International Journal on
Advanced Science
Engineering
Information Technology

Vol.3 (2013) No. 1
ISSN: 2088-5334

The Research for Supporting Sustainable Mangosteen (*Garcinia mangostana* L.) Production

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***Abstract*— Mangosteen has already given the highest contribution to Indonesian fruit export value in the recent ten years. Therefore, this commodity should be developing into commercial scale. This plant has many benefits, especially for health, processed food and cosmetics industries. Problems encountered in developing and marketing mangosteen are slow growth, poor quality of fruit by scars on fruit skin, and gamboge disorder on fruit skin and flesh. To support the sustainable production of mangosteen Indonesian Agency for Agricultural Research and Development (IAARD) has conducted a series of research to overcome the problems through safety, healthy and eco-friendly ways. The results including: technology to accelerate the growth of mangosteen through CO2 manipulation and the use of mycorrhizal fungi; eco-friendly technology to control fruit skin scars; the controlling of gamboge disorder through fertilization and irrigation, in which mangosteen variety is free from gamboge disorder.**

***Keywords*— Mangosteen; Production; Research; Sustainable**

I. INTRODUCTION

Indonesia is the second mangosteen producer and exporter country in the world after Thailand. In 2009 Indonesian mangosteen harvest area was about 12,000 ha with the production of about 105 558 tons [1]. Indonesian mangosteen production centers mainly located in Java and Sumatra with harvesting area of approximately 9.352 ha [2]. The highest harvesting area is West Java (1,471 ha), followed by West Sumatra (1.420 ha), East Java (752 ha), Bengkulu (728 ha), Banten (706 ha), and North Sumatra (669 ha). The productivity of each location is varied from 5 to 10.7 ton/ha [3] As export commodity Indonesian mangosteen have been shipped to 40 countries mainly to Taiwan, Hong Kong, Malaysia, Singapore, China, Saudi Arabia, United Arab Emirates, Netherlands, and Germany [4]. The largest Indonesian mangosteen importer countries are China and Hong Kong [5]

The problem in cultivating mangosteen is very slow growth of plant and it takes about 8-12 years to begin fruiting, depending on soil fertility and plant maintenance. The slow growth of mangosteen is caused by poor root system and the low photosynthetic rates. The lateral root is not complemented by root hairs which are needed for water and nutrients absorption. Low photosynthetic rate is caused by the low capacity of mangosteen leaves in capturing carbon dioxide (CO_2) [6],[7],[8]

The problem in marketing of mangosteen is the low percentage of eligible export fruits. From the total of Indonesian mangosteen production is less than 25% of fruits that fullfill export standard criteria. These conditions are caused by the existence of scars on fruit skin and fruit which is damaged by yellow gummy, commonly called as gamboge disorder (GD). Losses caused by scars and GD are very seriously, since the fruit with scars is unattractive and with GD is unfit for consumption and being processed. To overcome these problems as well as to support the sustainable mangosteen production IAARD has been conducting a series of research activities. The objective of the research program is to increase quality and productivity of mangosteen, in order to have competitiveness, to boost farmers' income and export as well.

II. RESEARCH FOR SUSTAINABLE MANGOSTEEN PRODUCTION

A. Technology to accelerate plant growth

1) Application of mycorrhizal fungi to accelerate mangosteen seedlings growth

The use of mycorrhizal fungi is intended to overcome the slow growth of mangosteen which is caused by poor root

system. Mangosteen, known as, the plant without root hairs that leads to poor of water and nutrient uptake. Arbuscular mycorrhizal fungi (AMF) is one of the obligate symbiotic fungi which is known to have beneficial effects for plant growth. The mycorrhiza makes a symbiotic with plant roots to enhance nutrient uptake, stimulate growth and improve plant resistance to drought and soil pathogens [9],10],[11].

An application of mycorrhiza was conducted through inoculation of about 100 mycorrhizal spores on 2 months of age of mangosteen seedling (Fig 1). The research was arranged on Randomized Block Design with eleven treatments and three replications. The treatments are without mycorrhiza (control), mycorrhyzal inoculums originated from 50 Kota District, Agam district, Sijunjung District, Padang, mixed inoculum (*Glomus manihotis, etunicatum Glomus, Gigaspora margarita, Acaulospora tuberculata), Glomus manihotis, Glomus etunicatum, Scutellospora heterogama, Acaulospora tuberculata* and *Gigaspora rosea*. The variables observed were plant height (cm), number of leaves (pieces), stem diameter (mm), plant dry weight (mg), root dry weight (mg), root infection (%), nutrient uptake (mg/plant) of Nitrogen (N) and phosphorus (P).

