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Effect of Girdling and Paclobutrazol on Growth, Plant Physiology, Inflorescence and Fruiting of *Mangifera indica* cv. Harumanis in Agroclimatic-Zone 3 of Peninsular Malaysia

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Authors' contributions

This work was carried out in collaboration among all authors. Author SSS designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors SAR and MAR conducted the study and collect the data. Authors MHMH and ZHMS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This experiment aimed to study the effect of girdling, paclobutrazol application, combined methods, and untreated trees on physiological response, vegetative and reproductive growth, and fruiting.
Place and Duration of Study: This study was carried out from August 2021 to June 2022 at MARDI, Serdang, Selangor, using five years of open-field *Mangifera indica* cv. Harumanis trees.
Study Design: The study was arranged in the frame of randomized complete block design with three replications and three samples per treatment; ANOVA and DMRT at p=0.05 were used for significance and post hoc comparison, respectively.
Methodology: Each replication consists of: 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. The girdling process was performed by removing a 10 mm width ring of bark at all primary branches. Paclobutrazol (25% active ingredient) of the commercial product was used. The treatments were performed concurrently on 1st December 2021 at the same morphological size as the tree subjected to similar light exposure.
Results: The combination method of girdling and paclobutrazol application had the most significant adverse effect on internode length. Nevertheless, no significant difference was observed in plant stem diameter, height, number of primary and secondary branches, and number of shoots generated from all treatments. The combined method reflected a significant reduction in stomatal conductance, rate of photosynthesis, and transpiration rate. However, the intercellular CO_2 concentration of this combined method and untreated tree is significantly higher. Flowering appears on the leaf buds 90 days after the treatment for paclobutrazol application and the combined method. These methods also resulted in yield per fruit and yield per tree.
Conclusion: Based on the results, the combination method of girdling and paclobutrazol application was effective to reduce vegetative growth, suppress physiological capacity, yet the most effective to produce flowering and fruit yield.

Keywords: Fruiting; girdling; growth; inflorescence; Mangifera indica cv.; harumanis; paclobutrazol; plant physiology; peninsular Malaysia.

1. INTRODUCTION

Harumanis continues to receive a sustained increasing demand annually [1]. The hectarage of Harumanis is currently at 1,575 hectares, and the yield production area is at 1,334.62 hectares, with the majority being found in Perlis and Kedah. Economically, the production value of Harumanis was RM66.72 million in 2020 [2]. However, Harumanis production is still unable to meet domestic demand, not to mention the previous export to Singapore and Japan has been halted since 2019 due to production limits [3,4].

In Peninsular Malaysia, there are three designated agro-climatic zones divided by elemental climatic data such as averaged minimum and maximum temperature, mean rainfall, number of rain days, humidity, and length of dry season per year [5]. The first region is agro-climatic zone 1 (ACZ1), a region with predictable clear dry seasons lasting up to four months [6]. ACZ1 represented only a small part of the area (Fig. 1), which includes Perlis, the northern part of Kedah, Kuala Sedeli, Johor, Marang, Kuala Nerus, and Kuala Terengganu, Terengganu [7]. Some locals say, Harumanis grown in ACZ1 will result in higher yields and better quality [8]. The second region is agro-climatic zone 2 (ACZ2), with a short, fairly regular dry season ranging from one to two months. Production of Harumanis in ACZ2 located in Jelebu, Negeri Sembilan, produced low yield in comparison to those planted in ACZ1 [9]. The third region is agro-climatic zone 3 (ACZ3), a region without a regular dry season. Attempts to produce Harumanis inside ACZ3 in Serdang, Selangor, failed to flower, thus bearing fruit.

Mass production of Harumanis could not be expanded beyond ACZ1 due to its sensitivity to climate change, such as seasonal patterns, temperature, precipitation, relative humidity, photoperiod, water stress, and solar radiation [10]. Harumanis requires a significant prolonged dry season period to initiate flowering [8,11]. Besides requiring high temperatures for vegetative stages, low rainfall distribution is the most significant factor for reproductive stages in

Harumanis that induce flowering, fruit set, fruit growth, and fruit development [12]. In contrast, high rainfall distribution with high humidity can force Harumanis to continuously grow and stay in its vegetative stage, adversely affecting on flowering and fruit development.

