostat [9] over seven days then samples were harvested at day 0, 1, 4, 5 and 7 and stored at -80°C for further molecular analysis. Physical and physiological analyses were performed to observe changes in peel colour and to measure peel to pulp ratio. In addition, gene expression analysis was also performed, a total of RNA was isolated from 1 gram sample [10], then qPCR was performed [8,11]. The qPCR result was analysed using relative analysis method with equations, $\Delta\Delta Ct = (Ct_{\text{target gene}} - Ct_{GAPDH})T_x - (Ct_{\text{gene target}} - Ct_{GAPDH})\,T_0$, where T was the treatment time [12].

3. Results

Effect of 3D clinorotation on fruit ripening was analysed using physical and physiological approaches. Peel colour changed gradually from green to yellow during seven days of fruit ripening process. The peel colour at closed chamber on clinostat changed slightly and took longer time compared to control and other treatments (figure 1(a)). Mass ratio of peel to pulp fruit ripening bananas was also measured, ripening process at closed chamber on clinostat treatment was slowest and resulted highest ratio of peel to pulp (figure 1(b)).



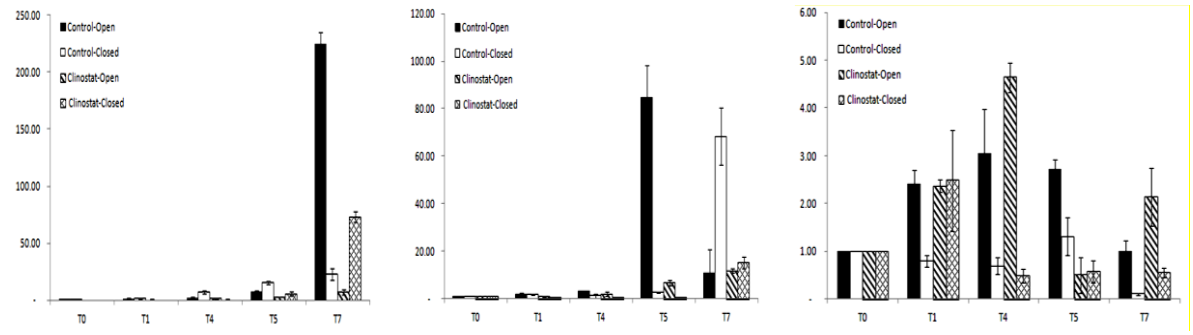
(a) (b)

Figure 1. (a) Peel colour changes, (b). Mass ratio of peel to pulp

In addition to physical and physiological analyses, analysis at molecular level was also performed using gene expression analysis. Total RNA was isolated from all banana samples. The expression of fruit ripening-related (*MaACS1*, *MaACS5* and *MaACO1*) genes expressions were characterized by qPCR method using gene specific primers. The result of *MaACS1* expression analysis during fruit ripening showed that gene expression increased in all conditions from day 0 until day 7 (2A). Lower gene expression of *MaACS1* was shown in all treated samples compared to control. On the other hand, the

expression of *MaACS5* in control condition increased from day 0 and reached the highest at day 5, whereas at other treatment still increasing after day 5 (figure 2(b)).

The expression of *MaACO1* gene during fruit ripening was shown in figure 2(c). The highest gene expression at closed chamber on clinostat was at day 1, then it decreased whereas in other treatments reached the highest expression later at day 4 then decreased. However, gene expression at open chamber on clinostat increased again at day 7.



(a) (b) (c)

Figure 2. Relative quantification analysis of gene expressions from banana pulp during ripening process in four storage conditions: control open, clinostat open, control closed, and clinostat close chamber. (a). *MaACS1*, (b). *MaACS5* and, (c). *MaACO1*.

4. Discussion

According to the results, longest self-life was obtained when banana was stored at microgravity simulation condition (closed chamber on clinostat), followed by closed chamber and open chamber on clinostat, and last was control.

In this study, changes in peel colour were slowest in banana placed at closed chamber on clinostat compared to other treatment. This phenomenon was confirmed by the pattern of chlorophyll content at the same treatment which was also the lowest (data not shown). The result might be related to the limited oxygen content around the fruit. Peel colour of banana commonly changed gradually from green into yellow during ripening process, which was caused by chlorophyll degradation [13].

Mass ratio of fruit peel to pulp is an indicator of bananas fruit ripening [14]. The mass ratios of peel to pulp for all conditions in this study were decrease during ripening process. However, closed chamber on clinostat treatment showed the highest ratio and slowest ripening process. The decrease in the mass ratio of peel to pulp might be associated with changes in water levels on both tissues.

