Polymorphic Marker Designed from Bioinformatics Sequences Related to Cell Wall Strength for Discrimination of Mangosteen (Garcinia Mangostana L.) Clones Resistant to Gamboge Disorder

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Abstract—Gamboge disorder (GD) or fruit damage by the yellow sap is a major problem in mangosteen. Mangosteen plants varied in the level of GD, from very low or non GD to low, moderate and high GD. However it was difficult to differentiate between GD and non GD plants because evaluation of the disorder is strongly influenced by environment. In this study we investigated the usefulness of primer designed from bioinformatics related to cell wall strength, termed as MCWS, to predict GD. Plant materials used were 28 mangosteen plants selected based on percentage of GD categorized as high, moderate, low and very low or non GD. The result showed that the specific DNA fragments were absent in the high GD accessions. The MCWS marker suggests as a novel polymorphic marker for GD in mangosteen as well as a marker for detect variability in mangosteen as apomictic plant.

Keywords—Bioinformatics, cell wall strength, gamboge disorder, mangosteen, polymorphic marker.

I. INTRODUCTION

MANGOSTEEN is prospective fruit of Indonesia as export commodity. Volume of fresh fruit export increases gradually particularly to European countries, China, Hongkong, and United Arab Emirates [1]. Of the total Indonesian mangosteen production only about 25% are eligible for export. One of the factors causing the low volume of mangosteen exports is fruit damage by yellow gummy, commonly called as gamboge disorder (GD). Losses caused by GD are very serious because the fruit with yellow gummy unfit for consumption or used for processed products. [2] suggested that the GD is a physiological disorder which showed symptoms of yellow fruit aril. One of the factors caused of GD is suspected by the destruction of the epithelial cells that surrounding the yellow latex secretory ducts. The rupture epithelial cell of yellow latex secretory ducts can be seen in the longitudinal section of the mangosteen fruit endocarp [3]. The turgor pressure of the cells contributed to the breaking pressure. Rupture occurs when the combination of cell turgor pressure exceeds the strength of the cell wall [4].

This information suggest that interaction between the cell wall strength and the release of the yellow sap in mangosteen fruits is probably the most important mechanisms of gamboge disorder. Mangosteen trees showed differences in resistance to gamboge disorder both among individuals and locations. Percentage of gamboge disorder of individual plants ranged from very low (0-1%) to very high (up to 69%). Observations at six locations in West Sumatra showed that gamboge disorder varied from 15% to 46% [5]. Reference [6] reported several trees with heavy bearing in Burma and susceptible to gamboge disorder. However it has remained difficult to differentiate between GD and non GD on the basis of the morphological findings. Beside that evaluation of the disorder is strongly influenced by environment. Previous work [5] reported that high rainfall contribute to the high percentage of GD. High turgor pressure induced by high water absorption can directly destruction epithelial cells and also release yellow gummy from the duct. Breaking pressures above turgor pressure were remarkably variable among organisms [4].

The strength of cell walls that responsible for allowing the persistence of GD is also the environmental and genetic interaction. The mechanisms that regulate the mechanical strength and cell wall biosynthesis is complex and requires coordination of a number of metabolic pathways involving gene [7]. Detection of cell wall strength by specific molecular markers could potentially be helpful in overcome GD. It has been accepted that mangosteen exhibits apomictic, where all sibling are believe as similar or uniform genetically. However, according to recent assessment using RAPD [8,9], RAF [10], AFLP [11] and ISSR markers [12] and also morphological traits [13] showed variation that suspected as different genetic. Based on the existence of genetic variation in mangosteen it is possible to develop reliable markers to access the variation and to detect gamboge disorder as well.

In order to enhance and coop with time frame to develop molecular markers, the marker related to cell wall strength was designed from NCBI sequences data base. The
availability of specific markers for cell wall strength provides a useful tool to detect GD in mangosteen. This study aims to 
a) determine the relationship between GD and the cell wall 
strength, and b) obtain specific markers for the detection GD 
in mangosteen.