Two common agronomic practices to induce flowering and fruiting are girdling [13] and paclobutrazol [14]. Girdling involves the removal of a thin strip of bark tissue encircling a trunk or branch [15], thereby obstructing the downward translocation of photosynthates and metabolites through the phloem [16]. It induces an immediate effect to stop the movement of assimilates, resulting in an accumulation of carbohydrates produced from photosynthesis along the phloem [17]. Flowering is induced by the increased accumulation of carbohydrates and natural hormones beneath the girdle [18]. Paclobutrazol (PBZ) application on the soil inhibits the biosynthesis of gibberellin responsible for regulating growth and development [19]. It induces the production of abscisic acid that confers stress tolerance in crop plants [20,21]. Under extreme stress, where plants cannot survive as individuals, they need to flower and produce seeds to survive as a species. Flowering is a stress response and is considered the ultimate stress adaptation [22]. Furthermore, the application of PBZ increases leaf carbohydrate accumulation to induce flowering [23].

Hence, this experiment aims to determine the effects of untreated tree, girdling, paclobutrazol, and the combination of these methods on the growth, plant physiology, inflorescence, and fruiting of *Mangifera indica* cv. Harumanis in ACZ3. We hypothesized that combination of girdling and paclobutrazol would decrease growth, suppress plant physiology, induce inflorescence, and fruiting of *Mangifera indica* cv. Harumanis.

