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Original Research Paper

Isolation and Characterization of Encoded Formaldehyde Responsive Gene from the Plant *Dieffenbachia compacta*

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ABSTRACT

Plants have several defence mechanisms to survive in a stressful environment. Formaldehyde is one of the indoor air pollutants that can cause cancer. Ornamental plant *Dieffenbachia compacta* can be used as biological control for pollutants in the indoor air environment. This study was conducted to isolate the genes that response for detoxifying formaldehyde in *Dieffenbachia compacta*. Methods used to isolate the gene is by using reverse transcription PCR (RT-PCR). A total of 735 base pairs was isolated using a primer from *Epipremnum aureum* FALDH genes. Nucleotide sequence analysis showed that the sequence similarities of 85% and 84% to the FALDH mRNA for *Epipremnum aureum* and *Populus trichocarpa* respectively. While for amino acid 92% similarities were found against *Epipremnum aureum* and *Populus trichocarpa*. A total of 87 amino acids that were encoded from 261 base pairs using the Open Reading Frame Finder. Our finding showed that the isolated *Dieffenbachia compacta* gene is the gene for glutathione-dependent formaldehyde dehydrogenase (FALDH). As a conclusion, formaldehyde metabolism in plants is regulated by the enzyme glutathione-dependent formaldehyde dehydrogenase (FALDH). The activity of FALDH indicates that this enzyme might be part of the universal metabolism pathway shared by a variety of organisms.

INTRODUCTION

According to the International Agency for Research on Cancer (IARC), formaldehyde is classified as a human carcinogen. Humans are exposed to formaldehyde in the indoor environment from wood-based products, rubber, paints, lubricants, cosmetics, electrical appliances and construction materials. Reduction of formaldehyde is important to improve indoor air quality and reduce public health risks.

Ornamental plants can be used as biological control for pollutants in indoor environments. *Dieffenbachia compacta* can reduce formaldehyde contamination (Aydogan & Montoya 2011). Due to the increasing air pollution in environment, plants also need to have a variety of resistance to environmental pollutants. The plants produce various defence mechanisms against environmental stress and the earliest change that can be observed is in the gene (Tamoaki et al. 2004).

The main enzyme that reacts to the stimulus of formaldehyde on the eukaryotic cell is gluthatione-dependent formaldehyde dehydrogenase (FALDH) (Tada et al. 2010). According to a study conducted on golden pothos (*Epipremnum aureum),* a class II chitinase gene responds to the stimulus from formaldehyde (Tada et al. 2010). This study was conducted to identify the genes that respond to formaldehyde in *Dieffenbachia compacta*.

MATERIALS AND METHODS

Formaldehyde treatment: For formaldehyde treatment, *Dieffenbachia compacta* was put into (30×30×30 cm) static environment airtight chamber containing 5µL of a 37% formaldehyde solution placed in a Petri dish. The leaves were sampled at time 3 hours and 24 hours after exposing to the formaldehyde. *Dieffenbachia compacta* without formaldehyde treatment was used as a control.

mRNA extraction and RT-PCR: Green leaves of *Dieffenbachia compacta* treated and untreated with formaldehyde were harvested and rapidly ground in nitrogen liquid. Total RNA was isolated using the RNeasy Plant Mini kit (QIAGEN, Tokyo Japan). Total RNA isolated was used for synthesis of first-strand cDNA. cDNA was synthesized using a QuantiTech Reverse Transcription kit (QIAGEN, Tokyo Japan) according to the protocol supplied. Reverse transcription was carried out by the incubation technique at 42°C in a final volume of 20µL. The RT-PCR was performed using PCR reagent (PROMEGA) in final volume 25µL. Reaction contained 1.5µL of cDNA, 5µL 10 × PCR buffer, 3µL

25mM $MgCl_2$, 1µL 10mM dNTP, 0.25µL 2.5U Taq Polymerase and 0.5µL forward and reverse primer. The specific forward and reverse primer used in this reaction as follows:

1. ACCTGGAGCGGCAAGGATCCTGA
2. GTTCCCAGCCCTTGTGGCAGC

PCR amplification was carried out for 30 cycles at 94°C for 1 min, followed by 72°C annealing for 1 min, 72°C for 1.5 min and final extension at 72°C for 5 min. The amplified PCR product was separated using 1.5% agarose gel electrophoresis. Expected band was extracted from the gel using a Qiaquick Gel Extraction kit (QIAGEN). Purified product was sent to NHK Bioscience Solution Sdn Bhd for sequencing.

