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Original research article

Expression Study of Banana Pathogenic Resistance Genes

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ABSTRACT

Banana is one of the world's most important trade commodities. However, infection of banana pathogenic fungi (*Fusarium oxysporum* race 4) is one of the major causes of decreasing production in Indonesia. Genetic engineering has become an alternative way to control this problem by isolating genes that involved in plant defense mechanism against pathogens. Two of the important genes are *API5* and *ChiI1*, each gene encodes apoptosis inhibitory protein and chitinase enzymes. The purpose of this study was to study the expression of *API5* and *ChiI1* genes as candidate pathogenic resistance genes. The amplified fragments were then cloned, sequenced, and confirmed with in silico studies. Based on sequence analysis, it is showed that partial *API5* gene has putative transactivation domain and *ChiI1* has 9 chitinase family GH19 protein motifs. Data obtained from this study will contribute in banana genetic improvement.

1. Introduction

Indonesia as one of the mega biodiversity country has been known as a fruit producer for local or global consumption. Indonesia is one of the main banana producer countries in the world. However, the export of fresh fruit was only about 10% of total local production (Purwadaria 2006). This is because of several problems that occur at preharvest and postharvest stages. One of the preharvest problems is susceptibility to pathogen attack, such as bacterial, viral, and fungal infections. One of major issues in banana disease is banana wilt caused by pathogenic fungi, *Fusarium oxysporum* f.sp. *cubense* (*Foc*) (Dimyati *et al.* 2001). As soilborne fungi, the spore of *Fusarium* stays on the soil and is hard to eradicate (Walduck and Daly 2006). Therefore, this disease has led to a loss harvest of 20.000 tons in Lampung province in 1993–1994 (Dimyati *et al.* 2001).

Foc as soilborne fungi infects plants through the roots and then colonizes the vascular tissues in the rhizome and pseudostem and finally causing plant wilt that can cause plant death (Beckman 1987, 1990). Wilting symptoms on infected plants usually occur in the same time with necrosis and rotting in the root, rhizome, and the vessels of pseudostem (Pérez-vicente 2004). Banana disease treatment so far still uses conventional methods such as conventional breeding, pesticide usage, optimization of bananas planting technical process, and utilization of biocontrol agents. However, most of the commercial banana possessing triploids (AAA) are sterile, thus hybridization is less effective (Heslop-Harrison 2011). Therefore, genetic engineering has become the most promising method to be an alternative solution to solve these diseases (Gurr and Rushton 2005). Transgenic methods in developing crops such as tobacco, potato, and cucumber have chosen to express pathogen resistance-related genes (Reimann-Philipp and Beachy 1993).

Previous studies have shown that transgene could result in *Foc* resistance in banana plants. For instance, antiapoptosis genes that are isolated from animal cells can be used as resistance gene to *Foc* infection in tobacco plants, tomatoes, and bananas (Li *et al.* 2010; Paul *et al.* 2011). As an alternative of antiapoptosis genes from animal cells, we found that plant antiapoptosis inhibitor 5 (*API5*) genes were also available at the genome database of *Arabidopsis thaliana*. In addition, class I chitinase gene (*ChiI1*) from *Musa* AB group (FJ858155.2) was also available at GenBank. This gene encodes chitinase, which is an enzyme that has a role in degrading chitin component on fungi cell wall. Therefore, this research was focused to study *A. thaliana API5* and local banana (pisang ambon lumut, *Musa acuminata* AAA group) *ChiI1 gene* expressions before isolation of full-length genes. The results are expected to be a reference for conducting overexpression of both genes to produce

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transgenic banana plants that are resistant to *Foc* attack. The resistance of bananas to *Fusarium* pathogens is expected to improve the quality and quantity of banana production in Indonesia. This can provide benefits for banana farmers in Indonesia, increasing export and support agroindustry by reducing production losses caused by pathogen attack.

2. Materials and Methods

2.1. Gene expression study

Gene expression study was done by reverse transcription polymerase chain reaction to amplify *ChiI1* from pisang ambon lumut *(M. acuminata* AAA group*)* leaf complementary DNA (cDNA) using the method of Handayani and Dwivany (2014) and Dwivany *et al.* (2014). Primers were designed based on sequences of class I chitinase gene (*ChiI1*) *Musa* AB group (FJ858155.2). The primers were 5′(GACTGCTAAGGAGGATGAAG)3' as forward primer and 5′(GGCCATGTACTAGTTCAGC)3′ as reverse primer. Meanwhile, to study expression of *API5*, root cDNA of *A. thaliana* (12 leaves stage plants) was used as template. Primers were designed based on sequences of *A. thaliana API5* (NM_128955.4). The primers were 5′(AATTTCATCAGAGATAAGGTGA)3' as forward primer and 5′(ACTTGTTATTACCAGTGACC)3' as reverse primer.

