Harumanis Mango: Perspectives in Disease Management and Advancement using Interdigitated Electrodes (IDE) Nano-Biosensor

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Abstract. The agriculture sector plays an important role in economic development in providing rural employment, uplifting rural incomes and ensuring national food security. Harumanis mango has been predominantly produced in the Perlis state of Malaysia, and it is the seasonal once-a-year. However, plant diseases became a serious issue that significantly affects both the quality and quantity of Harumanis mango production. The primary problem with Harumanis mango has experienced by farmers is a disease and the farmers are failed to complete the process of post-harvest for Harumanis mango. Further, in the case of Harumanis mango disease, the symptoms are often detected at the later stage, which makes it difficult to take any further action. Usually, the diseases are controlled by fungicides and the use of synthetic chemicals to eliminate the postharvest diseases and deterioration has their limit due to carcinogenicity, teratogenicity, environmental pollution, effects on food and other side-effects on humans. Up to date, the control of Harumanis mango disease is still challenging, although several studies have been conducted to eradicate and prevent its outbreak in the field. This overview is about Harumanis mango and the prospect in preventing a serious outbreak with Harumanis mango production using interdigitated electrodes (IDE) nano-biosensor.

1. Introduction
For several decades, the agriculture sector has been the backbone of the country’s economy by producing agricultural products for domestic consumption, as the earner of foreign exchange [1]. This sector has contributed significantly being recognized as the third engine to support the economic growth for the countries like Malaysia [2]. Therefore, agriculture sector should continue to sustain for the economic
development of the country [3]. In this flow, counties like Malaysia experience a tropical climate that undergoes hot and humid weather throughout the year. Hence, this weather allows for a wide variety of tropical fruits to grow. Among the tropical fruits that existed in Asian countries are durian, mangosteen, rambutan, papaya, starfruit, guava, ciku, banana and so on [4]. Apart from that, Harumanis mango (Magnifera Indica cv. Harumanis) also becomes the most popular fruit plants. It has a tremendous commercial demand and is growing rapidly in the northern state of Perlis, Malaysia. The uniqueness of the land Perlis has made Harumanis mango in the best Perlis introduced to other parts of the world.

Harumanis mango is a special tropical species breed, grows only in Perlis, the smallest Malaysian state near the Malaysia-Thai border. Harumanis mango is an ellipse and has tapered ends, the orchards are mainly found in Bukit Bintang, Paya Kelubi, and Chuping. The base of the fruit is a red-violet colour, but in another part is green. The skin is thick and covered with the wax layer, it has a thick pupil with yellow colour, soft, no fibre, and not contained much water. This fruit is sweet and fresh, but small of the part feels a little bit acid. The uniqueness of the land Perlis has made Harumanis mango acts as iconic and make the best Perlis variety introduced to the outside of the world [5].

Plant disease outbreak continues to be one of the most vital issues globally especially in the agriculture-based countries [6]–[9]. The biggest problem with Harumanis mango frequently faced by farmers is the lack of fruits and mango fruit caused by a known disease, called Anthracnose and the stem-end rot disease. Anthracnose and stem-end rot diseases are considered the major problems in storage and shelf-life of mango fruits. The diseases are controlled by fungicides such as benomyl, carbendazim or propiconazole [6]. The use of synthetic chemicals is to control postharvest diseases and deterioration has their limit due to carcinogenicity and other side-effects on humans. Up to date, the control of Harumanis mango disease is still a challenge, although many studies have been conducted to eradicate and prevent its outbreak in the field. As a result, a monitoring system is essential to provide and prevent a serious outbreak of Mango Harumanis production in Perlis.

1.1 History of Harumanis in Malaysia

Harumanis mango has been introduced by MARDI in 1980, also known as the MA 128. In the beginning, the Harumanis mango clone (MA 128) came from Sungai Nibong, Kuala Selangor, was registered on 28 May 1971 in Selangor, however, it was believed to be initiated and import from Indonesia before being planted in Malaysia [11]. The cultivation of these varieties has been practised in various states of Malaysia. At the beginning of the cultivation, these varieties show a good growth rate, but the yield is lesser. It was found that the cultivation of these verities was only successful in the state of Perlis with excellent production. Thus, Harumanis mango was launched in all cities of Perlis beginning in 1982, where the fruit clone was introduced by the Perlis Department of Agriculture (DOA) through the Input Subsidies and Diversification Program (AID Plan) under the Federal Government's allocation in the 2nd Malaysia Plan (RM2). From 1979 to 1982, the Grove Rehabilitation Project was introduced for the development of agricultural land in the village, where the unproductive trees such as rubber trees are cut down and replaced by the fruit crops with Harumanis mango. In addition to that, to support the Harumanis mango industry development, Perlis DOA has opened its Harumanis mango farm at the Bukit Temiang Agricultural Complex and the Bukit Bintang Agricultural Center as a demonstration farm for the farmers and visitors to gain knowledge and references regarding on Harumanis mango plantation [7].

