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FULL PAPER

Effect of Paclobutrazol Application on the Phenological Patterns of Flowering, Fruit Production and Cherelle Wilt Incidence in Cacao (*Theobroma cacao* L.)

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Cherelle wilting, a major constraint in cacao production, is caused by inefficient carbohydrate distribution in developing cherelles. Understanding carbohydrate metabolism in cacao can improve our knowledge of its physiology and help develop yield-enhancing strategies. Improving carbohydrate assimilation in cacao during critical flowering and pod setting stages requires thorough analysis of physiological, biochemical, and molecular processes, necessitating prompt intervention. Paclobutrazol (PBZ) by inhibiting gibberellin biosynthesis, can increase carbohydrate accumulation in sink tissues. This boosts photoassimilate partitioning to the reproductive structures, sustaining cherelle growth and improving cacao yield. In a separate study, two cacao varieties (UF667 and UF18) were applied with paclobutrazol to enhance flower, cherelle, and pod production. Ten-year-old trees were treated with 0.1% paclobutrazol, untreated trees served as controls. Vegetative growth, flower, cherelle, and pod production were recorded monthly. Carbohydrate content and leaf SPAD values were determined in UF667 leaves. Data were analyzed using Pearson Correlation and T-test at a 95% confidence interval.

Paclobutrazol enhanced chlorophyll (as represented by leaf SPAD value) but did not significantly affect carbohydrate accumulation in cacao leaves. Increased leaf SPAD value in mature leaves boosted flowering intensity and cherelle formation. PBZ increased the total number of flower cushions and flowers per tree in UF18, and the total flowers and flowers per cushion in UF667. Cherelle formation and pod retention in PBZ-treated trees were not significantly different from untreated trees. Flower production increased by over 100%, but the conversion rate to cherelles and pods was very low. Paclobutrazol increased dried bean yield from 1.18 to 2.84 kg per tree per year in cacao var UF18, much higher than the local production. Cherelle wilt increased with more cherelles and pods, indicating competition for photoassimilates. Overall, PBZ was effective in enhancing flower production in cacao. However, proper cultural management practices, such as pruning and pest control measures, should be implemented alongside paclobutrazol to sustain the growth of developing cherelles and improve pod retention.

Keywords: carbohydrate accumulation, cherelle formation, dried bean yield, leaf SPAD value, pod retention

INTRODUCTION

Assimilate partitioning between the competing organs (sink tissues) has become a limiting factor in cacao production. The regulation of carbon assimilation in cacao trees was identified as one significant gap, which could be considerably interlinked to loss of cherelles during early growth stages and low pod retention because of competition among sink tissues (Lahive et al. 2019). Cherelle wilt is one of the factors contributing to low cherelle formation during the early stages of growth. Cherelle wilt is a physiological thinning mechanism triggered by insufficient carbohydrate reserves during early stages of cherelle growth. Carbohydrates are crucial for regulating cherelle development, explaining why maximum wilting occurs during peak pod growth (Ten Hoopen et al. 2012), specifically at 50 and 70 days after pollination coinciding with active cell division and enlargement, respectively (Handa et al. 2012, Goudriaan and van Laar, 1994 and Ten Hoopen et al. 2012). The wilt phase ends when cell growth ceases.

Carbohydrate reserves are crucial for reproductive growth, determining the success of flower and fruit development. Starch accumulation in developing fruits supports growth energy needs. Starch synthesis in leaves and storage organs relies on ADP-glucose, regulated by the adenosine diphosphate glucose phosphorylase (AGPase; Preiss and Levi, 1980). AGPase is a key regulatory enzyme that determines the committed pathway toward starch biosynthesis (Smith et al. 1997; Mares et al. 1981) and it is highly influenced by gibberellin (GA) levels. Increased starch inhibits gibberellin biosynthesis (Paparelli et al. 2013) or vice versa suggesting potential manipulation of the carbohydrate metabolism in cacao during active reproductive growth. For example, reducing gibberellin with paclobutrazol (PBZ) in mango increases starch in stems, enhancing flower production and fruit yield (Protacio et al. 2000;2006). Adequate carbohydrate levels in fruits are necessary for successful fruit setting and reduced abscission (Mehouachi et al. 1995). Therefore, accumulation of carbohydrate reserves (i.e., stems) before the initiation of fruit formation is deemed necessary to sustain the energy required during cell division and expansion, and gibberellin level within the tree must be regulated.