2.2. Gene characterization

The cDNA fragments were then sequenced and analyzed through in silico studies. Web sites and software used are Softberry© (http://www.softberry.com), analysis of protein localization at sites CELLO (http://www.cello.life.nctu.edu.tw/cgi/main.cgi), analysis of local alignment (BLAST) from the GenBank Web site (http://www.ncbi.nlm.nih.gov), and multiple sequence alignment with ClustalW (http://www.srs.ebi.ac.uk). Gene annotations were also conducted with CLC Genomics Workbench v3.6.5© software.

3. Results

3.1. Gene expression study

The ~1178 bp *API5* was amplified from root tissue cDNA (Figures 1A and 1C). This result shows that *API5* was expressed in the root of *A. thaliana* plant. Meanwhile, ~1053 bp fragment was amplified using *ChiI1* gene-specific primers from leaf cDNA (Figures 1B and 1D). This result showed that the genes were expressed in banana leaf tissue.

3.2. Gene characterization

Motifs from each sequence were characterized with in silico studies. Figure 2A shows that putative API5 amino acid sequences have the same motifs with API5 from another species such as *Oryza sativa* and *Homo sapiens*. In the figure, one represent as LXXLL motif, two represent as transactivation domain, whereas three represent as nuclear localization sequence. From the alignment, putative API5 shares partial amino acid sequences characterized as transactivation domain.

As for the chitinase, it has been found that several motifs are based on the multiple sequence alignment result (Figure 2B). Motifs 1, 2, 3, 5, 12, and 13 are chitinase protein motifs found only in plants, whereas others may be found in another species, such as arthropods, purple bacteria, actinobacteria, and nematodes (Udaya Prakash *et al.* 2010).

4. Discussion

To identify putative genes, both cDNA fragments were cloned and sequenced, and the sequencing results were then analyzed with BLAST. The sequences were aligned to both nucleotide and protein database in GenBank. Alignment results showed that putative genes of *API5* share high homology with *API5* gene and protein from *A. thaliana*. Based on nucleotide alignment (BLASTn), putative *API5* gene shares 90% homology with *A. thaliana* apoptosis inhibitory protein 5 (*API5*) (NM_128955.4). In addition, putative *API5* gene sequence was also compared with protein database in GenBank using BLASTx alignment. Predicted amino acid sequence of putative *API5* gene shares 96% homology to API5 protein isolated from *A. thaliana*. Because of this sequence similarity, putative *API5* that has been successfully isolated is considered as one of the *API5* gene (NP_565777.1). *API5* genes were also compared for its amino acid motifs and protein domain using characteristics compared from other known *API5* gene family, specifically from *Oryza sativa* and *Homo sapiens* (Li *et al.* 2011). From Figure 2A, putative API5, both *Os*API5 and *Hs*API5 have conservative motifs, such as LxxLL

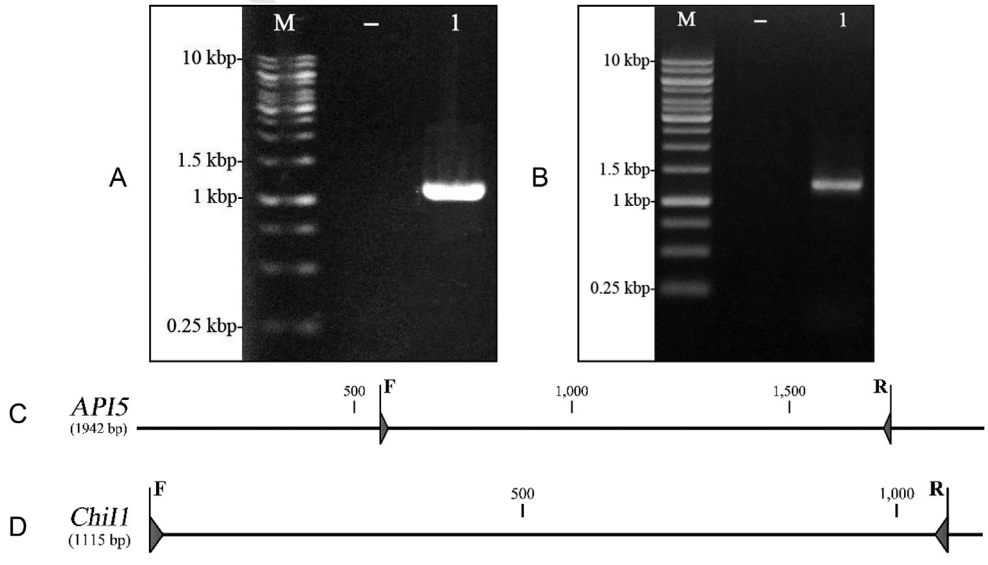

Figure 1. PCR result of *API5* gene amplification using touchdown (A) PCR method and (B) *ChiI1* gene from cDNA pisang ambon lumut. (C) and (D) show primers position for each gene amplification. PCR, polymerase gene reaction.

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