1.2 Overview on Harumanis mango Production

The development of Harumanis mango industry in Perlis is regarded as Perlis iconic agro-tourism product. It is due the attractive nutritional value, the production, market, and consumption of Harumanis mango. This fruit has a high economic demand not only for the domestic consumptions but also has potential for Malaysian export business especially the Perlis State. Based on the report Perlis State Agriculture Department It was a big success when in 1989, Perlis first imported Harumanis mango about 1 ton to Hong Kong. In 2010 Harumanis mango has also penetrated the Japanese market where successful export with 3.1 metric tons [13]. The current market price of Harumanis mango can reach 10
USD per kilogram, and this price is very high compared to other types of mango. Based on fruit crops statistic of Malaysia, there was 17,429.7 metric tonne of production with worth more than RM57 million where 5,816.4 hectares of mango cultivation in Malaysia including several varieties of mango grown in Malaysia such as Golek (MA 162), Masmuda (MA 204), Maha 65 (MA165), Chok Anan (MA 224), Nam Dok Mai (MA 223), Sala and Harumanis [14].

Until now, Harumanis mango production has been increased to accommodate a high demand both within inside and outside the country. As a result, Harumanis mango is among the mango varieties that are popular among Malaysians. Based on Federal Agricultural Marketing Authority (Fama), the total area of the Harumanis mango crop that has been producing in the state is 745 hectares, while the remaining 260 hectares of area involves new crops [15]. The Harumanis mango is only available once a year in the season is the best mango in terms of shape, the texture of content, taste, and demand in the market. Limited only north of the homeland, especially in Perlis, the Harumanis mango is not to be found in other parts of Malaysia but the long hot weather factors in the north promise the best fruit quality. As with its title, Harumanis mango has a fragrance that is so aromatic when it rips and its sweet content due to the less rainfall distribution factor besides the excellent sunshine there.

1.3 Texture
Ripen mango has the texture of smooth, firm flesh with no fibres. After a few days, the texture becomes fibrous. However, the texture of Harumanis mango is varying with varieties. Some of them have soft and juicy flesh with fibres texture during the ripening season. Harumanis mango has a thick skin and has a layer of wax on the surface of the fruit. It has thick yellow pulp, no fibre, and soft and contains little water in the fruit. Figure 1 shows the texture of Harumanis which has a green surface skin followed by the yellow flesh and seed at the middle of mango fruit [11].

![Figure 1](image1.png)

Figure 1. The texture of Harumanis mango has a green surface of the skin, followed by the yellow flesh and seed at the middle.

1.4 Appearance and Colour
Harumanis mango has a dark green background colour when developing on the tree that turns lighter green to yellow as it ripens. Some varieties develop a red background colour at fruit set that remains until the fruits ripen. Moreover, another kind of mango also has an orange, red, or burgundy blush that develops later in the fruit development when the skin is directly exposed to sunlight. Harumanis mango has vibrant green skin with large, light-yellow dots. The fruits still present with green colour although the fruit has ripe. Figure 2 shows the different colour of Harumanis mango at week 8 day 1 to day 7. It is still maintained in green colour after 7 days [16].
1.5 Shape and Size

*Harumanis* mango can be many shapes such as round, oval, heart-shaped and kidney-shaped. For the average of mango, it can be less than 50 g (0.35 lbs.) to over 2 kg (4.4 lbs.) (Shapiro, 2012). While for the total length of mango, the range is between 2.5 cm to 30 cm or more than 30 cm, depending on the cultivar, vary with the shape. *Harumanis* mango has a taper at the ends and ellipse in shape. It has small seed with the length between 13 –14 cm, but the fruit’s length is 15 cm with weight 300g- 650 g per piece. Figure 3 shows the size of *Harumanis* mango for 48 days and 56 days [12,13].

