

Relationship between Photosynthetic Capacity and Carbohydrate Content of *Mangifera indica* cv. Harumanis in Response to Girdling and Paclobutrazol

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Girdling and paclobutrazol have been related with the effects on the photosynthetic capacity and carbohydrate content of plants. The physiological changes caused by these methods distress the growth and development of plants in general. A field experiment was carried out from August 2021 to June 2022 on 5 years of open-field *Mangifera indica* cv. Harumanis trees grown at Malaysian Agriculture Research and Development Institute (MARDI), Serdang, Selangor. The objective of this experiment was to understand the relationship between girdling, paclobutrazol application, combined methods and untreated trees on plant photosynthetic performances and carbohydrate content in the leaves. The treatments were performed on 1st December 2021, and the measurements of leaf gas exchanges and carbohydrate content were performed 4th weeks later on fully expanded leaves shoot, experiencing similar light exposure. In the study, the combination of girdling and paclobutrazol application resulted in a significant decrease in photosynthetic rate (Pn), stomatal conductance (g_s) and transpiration rate (Tr) but significantly increase in intercellular CO₂ concentration (Ci) and carbohydrate content.

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1. INTRODUCTION

Harumanis are one of the best mangoes in Malaysia, and they are primarily grown in the Northern Peninsular Malaysian states of Perlis and Kedah [1,2]. Harumanis mango is only available once a year during the season and is only found north of the homeland due to its sensitivity to climate conditions that necessitates a clear and consistent dry season [3] that provides prolonged hot weather for 2 to 3 months [4]. Harumanis have a distinct flavour unlike any other mango. They have a slightly creamy and milky taste and a very strong and distinct aroma, making it the most popular and receiving a steady increase in demand year after year [3,5,6].

Harumanis production is frequently associated with physiological changes in the plant caused by endogenous factors such as hormones, genetic composition, carbohydrates, and stress conditions, as well as the age of the buds [7]. Furthermore, environmental factors, primarily temperature change [8], as well as management practises such as plant growth regulator use [9], pruning [10], and irrigation [11], are frequently associated with the changes. Endogenous and environmental factors are beyond our control; thus, under management practise, girdling [12] and paclobutrazol [13] application are quite common for fruit producers, and have also shown great results in improving crop growth, yield, and quality in increasing the productivity and quality of several agricultural crops [14,15,16].

Girdling is the removal of a thin strip of bark tissue encircling a trunk or branch, obstructing the downward translocation of photosynthates and metabolites through the phloem [17,18]. Its most immediate effect is to halt assimilate movement, resulting in an accumulation of carbohydrates produced by photosynthesis along the phloem [19]. The accumulation of carbohydrate reserves in the leaves reduces photosynthetic capacity [20,21]. Girdling has a significant impact on photosynthesis because it reduces stomatal conductance [22] which affecting the rate of gas exchange and transpiration rate [23] resulting in less leaf transpiration and thus a decrease in photosynthesis rate.

Paclobutrazol (PBZ) functions as a chemical growth and development stimulator by altering associated physiological processes [24]. The use of this plant growth inhibitor on soil inhibits the biosynthesis of gibberellin, which is responsible for regulating growth and development [25]. Previous research has shown that PBZ application alters crop plant photosynthetic capacity [26,27,28] and increases carbohydrate production in leaf [29,30]. Because gibberellins are involved in the biogenesis of chloroplasts, paclobutrazol alters photosynthetic potential and, as a result, plant gas exchange [31]. Paclobutrazol also has an effect on the efficiency of carbon use in leaves, which is determined by the balance of photosynthesis and respiration [32].

Therefore, the objective of this experiment was to investigate the relationship between photosynthetic capacity and carbohydrate content of *Mangifera indica* cv. Harumanis in response to 4 methods; (i) girdling, (ii) paclobutrazol, (iii) combined methods including, and (iv) untreated tree. We hypothesized that combination of girdling and paclobutrazol would decrease photosynthetic capacity inversely proportional to carbohydrate content of *Mangifera indica* cv. Harumanis.

2. MATERIALS AND METHODS

2.1 Planting Material

This study was conducted at MARDI, Serdang, Selangor, from August 2021 to June 2022, using five years of open-field *Mangifera indica* cv. Harumanis trees grown in sedentary soil and separated by 4 m x 4 m. Thirty-six healthy trees of nearly uniform shape and size are subjected to the same fertilisation programme and other agricultural practises chosen for this study. T1- No induction (Control); T2- girdling at primary branches; T3- soil drenching at 4 ml/l paclobutrazol; and T4- girdling at primary branches + soil drenching at 4 ml/l paclobutrazol were applied to selected trees. Girdling was accomplished by removing a 10 mm wide ring of bark above 30 cm from the base of all primary branches. The commercial product paclobutrazol (25% active ingredient) was used. The treatments were carried out simultaneously on December 1, 2021, at the same morphological

size of the tree that had been subjected to similar light exposure.

