ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/282441125

Optimatization of transient transformation methods to study gene expression in Musa acuminata (AAA group) cultivar Ambon...

Article · January 2014

DOI: 10.1063/1.4930752

CITATIONS
0

READS
130

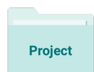
1 author:

Fenny MARTHA Dwivany
Institut Teknologi Bandung-Indonesia, Bali International Research Center for Banana (BIRCB)

33 PUBLICATIONS **33** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



1) fruit ripening process View project

All content following this page was uploaded by Fenny MARTHA Dwivany on 03 October 2015.

The user has requested enhancement of the downloaded file.

Optimatization of transient transformation methods to study gene expression in Musa acuminata (AAA group) cultivar Ambon Lumut

Kinasih Prayuni and Fenny M. Dwivany

Citation: AIP Conference Proceedings **1677**, 090007 (2015); doi: 10.1063/1.4930752
View online: http://dx.doi.org/10.1063/1.4930752
View Table of Contents: http://scitation.aip.org/content/aip/proceeding/aipcp/1677?ver=pdfcov
Published by the AIP Publishing

Articles you may be interested in

Cell growth, gene expression, and their interdependence
Phys. Today **64**, 21 (2011); 10.1063/1.3541936

The noisy expression of genes into proteins
Phys. Today **63**, 18 (2010); 10.1063/1.4796345

A microfluidic DNA computing processor for gene expression analysis and gene drug synthesis
Biomicrofluidics **3**, 044105 (2009); 10.1063/1.3259628

Growing Gene Network by Integration of Gene Expression, Motif Sequence and Metabolic Information
AIP Conf. Proc. **952**, 279 (2007); 10.1063/1.2816632

Stochastic regulation of gene expression
AIP Conf. Proc. **502**, 191 (2000); 10.1063/1.1302384

Optimatization of Transient Transformation Methods to Study Gene Expression in *Musa acuminata* (AAA group) Cultivar Ambon Lumut

Kinasih Prayuni[1, a)] and Fenny M. Dwivany[1, b)]

[1]*School of Life Sciences and Technology – Institute of Technology Bandung*
Jl. Ganesha 10 Bandung 40132
Indonesia

[a)]Corresponding author: prayunikinasih@gmail.com
[b)]fennymd@yahoo.com

Abstract. Banana is classified as a climateric fruit, whose ripening is regulated by ethylene. Ethylene is synthesized from ACC (1- aminocyclopropane-1-carboxylic acid) by ACC oxidase enzyme which is encoded by *ACO* gene. Controling an important gene expression in ethylene biosynthesis pathway has became a target to delay the ripening process. Therefore in the previous study we have designed a MaACO-RNAi construct to control *MaACO* gene expression. In this research, we study the effectiveness of different transient transformation methods to deliver the construct. Direct injection, with or no vaccum infiltration methods were used to deliver MaACO-RNAi construct. All of the methods succesfully deliver the construct into banana fruits based on RT-PCR result.

Keywords: pisang Ambon Lumut, *MaACO* gene, transient transformation

INTRODUCTION

Banana (Musa sp.) is a prominent crop in the world and has an important role in the economic sector of developing countries - not only as a staple food but also as an export commodity. Based on world production gross value, banana is fourth in the ranking for primary food after rice, wheat, and corn [1]. In 2012, based on datas from Statistic Indonesia, banana ranks as the largest amount of fruit produced in Indonesia. This placed Indonesia as the sixth largest banana-producing country in the world, although not the largest exporter [6, 14]. One of the factor of banana's high productivity rate is the fruit's physiology - which is classified to climateric fruit because its ripening process is influenced by ethylene. Ethylene will accelerate climateric fruit ripening so that it will ripen in a relatively short time [3]. This regulation causes a disadvantage in the fruit's distribution process, where any delay could damage the fruit up to the point where it's not fit for consumption [2].