Sequence analysis: Nucleotide and amino acid sequence were analysed using BLAST. BLASTn analysis was used to compare the identity sequence obtained with existing nucleotide sequence data available in GenBank. While BLASTx analysis was used to compare the identity percentage of amino acid obtained with existing amino acid sequence data available in GenBank.

RESULTS AND DISCUSSION

There are several studies which show that indoor plant can improve air quality by detoxifying indoor pollutant (Aydogan & Montoya 2011, Lim et al. 2009, Wolverton et al. 1984, Xu et al. 2011), however there are still a few studies about the effect of formaldehyde metabolism in plants. Glutathione-dependent formaldehyde dehydrogenase plays an important role of formaldehyde detoxification in living organisms. In this study, the isolation of genes encoding FALDH in *Dieffenbachia compacta* was conducted by using primer from *Epipremnum aureum* (Tada & Kidu 2011). Isolation of total RNA has been done and run on agarose gel showing sharp and clear band.

RT-PCR: Amplified PCR product, separated using 1.5% agarose gel electrophoresis, showed that putative gene had approximately 700 base pairs in size as shown in Fig. 1. There was no difference in size between all the three samples.

From the sequencing analysis, amplified PCR product showed that 735 base pairs were isolated. There are no differences in nucleotide sequence for all three samples. Fig. 2 shows the sequence of specific gene isolated using RT-PCR.

The method used to amplify a product 735 base pairs in length is by using RT-PCR. Partial sequence of gene has been isolated because the putative gene is smaller than expected gene from *Epipremnum aureum* which is 1362 base pairs in length (Tada et al. 2010). In addition, the difference in plant species also may cause difference in length and sequence of DNA.

BLASTn sequence analysis showed that putative gene of *Dieffenbachia compacta* has similarity with mRNA FALDH *Epipremnum aureum* and mRNA GSH FDH *Populus trichorpa*. For this study, there is no significant difference in nucleotide sequence of all the three samples because this gene that encoded for FALDH enzyme is universal to living organisms. This enzyme is naturally present in the plant without exposure to exogenous formaldehyde.

Sequence analysis: Putative gene isolated from *Dieffenbachia compacta* showed the highest sequence similarities of 85% and 84% to the FALDH mRNA for *Epipremnum aureum* (accession number AB 618795.1) and *Populus trichocarpa* (accession number XM_002301800.1) respectively. While for amino acid, 92% similarities were found against *Epipremnum aureum* and *Populus trichocarpa*.

A total of 87 amino acids have been encoded from 261 base pairs using the Open Reading Frame Finder. These amino acids were translated based on the longest open reading frame that fulfills the criteria that have started and stop codon.

The result of amino acid analysis by using the Open Reading Frame Finder (ORF) indicate that 87 amino acids have been encoded by 261 base pairs. This is because of 735 base pairs isolated, this longest ORF has fulfilled the criteria that had started and stop codon. No functional amino acid translated before start codon and after the stop codon because it did not fulfil the stated criteria.

In this study all the three samples have no difference in nucleotide sequence but there is difference in nucleic acid

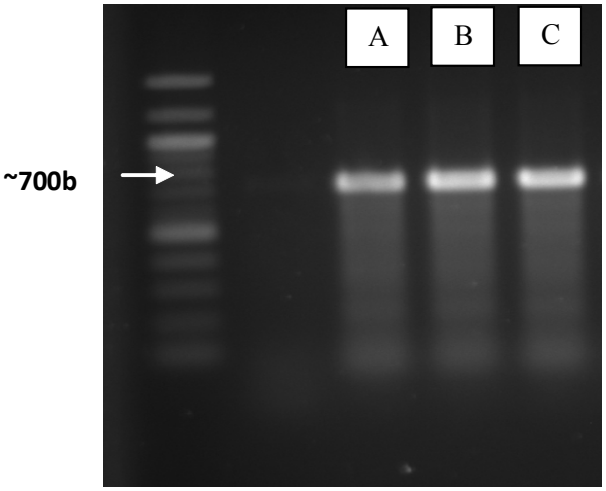

Fig. 1: Agarose gel showing the PCR product for control (A), 3 hours exposure (B) and 24 hours exposure (C).