Paclobutrazol (PBZ) and chlorocholine chloride (CCC) are triazoles that enhance plant growth and yield by altering hormonal biosynthetic pathways and biochemical components. These two gibberellin inhibitors (GA) target the oxidative steps that convert ent-kaurene to ent-kaurenoic acid, thereby reducing stem elongation (Rademacher, 2000). Reduction of gibberellin levels lead to increased starch accumulation by enhancing AGPase activity (Mares et al. 1981; Smith et al. 1997; Lloyd and Kossmann, 2019) and reducing invertase activity (Nielsen et al.1991) in the sink cells. PBZ has been identified in the xylem and phloem sap of some crops, demonstrating that triazoles can be transported both acropetally and basipetally with considerable stability (Desta and Amare, 2021). Consequently, this increases carbohydrate accumulation in the source tissues (stems in mango and trunks and branches in cacao trees), which then diverts assimilate partitioning in the sink tissues (i.e., reproductive tissues) where it is needed. That is why they are effective in enhancing growth in a wide range of plant species, including mango and cacao. Specifically, in some crops, PBZ increases the root to shoot ratio and directs assimilates to economically important parts such as bulbs, tubers, flowers, fruits, and grains. Additionally, PBZ increases chlorophyll content in tomato leaves (Sharma et al. 1998), improves photosynthetic capacity in *Dioscorea rotundata* (Berova and Zlatev, 2000; Jaleel et al. 2007), boosts crop yield by enhancing assimilate partitioning and improve flow toward reproductive structures (Setia et al. 1996). It also suppresses vegetative growth and reduces leaf area, thereby enhancing the accumulation of assimilates in sink tissues (Tekalign and Hammes, 2004). In this study, it is hypothesized that inhibiting gibberellin biosynthesis pathway through the application of the GA-inhibitor compound, PBZ, in cacao trees can increase the build up of starch in sink cells to sustain the growth of developing cherelles and enhance flowering during the dry season. Consequently, increasing cherelle formation and pod retention, improves the yield of the cacao bean.

METHODOLOGY

Experimental Materials and Maintenance

Ten cacao var UF18 trees of uniform age in two sites within the UPLB locations (Plantation Crops Demonstration Area (PCD) and Jamboree, ICROPS, UPLB), were used for this study. While eleven cacao var UF667 of uniform age trees at the PCD area also were used. The experimental setup was composed of five trees applied with PBZ (PACLO® 25 SC 250g L^{-1}) and another five were untreated trees at PCD and Jamboree sites. At the PCD experimental site for cacao var UF667, six trees were treated with PBZ, and five served as the controls.

PBZ was applied in March 2021, one month after fertilizer application. The PBZ treatment involved a soil drench at 3-L of 0.1% solution per tree (3 mL PBZ in 3 L of water) as per manufacturer's recommendation, in which 3 mL of PBZ was diluted to 3 L of water. Complete fertilizer was applied in split doses of 125 g $tree^{-1}$, following cacao fertilizer recommendations and soil nutrient status.

Measurement of Growth Morphology

The effect of PBZ on the vegetative growth such as the number of new shoots produced, shoot length, shoot diameter, and the number of leaves per new shoot was recorded monthly after paclobutrazol application until there were no new shoots formed. Fifteen new shoots were measured for the stem length, diameter, and the number of leaves in each tree representing five shoots from the top, middle and lower branches.

The total number of flower cushions (flowers borne in clusters) per tree, the total number of flowers per tree, and the average number of flowers per cushion per tree were also recorded. The total number of flower cushions per tree was individually counted. Measurements of stem length, stem diameter, and the number of leaves per new shoot were taken from 25 randomly selected cushions per tree at anthesis (when 50% of the flowers are open), with 5 cushions sampled per branch. The total number of cherelle developed and pod retention as well as the number of cherelle wilt for each tree were recorded.