Fig 1. Mycorrhizal inoculation on 2 months old of mangosteen seedling (100 spores / plant)

The result showed that indigenous AMF from Padang and Sijunjung district could accelerate the growth of mangosteen seedlings to 50% faster than the control. Mangosteen seedlings which were inoculated with AMF from Padang and Sijunjung showed the best growth. The plant height about 33.08 cm and 31.29 cm, number of leaves 19.84 and 19.90 , and dry weight of about 61.96 g and 62.63 g at 19 months old. The seedlings without mycorrhiza (control) showed the lowest growth by 21.13 cm height, 15.88 leaves, and dry weight 35.64 g (Fig 2). The seedlings with mycorrhiza from Padang and Sijunjung also show the better root dry weight, 26 and 28 g respectively. The mycorrhizal from Padang and Sijunjung could increase N and P nutrient uptake better than other treatments (Fig 3). The nutrient uptake of mangosteen seedlings which were inoculated by mycorrhizal from Padang and Sijunjung were 387.85 and 82.72 mg / plant for N and 151.45 and 138.07 mg/plant for P respectively [12].

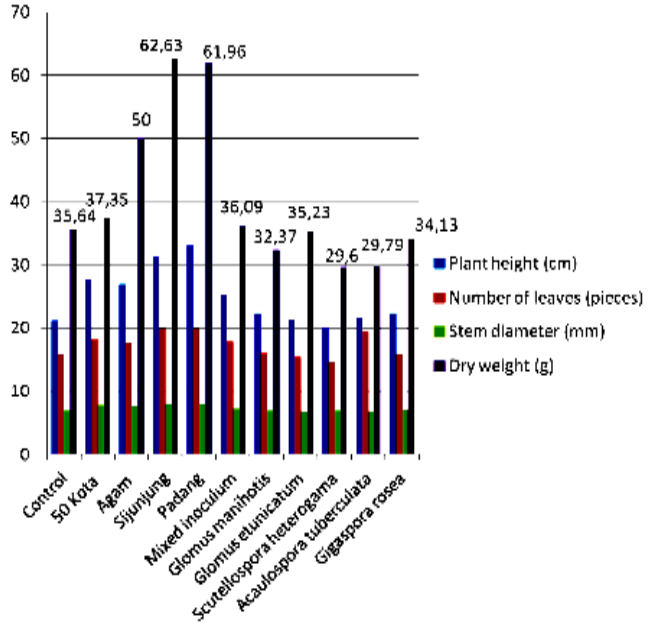

Fig 2. Plant height, number of leaves, stem diameter and dry weight of 19 months old mangosteen seedlings treated by different types of mycorrhizal inoculum

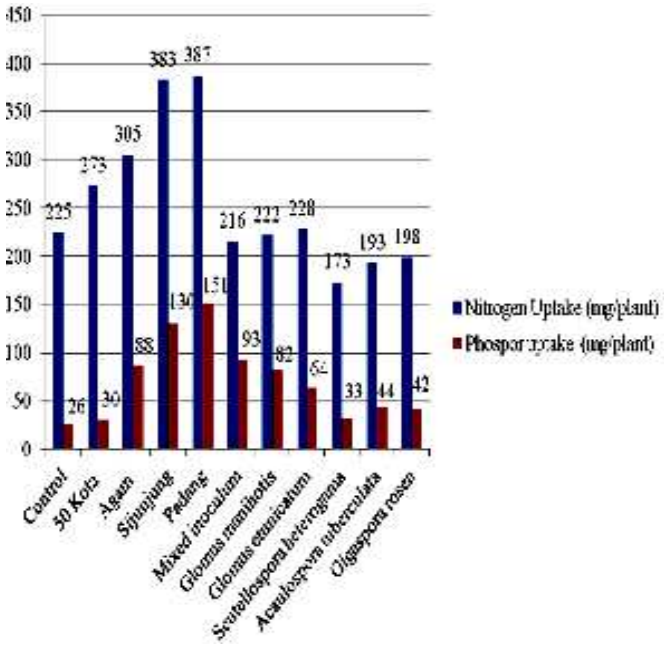

Fig 3. N and P nutrient uptake of 19 months old mangosteen seedlings treated by different types of mycorrhizal inoculum

