

## Assessment of Carbohydrate Contents in Perlis Harumanis Mango Leaves during Vegetative and Productive Growth

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**Abstract** - This study was aim to assess one of the flower inducing parameter of mango plant for further research and development to produce premium Perlis Harumanis mango in off-season. The carbohydrate content in mango leaves were known as the flower inducing parameter but there was no scientific research on the concentration variation of carbohydrates especially for Perlis Harumanis mango grown in the greenhouse. In this study, the elements of carbohydrate content such as glucose and starch concentration level in the leaves were analyzed using UV spectrophotometer observed during the vegetative and productive growth. The results of the study showed a higher concentration value of glucose and starch during productive growth. Meanwhile for the total carbohydrate content, the highest concentration value was obtained during the vegetative growth.

### 1. Introduction

Mango (*Mangifera Indica*) is one of the famous fruits grown in tropical and sub-tropical area especially in South East Asia countries. Malaysia is known as one of the mango producer and one of the iconic mango is known as the Perlis Harumanis mango. The Perlis Harumanis mango is very popular because of its aroma, rarity and sweet rich taste. The local demand for Perlis Harumanis mango is very high as the fruits usually fully booked even before it is ripen especially on the beginning of its season. The increasing demand for Perlis Harumanis mango also has been proven by the agreement on exporting the premium quality Perlis Harumanis mangoes into Japanese market by both government of Malaysia and Japan [1]. As the Perlis Harumanis mango is a seasonal fruit, thus the off-season fruit production may contribute to the high potential of extra income for the farmers as well as to the local community who involved in the agro-tourism sector.

The vegetative growth period for Perlis Harumanis mango trees is approximately from July to December. The productive growth period usually occurs on January to February. Meanwhile, during March to April, the Perlis Harumanis mango trees started to bear fruits and the harvesting season will started on April until June. The pre-flowering growth period is recognized as one of major stages in the physiologic phenomenon of a mango plant. The growth of Perlis Harumanis mango tree can be affected by the environmental and biological factors. It is very crucial that the final stems of the trees are let to be break for a period of time in the dormancy period after the last vegetative development so that the new reproductive shoots can be generated. This dormancy period is crucial to provide enough time for the stem to develop the perfect whorl, length and diameter [2].

During the vegetative and productive growth, flowering period is the transition phase from the vegetative to the productive growth of a tree [3]. Carbohydrates is the energy source that is the product of photosynthesis process are stored in different parts of the trees such as in the leaves. The higher level of carbohydrates concentration is desired during the floral development [4]. Thus, the understanding of carbohydrate content variation in the leaves is important to develop a guideline of floral induction procedure for the local farmers but there is lack of scientific report on the variation of carbohydrate content in the leaves especially for Perlis Harumanis mango trees. Thus, the objective of this study was to assess the variation in carbohydrate content in the Perlis Harumanis mango leaves during vegetative and productive growth.



## 2. Materials and Method

### 2.1 Study Area

This study was carried out in a greenhouse at Institute of Sustainable and Agrotechnology (INSAT), Universiti Malaysia Perlis (UniMAP), Padang Besar, Perlis. The greenhouse is planted with 212 Harumanis mango trees located at the GPS location of 6°39'45.439N and 100°19'17.994'E.

### 2.2 Leaves sample collection

The leaves samples were collected from 21 healthy branches from Harumanis mango trees where 9 branches were in a vegetative growth and 12 branches from productive growth in 4 weeks. The leaves sample for vegetative growth was collected from four different stages of vegetative growth and was labelled as V1, V2, V3 and V4. For productive growth stage, the leaves samples were collected at a flowering branch started from budding until the flowers were fully bloomed and was labelled as F1, F2, F3, and F4. The sample images of each stage for vegetative and productive growth is as shown in Figure 1 (a-b). All of the samples were sealed in plastic bags to be analysed in the laboratory. Table 1 shows the number of collected leaves samples for vegetative and productive growth in the greenhouse. In the laboratory, the leaves samples were composited with the respective growth stage were dried in the oven at 60°C for 24 hours. The dried samples were ground into powder form and kept in a cold room at 4°C.



**Figure 1 (a - b)** Stages in vegetative and productive growth

**Table 1** Leaves samples for vegetative and productive growth collected inside the greenhouse for 4 weeks

Growth	Stage	Number of Sample
Vegetative	V1	3
	V2	12
	V3	15
	V4	6
Reproductive	F1	3
	F2	12
	F3	18
	F4	15

### 2.3 Determination of Total Carbohydrate Content

The total carbohydrate content was determined using anthrone method. Leaves sample of 0.05g was added into a boiling tube. It was hydrolysed in a boiling water bath for 3 hours using 5mL 2.5 N hydrochloric acid. After 3 hours, it was let to be cooled to room temperature. Then, the solution was neutralised with solid sodium carbonate until the bubble formed. The solution was added with distilled water until the volume reached 50mL before it was centrifuged for 10 min. Then, the supernatant were collected and 1mL of the aliquot were taken for analysis. Meanwhile, the standard glucose was prepared by adding 0.1g of glucose in 100mL water as stock solution. The working standard was made from 10mL of stock solution that was diluted to 100mL water. The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard and let '0' serves as blank. The volume was made up to 1mL in all the tubes including the sample tubes by adding distilled water. Then, 4mL of Anthrone reagent was added into the tubes and was heated for 8 minutes in a boiling water bath. After that, the solution were cooled rapidly before it was placed in the UV spectrophotometer (Thermo Fisher Scientific Orion AquaMate 7000) and the light absorption was recorded at 630nm [4].