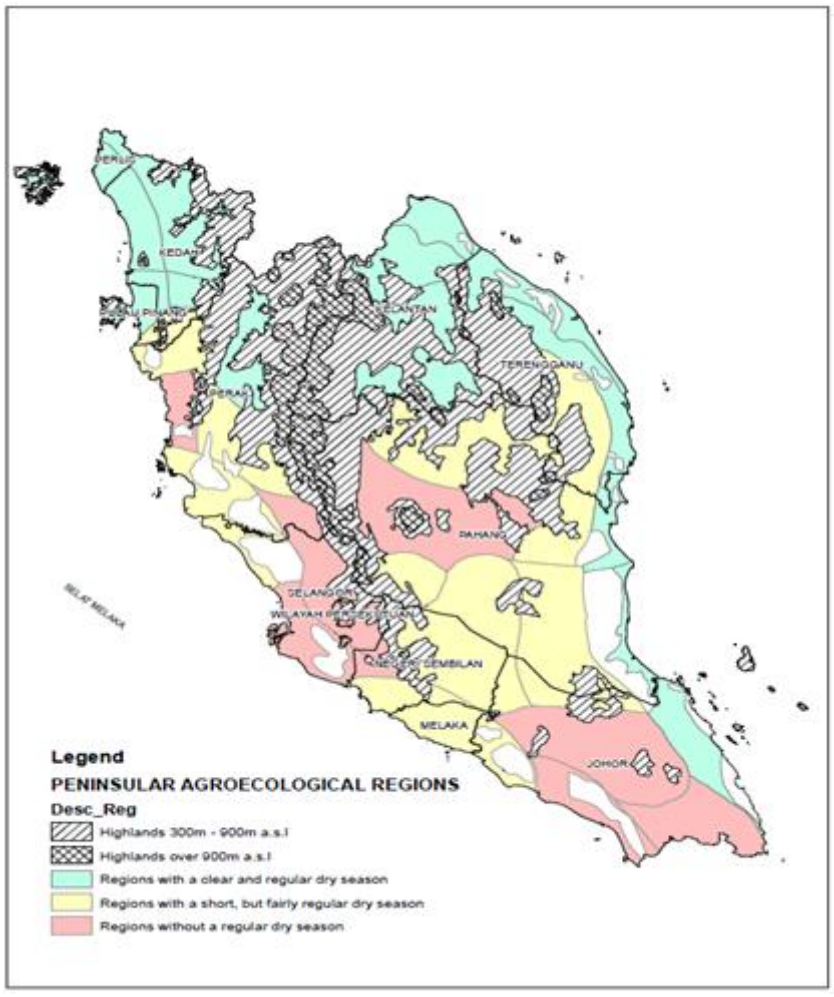

Fig. 1. Agro-climatic zone (ACZ) map of peninsular Malaysia

2. MATERIALS AND METHODS

2.1 Experimental Design and Treatments

The experiment was conducted using randomised complete block design (RCBD) with three replication where each replication had three samples per treatment. Each replication consists of the following treatments: 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. The girdling process was performed by removing a 10 mm width ring of bark at all primary branches. Paclobutrazol (25% active ingredient) of the commercial product was used. The treatments were performed concurrently on 1st December 2021 at the same morphological size as the tree subjected to similar light exposure.

2.2 Planting Materials

This study was carried out from August 2021 to June 2022 at MARDI, Serdang, Selangor, using five years of open-field *Mangifera indica* cv. Harumanis trees grown in sedentary soil (Fig. 2) is described as sandy clay soil and spaced 4 m × 4 m apart. Thirty-six healthy trees of nearly uniform in shape and size receive the same fertilisation programme and other agricultural practices selected for this study.

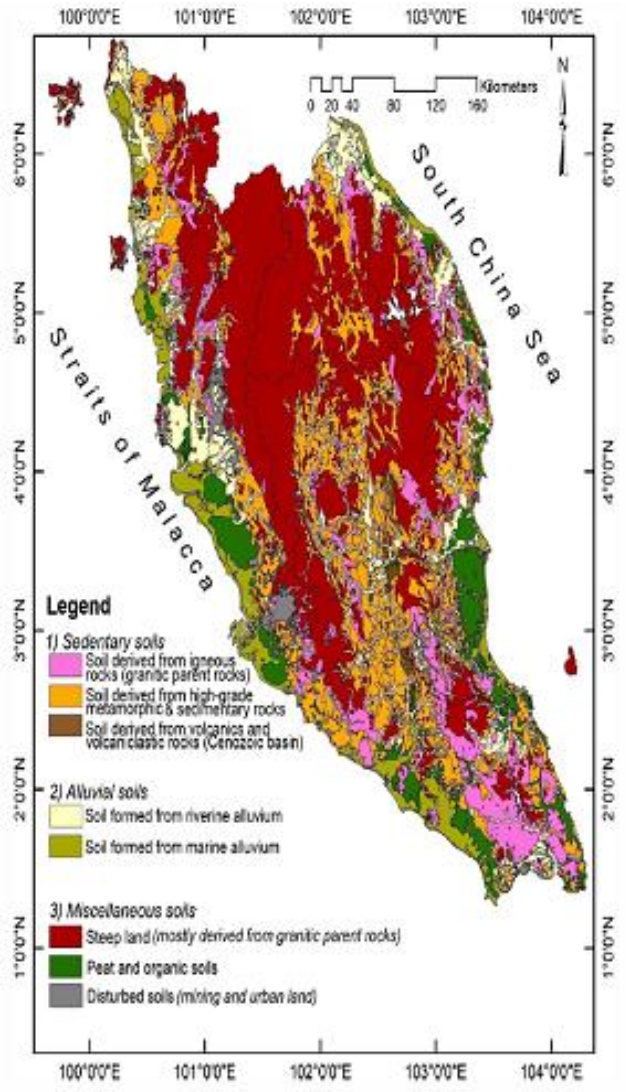

Fig. 2. Simplified distribution and classification of soils in Peninsular Malaysia
(Source: Map of soil types in Peninsular Malaysia, DOA, 2002.)

2.3 Maximum Temperature, Average Rainfall and Number of Rainy Days

This study collected meteorological information on the number of rainy days, maximum temperature (°C), and average rainfall (mm). Based on the data availability, the iMetos 2 weather station, Pessl Instruments (Austria) was utilised to analyse the climate change parameters (monthly time series) from August 2021 to June 2022.

