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Short Communication: Detection of lectin gene (MLL1 and M35) in mulberry plant (*Morus* spp.) from Bogor, West Java, Indonesia

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Abstract. *Wulandari YRE, Yogiara, Lizar M. 2018. Short Communication: Detection of lectin gene (MLL1 and M35) in mulberry plant (Morus spp.) from Bogor, West Java, Indonesia Biodiversitas 19: 2381-2384.* Plant species contains carbohydrate-binding protein known as lectin or agglutinin. Lectin binds to specific carbohydrates, such as monosaccharides or oligosaccharides and initiates agglutination process. Lectin plays an important role as plant defense, so that it can be used to prevent pest attacks. Mulberry leaf lectin 1 (MLL1) from young leaves of *Morus alba* can be used against phytopathogenic bacteria, *Pseudomonas syringae* pv. *mori.* Mannose-binding lectin (M35) was found on stems of *M. nigra* as protein storage. M35 is also produced on roots of *M. alba* and induced by mulberry stem cuttings. This research purpose was to isolate and analyze lectin gene expression (MLL1 and M35) in *M. alba* var. *multicaulis*, *M. cathayana*, *M. bombycis* var. *lembang*, and *M. alba* var. *kanva-2* from Bogor, West Java, Indonesia. Different plant organ including leaves, stems, and roots were used as source of samples and analyze using Reverse Transcription Polymerase Chain Reaction (RT-PCR). Our results showed that all of MLL1 genes were expressed in young leaves, but not expressed in stems and roots of mulberry plant samples. The M35 gene was expressed in young leaves, stems, and roots of all mulberry plant samples. Reverse Transcription PCR of MLL1 gene exhibited a 350 bp DNA band, while M35 gene exhibited a 99 bp DNA band.

Keywords: 1-deoxynojirimycin, antiherbivory, mulberry, *Morus*, plant-defense, RT-PCR

INTRODUCTION

Mulberry (*Morus* spp.) is a shurb and distributed worldwide, from tropical to temperate regions. Mulberry is traditionally used in Chinese medicines as a pharmaceutical and known for its nutritional benefits. Mulberry fruits are rich in phenolic compound, including flavonoids, anthocyanin, and carotenoids while the leaves are rich in chlorogenic acid, polyphenolic acid, quercetin, and 1-*deoxynojirimycin* (DNJ). The fruits can be consumed directly, while the leaves can be used as herbal tea for prevent diabetes. Mulberry leaves can also be used as feeds for ruminants because the leaves are digestible (Huang et al. 2013; Geniola 2016).

Mulberry is often associated with the development of sericulture industry. Sericulture is an agro-based industry and involves rearing of silkworms for the silk production. Mulberry silk comes from the silkworm, *Bombyx mori* Linnaeus. which solely feeds on the leaves of mulberry plant. Sericulture industry often disturbed by pest population. It causes extensive damage to host plants and fluctuation in cocoon production. The major insects orders known to be the pest of mulberry plant are Lepidoptera, Hemiptera, Coleoptera, Thysanoptera, Orthoptera, and Isoptera (Avhad and Hiware 2013). Pest populations can be managed by using chemical pesticides, but if used excessively can harm the plants itself, and other organisms as well. The high risk groups exposed to chemical pesticides includes production workers and agricultural farm workers. Pesticides also contaminate environment such as soil, air, and water (Aktar et al. 2009). Therefore, other compounds that can be used as a plant defense are needed so as to reduce the use of chemical pesticides.

Some studies have found that lectin content of plants can be used as antibacterial, antiinsecticidal, antiherbivory, and antifungal to prevent pest attacks (Peumans and Damme 1995). Lectin are carbohydrate-binding proteins present in most of the plants and in some animal. Lectin can be found at seeds, bulbs, leaves, stems, and roots of the plants. Mulberry leaf lectin 1 (MLL1) which is isolated from young leaves of *M. alba*. It has a specificity to N-glycolylneuraminic acid (NeuGc) and against a specific phytopatogenic bacteria, *Pseudomonas syringae* pv. *mori* (Ratanapo et al. 1998, 2001). Other studies (Van Damme et al. 2002; Du et al. 2016) reported that mannose-binding lectin (M35) has a binding specificity to mannose that was found on stems and roots of *Morus nigra* or Black Mulberry. This study aimed to isolate and analyze lectin gene expression (MLL1 and M35) on different organ from the mulberry plant species using Reverse Transcription Polymerase Chain Reaction (RT-PCR) method.

