BIODIVERSITAS
Volume 19, Number 6, November 2018
Pages: 2204-2212

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190628

Short Communication: Identification of *Mildew Locus O* (*MLO*) genes in *Durio zibethinus* genome corresponding with the Powdery Mildew disease

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Manuscript received: 4 March 2018. Revision accepted: 8 November 2018.

Abstract. *Kemal RA, Sandjaja EBL, Santosa AP, Ivan J. 2018. Short Communication: Identification of* Mildew Locus O (MLO*) genes in* Durio zibethinus *genome corresponding with the Powdery Mildew disease. Biodiversitas 19: 2204-2212.* Mildew Locus O (MLO) is a protein consisting of seven transmembrane domains and appears in the various type of plants. MLO proteins are classified into seven clades. It is known that specific clades have different roles in a plant. MLOs from Clades IV and V have been linked to plant's susceptibility to Powdery Mildew (PM) disease. This study aimed to provide an overview of *MLO* genes present in durian (*Durio zibethinus*) genome. Bioinformatic analyses were conducted to analyze the phylogeny and structure of MLO genes and proteins in durian. The result showed that there were 20 putative *DzMLO* genes in durian, encoding 39 putative DzMLO proteins. Durian MLOs belong to Clade I-VI with one protein belongs to Clade IV and five proteins belong to Clade V. Those six MLO proteins shared a common motif in C-terminal and second intracellular domains. Putative alternative splicing and differential expressions were observed among Clade V *DzMLO* genes. These findings will facilitate the functional characterization of MLO genes and proteins in durian. Functional studies, especially on C-terminal and second intracellular domains, need to be conducted to elucidate the role of MLO in PM susceptibility in durian.

Keywords: Bioinformatics, durian, *Durio zibethinus*, Mildew Locus O, powdery mildew

Abbreviations: CT: C-terminal, CaMBD: Calmodulin-binding domain, DzMLO: *Durio zibethinus* MLO, EC: Extracellular, IC: Intracellular, MLO: Mildew Locus O, NT: N-terminal, PM: Powdery Mildew, TM: Transmembrane

INTRODUCTION

Mildew Locus O (MLO) is a highly conserved protein containing seven transmembrane domains (Acevedo-Garcia et al. 2014; Rispail and Rubiales 2016). MLO proteins are classified into at least seven clades (Kusch et al. 2016) and members of clades IV and V have been linked to plant's susceptibility to Powdery Mildew (PM) disease (Appiano et al. 2015a). Powdery mildew is a plant disease caused by fungal infection from the order of Erysiphales (Kuhn et al. 2016). Fungal growth on the epidermal cells of the leaves can cover the leaves' surface area and lead to the declining photosynthesis rate (Heffer et al. 2006; Berg et al. 2017). The disease has been reported in some plants such as grapevines (Feechan et al. 2011), cherry (Hubert et al. 2012), and legumes (Rubiales et al. 2015).

MLO has also been reported to have another role in phytohormones signaling, stress response processes, and root thigmomorphogenesis (Chen et al. 2009; Acevedo-Garcia et al. 2014). Some reports suggested that knockout of *MLO* gene should be performed to prevent the Powdery Mildew diseases (Pessina et al. 2016), but there are possibilities that the disruption or knockout of *MLO* genes may hamper the regulation or process of phytohormones and stress responses. However, the biochemical function of MLO proteins remains unknown (Kusch et al. 2016; Zheng et al. 2016). Therefore, functional study of MLO proteins becomes interesting. To study them, the first step is to identify *MLO* genes.

Analysis of *MLO* gene can be conducted by cloning and characterization of a single gene (Cheng et al. 2012; Cheng et al. 2013; Qin et al. 2015). Currently there are some reports analyzing *MLO* genes on the whole genome level, for example in cucumber (Zhou et al. 2013; Schouten et al. 2014), rose (Qiu et al. 2015), pea (Mohapatra et al. 2016), legumes (Rispail and Rubiales 2016), sweet orange (Liu et al. 2017), barrel clover, and chickpea (Deshmukh et al. 2017). Durian (*Durio zibethinus*) is a native fruit to South-East Asia region which draft genome was recently published (Teh et al. 2017). This fruit has been known for its sweet taste with a strong penetrating odor, and this fruit is deeply appreciated in South-East Asia as the 'king of fruit' (Li et al. 2012). There is a report that *Durio zibethinus* can act as a host of Powdery Mildew diseases (AQIS 1999; Siahaan et al. 2016) which might be correlated with the activity of *MLO* genes. This study

aimed to analyze the number, structure, and pattern of *MLO* gene family in durian genome. Results from this analysis are expected to provide insight on further functional studies of the *MLO* genes in durian.