2.4 Vegetative Growth

The height, the number of primary and secondary branches, stem diameter, the number of shoots, and internode length of the plant were determined by measuring the vegetative growth after the treatment was applied for 90 days. A measuring tape was used to measure the plant height – the distance from the highest shoot tip to the soil's surface. Meanwhile, a pair of the digital caliper was used at the base of the stem to measure the stem diameter. The branches attached to the stem were enumerated to obtain the number of primary branches, whereas the secondary branches were recorded as the number of branches attached to the primary branches. The fully expanded leaves and shoots were counted to determine the number of shoots. Lastly, a measuring tape was applied to measure the internode length.

2.5 Plant Physiology

A portable photosynthesis system (LI-6400XT; LI-COR, Lincoln, NE, the United States) was used to compute the intercellular CO2 concentration (Ci), transpiration rate (Tr) photosynthetic rate (Pn), and stomatal conductance (gs). A portable fluorescence system PAM-2500 Chlorophyll Fluorometer (WALZ, Effeltrich, Germany) was utilised to measure the Chlorophyll fluorescence parameters, whereas a SPAD502 metre (Konica Minolta Optic Inc., Tokyo, Japan) was applied to measure the relative chlorophyll content. All the measurements were recorded on the 5th completely expanded leaves at the plant apex from 8:00 to 10:00 a.m. on the 4th-week post-treatment.

2.6 Reproductive Growth

Reproductive growth was measured starting from the flowering phase, and the collected data comprised the percentage of inflorescence trees (%) and time to inflorescence (days). The time to inflorescence was measured from the day the tree was treated until it produced flowers. The percentage of inflorescence trees was calculated by dividing the percentage of inflorescence produced by the number of shoots produced by each tree.

2.7 Fruit Yield

The diameter, weight, and length of each fruit were measured to determine the overall fruit yield. At a 12-week post-treatment of the fruit set, the fruits were harvested, and three fruits were selected for evaluation treatment.

2.8 Statistical Analysis

All the analyses were performed in SAS software (Version 9.4, SAS Institute Inc. Cary, North Carolina, USA) using Analysis of variance (ANOVA). Post-hoc analysis was conducted using the Duncan Multiple Range Test Difference (DMRT) and differences between treatments were compared at P= 0.05.

3. RESULTS AND DISCUSSION

3.1 Maximum Temperature, Average Rainfall and Number of Rainy Days

An overview of maximum temperature (°C) is presented with the highest maximum temperature recorded in March at 35.9 °C while the lowest maximum temperature was recorded in January at 33.7 °C (Fig. 3). This implies that the trees received the maximum temperature range (30 °C to 38 °C) for Harumanis growth [24]. During the eleven-month study period, March 2022 had the highest average rainfall of 489.8 mm, while May 2022 had the lowest average rainfall of 143.2 mm (Fig. 4). These findings are linked to a transitional phase of the monsoon beginning in March and lasting until mid-May, signaling the end of the North-east Monsoon and resulting in brief periods of heavy rain [25]. Despite the wide range and varying amounts of total rainfall, these factors did not affect the Harumanis' growth since the plants require at least a maximum 300 to 380 mm of rainfall monthly [7]. September 2021 received the greatest number of rainy days in a month which was 24 days, while October 2021 received the least number of rainy days in a month which was 16 days (Fig. 5). This study was conducted in

ACZ3, which was proven not to have a consistent dry season (Fig. 1). Studies on the trends of rainfall on the East coast of Peninsular Malaysia reported a significant increment in yearly rainfall during the significant East Monsoon (NEM) transition, which was observed between November and March to South West Monsoon (SWM) occurring between May and September, including the increased frequency of rainy days [26,27]. In addition, the Northeast Monsoon Winds that hit the South China Sea and Peninsular Malaysia simultaneously caused heavy rains for several days in a row (Malaysian Meteorological Department [28].