MATERIALS AND METHODS

Sample preparation

A total of 10 mulberry stem cuttings from *M. alba* var. *multicaulis*, *M. cathayana*, *M. bombycis* var. *lembang*, and

M. alba var. *kanva-2* as the mother plant were taken from University Farm belong to Bogor Agricultural University (IPB) in Sukamantri, Bogor, West Java, Indonesia and grown in the green house of Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia.

RNA isolation

Samples were taken and weighed as much as 100 mg from young leaves, stems, and roots organ from different species of mulberry plants. Total RNA was isolated from these samplesusing GeneJET Plant RNA Purification Mini Kit (ThermoScientific, USA) following manufacture's protocol. The quality of isolated RNA was checked for by using agarose gel electrophoresis with 1 % agarose concentration and run at 90 volt for 60 min. The concentration and purity of RNA isolates were measured by using NanoDrop 2000 UV-Vis Spectrophotometer (ThermoScientific, USA).

cDNA synthesis

Synthesis of first strand cDNA was done by using RevertAid First Strand cDNA Synthesis Kit (ThermoScientific, USA). The cDNA synthesis was performed for all RNA samples and reference (GAPDH RNA). The reaction mixtures were incubated at 42 °C for 60 min. The reaction was terminated by incubation at 70 °C for 5 min. The reaction product can be directly used for PCR or can be stored at a temperature of -20 °C at freezer, or it can be stored at a -80 °C freezer. The cDNA synthesis were completed by mixing first strand cDNA with each of MLL1 and M35 primer pairs (Table 1), and KAPA2G Fast Ready Mix PCR Kit (KAPABiosystems, USA).

The optimum condition for PCR of MLL1 and M35 consisted of initial denaturation 94 ºC, 5 min; denaturation 94 ºC, 30 sec; annealing 52 ºC, 1 min for MLL1 and 55 ºC, 1 min for M35; extension 72 ºC, 1 min; final extension 72 ºC, 7 min, and total 35 cycles. The cDNA synthesis was confirmed by amplification of GAPDH, 18S rRNA, and *rbc*L. 18S rRNA and *rbc*L genes were plant genes internal control. The condition PCR of GAPDH, 18S rRNA, and *rbc*L were as follow: initial denaturation 94 ºC, 5 min; denaturation 94 ºC, 30 sec; annealing 58 ºC, 30 sec for GAPDH or 51 ºC, 30 sec for 18S rRNA and *rbc*L; extension 72 ºC, 2 min; final extension 72 ºC, 7 min, and total 35 cycles.

Verification of the PCR products was performed using agarose gel electrophoresis with 2 % agarose. Electrophoresis was run at 80 Volt for 90 min followed by EtBr staining for 30 min and destained in ddH_2O for 10 min. The agarose gel is exposed to Ultraviolet (UV) light on the Gel Doc ™ EQ System (BioRad, US) for visualization.

RESULTS AND DISCUSSION

RNA isolation

RNA was isolated from roots sample were visualized by using agarose gel electrophoresis yielded two bands 28S rRNA and 18S rRNA (Figure 1). The presence of these two bands indicates that RNA is isolated intact and can be used for first strand synthesis of cDNA.

Based on the results of the measurement of the quantity and purity of RNA can be seen that the young leaves organ might yield RNA concentration higher than stems and roots organ (Table 2).

First strand synthesis of cDNA

RNA isolates were synthesized into cDNA and verification of cDNA synthesis is amplified by 18S rRNA and *rbc*L primer. The cDNA amplification of samples with 18S rRNA (Figure 2) and *rbc*L (Figure 3) primer as an internal control of plant RNA is done. All samples were successfully amplified by 18S and *rcb*L primer and produce a 500 bp band. The results obtained are in accordance with the previous study that *rbc*L gene is about 599 bp (Smit et al. 1999; Marghali et al. 2014).

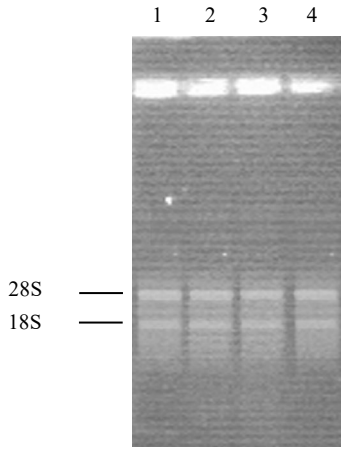

Figure 1. RNA isolation visualization results from roots of: 1. *M. alba* var. *multicaulis*, 2. *M. cathayana*, 3. *M. bombycis* var. *lembang*, 4. *M. alba* var. *kanva-2*