A standard graph was developed by plotting the concentration of the standard glucose on the X-axis versus absorbance on the Y-axis in order to determine the concentration of glucose of each test sample. From the graph, the amount of carbohydrate present in the sample tube was calculated using Equation 1:

$$\begin{aligned} & \text{Amount of carbohydrate content present in 100 mg of the sample} \\ &= \frac{\text{mass of glucose (mg)}}{\text{Volume of test sample}} \times 100 \end{aligned} \quad \text{(Equation 1)}$$

### 2.4 Determination of Glucose and Starch Content

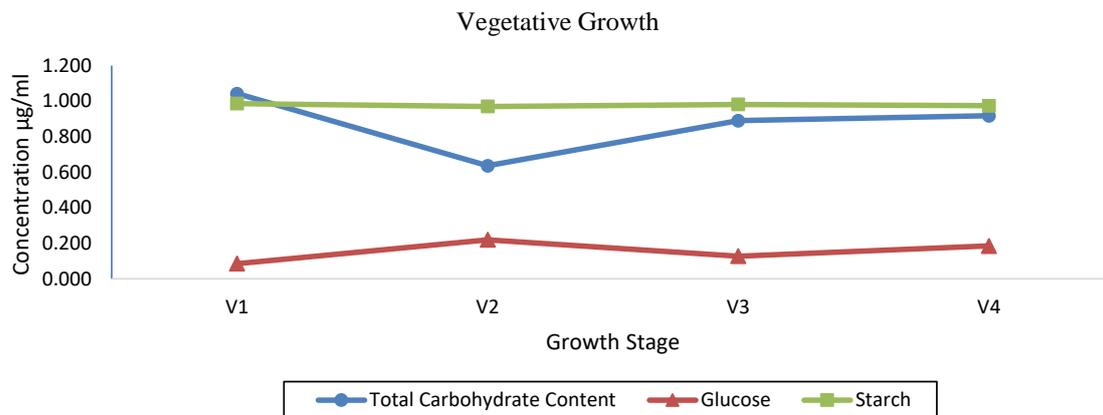
To determine the total glucose and starch content in Perlis Harumanis mango leaves, 1g of finely ground sample was put into 50mL centrifuge tube and 10 mL of hot 80% ethanol were added. The tubes were let to vortex for 2 min and it was centrifuged at 3000 rpm for 10 min in a bench top centrifuge. After centrifuging, the supernatant were decanted. These steps were repeated until the solution did not give any changes in colour when tested with anthrone reagent. As the solution gave no reaction in term of colour changes the residue was cooled in ice water. A 6.5 mL of 52% perchloric acid were added to the residue while stirring the content and then it was centrifuge at 4°C in refrigerated centrifuge. The supernatant were used and made up the volume to 100mL with distilled water. The 0.2mL supernatants were pipetted out and the volumes were made up to 1 mL with distilled water for sample solution. The standards glucose solutions was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard and the volume were increased to 1 mL with water in each tube. A 4 mL anthrone reagent were added into each tube and were heated up for 8 min in a boiling water bath. After that, the tubes were cooled under running tap water and the sample was read at 630 nm light absorption using the UV spectrophotometer. A standard curve was prepared to determine the glucose content in each samples. The obtained value was calculated using Equation 2 and 3 to convert the glucose into starch concentration value [5].

$$\begin{aligned} & \text{starch value in sample solution} \\ &= 0.9 \times \text{glucose absorbance of sample} \end{aligned} \quad \text{(Equation 2)}$$