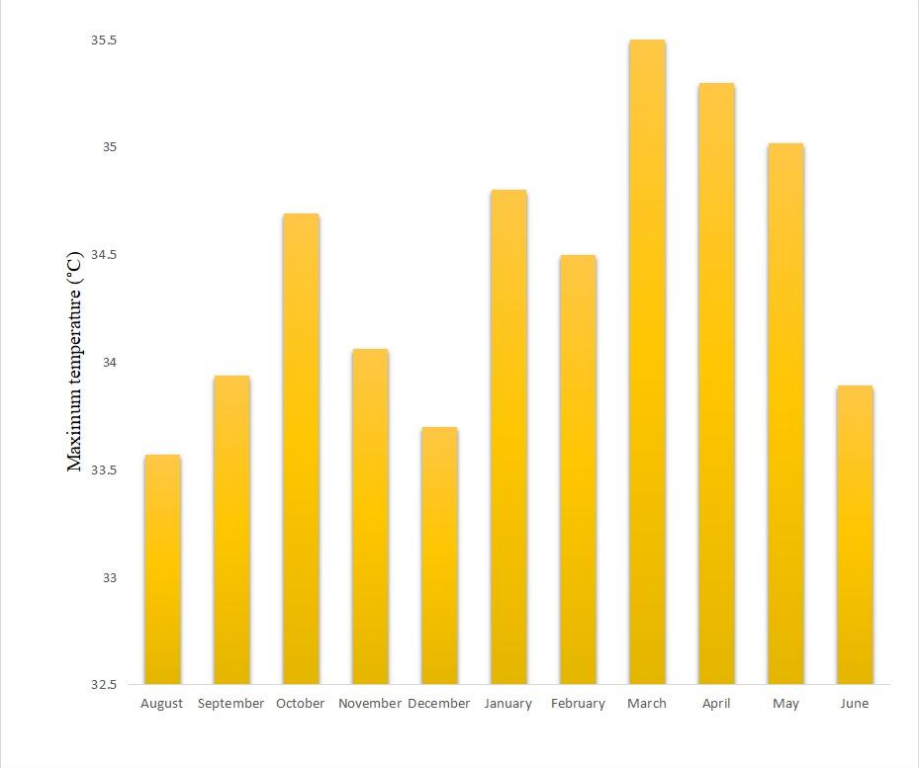

Fig. 3. Maximum temperature (°C) from August 2021 to June 2022

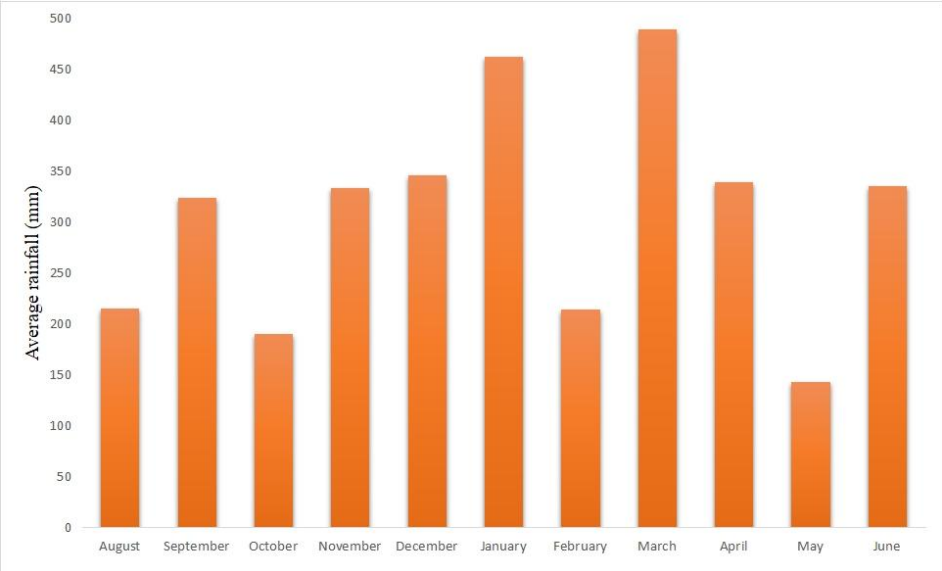

Fig. 4. Average rainfall (mm) from August 2021 to June 2022

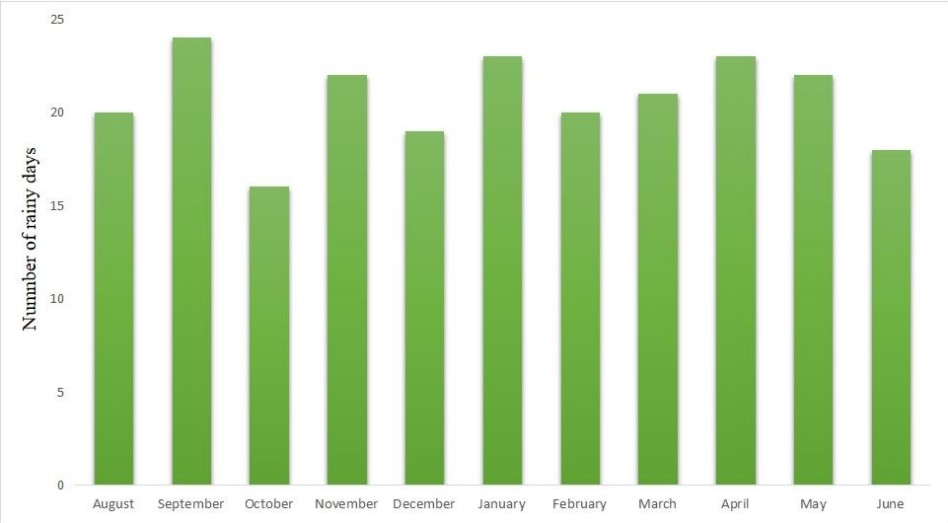

Fig. 5. Number of rainy days in a month from August 2021 to June 2022

3.2 Vegetative Growth

Table 1 indicates that the combination method of girdling and paclobutrazol application (T4) had the most significant adverse effect on internode length, followed by paclobutrazol application (T3), girdling (T2), and the untreated tree (T1). Paclobutrazol, the growth retardant, inhibits and reduces the levels of gibberellins in fruit plants. Gibberellins play an essential role in stimulating cell elongation in trees. The inhibition of gibberellin production does not interfere with cell division. However, the cell elongation is interrupted. As a result, the synthesized internode is compressed and shorter in length [29-31]. The same is true for girdling; when photoassimilates are restricted on girdled branches, only changes in shoot length are visible. The effect of stem girdling at the base of the stem differs from the effect of the upper girdled branch because it prevents photoassimilates accumulation above the girdle phloem from being transported to the roots [32].

3.3 Plant Physiology

The combined method of PBZ application and girdling is presented in Table 2, and the result reflected a significant reduction in stomatal conductance, rate of photosynthesis, and transpiration rate. However, the intercellular CO_2 concentration of this combined method (T4) and untreated tree (T1) is significantly higher. This combination method also results in a slight increase in relative chlorophyll content. Paclobutrazol application (T2), on the other hand, has the highest chlorophyll fluorescent values.