Analysis of Carbohydrates

The total carbohydrates in leaf samples of cacao var UF667 were analyzed using the Anthrone method at the Analytical Services Laboratory, Institute of Chemistry, University of the Philippines, Los Baños, Laguna. The 3rd or 4th leaf samples from the youngest leaf were collected from 9:00 AM to 11:00 AM and oven dried at 70 °C for 48 hrs or until the weight of the samples had stabilized.

For sample extraction,10 mL of distilled water were added to 1.0 g of dried, powdered sample, stirred thoroughly. Then, 13 mL of 52% perchloric acid was added and stirred for at least 20 mins. The mixture was diluted to approximately 100 mL, filtered into a 250 mL volumetric flask, and the remaining washings were added to the flask.

The colorimetric analysis involved diluting 10 mL of the sample extract to 100 mL of distilled water. Then, 1.0 mL of the solution was placed in a test tube and cooled in an ice bath. Then, 5 mL of Anthrone reagent was added, mixed thoroughly, and heated in a boiling water bath for 12 mins. The solution was cooled to room temperature. Absorbance was measured at 630 nm using a Shimadzu UV mini 1240 UV-Vis Spectrophotometer with a blank solution prepared using 1.0 mL distilled water.

The total available carbohydrates as % glucose and starch were calculated using a calibration curve. Standard solutions (0.01 mg mL^{-1} to 0.15 mg mL^{-1}) were prepared, and 1 mL of each was measured for absorbance at 630 nm, with 1 mL of water as a blank. The % glucose was derived from the standard curve and as % starch was calculated by multiplying the % glucose by a conversion factor of 0.9.

Measurement of leaf SPAD value

Leaf SPAD value in leaf samples from the lower, middle, and upper canopy of cacao var. UF667 trees was monitored using a SPAD 502 Plus® chlorophyll meter (KONICA MINOLTA) from one month after PBZ application until reproductive growth. The SPAD 502 Plus® chlorophyll meter was used for rapid, nondestructive in situ reference measurements, showing results comparable to wet laboratory methods for chlorophyll analysis (Sub et al. 2015; Vishwakarma et al. 2023).

Statistical Analysis

The data were analyzed using Pearson Correlation Analysis and the difference between treatment means was compared using t-test at 95% confidence interval.

RESULTS AND DISCUSSION

Influence of paclobutrazol application on the production of flowers, fruits, and leaf flushing and the regulation of cherelle wilt in cacao var UF667

PBZ on leaf SPAD value. The t-test analysis for comparison between treatment means revealed that leaf SPAD value in mature leaves from the middle and lower canopy of the two treatments were highly significant ($p < 0.0001$ and $p < 0.003$, respectively; Table 1). Specifically, the leaf SPAD value in leaves from the upper canopy between the untreated and PBZ-treated trees did not vary significantly ($p > 0.05$). Overall, statistical analysis revealed a significant difference ($p = 0.012$) between the untreated and PBZ-treated trees, with higher leaf SPAD value in PBZ-treated trees than in untreated trees. Although the PBZ-treated cacao trees had higher leaf SPAD value, this effect remains unclear. Both PBZ-treated and untreated trees maintained the leaf SPAD value during the wet season (July to August), showing a minimal reduction in PBZ-treated trees while untreated trees consistently retained their leaf SPAD value. This reduction was not significantly different from the control trees and can be attributed to climatic factors, specifically rainfall distribution during seasonal changes. Consistent rainfall during the wet-season maintained leaf SPAD value. Additionally, PBZ's effect on leaf SPAD value may be linked to its role as triazole compound, stimulating cytokinin synthesis, which enhances chloroplast differentiation (Fletcher et al. 2000) in various crops such as cucumber (Visser et al. 1992), *Camelina* (Kumar et al. 2012), black rice (Dewi et al. 2016) and grape vines (Hunter and Proctor, 1994).