1.6 Identification of Harumanis mango Maturity for Harvesting

Harvesting the mature fruits without any damage is critical to ensure a better quality of ripened fruits and to fetch the higher price. When flowering occurs in several branches, not all the fruits will be ready for harvesting at one time. In such a situation, the selective harvesting of mature fruits should be carried out. The identification of mature fruits on the trees is often difficult. However, most farmers used “plant calendars” technique in plucking the fruits where each fruit will be wrapped with paper and marked with color after flowering as shown in figure 4. As the fruit reaches the final stage of maturity in 56-120 days after flowering, the shoulder portion starts bulging forming a depression at the point where the fruit is connected to the stem [14]. This is a reliable test to identify the mature fruit which is ready for ripening. Once harvested the *Harumanis* mango, the fruits were ripened by manually using calcium carbide for 3 days before ready to be market [7].
1.7 Harumanis Growth and Reproductive Phase

Harumanis mangoes are normally ready to harvest from the end of April until the middle of June in Perlis, Malaysia. Normally, they are harvested between 60 until 120 days after blooming or around 8 weeks after the fruits achieve the size of roughly 4 cm in diameter [16]. Hence, the mangoes will be described by personnel trained into six different classes in view of physical size and appearance as shown in table 1.

Table 1: Description of maturity stages and ripeness level of Harumanis mangoes

| Stage | Age            | Description                                                                 |
|-------|----------------|------------------------------------------------------------------------------|
| 0     | Week 0–7       | Pre-mature; Not yet harvested                                                |
| 1     | Week 8 (day 1) | Mature; Can start harvesting                                                 |
| 2     | Week 8 (day 2) | Mature; Right after being harvested, cleaned, warm water treatments, and sorted based on weight and skin cleanliness. |
| 3     | Week 8 (day 6) | Early ripening; Artificial ripening with calcium carbide. Smooth skin, and slightly bleak. |
| 4     | Week 8 (day 7) | Nearly ripe; Smooth, slightly soft skin and having slightly sweet aromas.    |
| 5     | Week 9 (day 1) | Optimum ripeness; Smooth skin, soft and strong sweet aromas                 |

Harumanis mango tree is a seasonal fruit grown in tropical countries and the reproductive phase of the mango trees often to start from January and ends nearly in June. Harumanis mango can be plant in various type of soil but it can produce fruits only location with hot weather between two or three months under maximum temperature up to 30 to 38 °C. Moreover, minimum rainfall of up to 2700 mm a year is enough to induce flowering. Deep soils, lush and high mineral content with appropriate soil pH value of 5.5 to 6.5 and good drainage system help in producing a good fruit quality and only available in the state of Perlis.
Figure 5. Harumanis mango plantation development phases

Figure 5 describe the phase Harumanis mango plant development, which consists of the vegetative growth, pre-flowering, flowering, fruit-bearing and harvest. Usually, the seasonal fruiting plant has the same phases for each cycle but might differ in its specific period depending on the type of plant. The vegetative growth period is approximately from July to December where the period of development between germination and before pre-flowering. During the vegetative phase, plants are a focus on carrying out photosynthesis process and accumulating resources that will be needed for flowering and reproduction [18]. December to January is considered as pre-flowering phases where the flower induction process is stimulated.

This phase requires having a low temperature and a low rainfall for at least two (2) or more weeks periodically to induce flowering where it is call environment stress. January to February month is the period when the flowers grow and bloom. The rainfall should not be too heavy to prevent the flowers from falling. This is difficult to control if the plant is in an open environment. March to April is the fruit-bearing period. In annual crops, botanical fruits and seeds are form during this developmental stage. May until June is the Harumanis mango harvest time. This is the best period of the year to pick the fruits and collect from the Harumanis mango plantation. To ensure that the fruit is ready to be harvest in perfect condition, it should be consider within a specified period. If it is too early or too late when the fruit is separated from the tree (the fruit is taken from the ground) will most likely change the taste and texture of the fruit and will not reach the desired quality [13].