Table 2. RNA quantity and purity measurements

Species	Organ	[RNA] ng/µL	260/280	260/230
M. alba var. *multicaulis*	Leaves	163.8	1.95	1.80
	Stems	102	2.10	1.77
	Roots	50.8	2.01	1.65
M. cathayana	Leaves	246.8	1.87	2.00
	Stems	71.1	2.15	1.75
	Roots	96.3	2.02	1.67
M. bombycis var. *lembang*	Leaves	296.6	1.89	2.18
	Stems	158.2	2.00	1.81
	Roots	154.1	1.95	1.97
M. alba var. *kanva-2*	Leaves	173.3	1.81	2.07
	Stems	78.4	2.11	1.71
	Roots	68.4	2.03	1.60

Table 1. List of primer for cDNA synthesis

Gene name	Genbank accession Number	Forward/reverse primer sequence (5′-3′)	Size	Source
MLL1	JF745131.1	GG (A/C)GT (G/C)GC (A/C)TT (C/T)GA (C/T)G A (C/T)GG / CCTTTGAATCC (A/G) (A/G)C (A/G)AT (C/T) (A/T) (A/T)GC	350 bp	(Kankamol et al. 2012)
M35	AAL10685.1	ATCCATAAGAGCAAGCA CC / GTTGGCTGAGGATAGG TTC	99 bp	(Du et al. 2016)

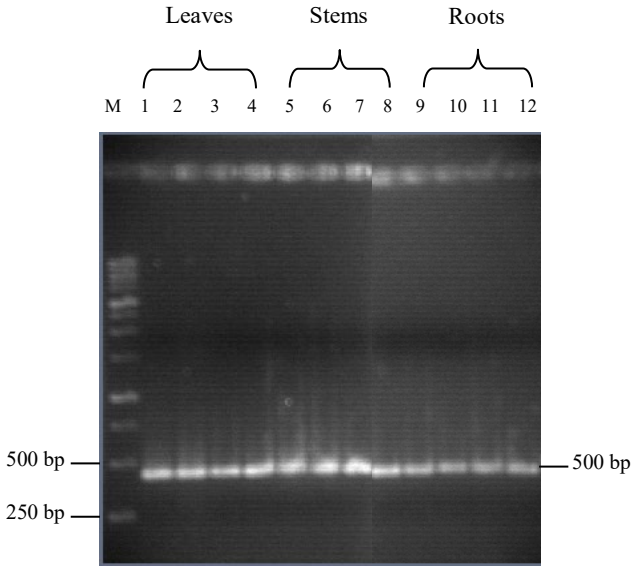

Figure 2. 18S rRNA gene visualization results. 1Kb DNA ladder (M); cDNA sample from *M. alba* var. *multicaulis* (1, 5, 9), *M. cathayana* (2, 6, 10), *M. bombycis* var. *lembang* (3, 7, 11), and *M. alba* var. *kanva-2* (4, 8, 12)

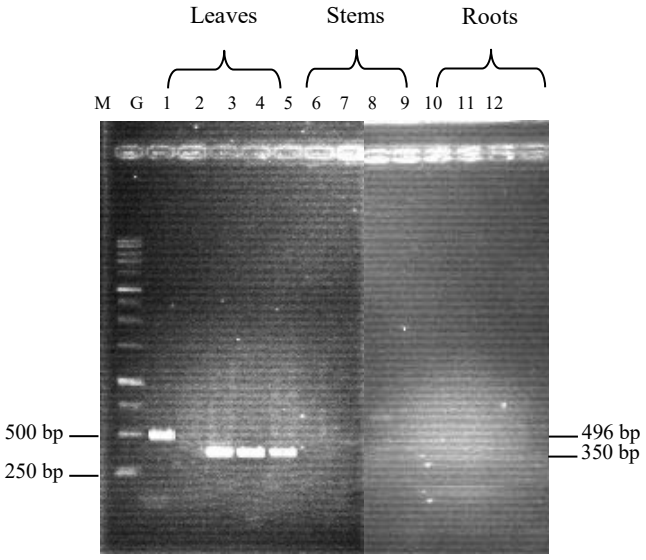

Figure 4. MLL1 gene visualization results. 1Kb DNA ladder (M); GAPDH as the control kit (G); cDNA sample from *M. alba* var. *multicaulis* (1, 5, 9), *M. cathayana* (2, 6, 10), *M. bombycis* var. *lembang* (3, 7, 11), and *M. alba* var. *kanva-2* (4, 8, 12)

