Original Research Article

Relationship between photosynthetic capacity and carbohydrate content of *Mangifera indica* cv. Harumanis in response to girdling and paclobutrazol

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 CO_2 concentration (Ci) and carbohydrate content.

KEYWORDS

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Carbohydrate content, Girdling, *Mangifera indica* cv. Harumanis, Paclobutrazol, Photosynthetic capacity

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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 (Nasron et al. 2020; Sani et al. 2018). 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 (Shaidatul Azdawiyah et al.. 2020) that provides prolonged hot weather for 2 to 3 months (Uda et al.. 2020). 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 (Muhamad Hafiz et al. 2019; Nur Afiqah 2015; Rosidah et al. 2010).

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 (Cho et al. 2017). Furthermore, environmental factors, primarily temperature change (Halder and Bandyopadhyay 2021), as well as management practises such as plant growth regulator use (Malshe et al. 2020), pruning (Hahn et al. 2022), and irrigation (Venkata Subbaiah et al. 2017), are frequently associated with the changes. Endogenous and environmental factors are beyond our control; thus, under management practise, girdling (Prates et al. 2021) and paclobutrazol (Kumar et al. 2020) application are quite common for fruit producers, and have also shown great results in improving

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crop growth, yield, and quality @ in increasing the productivity and quality of several agricultural crops (Azizi et al. 2022; Ran et al. 2022; Shivashankara et al. 2019).

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 (Bottcher et al. 2018; Isogimi et al. 2014). Its most immediate effect is to halt assimilate movement, resulting in an accumulation of carbohydrates produced by photosynthesis along the phloem (Gawankar et al. 2019). The accumulation of carbohydrate reserves in the leaves reduces photosynthetic capacity (Zepeda et al. 2022; Asao and Ryan 2015). Girdling has a significant impact on photosynthesis because it reduces stomatal conductance (William et al. 2000) which affecting the rate of gas exchange and transpiration rate (Lopez et al. 2015) resulting in less leaf transpiration and thus a decrease in photosynthesis rate.

Paclobutrazol functions as a chemical growth and development stimulator by altering associated physiological processes (Abdalla et al. 2021). The use of this plant growth inhibitor on soil inhibits the biosynthesis of gibberellin, which is responsible for regulating growth and development (Desta and Amare 2021). Previous research has shown that PBZ application alters crop plant photosynthetic capacity (Zhao et al. 2022; Nyan et al. 2017; Roseli et al. 2012) and increases carbohydrate production in leaf (Xu et al. 2020; Upreti et al. 2014). Because gibberellins are involved in the biogenesis of chloroplasts, paclobutrazol alters photosynthetic potential and, as a

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result, plant gas exchange (Rodrigues et al. 2016). Paclobutrazol also has an effect on the efficiency of carbon use in leaves, which is determined by the balance of photosynthesis and respiration (Christiaens 2014).

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.

MATERIALS AND METHODS

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 ×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

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on December 1, 2021, at the same morphological size of the tree that had been subjected to similar light exposure.

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.

Determination of Total Carbohydrate Content

The anthrone method was used to determine the total carbohydrate content (Hedge and Hofreiter 1962). 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 and 1ml 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

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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:

Amount of carbohydrate present in 100 mg of the sample =(mg of glucose ÷ Volume of test sample) X 100

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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 $P \leq 0.05\%$.

RESULTS AND DISCUSSION

The treatment reduced the photosynthetic capacity of the *Mangifera indica* cv. Harumanis' plants while increasing carbohydrate content compared to the control (Figure 1). The combination method of girdling and paclobutrazol application resulted in the lowest net photosynthesis rate of 2.71 µmol CO_2 m/^{2}/s^1. This combination also reduced stomatal conductance, with the lowest result being 0.07 µmolH_2O/m^2/s. Furthermore, the combination method yielded the lowest transpiration rate of 1.66 µmolH_20/m^2/s. However, the intercellular CO_2 concentration of this combined method was significantly higher than the other treatments, at 312.48 µmol CO_2 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 (Sarker and Rahim 2018). It is well known that paclobutrazol interferes with the hormone abscisic acid's production (Opio et al., 2020). A stress hormone that causes stomatal closure is one of abscisic acid's most crucial roles (Bharath et al. 2021). 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 CO_2 it can absorb. In plants, CO_2 is an essential component of the process of photosynthesis. Therefore, a decrease in CO_2 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 (Agurla et al. 2018). Similar to the present study, the removal

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of the sink demand of roots typically results in decreased stomatal conductance and transpiration, and accumulation of abscisic acid in leaves (Tang et al. 2016), which affects carbon status and leads to a decrease in photosynthetic results (Urban and Alphonsout 2007).

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 (Sousa et al. 2022; Wu et al. 2018; Hua et al. 2014). 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 (Ashraf and Ashraf 2020; Wieland and Wample 1985). 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 (Chai et al. 2021). In addition, when the vegetative sink decreased, the transfer of carbohydrates to the shoot increased (Quentin et al. 2013).