The comparison of the growth of mangosteen seedlings inoculated by mycorrhizal from Padang and control is presented in Fig 4. Mangosteen seedlings inoculated with mycorrhiza from Padang has emerged first lateral branch faster than control. By this treatment the mangosteen seedlings emerged the lateral branch after they have nine pairs of leaves at 16 months old, while the control still have 8 pairs of leaves without lateral branch. Normally mangosteen seedlings emerged first lateral branch after 13 pairs of leaves (26 months old) this information suggests that the mycorrhyza from Padang could accelerate the growth of mangosteen seedlings to reach a suitable condition for

transplanting in the field. The prerequisite of good mangosteen seedlings for transplanting in the field is when it's height reach 30 to 50 cm and has at least one lateral branch.

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Fig 4. The comparison between mangosteen seedling with mycorrhiza from Padang (left) and without mycorrhiza (right) at 19 months old .

2) Enriching mangosteen seedlings environment using Carbondioxide (CO2)

Reference [13] reported that mangosteen plant grows naturally in the field has low photosynthesis rates, from 1.0 to 4.8 µmol m-2. s-1, which is associated with slow growth rates of this species. In general, tropical fruit crops have higher photosynthetic rate, from 10 to 20 µmol m-2. s-1.

Enriching the mangosteen seedlings environment by using CO_2 is intended to increase the rate of photosynthetic in plants. In this study we used straw as sources of CO_2. The research was arranged in a Randomized Block Design with 3 treatments and 4 replications. Plant materials used were two month olds mangosteen seedlings The three treatments are 1) Control 2). Mangosteen seedlings covered by plastic box, and 3) mangosteen seedling with straw mulching covered by plastic box

The results indicated that mangosteen seedlings grown under environmental conditions enriched with CO_2 from straw and covered by plastic box showed the best growth, followed by treatment of the plastic box only (Fig 5). Plant heights, leaf number, stem diameters and dry weight of both treatments was significantly different from the control. In addition, the seedlings based by straw and placed in plastic box, in which the lateral branch raised faster than the two other treatments that only have 8 pairs of leaves (Fig 6). This condition caused by their environment contains a higher CO_2 concentation which can increase the rate of photosynthetic of plants. Analysis of CO_2 content in the plastic box was much higher, at 78.17 mg CO_2/m3and 144.32 mg CO_2/m3, than CO_2 content at the outer plastic box which is about 6.01 mg CO_2/m3 [14]. Reference {7} reported that photosynthesis and growth of mangosteen at nursery can be greatly increased 60–80% if supplementary carbon dioxide is provided.

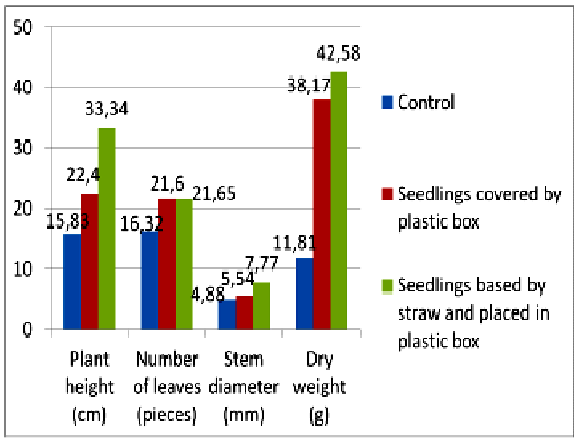

Fig 5. Plant height, number of leaves, stem diameter and dry weight of mangosteen seedlings grown in different environtmental conditions.

Fig 6. Mangosteen seedlings grown in different environtmental coditions: a. Control; b. Covered with plastic box; and c. Straw mulched and covered with plastic box

B. Improvement of fruit quality

1) Controlling yellow latex (Gamboge disorder) on mangosteen fruits

Gamboge disorder (GD) or fruit damaged by the yellow latex is a major problem in mangosteen**.** The previous research showed that yellow latex on mangosteen could be differentiated as GD at outer part of fruit skin (exocarp) and inside fruit (endocarp and fruit flesh) (Figure 7). Reference [15] and [16] reported that there was no correlation between GD on exocarp and endocarp.