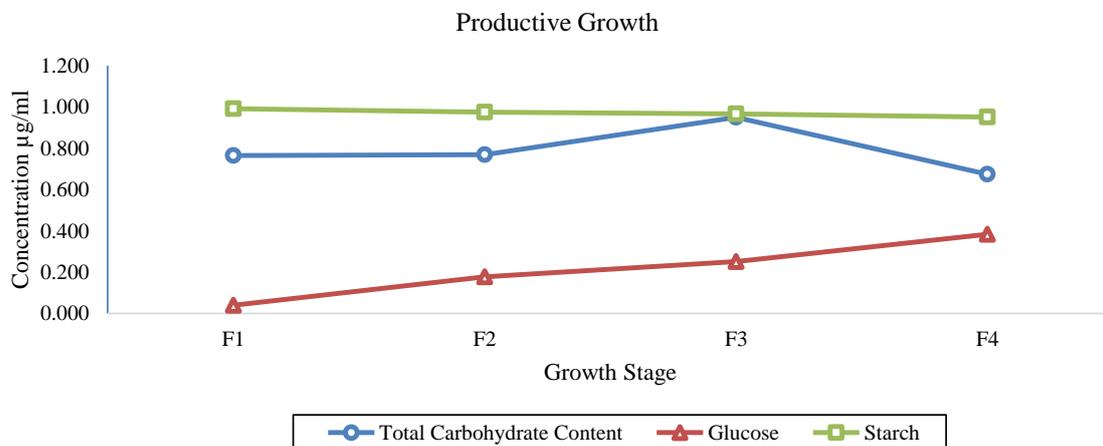
$$\begin{aligned} & \text{starch value in 1 g of powdered sample} \\ &= \text{1g of sample} - \text{starch value in sample solution} \end{aligned} \quad \text{(Equation 3)}$$

## 3. Results and Discussion

Total carbohydrate content, glucose and starch level for Perlis Harumanis mango leaves consisted of 84 samples based on different stages of vegetative and productive growth. Figure 2 and Figure 3 illustrates the average of total carbohydrate content, glucose and starch concentration levels for vegetative and productive growth respectively.



**Figure 2** Total carbohydrate content, glucose and starch concentration levels at vegetative growth



**Figure 3** Total carbohydrate content, glucose and starch concentration levels at productive growth

The total carbohydrate content during the vegetative growth was decreased at V1 to V2 and steadily increased at V2 to V4. Meanwhile for productive growth, the total carbohydrate content were steadily increased at F1 to F3 and decreased back from F3 to F4. The highest concentration level of total carbohydrate content for vegetative and productive growth was 1.042 µg/ml and 0.950 µg/ml respectively. The total carbohydrate content was assumed to be high during the vegetative growth. As supported by Helaly [6], the high level of differences in carbohydrate content may be caused by the water stress in plant thus it show the changes of carbohydrate content throughout the stages in vegetative growth.

The glucose concentration during vegetative growth increased from V1 to V2 and decreased back from V2 to V3. However, the glucose values started to increase back from V3 to V4. Meanwhile, for productive growth, the glucose values showed a steady increment from F1 until F4. From the graph, the highest glucose values were obtained during productive growth which was 0.383 µg/ml as compared to 0.220 µg/ml in vegetative growth. These differences may be due to the movement of sugars from leaf to the tissue as the result from requirement of higher photosynthesis activity process as the trees require more energy during productive growth. The findings was conform with the study conducted by Sandip [7], where the high energy consumption are required in the form of high soluble sugars and it was important in floral activity during productive growth.

The starch concentration trend in Figure 2 does not showed significant different for both vegetative and productive growth. The highest starch concentration obtained in productive and vegetative growth were at the concentration of 0.991 µg/ml and 0.986 µg/ml respectively. The starch value in the productive growth is

higher as compared to vegetative growth although there a small difference in the concentration value. It is may due to the reduction in photosynthesis rate [8].

#### 4. Conclusion

This study showed that there were differences of concentration level for carbohydrate, glucose and starch content in Perlis Harumanis mango leaves. The finding of this study provides a reference for further research to identify the possible flower inducing parameter for off-season Harumanis mango production in the greenhouse.

#### References

- [1] F A S Syahir, A y m Shakaff, A Zakaria, M Z Abdullah and A H Adom 2012 Bio-inspired vision fusion for quality assessment of harumanis mangoes *Proc. - 3rd Int. Conf. Intell. Syst. Model. Simulation, ISMS 2012* **6** 317–324
- [2] R S M Farook *et al* 2013 Harumanis Mango Flowering Stem Prediction using Machine Learning Techniques *Res. Notes Inf. Sci.* **13** 46–51
- [3] P Shivakrishna, K Ashok Reddy and D Manohar Rao 2016 Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots *Saudi J. Biol. Sci.* 8–12
- [4] J E Hedge and B T Hofreiter 1962 Carbohydrates chemistry *Biochem. Methods* 19
- [5] K Mesa, S Serra, A Masia, F Gagliardi, D Bucci and S Musacchi 2016 Seasonal trends of starch and soluble carbohydrates in fruits and leaves of ‘Abbé Fétel’ pear trees and their relationship to fruit quality parameters *Sci. Hortic. (Amsterdam)*. **211** 60–69
- [6] M N Helaly, H El-Hoseiny, N I El-Sheery, A Rastogi, and H M Kalaji 2017 Regulation and physiological role of silicon in alleviating drought stress of mango *Plant Physiol. Biochem.* **118** pp. 31-44
- [7] M Sandip, A N Makwana, A V Barad and B D Nawade 2015 Physiology of Flowering- The Case of Mango **1** 11 1008–1012
- [8] P Wu, C Wu and B Zhou 2017 Drought Stress Induces Flowering and Enhances Carbohydrate Accumulation in Averrhoa Carambola *Hortic. Plant J.* **3** 2 60–66