MATERIALS AND METHODS

Retrieval of putative *MLO* genes from *Durio zibethinus*

The genome of *Durio zibethinus* was retrieved from NCBI (Genome ID: 57226). Using Genome BLAST tool, a blast was conducted on the genome using *Arabidopsis thaliana* MLO 2 (AtMLO2) protein sequence. Protein and its corresponding gene and mRNA sequences were downloaded for these analyses. Retrieved proteins were checked for the presence of MLO domain using Pfam (http://pfam.xfam.org/). Sequences without MLO domain were omitted. Gene and mRNA sequences were checked for the locus and mRNA products in NCBI. Locus of the respective gene was obtained from the GeneID site, while the mRNA product was obtained from the CDS in the GenBank. Proteins from the same gene locus but resulted from different mRNA were grouped as putative isoforms. mRNA coding for miscellaneous mRNA was omitted from the analysis. Information on coding region length, number of exon and intron, and amino acid residues were recorded. Proteins were analyzed for putative molecular weight and isoelectric point using ExPASy (https://web.expasy.org/compute_pi/).

Protein phylogenetic analysis

The longest isoform of each durian MLO (DzMLO) protein was selected for phylogenetic analysis. For comparison and clade determination, MLO protein sequences from other dicots were retrieved from the NCBI database. These were of *A. thaliana* (AtMLO1-AtMLO15), *Aquilegia coerulea* (AcMLO1, AcMLO3, AcMLO4, AcMLO5, AcMLO9, AcMLO10, AcMLO12, AcMLO13), *Fragaria vesca* (FvMLO1, FvMLO7, FvMLO9, FvMLO10, FvMLO13, FvMLO14, FvMLO17), *Glycine max* (GmMLO4, GmMLO7, GmMLO8, GmMLO24, GmMLO28, GmMLO30, GmMLO31, GmMLO33), *Nelumbo nucifera* (NnMLO1, NnMLO5, NnMLO3, NnMLO6, NnMLO8, NnMLO11, NnMLO12, NnMLO13). MLO protein sequences were also retrieved from monocots *Hordeum vulgare* (HvMLO1, HvMLO2, HvMLO7, HvMLO8), *Oryza sativa* (OsMLO3, OsMLO4, OsMLO8, OsMLO12), *Triticum aestivum* (TaMLO B1, TaMLO4, TaMLO5, TaMLO6), *Zea mays* (ZmMLO1, ZmMLO2, ZmMLO11, ZmMLO13). Multiple sequence alignment (MSA) of MLO protein sequences was conducted using MUSCLE (Edgar 2004), and Maximum Likelihood phylogenetic tree was constructed with 1000 times bootstrap replicates (Hall 2013).

Phylogenetic and structure analysis and *DzMLO* genes

DzMLO genes were aligned using CLUSTALW, and the phylogenetic tree was constructed by MEGA 5 (Tamura et al. 2011) using Maximum Likelihood method with Tamura-Nei model (Tamura and Nei 1993) and 1000 times bootstrap (Hall 2013). Branches supported with less than 50 bootstrap value were collapsed. The resulting tree was exported as Newick file, and the gene structure was drawn with Gene Structure Display Server 2.0 (GSDS 2.0: http://gsds.cbi.pku.edu.cn/).

Protein motif and gene ontology prediction

To enable motif comparison within and between clades, all 39 putative DzMLO proteins were aligned using CLUSTALW, and the phylogenetic tree was constructed by MEGA 5 (Tamura et al. 2011) using Maximum Likelihood method JTT matrix-based model (Jones et al. 1992) and 1000 times bootstrap replicates (Hall 2013). Branches supported with less than 50% bootstrap value were collapsed. Conserved motifs in amino acid sequences were analyzed using the MEME algorithm (Bailey et al. 2009). The parameters were set to search a maximum of 20 motifs with 25-50 residues wide. Several identified motifs were analyzed using FIMO search tool (Grant et al. 2011) against the Ensembl Genomes database for *Saccharomyces cerevisiae* version 86. Gene list obtained was analyzed using Generic GO Term Finder (Boyle et al. 2004) and annotated using *Saccharomyces cerevisiae* SGD for processing ontological aspect.

Expression pattern analysis

mRNA sequences belonged to Clades IV, and V were analyzed using BLAST in Sequence Read Archive (SRA) database. The RNA's sequences of *Durio zibethinus* Musang King isolated from leaf, root, stem, and arils were obtained by Teh et al. (2017) served as a template. Putative expression pattern was obtained based on the coverage of the sequence. Sequences that are covered by the short reads with less than 200 bp gaps are coded as expressed (+).