II. MATERIALS AND METHODS

A. Plant Materials

Plant materials used were 28 mangosteen accessions (Table 
1) from Leuwiwian and Tajur (Bogor, West Java), Tembilahan 
(Riau), Indonesian Tropical Fruits Institute (ITFRI) collection  
(West Sumatera), and Bulukumba, Bantaeng and Manado  
(South Sulawesi). The plants different in gamboge disorder 
percentage categorized as non GD or very low (0-1%), low 
GD (<20%), moderate GD (20-30%) and high GD (>30%).  
An example of mangosteen fruits with GD and non GD 
presented in Fig. 1.

B. Primer Designed

DNA sequences associated with cell wall strength are 
determined by using bioinformatics data base of NCBI  
(National Center for Biotechnology Information) at  
were then analyzed by using online analysis tools consists of  
several software for genome analysis including Genebee  
ClustalW 1.83 and GeneBee BLAST 2.2.8 and Services (http://www.genenec.msu.su). ClustalW analysis is used to  
obtain multiple sequence alignment data and BLAST analysis  
(Basic Local Alignment Search Tools) to determine the  
sequence similarity with the plant cell wall strength or other  
organisms. A pair of primer was designed based on conserve 
region of multiple sequence alignment data. The simple  
method for design of primer sequences are based on [14] as  
follows: 17-28 bases long, G + C composition of 50-60%, 3' 
end is G or C, or CG or GC, melting temperature between 
55-80 °C, and avoid the three or more C or G at the 3' end of 
primer.

C. DNA Extraction, Purification and Amplification

DNA was isolated from 28 mangosteen accessions (Table 
1). About 0.1 mg fresh leaflets were grind for DNA extraction.  
Total DNA was extracted according to the modified CTAB  
protocol [15] by addition 1% of PVPP (polyvinyl 
polypyrrolidone). DNA concentrations were determined with 
electrophoresis in agarose gel, ethidium bromide staining 
solution and visualization on UV transiluminator. DNA was  
then PCR-amplified by using the primer designed from  
bioinformatics sequences in a 96-well Applied Bysystems  
2720 thermal cycler. Reactions were carried out in a total  
volume of 25 μl consisting of 2 μl (20 ng) of template DNA, 
12.5 μl Go Taq Green Master Mix (Promega M7122), 1 μl  
primer (20 μM), and 9.5 μl free nuclease water. Amplification  
was performed under the following conditions: 4 min at 94°C  
for 1 cycle, followed by 0.5 min at 94°C, 0.5 min at 42°C  
and 1 min at 72°C for 35 cycles, and 5 min at 72°C for final  
extension. PCR amplification was repeated twice with similar  
conditions to ensure the stability of the DNA banding patterns 
produced. PCR products were then separated on 1.2% agarose  
gel and 1X TAE buffer solution, stained with ethidium  
bromide and visualized with UV light.

D. Data Interpretation

Genotypic data obtained from the gel compared with a  
molecular weight standard 1 kb DNA ladder. Observations  
made on the level of polymorphism and variability of DNA  
banding patterns produced. Presence or absence of the target  
trait (cell wall strength) in the plant samples estimated by the  
presence of specific bands generated and the degree of GD  

III. RESULTS AND DISCUSSION

A. Primer Design

The results of BLAST analysis obtained five GenBank  
accessions having high homology with the genes related to the  
cell wall strength. The five accessions were AY158083.1 from  
Arabidopsis thaliana kinesin-like protein (FRA1) mRNA
complete cds, NM_180820.1 from Arabidopsis thaliana FRA1 (Fragile FIBER 1); microtubule motor (FRA1) mRNA, complete cds, NM_124156.4 from Arabidopsis thaliana FRA1 (Fragile FIBER 1); microtubule motor (FRA1) mRNA, complete cds, and AY158084.1 from Arabidopsis thaliana kinesin-like protein (FRA1) gene, complete cds [16], and XM_002510131.1 from Ricinus communis Kinesin heavy chain, putative, mRNA [17]. The sequences were then analyzed using ClustalW 1.83 Genebee and the results were used to design a pair of reverse and forward primers associated with the cell wall strength termed as MCWS (Mangosteen Cell wall Strength). Part of multiple sequence alignment of five DNA sequences presented in Fig. 2.