Table 1. Effect of girdling, paclobutrazol, combined methods, and untreated trees on plant height (cm), stem diameter (cm), number of primary branches, number of secondary branches, number of shoots and internode length of *Mangifera indica* cv. Harumanis

Treatment	Vegetative growth					
	Plant height (cm)	Stem diameter (cm)	Number of primary branches	Number of secondary branches	Number of shoots	Internode length (cm)
T1	159.83a	8.61a	2.83a	8.00a	49.67a	12.97a
T2	153.25a	8.44a	3.13a	8.13a	50.25a	9.44b
T3	157.86a	8.14a	3.43a	8.86a	49.71a	8.37c
T4	158.57a	8.84a	2.71a	7.00a	49.29a	7.19d

Means with different letters within each column are significantly different at P= 0.05 using DMRT. T1: No induction (control); T2: girdling at primary branches; T3: soil drenching at 4 ml/l PBZ; T4: girdling at primary branches + soil drenching at 4 ml/l PBZ

An earlier study has demonstrated the effect of paclobutrazol on the production of abscisic acid [33]. One of the most critical functions of abscisic acid is a stress hormone that causes stomatal closure [34]. As a result, trees will absorb CO_2 at a slower pace. The process of photosynthesis depends heavily on CO_2. Carbon dioxide and photosynthesis are equally correlated. A shortage of CO_2 will reduce photosynthesis. The rate of transpiration will decrease due to stomatal closure in the same way. Under stress, a plant may close its leaf stomata in an effort to conserve water and reduce water loss. Furthermore, along with the application of paclobutrazol to the girdled branches, a general decline in this typical plant adaptation response to the commencement of artificial stress followed a pattern comparable to photosynthetic activity [35].