CONCLUSION

In conclusion, girdling and paclobutrazol together dramatically lower stomatal conductance and transpiration, which affect net photosynthesis rate, while

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simultaneously raising the carbohydrate content in the leaves of *Mangifera indica* cv. Harumanis.

CONFLICT OF INTEREST STATEMENT

All authors contributed to the (a) conception and design, (b) writing of the article or its critical revision for essential intellectual content, and (c) approval of the final version. This paper has not been sent to another journal or other publishing outlet, and neither is it being reviewed there.

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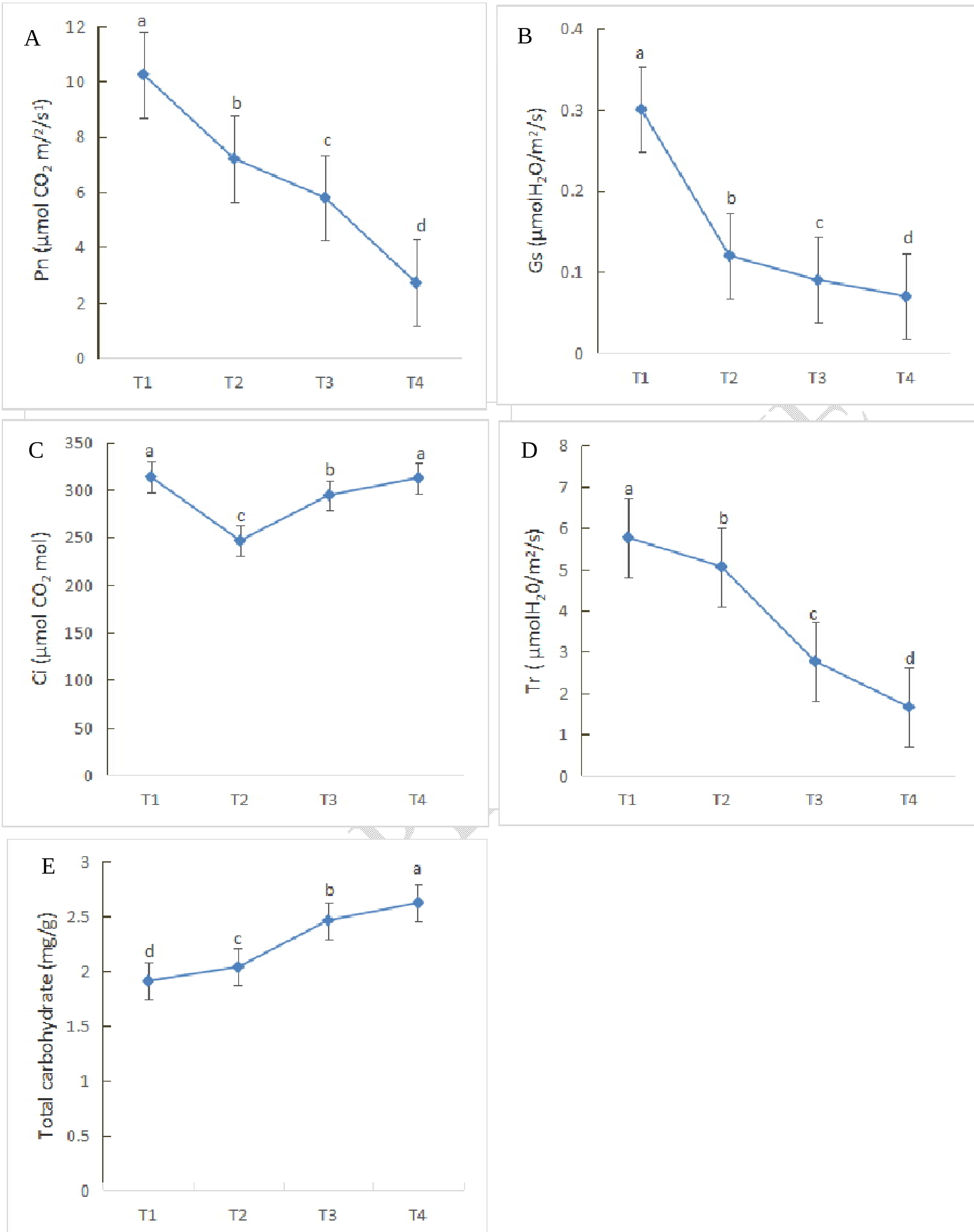

Figure 1: Net photosynthesis rate (A), stomatal conductance, intercellular CO_2 levels (C), transpiration rate (D) and total carbohydrate (E) at T1- No induction (Control); T2- girdling at primary branches; T3- soil drenching at 4 mL/L PBZ and T4- girdling at primary branches + soil drenching at 4 mL/L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