AY158083.1
AAGGTTGCTGTTCACATCAGACCACTCATCGGCGAC
GAAAGGATTCAAGGTTGTCAAGAT
** ***********  ******************* *** * ****** **********

AY158084.1
ATGGGTAACGGCTCTGCGAGCAGACCCAGACTGTG
ATCATCCTGTCAGTTGAAGCCT
XM_002510131.1

AY158084.1
ATGGGCACCGAGATTTAAGATGTTGCCAAACAGGA
ATAATCTCCTAGTCATGAGTTT
****** ** *** * ****** ** ** **********
****** ** **

AY158083.1
CTTTTCAGAATAGAAGCTTGAAGCAGCAGATT
GATTTTCAGATCATGTTTCTTT
NM_180820.1

AY158083.1
GGATCTGTACTGTTGATAAACATAGACACTAAACT
ACTGGACATGGTTGAAGATGAC
NM_180820.1

AY158083.1
CAAGGCTTCAATGGTTTTGGCTTATGGACAAA
CGGTTGCGTAAACACATATACA
NM_180820.1

AY158083.1
CAAGGCTTCAATGGTTTTGGCTTATGGACAAA
CGGTTGCGTAAACACATATACA
NM_124156.4

AY158083.1
CAAGGCTTCAATGGTTTTGGCTTATGGACAAA
CGGTTGCGTAAACACATATACA
AY158084.1

AY158083.1
CAAGGCTTCAATGGTTTTGGCTTATGGACAAA
CGGTTGCGTAAACACATATACA
AY158084.1

AY158083.1
CAAGGCTTCAATGGTTTTGGCTTATGGACAAA
CGGTTGCGTAAACACATATACA
AY158084.1

AY158083.1
CAAGGCTTCAATGGTTTTGGCTTATGGACAAA
CGGTTGCGTAAACACATATACA
AY158084.1

AY158083.1
CAAGGCTTCAATGGTTTTGGCTTATGGACAAA
CGGTTGCGTAAACACATATACA
AY158084.1

AY158083.1
CAAGGCTTCAATGGTTTTGGCTTATGGACAAA
CGGTTGCGTAAACACATATACA
AY158084.1

AY158083.1
CAAGGCTTCAATGGTTTTGGCTTATGGACAAA
CGGTTGCGTAAACACATATAC-
**Fig. 2** Part of multiple sequence alignment of five DNA sequences for cell wall strength based on ClustalW 1.83 software (http://www.genebee.msu.su). The underlined sequences are MCWS Forward and Reverse primers.

**B. Polimorphism of MCWS Primer**

The MCWS primer was amplified successfully, produced 10 DNA bands ranged in size from 200 to 1200 bp. Amplified product consisting of 2 (20%) monomorphic bands and eight (80%) polymorphic bands (Fig. 3). This result inform that mangosteen shows diversity in genetic.
genetic diversity in apomictic plants is the result of point mutation, insertion, deletion, inversion, non-disjunction, somatic recombination by transposon activity, and mutations or chromosomal changes in the maternal genome [18]. Previous study [8,9,10,11,12] have reported the variability of DNA banding patterns in mangosteen, and concluded that the mangosteen varied in genetics.