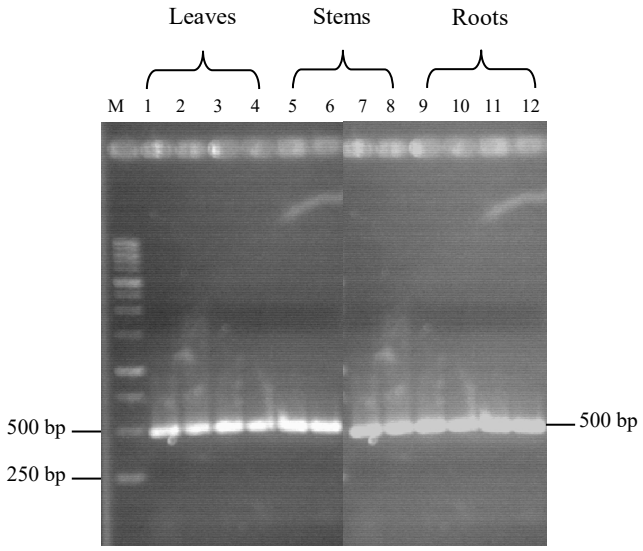

Figure 3. *rcb*L gene visualization results. 1Kb DNA ladder (M); cDNA sample from *M. alba* var. *multicaulis* (1, 5, 9), *M. cathayana* (2, 6, 10), *M. bombycis* var. *lembang* (3, 7, 11), and *M. alba* var. *kanva-2* (4, 8, 12)

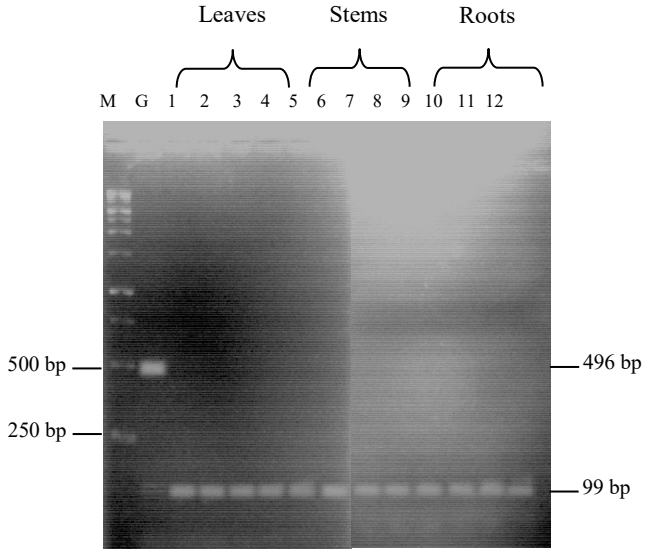

Figure 5. M35 gene visualization results. 1Kb DNA ladder (M); GAPDH as the control kit (G); cDNA sample from *M. alba* var. *multicaulis* (1, 5, 9), *M. cathayana* (2, 6, 10), *M. bombycis* var. *lembang* (3, 7, 11), and *M. alba* var. *kanva-2* (4, 8, 12)

cDNA amplification and verification

cDNA amplification of lectine genes were done by using MLL1 (Figure 4) and M35 (Figure 5) primer as the gene target. All samples were successfully amplified by MLL1 primer and produce a 350 bp band, while by M35 primer produce a 99 bp band.

cDNA of GAPDH were successfully amplified with MLL1 and M35 primer as shown by the appearance of a 496 bp DNA band of control and all samples. Young leaves samples from *M. cathayana*, *M. bombycis* var. *lembang*, and *M. alba* var. *kanva-2* produced a 350 bp band. However, young leaves sample from *M. alba* var. *multicaulis* also stems and roots from *M. alba* var. *multicaulis*, *M. cathayana*, *M. bombycis* var. *lembang*, and *M. alba* var. *kanva-2* with MLL1 primer did not show any bands. This indicated that cDNA samples can not be amplified using MLL1 primer because there was no match of cDNA samples with sequence of primer (Slack 2006). The results were similiar with the previous study that MLL1 gene size is 350 bp (Kankamol et al. 2012). In addition, all samples were successfully amplified with M35 primer and produce a 99 bp band. This indicates all samples are compatible with the primer sequences so that they can be well amplified. The results obtained are in accordance with the literature that M35 gene size is 99 bp (Du et al. 2016). This preliminary study successfully detected MLL1 and M35 gene through PCR amplification. To confirm that the amplicons were correctly belongs to MLL1 and M35, DNA sequencing should be performed.

Based on PCR detection method, we conclude that M35 and MLL1 gene were found in mulberry species from Bogor, West Java. MLL1 gene might be expressed on the young leaves organ of *Morus cathayana*, *M. bombycis* var. *lembang*, and *M. alba* var. *kanva-2*. However, MLL1 might not be expressed in young leaves of *M. alba* var. *multicaulis*, and in the stems and roots organ of all mulberry plant species. Meanwhile, M35 gene might not be expressed on the young leaves, stems, and roots organ in all mulberry plant species.

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