2.2 Photosynthetic

A portable photosynthesis system was used to measure photosynthetic rate (Pn), intercellular CO₂ concentration (Ci), stomatal conductance (gs), and transpiration rate (Tr) (LI-6400XT; LI-COR, Lincoln, NE, USA). The measurements were taken on the fifth fully expanded leaves from the plant apex on the fourth week after treatment between 8:00 and 10:00 a.m.

2.3 Determination of Total Carbohydrate Content

The anthrone method was used to determine the total carbohydrate content [33]. A 100 mg sample of leaves was placed in a boiling tube. It was hydrolyzed for 3 hours in a boiling water bath with 5ml 2.5 N hydrochloric acid. It was allowed to cool to room temperature after 3 hours. The solution was then neutralised with solid sodium carbonate until the effervescence was stopped. The solution was diluted with distilled water until it reached 100 ml before being centrifuged for 10 minutes. The 0.5 ml and 1 ml supernatant were then collected, and an aliquot was taken for analysis. Meanwhile, the standard was made by taking 0 ml, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1.0 ml of the working standard and leaving '0' as a blank. By adding distilled water to all of the tubes, including the sample tubes, the volume was increased to 1 ml. The tubes were then filled with 4 ml of anthrone reagent and heated in a boiling water bath for 8 minutes. After that, the solution was rapidly cooled before being placed in a UV spectrophotometer (Thermo Fisher Scientific Orion AquaMate 7000) and the light absorption at 630 nm was measured. To determine the concentration of glucose in each test sample, a standard graph was created by plotting the concentration of the standard glucose on the X-axis versus absorbance on the Y-axis. Equation 1 was used to calculate the amount of carbohydrate present in the sample tube based on the graph:

$$\text{Amount of carbohydrate present in 100 mg of the sample} = (\text{mg of glucose} \div \text{Volume of test sample}) \times 100$$

2.4 Statistical Analysis

The experiment was laid out in randomized complete block design (RCBD). Each treatment was replicated three times and each replicate

was represented by three trees. The data obtained was analysed using ANOVA in SAS software (Version 9.4, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatments means were compared using Duncan Multiple Range Test Difference (DMRT) at $P \leq 0.05\%$.

3. RESULTS AND DISCUSSION

The treatment reduced the photosynthetic capacity of the *Mangifera indica* cv. Harumanis' plants while increasing carbohydrate content compared to the control (Fig. 1). The combination method of girdling and paclobutrazol application resulted in the lowest net photosynthesis rate of 2.71 $\mu\text{mol CO}_2 \text{ m}^2/\text{s}^1$. This combination also reduced stomatal conductance, with the lowest result being 0.07 $\mu\text{molH}_2\text{O}/\text{m}^2/\text{s}$. Furthermore, the combination method yielded the lowest transpiration rate of 1.66 $\mu\text{molH}_2\text{O}/\text{m}^2/\text{s}$. However, the intercellular CO₂ concentration of this combined method was significantly higher than the other treatments, at 312.48 $\mu\text{mol CO}_2 \text{ mol}$. The combination method of girdling and paclobutrazol application increased total carbohydrate content. The highest carbohydrate content was found to be 2.62267 mg/g.

This finding is partially agrees with previous reports on mango [34]. It is well known that paclobutrazol interferes with the hormone abscisic acid's production [35]. A stress hormone that causes stomatal closure is one of abscisic acid's most crucial roles [36]. Based on the degree of stomatal aperture, stomatal conductance calculates the rate of gas exchange through the leaf stomata. A plant may close its leaf stomata under stress in an effort to preserve water and minimise water loss. But in doing so, the plant also limits how much CO₂ it can absorb. In plants, CO₂ is an essential component of the process of photosynthesis. Therefore, a decrease in CO₂ restricts photosynthetic. Furthermore, in addition to this treatment with the girdled branches, which increase in a general decrease in this common adaptation response of plants to the onset of artificial stress by stem girdling conditions, which followed a trend similar to photosynthetic activity [37]. Similar to the present study, the removal of the sink demand of roots typically results in decreased stomatal conductance and transpiration, and accumulation of abscisic acid in leaves [38], which affects carbon status and leads to a decrease in photosynthetic results [39].