Many efforts has been made to overcome the distribution problem, mostly having to do with a way to control fruit ripening, such as distribution with refrigerated container, insulated packaging, and combination of atmosphere modification and cold temperature (usually 10% CO_2 , 1-2% O_2, 14°C). But the results are still deemed in adequate, as it only lengthen the distribution time to a maximum of 14 days. However, mentioned methods are also expensive and space consuming [2]. Alternative methods would have to be made to overcome these problems. One of them is genetic manipulation to delay food ripening by controlling the pathways for ripening procces, specifically by manipulating the ethylene. *1-aminocyclopropane-1-carboxylic-acid* (ACC) oxidase (ACO) is the genes which encodes ethylene biosynthesis. Our previous study has produced ACO-RNAi construct to control *MaACO* gene expression (unpublished data).

In this research we are trying to develop different transient transformation from normal methods such as *Agrobacterium* mediated transformation and physical transformation such as particle bombartment [10, 13]. We used MaACO-RNAi construct with constitutive promoter, CaMV 35S and inducible promoter, MaACO promoter with direct injection, with or no vaccum infiltration methods.

The 5th International Conference on Mathematics and Natural Sciences
AIP Conf. Proc. 1677, 090007-1–090007-4; doi: 10.1063/1.4930752
© 2015 AIP Publishing LLC 978-0-7354-1324-5/$30.00

MATERIALS AND METHODS

Materials

We used 'Ambon Lumut' banana fruit as plant material and the MaACO-RNAi construct from previous research which have constitutive promoter, CaMV 35S and MaACO promoter.

Methods

Transfer of MaACO-RNAi Construct into Ambon Lumut Banana Sample

1) Injection method
The Ambon Lumut banana used in this research were taken from the same bunch. The bananas were treated with: injection of 1 mL of 10 µg plasmid solution (the MaACO-RNAi construct +35S promoter, the MaACO-RNAi construct + MaACO promoter).

2) Vacuum Method
Fruit from the same bunch were sliced into three 2-3 cm thick pieces from the middle part of fruit and then peel was removed. The pieces were then submerged in 25 mL solutions of TE+plasmid containing 10 µg plasmid MaACO- RNAi plasmid construct inside a petri dish. Submerged banana samples were vacuumed for 10 minutes while the control ones were left with no vaccuum. Sample were then collected on days 0, 1, and 3 for RNA isolation.

Semi-quantitative Analysis of *MaACO* Gene Expression

The analysis *MaACO* gene expression was performed by RT-PCR. RNA isolation was done with the Zhuang *et al.* (2006) method that has been modified (Robertlee, 2012). The cDNA synthesis was conducted using iScript cDNA synthesis KIT (BioRad) after RNA samples were treated with DNAse (DNAseI kit, Thermo Scientific). The interest fragment was amplified using specific *MaACO* and *MaGAPDH* primers using Handayani protocol (2010). Analysis of the electropherogram was conducted using ImageJ software.

RESULT AND DISCUSSION

Three methods of gene transfer using injection, vacuum, and without vacuum have been conducted in banana fruit. The RT-PCR analysis was conducted to measure effectiveness of different transient transformation methods to deliver the construct. The result from all treatment showed that *MaACO* gene expression was lower in all treated samples compared to control with different level of expression (Fig. 1). There was a decrease in *MaACO* gene expression on first day compare to control but increases on day 3 possessed similar amount of expression levels with control. This phenomenon was most likely caused by the disappearance of the silencing effect, which in turn was caused by the effect of transient expression.These findings are in line with Yan *et al.* (2012), who claimed that transient expressions of transgenes will reach its highest level on day 2-3 and will decrease afterwards.

Sharp increases found in sample treated with vaccum on day 3. This phenomenon is most likely caused by the presence of stress effect (wounding) from the vacuum treatment itself. Despite happening in only small numbers, the repression of gene expression happened due to the overabundance of *MaACO* expression caused by the induction of the wounding. The wounding made it so that when the effect of silencing is gone, the expression rate increased sharply. Wounding also has the role as one of the factor to induct genes related to ripening, including the *MaACO* gene. This is also bolstered by the fact that the ACO's promotor possesses a W box motif that is activated when it's inducted by wounding [4, 10].