TTGCTTTTCT GTTCTTGGTC ATGAGGCTGC TGGGATTGTT GAAAGTGTTG
GTGAAGGAGT AACAGAGGTT CAGCCAGGAG ATCATGTTAT TCCTTGTTAC
CAGGCAGAAT GCAGGGAATG CAAGTTCTGC AAGTCAGGGA AGACCAACCT
CTGTGGGAAA GTTAGAGCAG CTACTGGTGT TGGGGTCATG ATGAATGACC
GAAAAAGTCG ATTCTCAGTA AATGGAAAGC CAATCTATCA TTTCATGGGG
ACATCTACAT TTAGTCAGTA CACGGTTGTT CATGACGTCA GTGTTGCAAA
AATTGATCCC CAAGCTCCTC TGGATAAGGT CTGTCTGCTG GGGTGTGGTG
TTCCAACAGG TCTTGGAGCA GTTTGGAATA CAGCAAAGGT AGAAGCAGGT
TCGAATGTTG CTGTTTTTGG CCTTGGAACT GTTGGACTTG CTGTTGCGGA
GGGGGCTAAG GCAGCTGGTG CATCTCGGAT AATTGGTATA GATATTGACA
GCAAAAAGTT TGATAGAGCT AAGAATTTTG GAGTCACTGA ATTCATCAAC
CCAAAGGACC ATGACAAACC GATACAGCAA GTCATTGTCG ATCAAACTGA
TGGTGGAGTT GACTACAGTT TTGAGTGCAT CGGAAATGTG GCAGTGATGA
GGTCTGCTCT AGAATGCTGC CACAAGGGCT TGGGGAACAA GAAAAAACCA
GGCCCCCAGC AGACAGACCT TTTTCCAGAG GAGCT

Fig. 2: Sequence of the putative gene isolated from *Dieffenbachia compacta* amplified by RT-PCR with the forward and reverse primer stated in the methodology.

tttgggttgatgaattcagtgactccaaaattcttagctctatcaaacttttgctgtca
F G L M N S V T P K F L A L S N F L L S
atatctataccaattatccgagatgcaccagctgccttagccccctccgcaacagcaagt
I S I P I I R D A P A A L A P S A T A S
ccaacagttccaaggccaaaaacagcaacattcgaacctgcttctacctttgctgtattc
P T V P R P K T A T F E P A S T F A V F
caaactgctccaagacctgttggaacaccacaccccagcagacagaccttatccagagga
Q T A P R P V G T P H P S R Q T L S R G
gcttggggatcaatttttgcaacactgacgtcatgaacaaccgtgtactgactaaatgta
A W G S I F A T L T S

Fig. 3: Amino acid analysis using open reading frame finder.

intensity isolated from agarose gel electrophoresis. Sample for 3 hour formaldehyde treatment showed the highest intensity compared to the control and 24 hour treatment. This is because after exposure to exogenous formaldehyde, this glutathione-dependent formaldehyde dehydrogenase enzyme would be present to detoxify exogenous formaldehyde. After 24 hours exposure, the level of this enzyme reduced because the exogenous formaldehyde had been detoxified.

CONCLUSION

This finding showed that the isolated *Dieffenbachia compacta* gene is the gene for glutathione-dependent formaldehyde dehydrogenase (FALDH). As a conclusion, formaldehyde metabolism in plants is regulated by the enzyme glutathione-dependent formaldehyde dehydrogenase (FALDH). The activity of FALDH indicates that this enzyme might be part of the universal metabolic pathway shared by a variety of organisms. However, in this study, RT-PCR can express the presence of the gene but the level of expression has not been proven yet and can be determined by using quantitative real time PCR.

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