RESULTS AND DISCUSSION

Identification of durian *MLO* (*DzMLO*) gene family

Analysis showed that there are 20 putative *MLO* genes in durian genome (Table 1). This number is in line with other eudicot members that have 13-39 genes with a mean of 19.5 MLO/species (Kusch et al. 2016). *MLO* gene structure such as number of predicted introns that seems to be relatively conserved even among phylogenetically distant plant species such as potato *Solanum tuberosum* (Appiano et al. 2015b), tomato *S. lycopersium* (Zheng et al. 2016), thale cress *A. thaliana*, barley *H. vulgare*, rice *O. sativa* (Devoto 2003), barrel clover *Medicago truncatula*, and chickpea *Cicer arietinum* (Deshmukh et al. 2017). Eight (40%) genes are predicted to code for isoform proteins. Four genes (*DzMLO4*, *DzMLO13*, *DzMLO15*, *DzMLO17*) produce two isoforms, two genes (*DzMLO3*, *DzMLO19*) produce three isoforms, one gene (*DzMLO16*) produces five isoforms, and one gene (*DzMLO18*) produces eight isoforms. Therefore, it is predicted that durian has 39 MLO proteins. The predicted molecular weight ranges from 38.99 kDa (DzMLO18 X8) to 67.14 kDa (DzMLO12). All proteins are predicted to be alkaline (Table 1) which is similar to all predicted MLO proteins such as those from *S. lycopersicum* (Chen et al. 2014), *M. truncatula*, and *C. arietinum* (Deshmukh et al. 2017).

Table 1. Putative *MLO* genes from *D. zibethinus* and their corresponding products

Name	Gene	Isoform	Coding region (bp)	Exon number	Intron number	Amino acid residues	Protein Accession Number	The molecular weight (kDa)	Isoelectric point
DzMLO1	LOC111315043	-	1470	14	13	489	XP_022772397.1	56.13	9.23
DzMLO2	LOC111294434	-	1515	13	12	504	XP_022743467.1	57.15	8.95
DzMLO3	LOC111277785	X1	1509	13	12	502	XP_022719935.1	56.99	8.86
		X2	1434	12	11	477	XP_022719936.1	54.02	8.69
		X3	1320	12	11	439	XP_022719937.1	50.18	8.85
DzMLO4	LOC111276721	X1	1509	14	13	502	XP_022718216.1	57.68	8.43
		X2	1506	14	13	501	XP_022718217.1	57.61	8.43
DzMLO5	LOC111310601	-	1359	13	12	452	XP_022765807.1	51.84	7.66
DzMLO6	LOC111304402	-	1590	15	14	529	XP_022756687.1	60.08	9.09
DzMLO7	LOC111300107	-	1677	15	14	558	XP_022751467.1	64.66	8.82
DzMLO8	LOC111296618	-	1704	15	14	567	XP_022746752.1	65.03	8.81
DzMLO9	LOC111292305	-	1707	15	14	568	XP_022740337.1	65.76	8.29
DzMLO10	LOC111310303	-	1713	15	14	570	XP_022765350.1	66.16	9.31
DzMLO11	LOC111304404	-	1716	15	14	571	XP_022756688.1	66.28	9.64
DzMLO12	LOC111284736	-	1746	15	14	581	XP_022729353.1	67.14	8.03
DzMLO13	LOC111311041	X1	1680	15	14	559	XP_022766152.1	64.52	9.37
		X2	1674	15	14	557	XP_022766153.1	64.23	9.42
DzMLO14	LOC111310872	-	1563	15	14	520	XP_022765985.1	58.91	9.15
DzMLO15	LOC111292943	X1	1713	15	14	570	XP_022741334.1	65.15	9.37
		X2	1338	14	13	445	(XP_022741335.1	51.37	9.47
DzMLO16	LOC111301676	X1	1704	15	14	567	XP_022753242.1	65.23	8.68
		X2	1701	15	14	566	XP_022753243.1	65.10	8.68
		X3	1668	14	13	555	XP_022753244.1	63.68	8.80
		X4	1587	14	13	528	XP_022753245.1	60.76	8.87
		X5	1494	12	11	497	XP_022753246.1	56.83	8.14
DzMLO17	LOC111296812	X1	1695	15	14	564	XP_022747015.1	64.89	9.20
		X2	1287	14	13	428	XP_022747016.1	49.50	9.21
DzMLO18	LOC111284848	X1	1392	13	12	463	XP_022729613.1	53.54	8.60
		X2	1296	14	13	431	XP_022729615.1	49.87	8.61
		X3	1215	11	10	404	XP_022729617.1	46.94	8.56
		X4	1182	12	11	393	XP_022729619.1	45.70	8.78
		X5	1182	12	11	393	XP_022729620.1	45.00	8.75
		X6	1119	11	10	372	XP_022729621.1	43.22	8.68
		X7	1053	10	9	350	XP_022729622.1	40.16	8.65
		X8	1002	10	9	333	XP_022729623.1	38.99	8.91
DzMLO19	LOC111275322	X1	1461	14	13	486	XP_022716339.1	56.84	8.70
		X2	1344	13	12	447	XP_022716340.1	52.62	8.62
		X3	1191	13	12	396	XP_022716341.1	46.16	8.81
DzMLO20	LOC111312931	-	1692	14	13	563	XP_022769423.1	64.27	9.27