Fig 7. Gamboge disorder on mangosten: (a) on fuit skin (exocarp); and (b) inside fruit (endocarp and flesh)

Reference [17] suggested that the GD is a physiological disorder which showed symptoms of yellow fruit aril. One of the factors caused by GD is suspected by the destruction of the epithelial cells 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 [18]. The turgor pressure of the cells contributes to the breaking pressure. Rupture occurs when the combination of cell turgor pressure exceeds the strength of the cell wall [19]. This information suggests 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.

Environmental factors that influence the strength and structure of the cell wall will determine the level of GD. The previous study [18] revealed that GD inside fruits arise when it is stimulated by high intensity and fluctuation of rainfall and low soil Calcium content. GD in endocarp was positively correlated to the rainfall and relative humidity in which the higher the rainfall and relative humidity, the higher percentage of GD in endocarp. This is presumably due to the possible role of cell wall strength in response to unvafourable environtmental conditions.

Based on the previous results a further research to reduce yellow latex on mangosteen was done through application of continuously drift irrigation during fruit season and being combined with application of N, P, K, Mg and Ca fertilizer. The research was conducted at the farmer orchard in Lima Puluh Kota District, a mangosteen production center of West Sumatra Province. Factorial experiment in a Randomized Block Design with 2 factors and 3 replications was used in this study. The first factor was kind of irrigation (without irrigation and with irrigation) and the second factor was a kind of fertilizer (without fertilizer, NPK fertilizer, NPK+Cal (1000 g Urea + 500 g SP-36 + 1000 g KCl + 1000 g $CaCo_3$), NPK+Ca2 (1000 g Urea + 500 g SP-36 + 1000 g KCl + 1500 g $CaCo_3$), NPK+Mg+Ca1 and NPK+Mg+Ca2. Continuously water drift irrigation was applied at early flowering stage until harvesting time and fertilizer was given at early flowering stage. The variables observed were percentage of GD at exocarp and endocarp.

a. Gamboge Disorder on fuit skin (exocarp)

The result indicated that percentage of GD on exocarp was not significantly influenced by the treatment of irrigation and fertilization [20] The percentage of GD on exocarp was presented in Fig 8. Reference [16] reported that GD on exocarp was caused by pest, since the fruit skin that has latex owning small hole that seems to be made by insect bite. Based on this information it was known that the release of yellow latex on fruit skin caused by mechanical damage and insect bites. It is strengthened by the data showed that the GD on exocarp showed positively correlation with scars which is confirmed that GD in exocarp was caused by insect bite.

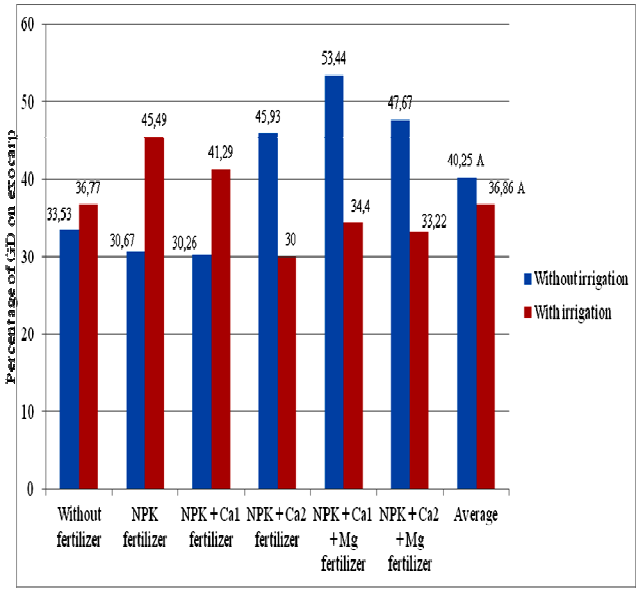

Fig 8 Percentage of GD on mangosteen fruit skin treated by combination of fertilization and irrigation

b. Gamboge Disorder (GD) inside fuit (Endocarp and flesh)

The highest percentage of GD in mangosteen fruit endocarp was found on the plants without fertilization and irrigation of about 48, 87% the combination of irrigation and NPK+Ca2 fertilizer gave the best result with the lowest percentage of GD about 16.9%. There was an interaction effect between application of continously drift irrigation and fertilization on percentage of GD on fruit endocarp. In general application of continuously drift irrigation during fruit development phase untill harvesting time can reduce percentage of GD in mangosteen fruit endocarp from 33,02% to 22,37% [20]. Percentage of GD on mangosteen fruit skin treated by combination of fertilization and irrigation was presented in Fig 9. This result is in accordance to [21] revealed that application of continously drift irrigation during fruit development stage significantly decreased percentage of GD on mangosteen endocarp.