Nevertheless, no significant difference was observed in plant stem diameter, height, number of primary and secondary branches, and number of shoots generated from all treatments (Table 1). Paclobutrazol elevates abscisic acid production in plants involved in developmental processes, mainly through environmental stress response produced in the roots of the plant and the terminal buds at the top of the plant [36]. Due to ABA's impact on the reproductive phases, gibberellin levels decreased. As a result, there has been no vegetative development, such as an increase in primary and secondary branches or the production of new shoots, and the stem diameter has not been impacted. The Harumanis tree's design is droopy. Thus, any consequences of growth changes cannot be observed from the tree's height. Harumanis' farms in Perlis, Kedah, and Johor employ droop architecture [37].

3.4 Reproductive Growth and Fruit yield

The trees react differently to the effects of the treatments given four weeks later. No new shoot production was seen in trees treated with paclobutrazol application (T3) and combination method of girdling and paclobutrazol application (T4). The leaves appear dark green on the entire plant. This is demonstrated by the highest relative chlorophyll content for the combined method (T4). This indicates that the tree has completed the vegetative stage and is ready to enter the reproductive stage. This is confirmed when flowering appears on the leaf buds 90 days after the treatment for paclobutrazol application (T3) and the combined method (T4) (Table 3). Harumanis flowers and begins to produce fruit three months after treatment [38].

The transition from the vegetative to the reproductive stage is marked by the forming of new reproductive structures, such as flowers [39-41]. Furthermore, Table 3 shows the combined method of paclobutrazol and girdling (T4) produced the highest percentage of inflorescence. When growth inhibitors inhibit gibberellin biosynthesis, photosynthate can be redirected into the development of reproductive parts, resulting in accelerated flowering and increased inflorescence [42]. Girdling has been proposed as a method to improve flowering [43], and the inflorescence intensity increased when combined with the application of paclobutrazol (T4) [44]. T4 increased the tree's stress. The chlorophyll fluorescent for T4 proves this. Given that chlorophyll fluorescence is directly related to the rate of energy flow, any perturbation in plant metabolism caused by artificial stress in this experiment will affect fluorescence parameters [45,46].

In the first and second weeks, girdling (T2) treatment produced nearly the same reaction as paclobutrazol application (T3) and combination method of girdling and paclobutrazol application (T4) treatments, but by the end of the third week, T2 treatment began to produce new shoots. Wallerstein [47] found that new callus bridges form about three weeks after ringing in citrus, but it takes two weeks in grapes. Ran et al. [48] discovered that new callus bridges form about 30 days after ringing in jujube tree, allowing the tree's vascular system, which transports water, nutrients, and other compounds, to function normally [49]. Furthermore, girdling (T2) produced the highest chlorophyll inflorescent in the fourth week. This is the effect of recovered girdled branches, which allows the tree's vascular system, which transports water, nutrients, and other compounds, to function normally. Trees receive their essential needs for growth, such as light, water, air, nutrients, and a suitable temperature. Trees with low and moderate girdling levels maintained high crown vigor five years after girdling, similar to untreated trees, whereas trees with severe girdling had significantly lower crown vigor [50].

In contrast to the tree's reaction to untreated tree (T1), the tree retained in the vegetative stage since no treatment that represents stress was given to the tree. Since it constantly produces new shoots, T1 had the lowest relative chlorophyll content due to low chlorophyll in the young leaves. Moreover, due to the limited growth factor on the young leaves, T1 showed the lowest chlorophyll fluorescence. The lower

the chlorophyll fluorescence value, the more fluorescence there is at saturating light, and therefore, the lower capacity for photosynthesis. It has been demonstrated that compared to other treatments, the measured leaves of T1 have a significantly higher photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (Tr), and intercellular CO2 concentration (Ci). As the relative chlorophyll content and chlorophyll fluorescent of the young leaves are significantly lower than those of the old, the rate of photosynthesis is approximately 4-5 times higher in the young leaves than in the old [51]. These responses have found that the common factor responsible for such continuous growing could be living in suitable conditions regarded as no stress applied. [52].