Table 2. Clades classification of *DzMLO*

Clade	DzMLO	Amount (%)
I	7, 8, 9, 16	4 (20%)
II	1, 2, 3, 4, 18, 19	6 (30%)
III	14, 15	2 (10%)
IV	20	1 (5%)
V	10, 11, 12, 13, 17	5 (25%)
VI	5, 6	2 (10%)
VII	-	0 (0%)

Phylogenetic of durian MLO (DzMLO) protein

The result of phylogenetic analysis of durian MLO protein family showed separation of MLOs into several clades (Figure 1). Clades numbering was based on *Arabidopsis* MLO protein classification (Devoto et al. 2003, Appiano et al. 2015b) (Figure 1). The results showed that MLO proteins from durian belong to Clades I-VI (Table 2). Majority of durian MLO (6 of 20, 30%) are members of Clade II. The clade distribution resembles other dicots (Kusch et al. 2016).

***MLO* gene structure analysis**

The number of exons in *DzMLO* genes ranges from 10 to 15, while the number of introns ranges from 9 to 14. The majority (60%) of the longest isoform genes have 15 exons and 14 introns (Table 1). Gene structure analysis (Figure 2) showed the differences in gene structure between *DzMLO* genes. The phylogenetic tree and gene structure support the presence of 20 *MLO* genes. The grouping of *DzMLO* genes supports the classification of DzMLO protein family in Figure 1. Some possible types of alternative splicing

occurred are exon skipping, intron retention, alternative promoter, and alternative polyadenylation sites (Black 2003, Keren et al. 2010). In an exon-skipping mechanism, an exon can be regulated to be excluded in the mature mRNA. The fourth exon of *DzMLO19* might be an example of this mechanism. The inclusion of the exon results in the longest X1 isoform, while exon skipping results in shorter X2 isoform. An intron can be retained in the mature mRNA; as in *DzMLO18* X1 and X2 isoforms which difference might have resulted from intron retention. The intron between the ninth and tenth exons of *DzMLO18 X2* might be retained to produce longer ninth exon of *DzMLO18 X1*. Alternative promoters can result in different transcription start, therefore different 5'-end exon. Difference between *DzMLO18* X1 and X3 isoforms might be produced by different promoter use, making *DzMLO18* X3 isoform has longer untranslated upstream and lacks the first two exons of *DzMLO18* X1. Alternative polyadenylation site can result in different transcription termination, therefore different 3'-end exon. Difference between *DzMLO17* X1 and X2 isoforms might be produced by the use of different polyadenylation site, making *DzMLO17* X2 isoform has longer untranslated downstream and lacks the last exon of *DzMLO17* X1. Two members of Clade V MLO, DzMLO13 and DzMLO17, are suggested to undergo alternative splicing. Alternative splicing of MLO genes has also been observed in tomato (Zheng et al. 2016). Data mining on Plant Alternative Splicing Database (http://proteomics.ysu.edu/altsplice/) also showed the presence of alternative splicing in several Clade V *MLO*, such as *Nelumbo nucifera NnMLO7* and *Malus domestica MdMLO19*, as well as Clade IV *MLO Zea mays ZmMLO1*.

MLO protein motif

Most conserved MLO protein motifs (Motifs 1-5, 7-8) are located in transmembrane (TM) and intracellular (IC) domains (Figure 3). When DzMLO proteins were analyzed using only one longest isoform for each gene, a motif that contains a calmodulin-binding domain (CaMBD) was found in all DzMLO except DzMLO18 (data not shown). When all putative MLO was analyzed, a motif that contains CaMBD (Motif 9) was detected in all DzMLO including DzMLO18 (Figure 3). CaMBD in DzMLO18 is located in the second intracellular domain (IC2), but in other DzMLOs, it is located in C-terminal (CT).