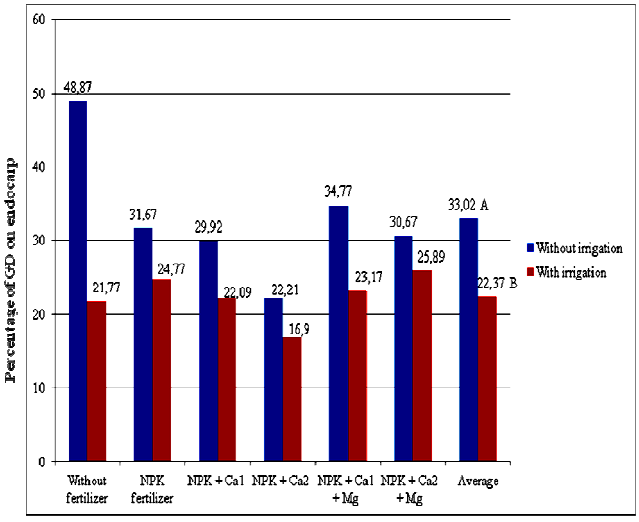

Fig 9. Percentage of GD inside fruit treated by combination of fertilization and irrigation

2). The control of Scar on mangosteen fruits skin

The other prerequisite needed for mangosteen export markets is a good performance of fruit that is free from scars. The existence of scars on fruit skin causes unattractive appearance and not eligible to export (Figure 10). The previous research [22]. reported that the scars caused by thrips (*Scirtothtrips sp*.) and mites (*Tetranychus spp*.). Thrips began attack from flower buds phase until harvest time. Increasing relative humidity and temperature could be increasing the trips populations. Mites can attack the leaves, flower buds and fruits yielding spots or scars on the surface of the skin. There were two species of thrips associated with mangosteen fuits, *Scirtothrips dorsalis* (Hood) and *Selenothrips rubrocintus* Giard. [23]

Fig 10. Scars on mangosteen fruit skin

The research to control scar was conducted at a farmer's mangosteen orchard at Lima Puluh Kota, West Sumatra. The mangosteen tree was planted in a polyculture system with cacao and coconut. The mangosteen trees are 10-15 years-old with approximately of 4-7 m height.. Four treatments studied were SNT (sanitation), YST (Yellow Fluorescent Sticky Trap), combination of SNT+YST, and control.The experiment of sanitation (SNT) and (YST) application was designed in a Completely Randomized Design with six replications, and replicated three times. The sanitation was applied by removing all weeds under the canopy of mangosteen trees followed by soil tillage and fogging. The sticky trap was nailed on woody sticks and placed on 3 m above the ground of four different positions around the tree canopy. Variable observed were the percentage and intensity of scars and number of thrips caught on the sticky traps.

The results showed that application of SNT, YST, and SNT+YST were effective to reduce intensity and percentage of scars (Figure 11). The combination of SNT+YST demonstrated the best results in reducing the scar intensity from 100% to 58,19% and scar percentage from 41,93% to 9,79%. The present study implies the importance of sanitation, and sticky trap application in protecting mangosteen fruits from scars [23].

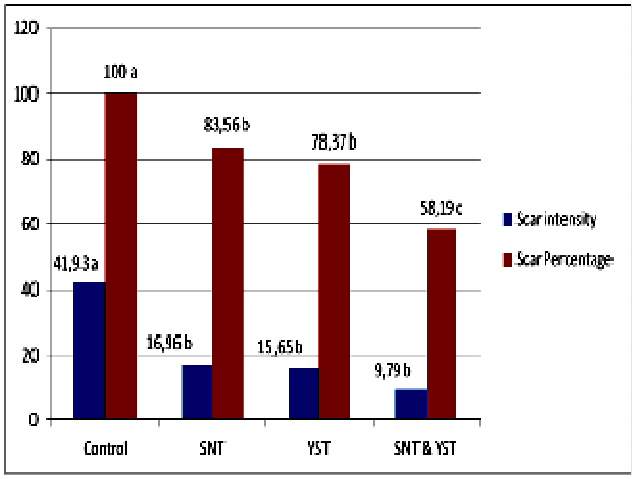

Fig 11. Intensity and percentage of scars on mangosteen fruits treated by sanitation (SNT), Yellow Fluorescent Sticky Trap (YST), and combination of SNT+YST