1.8 Harumanis Disease Outbreaks
Harumanis mango diseases become major problem and dilemma for the most farmer in the Perlis due to its will affect the growth and production of quality fruit. Among major Harumanis familiar disease had be describes on table 2 where the infection was occur mainly in tender leaves, stems, flowers and fruit.
### Table 2. Types of Harumanis Disease and Symptoms of Infection

| Types of Disease | Symptoms of Infection | References |
|------------------|-----------------------|------------|
| Anthracnose (Colletotrichum gleosporide) | The symptoms occur on leaves, flower clusters and fruits. The infection on leaves will show brown spots at the beginning to black spots and become severe extensive dead area. While on the flower cluster, the symptoms begin with small black or dark-brown spots then the flowers become dry and fall before fruits are matured thus reducing fruit yield. Lastly, an infected fruit will be appearing before or after matured fruit where the symptoms will start with black small spot and enlarge around the surface of the fruit. | [19] |
| Mango Sooty mould (Meliola mangiferae Eark) | The infections are caused by a parasite such as a mango hopper, scales, coccids and mealybugs where it depends on the honeydew secretions by the parasite which provides a suitable medium for fungal to growth. The symptoms will appear where the leaf surface covered with a black velvety coating. In worst cases, the whole tree turns completely covered with black coating due to the presence of the mould. In dry weather condition, infected leaves become curl and shrivel. | [20] |
| Pink Disease (Cortitium Salmonicolor) | Most farmers scared this disease because it will infect Harumanis mango trees where the infected of this disease mainly occurs at rod and tree branches. It can reduce the ability of trees to produce fruits. The infection will appear where white marked in the branches or groin trees. This thread will be pink and when this layer of will cover spreading the whole skin the fungus then destroyed the entire part of tree branches. | [21] |
| Insidious Fruit Rot (IFR) | The disease is caused by tissue breakdown in the fruit, where it has shown to be high in N and low in Ca. Nothing obvious symptoms were observed from the outer layer of fruits but the contents of the fruit are totally damaged and the fruits are only suitable for producing water juice. | [22] |
| Stem End Rot (Botryodiplodia theobromae) | This problem occurred during postharvest handling due to temperature and relative humidity is not properly maintained and controlled well. The bottom of the Mango will turn into a black casing. Fruit tissue strikes soft and juicy. The fruit is attacked will be completely damaged in time 3-14 days. | [23] |

### 2. Methodology

Our Malaysia government has recognized fruits production as one of the potential industries for the country where the government aimed to increase the high quality of fruits to penetrate the export market in Europe and the Middle East [24]. Therefore, our government will support any program focused on the production of fruits to ensure the high quality and safety fruit is provided to the consumer. However, plant diseases become the most barriers, especially in agriculture-based countries. Lately, plant disease became dilemma to the most farmers due to its effects toward the yield for both quality and quantity of agricultural products and give a vital problem to the economy as alarming to the food security. Controlling plant pathogens are the major challenge not only for farmers but also for the researchers such as difficulty to differentiate between non-pathogenic disorders occurred so that identification of the
cause, problem, and remedies should be defined clearly before being severe. Presently, IDE biosensor becomes one of the promising tools to be used to detect any pathogen occurred for harumanis plantation at early stage. Figure 6 shows the fabrication process of the Aluminium Interdigitated Electrode for biosensor development. First, the thin film was used as a silicon substrate for Al electrodes fabrication (Fig. 6). Deposited of Al coil onto the fabricated thin films was proceed with using a thermal evaporator. Next, the deposited Al metal was deposited by using a conventional lithography process where photoresist process was spin using spin coater at 3000 rpm for 30 seconds and coated on top of the substrate. Lastly, UV light was used to pattern and transfer process from the chrome mask onto the substrate for mass production. As a fabricated is completed, the electrical characterization was carried out by using Keithley and LabVIEW software for future analysis using bare IDE.

3. Results and Discussion
Fabricated IDE or Bare IDE must undergo surface modification for “capture” any pathogens for detection such as bacteria, viruses or fungus. At first, a suitable linker needs to adopt at the surface of the IDE platform sensor.

This linker is acting to speed up the reaction occurred between probe and target where the reading can be detailed after both are completement with each other[25]–[30]. Next, a sensitivity of IDE detection depends on the specific probe that has been used for each measurement where this probe can lock any specific pathogens as a target as shown in figure 7. A significant change in (I-V) was expected after immobilization onto the IDE sensor as shown in figure 8 after measurement occurred.
4. Conclusion
In conclusion, a simple and label-free sensing platform using IDE for detection for any pathogen occurred in Harumanis disease can be utilized not only beneficial to the farmers but also researcher as promising tools for early warning signals in plant disease outbreaks and secure and protect plant health before getting worst.

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