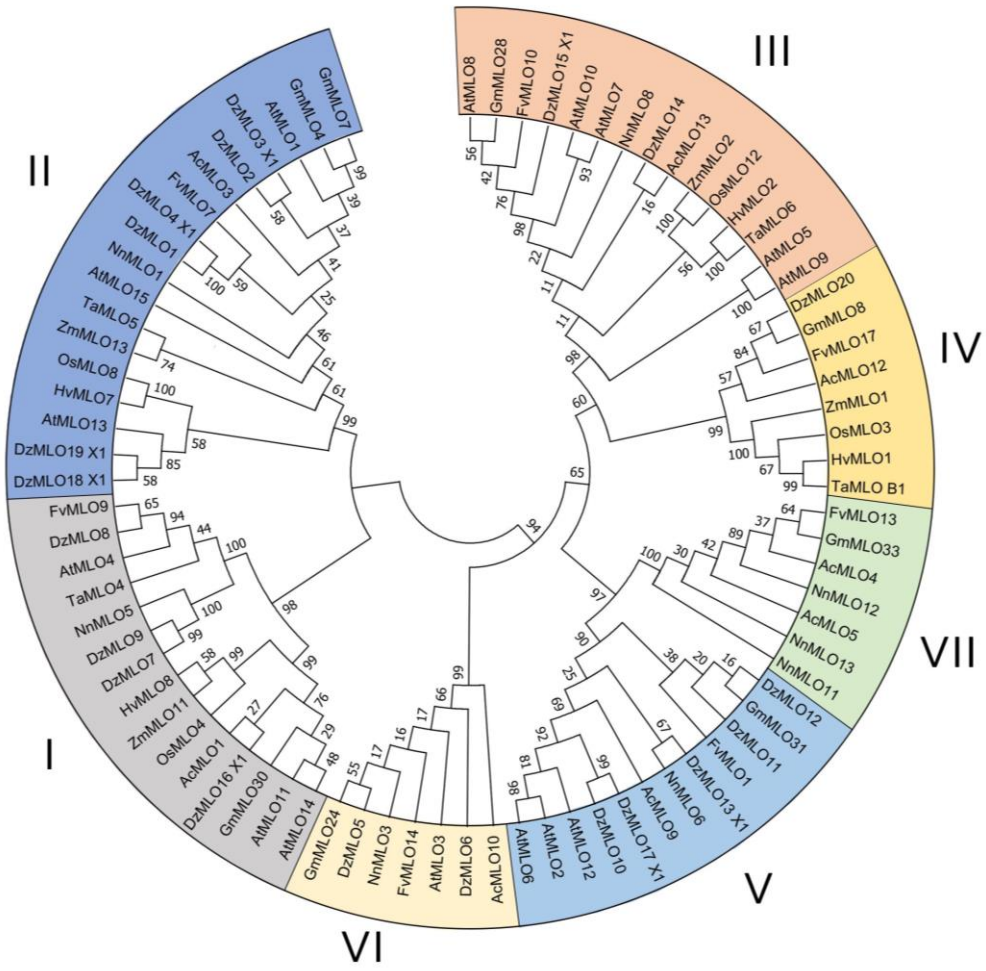

Figure 1. Phylogenetic tree of MLO protein from durian and other angiosperms showed seven clades. Members of durian MLO were distributed in all clades except for Clade VII. The number on the branches indicates the percentage of 1000 bootstrap replicates that support the node. The tree was constructed using MEGA 5 software (Tamura et al. 2011)