3.5 Fruit Yield

Table 4 depicts that both paclobutrazol alone (T3) and the combination of paclobutrazol and

Table 2. Effect of girdling, paclobutrazol, combined methods, and untreated trees on photosynthesis rate (A), stomatal conductance (gs), intercellular CO_2 concentration (Ci), transpiration rate (Tr), relative chlorophyll content (SPAD), and chlorophyll fluorescence of *Mangifera indica* cv. Harumanis

Treatment	Plant physiology					
	Photosynthesis rate (A)	Stomatal conductance (gs)	Intercellular CO_2 concentration (Ci)	Transpiration rate (Tr)	Relative chlorophyll content (SPAD)	Chlorophyll fluorescence
T1	10.25a	0.30a	313.50a	5.77a	37.08d	0.75c
T2	7.20b	0.12b	246.57c	5.06b	47.65c	0.81a
T3	5.79c	0.09c	294.47b	2.77c	58.79b	0.78b
T4	2.71d	0.07d	312.48a	1.66d	66.21a	0.75c

Means with different letters within each column are significantly different at P= 0.05 using DMRT. T1: No induction (control); T2: girdling at primary branches; T3: soil drenching at 4 ml/l PBZ; T4: girdling at primary branches + soil drenching at 4 ml/l PBZ

Table 3. Effect of girdling, paclobutrazol, combined methods, and untreated trees on time to inflorescence, number of inflorescence and percentage of the inflorescence of *Mangifera indica* cv. Harumanis

Treatment	Reproductive growth	
	Time to inflorescence	Percentage of inflorescence
T1	0b	0c
T2	0b	0c
T3	90a	34.61b
T4	90a	50.08a

Means with different letters within each column are significantly different at P= 0.05 using DMRT. T1: No induction (control); T2: girdling at primary branches; T3: soil drenching at 4 ml/l PBZ; T4: girdling at primary branches + soil drenching at 4 ml/l PBZ

Table 4. Effect of girdling, paclobutrazol, combined methods, and untreated trees on yield per fruit and yield per tree of *Mangifera indica* cv. Harumanis

Treatment	Yield per fruit			Yield per tree	
	Fruit weight (kg)	Fruit length (cm)	Fruit diameter (cm)	Number of fruits	Fruit weight (kg)
T1	0b	0b	0b	0b	0b
T2	0b	0b	0b	0b	0b
T3	0.75a	15.13a	8.88a	4.00a	3.00a
T4	0.79a	15.38a	9.00a	4.33a	3.41a

Means with different letters within each column are significantly different at P≤ 0.05 using DMRT. T1: No induction (control); T2: girdling at primary branches; T3: soil drenching at 4 ml/l PBZ; T4: girdling at primary branches + soil drenching at 4 ml/l PBZ

girdling (T4) resulted in yield per fruit and yield per tree of Mangifera indica cv. Harumanis. Paclobutrazol is a growth inhibitor that plays a vital role in stress responses and adaptation [53]. Plants subjected to stress expressed specific changes in plant development [54]. Under extreme stress, where plants are unable to survive as individuals, they need to flower and produce seeds to survive as a species [22]. Girding improves fruit set and fruit size in horticulture plants by stopping the downward flow of food material through phloem. Experiments on citrus girdling demonstrated that increasing chlorophyll content increased citrus fruiting [55]. The flowering and fruiting per branchlet were improved by girdling in navel oranges [56] and jamun trees [57] compared to un-girded trees.

4. CONCLUSION

The absence of a consistent dry season in agro-climatic zone 3 was widely known. The combination method of girdling and paclobutrazol had the most significant adverse effects on the plant's vegetative stage and physiological capacity. Still, it was most effective in triggering and intensifying inflorescence development and boosting fruit yield in ACZ3. Since it has been demonstrated that Harumanis may flower and bear fruit in ACZ3, further evaluation needs to be done regarding to increase the percentage of the flowering and fruiting of Harumanis in ACZ3 in future.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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