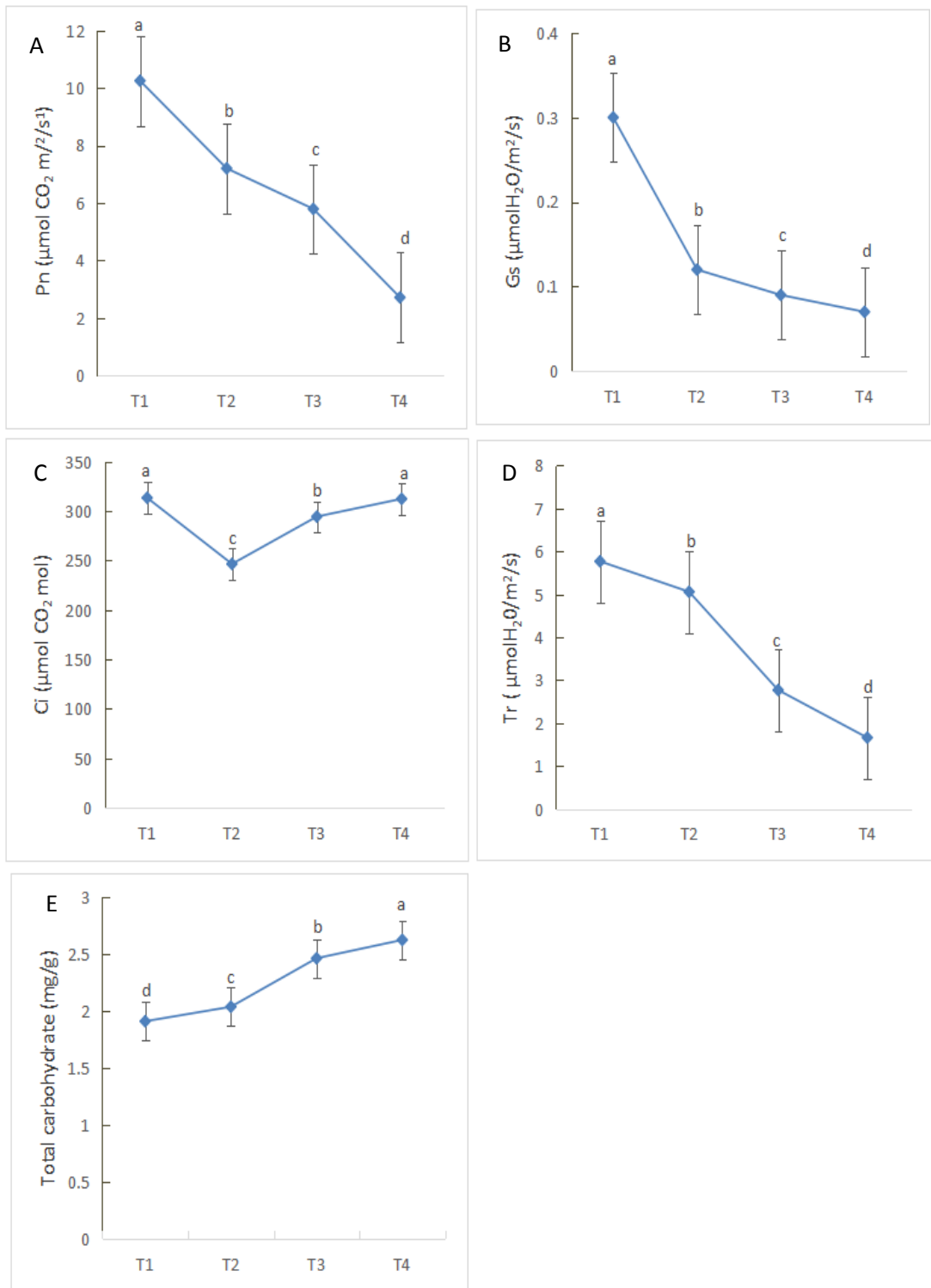


Fig. 1. Net photosynthesis rate (A), stomatal conductance, intercellular CO_2 levels (C), transpiration rate (D) and total carbohydrate (E) at T1- No induction (Control); T2- girdling at primary branches; T3- soil drenching at 4 ml/l PBZ and T4- girdling at primary branches + soil drenching at 4 ml/l PBZ. Bars are mean for standard error. Different letters above bars indicate significant differences using Duncan Multiple Range Test Difference (DMRT) at $P \leq 0.05$

The findings of this study, which are consistent with earlier reports, strongly suggested that girdling and paclobutrazol can increase the production of carbohydrates in the leaf [40,41] (Wu et al. 2018). The early termination of growth caused by the action of paclobutrazol as a gibberellin biosynthesis inhibitor is known to reduce vegetative growth rate, which leads to the buildup of carbohydrates [42,43]. Carbohydrates serve as a main source of energy for growth and differentiation of plant organs during flowering and fruit development (Hazis et al. 2018). Girdling alters the distribution of carbohydrates in the body by preventing assimilates from passing through the phloem. The buildup and depletion of carbohydrates above and below the girdling zone, respectively, cause a number of changes in growth and development [44]. In addition, when the vegetative sink decreased, the transfer of carbohydrates to the shoot increased [45-48].

4. CONCLUSION

In conclusion, girdling and paclobutrazol together dramatically lower stomatal conductance and transpiration, which affect net photosynthesis rate, while simultaneously raising the carbohydrate content in the leaves of *Mangifera indica* cv. Harumanis.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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