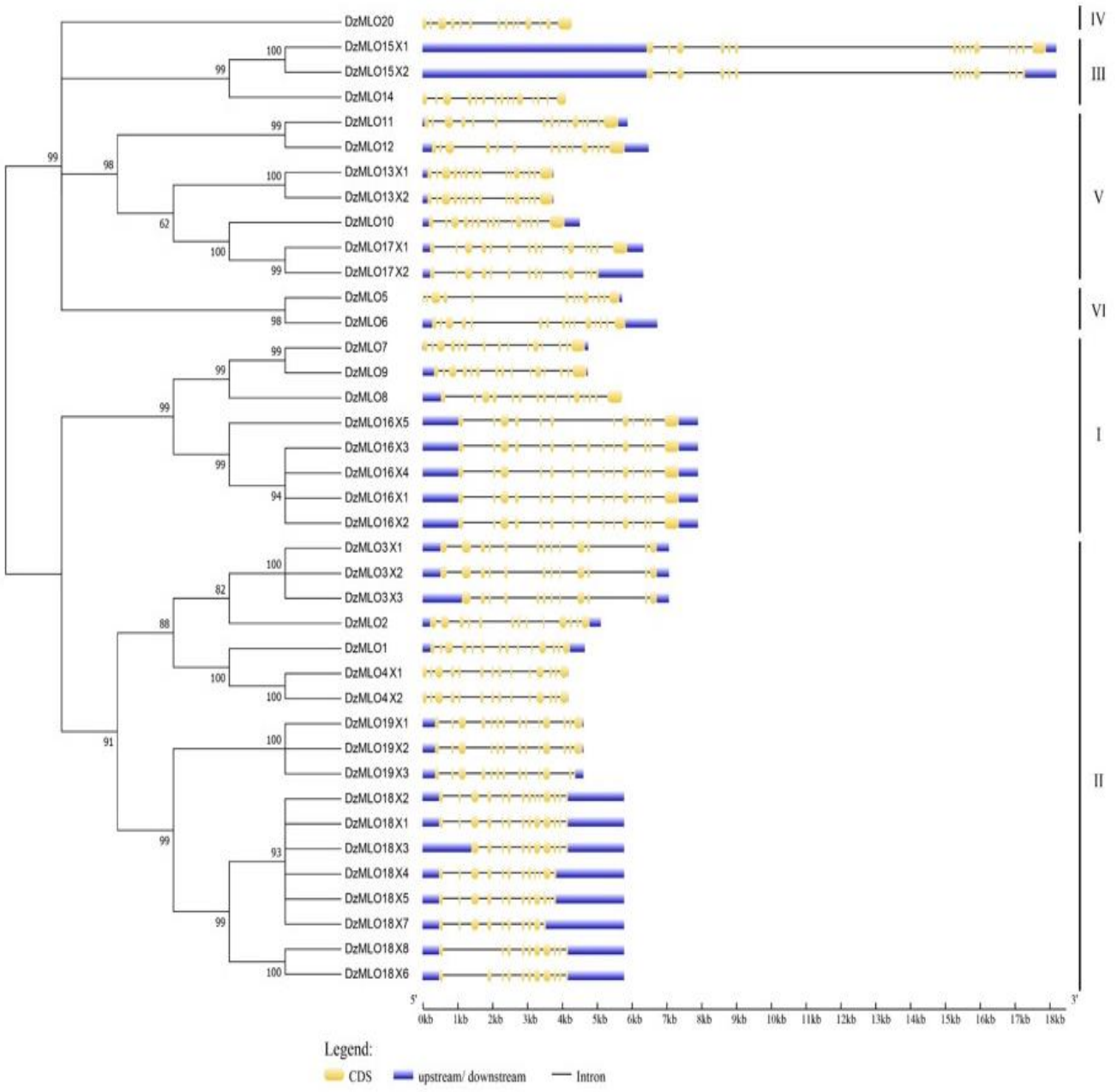

Figure 2. Phylogenetic tree and structure of *DzMLO* genes. There were variations among genes and similarity among isoforms in term of number, position, and length of exon, intron, and upstream/downstream regions. Gene structure was constructed using GSDS 2.0, and the accompanying dendogram was constructed using MEGA 5 (Tamura et al. 2011)

Other variable motifs showed association with clades (Table 3). A motif in IC2 is different between DzMLO Clade I and other clades. Motif 13 is only detected in Clade I but seems to be replaced by Motif 6 in other clades. Motifs 6 and 10 are found in DzMLO Clades II-VI while Motif 20 is only found in Clades III-VI. Motif 17 is found in first extracellular (EC1) domain of Clades IV and V as well as in CT of two members of Clade I (DzMLO9 and DzMLO16). Motif 15 is only found in C-terminal of Clades IV and V. Two members of Clade V, DzMLO 13 and DzMLO17, are predicted to undergo alternative splicing. Both DzMLO13 isoforms have identical motif organization. However, isoform 2 of DzMLO17 lacks motifs 9 and 15 which contain CaMBD motif and C-terminal respectively (Figure 3). Clade-specific motif diversification was also observed by Kusch et al. (2016).

Protein motifs supported the gene structure analysis. Motif variations between isoforms suggested the alternative splicing events. Analysis of three isoforms DzMLO3 can serve as an example. Gene structure difference between *DzMLO3* X1 dan X2 is the lack of the sixth exon in *DzMLO3* X2 (Figure 2). This is supported by the lack of

the sixth motif, containing Motif 6, in DzMLO3 X2 protein (Figure 3). Gene structure difference between *DzMLO3* X1 dan X3 is the longer untranslated upstream region in *DzMLO3* X3 (Figure 2). This is supported by the lack of first two motifs, containing Motifs 3 and 8, in DzMLO3 X3 protein (Figure 3).

Motifs found in Clades IV and V predict the function of the protein in molecule transport and cellular process regulation (Table 3). This functional assignment was also predicted by Kusch et al. (2016). The *Saccharomyces cerevisiae* is distantly related to plants and lacks MLO proteins. However, it has one of the best-annotated genomes, therefore enabling detailed and comprehensive gene ontology analysis (Kusch et al. 2016).

Members of MLO Clade IV and V are suspected to confer susceptibility to PM in monocots and dicots, respectively (Pessina et al. 2016). There are 1 and five members of durian MLO that belong to Clade IV and V, respectively. Those MLOs were clustered together with MLOs that have been linked to susceptibility to PM such as AtMLO02, AtMLO06, and AtMLO12 (Appiano et al. 2015a). It has been shown that Clade IV and V are complimentary for PM resistance (Appiano et al. 2015a). Interestingly, members of Clade IV and V have shared motif in C-terminal (Motif 20). This motif is predicted to be associated with signal transduction and response to stimulus (Table 3).