![Fig. 3 Polymorphisms in 28 mangosteen clones by primer MCWS. * = non GD accessions, ** = high GD accessions](image)

C. Relationships between Cell Wall Strength and Gambose Disorder

The polymorphism of MCWS primer indicated that the mangosteen not only has a diversity in genetics but also in the strength of the cell wall. The genetic diversity of cell wall strength has also been found in barley (Hordeum vulgare L.) which is known from the differences of its brittleness. Cell walls of brittle culms had 6 to 64% as much cellulose content as those of nonbrittle culms. Maximum bending stress correlated significantly with cellulose content of the cell walls (r=0.93), but not with the contents of noncellulosic compounds. The lower cellulose content of the brittle culm was significantly correlated with brittleness [19].

Among the polymorphic bands generated by MCWS primer there were two specific bands about 1200 and 1000 bp in size. The two DNA fragments present in non-GD, low and moderate GD accessions and absent in two high GD accessions (B2 and BT). Based on this result can be assumed that the two DNA fragments associated with the cell walls strength of mangosteen and suggest as a putative marker for mangosteen accessions resistance to GD. Accessions B2 and BT is suspected to have a weak cell wall indicated by the absence of 1200 bp and 1000 bp and the high percentage of GD. Accession T14 does not have a 1200 bp band but has a 1000 bp band with a moderate level of GD. This data suggested that 1000 bp fragment contribute more to the the cell wall strength.

The strength of plant cells is important traits to prevent the GD in mangosteen. Mechanisms regulated the mechanical strength and cell wall biosynthesis is very complex and requires coordination of a number of metabolic pathways that involved genes. Reference [16] showed that the cellulose content correlated with mechanical strength of the plant body. Fragile FIBER1 (FRA1), a kinesin-like protein, is essential for the deposition of cellulose microfibril orientation and cell wall strength. Gen FRA1 encodes kinesin-like protein with the N-terminal microtubule binding motor domain. Protein FRA1 concentrated around the periphery cytoplasm but absent in the nucleus. Based on these findings concluded that the kinesin-like protein FRA1 involved in the microtubule control of cellulose microfibril. FRA1 showed dominant role in the orientation of cellulose microfibril deposition during secondary wall thickening of fiber cells. Since the MCWS primer designed from sequences associated with the sequences involved in cell wall strength, it is possible to use the primer in a suspected cell wall strength and gambose disorder in mangosteen.

The interesting thing of this result is that there were several accessions having both the two bands but showed a different response to GD including non-GD, moderate GD and low GD. This is presumably due to the possible role of cell wall strength in response to unfavourable environmental conditions. Environmental factors that influence the strength and structure of the cell wall will determine the level of GD. Previous study [5] revealed that GD arise when stimulated by high intensity and fluctuation of rainfall and low soil Calcium content. Reference [20] stated that environmental conditions such as temperature, humidity, shade, and irrigation water salinity greatly affect the hardness of the fruit as a potential effect of cell wall structure and integrity. Other factors include the synthesis and metabolism of cell wall polysaccharides such as pectin. Increase in pH will stop the extension of the cells through the inhibition of protein and activation of enzymes involved in the relationship between cell wall by other factors.

Polymorphic marker for GD, here termed MCWS (Mangosteen Cell wall Strength), was detected in mangosteen accessions. The fragments 1200 bp and 1000 bp correspond to putative markers for cell wall strength and non GD to moderate GD accessions. Thus this study presents MCWS as a novel polymorphic marker for the cell wall strength as well as a marker for detects GD in mangosteen. The MCWS primer potentially is used as a tool for the detection of genotypes that are easily damaged by GD. This is the first study to differentiate GD from non-GD mangosteen, and as a marker of the severity of cell wall strength in causing GD on mangosteen fruits. The next activity needed is the sequencing analysis of the predicted fragments to determine its DNA sequence and then designed to more specific primers.

IV. CONCLUSION

Gambose disorder in mangosteen fruits associated with the cell wall strength, indicated by the absence of two specific bands, about 1000 and 1200 bp in length, for high GD accessions by MCWS primer designed for cell wall strength. This results represent the initial information linkage between molecular markers specific to cell wall strength on mangosteen and its potential to be developed as a new approach for the identification of mangosteen accessions sensitive to gambose disorder.

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REFERENCE