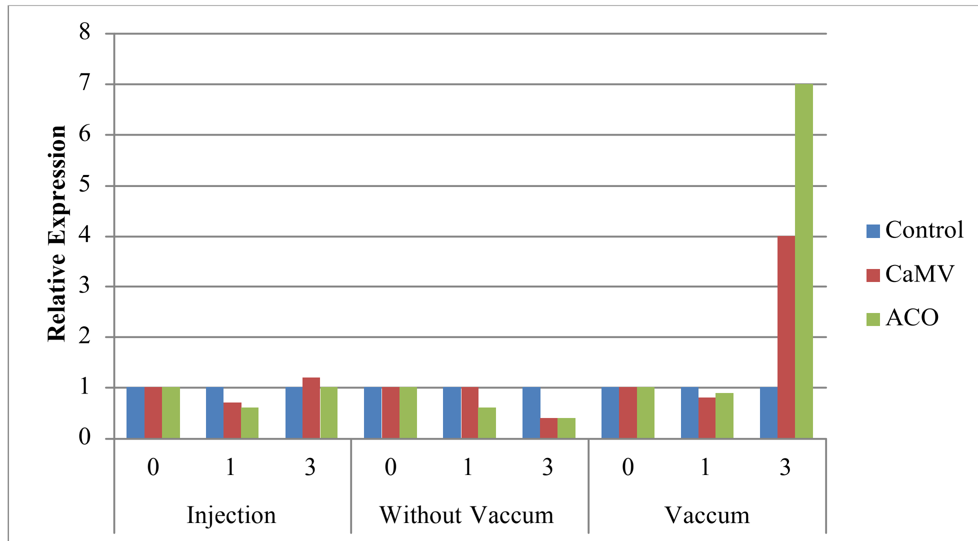


Figure 1. Semiquantitative *MaACO* gene expression graph. 0: day 0; 1: day 1; 3 day .
Control: treatment with TE buffer; CaMV: Treatment with MaACO-RNAi construct + CaMV 35S promoter;
ACO: Treatment with MaACO-RNAi construct + MaACO promoter.

Based on the collected data, we found that MaACO-RNAi constructs are effectively working in injection treatments, although during a short time period. It seemed that the dossage was influence in this treatment, also it is to be noted that the usage of this method did not utilize samples from the same individual banana to be collected on day 0, 1, and 3. Therefore, the decrease of gene expressions could only be compared to the control samples, which RNA was taken at the same day. Injection method has been applied in *Caenorhabditis elegans* [7], Shrimp [9], and *Drosophila* [5], but limited in plant. Through this research we want to showed that it can be promising new method which is cheap and easy for alternative RNAi delivery in plant.

The construct was aimed to silence mRNA *MaACO* since it will form hairpin RNA when transcripted. The hairpin structure of RNA is predicted to have efficient silencing mechanism in plants. This RNA consists of an inverted repeat of a gene sequence fragment and is separated by a spacer to increase the effect of silencing. The effectiveness will increase to 100 % when added introns as connecting sense and antisense strand, called ihpRNA (intron - hairpin RNA) [13,16]. The intron sequence in ihpRNA construct will drive the sense and antisense arm to be self-complementary more easily, also, the loop region of intron will stabilizes the hairpin structure [16]. It made this as a highly efficient construct if compared to other construct such as antisense RNA or cosuppression. However, further study using quantitative PCR (qPCR) is needed. Real Time PCR is capable of detecting the expression of the *MaACO* gene more accurately because it could detect amplicons during the PCR reactions. The resulting data would be measured in the exponential phase of the PCR reaction.

ACKNOWLEDGMENTS

This research is supported by Banana Group, ITB and Lembaga Pengelolaan Dana Pendidikan (LPDP) for Kinasih Prayuni.