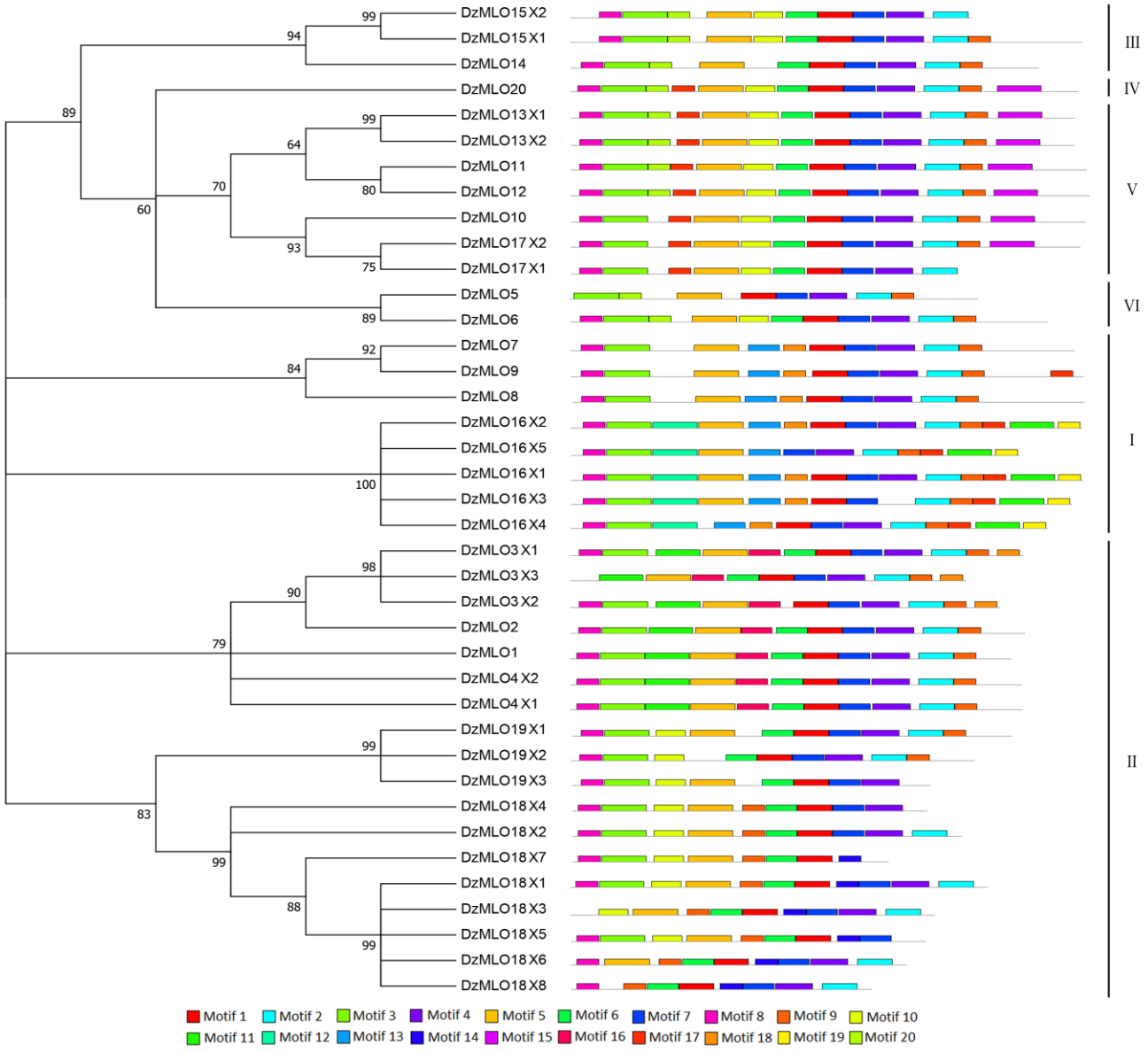

Figure 3. Motifs distribution in DzMLO protein family. Different motifs are colored differently and numbered from 1 to 20. All motifs were identified using the MEME algorithm. The accompanying dendogram of DzMLO protein was constructed using MEGA 5 (Tamura et al. 2011)

Table 3. Gene ontology prediction of several protein motifs found in DzMLO Clades IV and V

Motif	Clade	DzMLO	Location	Top GO Term
6	II-VI	1-6, 10-15, 17-20	IC2	Localization, Transport (nitrogen compound, ion, organic substance)
10	II	18 (X1-X5, X7), 19	EC1	The nucleic acid metabolic process, transposition, cellular response to DNA damage
	III	(X1-X2)	IC2	
	IV	15	IC2	
	V	20	IC2	
	VI	10, 11, 12, 13, 17	IC2	
		6		
13	I	7, 8, 9, 16 (X1-X2)	IC2	Regulation of cellular process (cellular component organization, organelle organization, cell cycle process)
15	IV	20	CT	Transposition, DNA integration, cell communication (signal transduction, response to stimulus)
	V	10, 11, 12, 13, 17 (X1)	CT	
17	I	9, 16	CT	Regulation of biological process (phosphorylation, intracellular signal transduction), localisation (macromolecule), transport (organic substance), regulation of cellular process
	IV	20	EC1	
	V	10, 11, 12, 13, 17	EC1	
20	III	14	EC1	Cellular component organisation, regulation of gene expression, chromatin organisation
	III	15	EC2	
	IV	20	EC1	
	V	11, 12, 13	EC1	
	VI	5	NT	
	VI	6	EC1	

Certain motif related to response to the stimulus was also observed by Kusch et al. (2016) in C-terminal of Clade V. As C-terminal plays a role in MLO functionality (Elliott et al. 2005), the motif might explain the overlapping function of Clades IV and V in PM disease. Elliott et al. (2005) also showed the importance of IC2 in MLO proteins. Domain swap between HvMLO and TaMLO-B1 in IC2 and IC2 + CT domain impaired MLO functionality, demonstrated by reduced penetration success of powdery mildew fungus. Presence of Motif 10 in IC2 related to cellular transport and DNA damage response might give further insight into MLO role in PM disease.