Molecular analysis showed similar patterns of *MaACS1* gene expression found in all conditions. *MaACS1* showed an increase in gene expression but the averages of gene expression were lower in other conditions compared to control. On the other hand, *MaACS5* gene expression patterns in treated conditions were different to control. In control condition, the gene expression increased and reached the peak at day 5 then decreased, whereas gene expression at treated conditions has not reached the peak and increased continually after day 5. Further study to obtain the peak of *MaACS5* expression in treated conditions was needed. The gene expression pattern of *MaACO1* showed that the peak of gene expression at control was attained earlier compared to other condition.

This study showed that *MaACS1*, *MaACS5* and *MaACO1* gene expression pattern were affected by different chamber condition during fruit ripening. Analysis on *MaACS1* showed that lower gene expression resulted in slower ripening process compared to control. Delayed activity of *MaACS5* gene expression might correlate with slower ripening process at treated condition compared to control. Interestingly, longest shelf-life of bananas stored at closed chamber on clinostat condition might correlate with *MaACO1* gene expression. In this condition, the gene expression of *MaACO* decreased drastically after day one, while in other conditions increased continually until day 4. Since these genes belong to gene families which encode ACC synthase and ACC oxidase, their activity all together might

affect fruit ripening. It has been known that the decrease of O_2 concentration in environment result in inhibition of ACC oxidase activity [5].

Our previous study showed that banana fruit ripening in bamboo closed chamber was also delayed compared to control [15]. It was reported that banana stored at closed chamber filled with 5% carbon dioxide showed lower *MaACS5* and *MaACS6* gene expressions [8]. Several other studies also reported a correlation between delay in banana ripening and high carbon dioxide composition around the fruit [16,17,18]. It has been known that ethylene synthesis from ACC is induced by the presence of oxygen and is inhibited at high concentrations of carbon dioxide [5].

5. Conclusion

Simulated microgravity conditions using closed chamber on clinostat condition resulted in delaying of banana fruit ripening. In this study, we also found that *MaACS1*, *MaACS5* and *MaACO1* gene expression were affected when banana was placed in such condition.

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References

[1] Musgrave ME, Kuang A and Matthews SW 1997 *Planta* **203** 1-19
[2] Monje O, Stutte GW Goins GD and Porterfield GE 2003 *Adv. Space Res.* **31(1)** 151-67
[3] Prassana V, Prabha TN, and Tharanathan 2007 *Critic. Rev. in Food Sci. and Nut.* **47** 1-19
[4] Yang SF and Hoffman NE 1984 *Ann. Rev. of Plant Physiol.* **35** 155-89
[5] Taiz L and Zeiger E 2002 *Plant Physiology* (Los Angeles: Sinauer Associates, Inc.)
[6] Liu X, Shiomi A, Nakatsuka Y, Kubo R, Nakamura and Inaba A 1999 *Plant Physiol.* **121** 1257-65
[7] Choudhury SR, Roy S, Saha PP, Singh SK, and Sengupta DN 2008 *Plant Cell Rep.* **27** 1235-49
[8] Dwivany F, Esyanti RR, Robertlee J, Paramaputra IC, Permatadewi RK, Tambun DH, Handayani RU, Pratiwi AS and Zaskia H 2014 *AIP Conference Proceedings **1589***, 285 doi:10.1063/1.4868801
[9] Swandjaja L, Esyanti RR, Khairurrijal, Dwivany FM, and Latief C 2014 *Trans. JSASS Aerospace Tech. Japan.* **12** ists 29 Th 5-10
[10] Zhuang J, Su J, and Chen W 2006 *Mol. Plant Breeding* **4(1)** 143-6
[11] Handayani RU and Dwivany F 2014 *J. Math Fund. Sci.* **46(2)** 194-3
[12] Livak KJ, and Schmittgen TD 2001 *J. Methods* **25** 402-8
[13] Yang X, Pang X, Xu L, Fang R, Huang X, Guan P, Lu W and Zhang Z 2009 *J. Experimental Bot.***60(14)** 4051-62
[14] Dadzie BK and Orchard JE 1997 *Routine postharvest screening of banana/plantain hybrids criteria and methods* International network for banana and plantain (Inibap) technical guidelines
[15] Pratiwi A, Dwivany F, Larasati D, Islamia HC and Martien R 2015 *AIP Conference Proceedings **1677***, 100005 doi:10.1063/1.4930763
[16] Mapson LW and Robinson JE 1966 *Food Tech.* **1** 215-25
[17] Kanellis AK, Solomos T, and Mattoo AK 1989 *Plant Phy.* **90** 251-25
[18] Kittur FS, Saroja N, Habibunnisa, and Tharanathan RN 2001 *Eur. Food Res. Tech.* **213** 306-11