REFERENCES

1. Arias, P., C. Dankers, P. Liu, and P. Pilkauskas (ed), Rome, Food and Agriculture Organization of the United Nations, Page 1, 4 (2003).
2. Badan Penelitian dan Pengembangan pertanian (Litbang Deptan). Prospek dan pengembangan arah agribisnis: Dukungan aspek teknologi pasca panen. Departemen Pertanian Jakarta. (2007).
3. Barry, C. S. and J. J. Giovannoni. Ethylene and Fruit Ripening. *Journal of Plant Growth Regulation,* **26**:143-159 (2007).
4. Choudhurya, S. R., S. Royb, and D. N. Senguptaa. 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. (2008).
5. Dzitoyeva, S., N. Dimitrijevic and H. Manev. Intra-abdominal injection of double-stranded RNA into anesthetized adult *Drosophila* triggers RNA interference in the central nervous system. Molecular Psychiatry,**6(6)**, 665-670. (2001).
6. FAOSTAT. Production and Trade 2012. http://faostat.fao.org/.
7. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, and Mello CC: Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature, **391**, 806-811 (1998).
8. Handayani, R.U. Studi Pola Ekspresi Keluarga Multigen MaACS dan gen MaACO pada Tahap Perkembangan Vegetatif dan Proses Pematangan Buah Pisang Ambon (Musa sp. AAA group) Menggunakan Metode Real- Tima PCR (qPCR). Tesis Program Magister Bioteknologi, Institut Teknologi Bandung (2012).
9. Loy, J.D., M. A. Mogler, D. S. Loy, B. Janke, K. Kamrud, E. D. Scura, D. L. H. Harris and L. C. Litopenaeus vannamei. Journal of General Virology, **93**, 880–888 (2012).
10. Moreno, A.B., Peñas, G., Rufat, M., Bravo, J.M., Estopà, M., Messeguer, J., dan San Segundo, B. Pathogen- Induced Production of the Antifungal AFP Protein from *Aspergillus giganteus* Confers Resistance to the Blast Fungus *Magnaporthe grisea* in Transgenic Rice. *Molecular Plant Microbe Interaction Journal*, **18 (9)**, 960–972 (2005).
11. Potrykus, I. Annual Reviews. *Plant Physiology Plant Molecular Biology*, **42**, 205-225 (1991).
12. Robertlee, J. Profil Ekspresi Gen MaACS1 , MaACS2 , dan MaACO1 selama Proses Pematangan Buah Pisang Cavendish (Musa acuminata AAA . Group) dalam Fruit Storage Chamber. Skripsi Program Sarjana Biologi, Institut Teknologi Bandung (2012).
13. Singh, S., A. Green, P. Stoutjesdijk and Q. Liu. ”Inverted-Rrepeat DNA: A New Gene-Silencing Tool for Seed Lipid Modification” 14th International Symposium on Plant Lipids. Cardiff University (23–28 July 2000).
14. Statistic Indonesia (BPS). Production of Fruits 2012. http://www.bps.go.id.
15. Watson, J. M., A. F. Fusaro, Ming Bo Wang, P. M. Waterhouse. RNA silencing platforms in plants. *Federation of European Biochemical Societies (FEBS) Letters*, 1-6 (2005)
16. Wesley, S. V., C.A. Helliwell1, N.A. Smith, M. Wang, D.T. Rouse, Q. Liu,P.S. Gooding, S. P. Singh, D. Abbott, P. A. Stoutjesdijk, S. P. Robinson, A. P. Gleave, Allan G. Green dan Peter M. Waterhouse. Construct design for efficient, effective and high throughput gene silencing in plants. *The Plant Journal.* **27(6)**, 581-590 (2001).
17. Yan, P., W. Yen., XinZheng Gao, Xiaoying Li, Peng Zhou, & Jun Duan. High-Throughput Construction of Intron-Containing Hairpin RNA Vectors for RNAi in Plants. *Plos One*, **7**: 1- 8 (2012).
18. Zhuang J., Su J., & Chen W. An effective method for High-quality RNA Isolation from Banana Fruit. *Molecular Plant Breeding*, **4(1)**, 143 – 146 (2006).

View publication stats