It is interesting to note that our analysis predicted *DzMLO17*, a member of Clade V, to undergo alternative splicing resulting in a truncated protein. The isoform lacks motifs 9 and 15 that correspond to CaMBD and response to a stimulus, respectively. Apple (*Malus domestica*) has been found to have a natural truncated *MdMLO19* allele that lacks CaMBD. It was hypothesized that such allele would be non-functional and support PM resistance (Pessina et al. 2017). A comparison can be drawn from alternative splicing of plant's resistance (R) proteins that contain C-terminal leucine-rich repeat (LRR) domain. Majority of truncated R protein isoforms lacking LRR domain are presumably unstable and/or lose the protein's autoinhibition ability (Yang et al. 2014). However, truncated R protein might also confer disease resistance by amplifying plant defense response. This might be achieved through intermolecular interactions between truncated and regular R proteins to increase active R protein dimerization. (Yang et al. 2014). Homooligomerisation and C-terminal have both been shown to contribute to AtMLO7 protein activity during pollen tube reception (Jones et al. 2017). Therefore interaction between isoforms to stabilize oligomerization might play a part in MLO activity. However, Pessina et al. (2017) observed that while there were four apple individuals homozygous for *MdMLO19* truncated allele that were very resistant to PM, there was a homozygous apple individual that was very susceptible to PM. Therefore mechanism of MLO role in PM susceptibility is still poorly understood. The identification of alternative splicing events in *MLO* should allow further functional characterization to understand their role in a plant's physiological events (Sablok et al. 2017).

Expression pattern analysis

Mining from transcriptomic short reads showed differential expression patterns among *DzMLO* Clades IV and V (Table 4). *DzMLO20*, a Clade IV member, was not detected in any tissue sample. *DzMLO12* was detected in all tissue samples. *DzMLO10* and *DzMLO17* were detected in leaf, root, stem, and aril 1. *DzMLO13* was only detected in aril 1, while *DzMLO11* was only detected in the root. There was no pattern difference between isoforms of *DzMLO13* and *DzMLO17*.

Table 4. *DzMLO* gene expression pattern

Clade	DzMLO	Leaf	Root	Stem	Aril 1	Aril 2	Aril 3
IV	20	-	-	-	-	-	-
V	10	+	+	+	+	-	-
	11	-	+	-	-	-	-
	12	+	+	+	+	+	+
	13 X1	-	-	-	+	-	-
	13 X2	-	-	-	+	-	-
	17 X1	+	+	+	+	-	-
	17 X2	+	+	+	+	-	-

MLO displays unequal redundancy (Consonni et al. 2006). Presence of *DzMLO12* transcript in all tissue samples might indicate its role as major PM MLO. Higher expression in the absence of PM infection has been analyzed in major PM *MLO* genes such as *A. thaliana AtMLO2* (Chen et al. 2006), tomato *SlMLO1* (Zheng et al. 2016), cucumber *CsaMLO8* (Berg et al. 2017), and rice *OsMLO3* (Nguyen et al. 2016). Those genes are expressed in leaf, root, and flower. *AtMLO2*, *CsaMLO8*, and *OsMLO3* are also shown to be expressed in the stem. This differential expression might contribute in unequal redundancy of MLO in PM disease as observed in *A. thaliana* (Consonni et al. 2006), tomato (Zheng et al. 2016), and cucumber (Berg et al. 2017).

In conclusion, we have analyzed 20 putative *DzMLO* genes encoding 39 putative DzMLO proteins in durian genome. Functional studies, especially on C-terminal and IC2, need to be conducted to elucidate the role of those DzMLOs in PM susceptibility in durian. After characterization of DzMLO as a susceptibility factor for the PM in durian, genome editing can be conducted to produce PM resistant durian. Nekrasov et al. (2017) have successfully produced transgene-free PM resistant tomato using CRISPR/Cas9 to cause mutation in tomato's primary PM MLO, *SlMLO1*. This transgene-free mutant is indistinguishable from natural mutant; therefore resistant durian variety will not be categorized nor regulated as genetically modified organism (GMO). The PM resistant durian can be produced and adapted to meet the fruit demands and promote its competitiveness by reducing chemical fungicide input.

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