C. Variety improvement

a. 1). Selection of mangosteen superior genotype that free from Gamboge Disorder

Mangosteen trees show differences in resistance to gamboge disorder both among individuals and locations. Percentage of gamboge disorder of individual plants are ranged from very low (0-1%) to very high (up to 69%) observations at six locations in West Sumatra. Reference [24] reported that 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.

The efforts to improve the quality of mangosteen can be done through the use of appropriate ideotype varieties without yellow gummy in its flesh. In 2007, IAARD has released non GD variety, Ratu Tembilahan, derived from Pulau Palas, Indragiri Hilir District, Riau Province. Unlike most other mangosteen that is generally found on dry land, the mangosteen is growing at a specific location of tidal swamp area. This mangosteen has unique characters that are not usually found in the mangosteen in general. The characters are the number of segment, ranging from 5 to 11, elliptical stigma lobes shape, and a short stalk [25]. The advantage of this variety is free from GD, especially inside of the fruit. Morphology and stability properties non GD traits of the Ratu Tembilahan were, then observed and evaluated during five fruiting seasons, from 2003 to 2007. The result showed that this variety consistent free from GD of inside fruits. Morphological and genetic differences between Ratu Tembilahan variety compared to Ratu Kamang variety was presented in Fig 12 and 13.

Fig 12. Morphological differences between free of GD mangosteen variety (Ratu Tembilahan) and the common variety (Ratu Kamang)

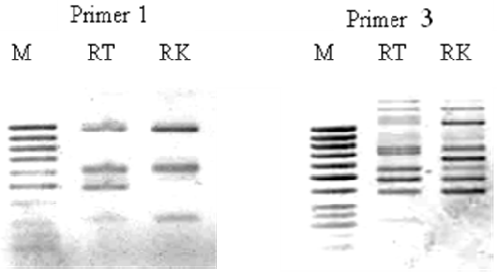

Fig.13. DNA banding patterns differences between free of GD mangosteen variety (RT) and the common variety (RK) by Primer 1 (GGTGCGGGAA) and Primer 3 (GTAGACCCGT).M= Marker

2). Application of molecular marker for discriminating GD and non GD mangosteen accessions

It has been accepted that mangosteen exhibits apomictic, where all sibling are believed as similar or uniform genetically. However, according to recent assessment using RAF (Randomly Amplified DNA Fingerprinting) [26], RAPD (Random Amplified Polymorphysm DNA [27], [28], AFLP (Amplified Fragment Lenght Polymorphysm) [29} and ISSR (Intersimple Simple Sequence Repeat) [30] and also morphological traits [31] 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.

The previous work [16] 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 [19]. The strength of cell walls that responsible for allowing the persistence of GD is also the environtmental 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 [32]. Detection of cell wall strength by specific molecular markers could potentially be helpful in overcoming GD

In this study, we investigated the usefulness of primer designed from bioinformatics or NCBI sequences data base related to cell wall strength. The primer termed as MCWS. This study aims obtain specific markers for the detection GD in mangosteen. 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 the *MCWS* primer was amplified successfully, produced 10 DNA bands ranged in size from 200 to 1200 bp. Among the bands, specific DNA fragments about 1000 and 1200 bp in length, were absent in the high GD accessions . (Fig 14). 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 [33].

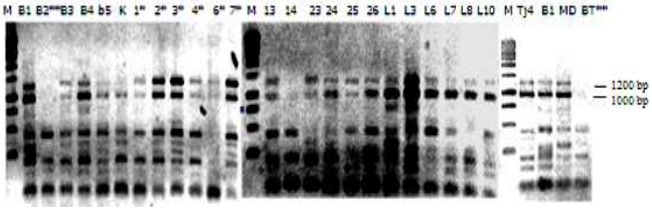

Fig 14. Polymorphisms in 28 mangosteen accessions by primer MCWS. *= non GD acessions, **= high GD accessions

This results represent the initial information linkage between molecular markers specific to GD on mangosteen and its potential to be developed as a new approach for the identification of mangosteen accessions sensitive to gamboge disorder.

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