PBZ on the carbohydrate content. Slightly more elevated levels of carbohydrates were observed in PBZ-treated trees compared to the controls, however, the difference in values were not big enough to be deemed statistically significant (Table 1). But the trend was clearly consistent when observed across different months. The physiological performance of PBZ-treated trees may be influenced by factors such as climatic conditions (Protacio et al. 2000) and the physical and health status of the tree. For instance, PBZ-treated mango trees grown under limited irradiance showed delayed flowering due to decreased photosynthetic activity, which delayed starch accumulation (Protacio et al. 2000). Unlike mango, cacao is a shade-tolerant species. It typically exhibits saturated net carbon assimilation rates at PAR ranging from 200 to 750 μmol/m^2/s^1 and a low light compensation point between 5 and 57 μmol/m^2/s^1 (Almeida et al. 2014). Cacao's tolerance to light intensities varies genetically. Suarez et al. (2018) found that cacao trees exposed to high mean daily incident radiation (PAR at 700 μmolm^2/s^1 with midday values of 1300 μmol/m^2/s^1) performed better than those exposed to mid- and low mean daily incident radiation (PAR at 400 μmolm^2/s^1 with 900 μmol/m^2/s^1 midday values and PAR at 300 μmol/m^2/s^1 with 500 μmol/m^2/s^1 midday values), improving photosynthetic activity. In the experimental area, some cacao trees experienced reduced solar radiation intensity due to canopy shading, which may have directly impacted their photosynthetic activity potentially contributing to the minimal response of cacao var UF667 to PBZ treatment. It indicated that cacao UF667 respond better to high light intensity for optimum growth.

Table 1. Summary of t-test results for comparison between the control and paclobutrazol treated trees of cacao (*Theobroma cacao* L.) var UF667 in relation to the morphological, biochemical, and physiological characteristics as influenced by paclobutrazol application.

Parameters	95% Confidence Interval	T	p-value
Leaf SPAD value (Upper leaves)	(-0.53 , 3.172)	1.4	0.161^{ns}
Leaf SPAD value (Middle leaves)	(-6.07 , -2.5)	-4.71	$<0.0001^{**}$
Leaf SPAD value (Lower leaves)	(-4.88 , -0.973)	-2.94	0.003^{*}
Total Leaf SPAD value	(-3.49, -0.436)	-2.52	0.012^{*}
Carbohydrates	(-0.63 , 0.309)	-0.69	0.493^{ns}
Total Cushions	(-98.39 , 91.019)	-0.08	0.938^{ns}
Total Flowers	(-61.439, -5.244)	-2.39	0.021^{*}
Flowers per Cushion	(-3.143, -0.561)	-2.87	0.006^{*}
Cherelle Number	(-2.48 , 2.279)	-0.08	0.933^{ns}
Cherelle Wilt	(-2.79 , 0.443)	-0.145	0.152^{ns}
Pod Number	(2.096, 10.813)	2.98	0.005^{*}
Total Number of Shoots	(-13.92, 60.16)	1.25	0.216^{ns}
Shoot Length	(31.3, 60.005)	6.29	$<0.0001^{**}$
Shoot Diameter	(-0.02 , 0.547)	1.86	0.064^{ns}
No. of Leaves	(0.29 , 1.144)	3.29	0.001^{*}

Table 2. Summary of correlation results between the biochemical, physiological and morphological characteristics in cacao (*Theobroma cacao* L.) var UF667.

Parameters	Correlation Coefficient	p-value	Result	Interpretation
Leaf SPAD value and flower production	-0.593	0.042	*	moderate
Carbohydrate content and flower production	0.431	0.162	ns	n/a
Cherelle number and cherelle wilt	0.614	0.004	*	strong

Leaf SPAD value and carbohydrate contents on flower production. Leaf SPAD values displayed a moderate correlation ($p < 0.042$; Table 2) with flower production in cacao var UF667, whereas accumulation of carbohydrates had no significant correlation with flower production ($p > 0.05$). Figure 1 shows the trend of

Table 3. Leaf SPAD value and carbohydrate contents in mature leaves of cacao (*Theobroma cacao* L.) var UF667 after paclobutrazol treatment.

	Leaf SPAD value		Carbohydrates (% w/w starch)	
	Control	PBZ	Control	PBZ
Month	74.95	72.5	2.018	2.10
March	69.26	69.37	1.98	1.97
April	69.26	69.37	1.98	1.97
May	57.65	62.88	1.76	1.78
June	48.81	54.64	2.96	2.96
July	45.59	45.52	3.44	3.68
August	44.84	43.3	1.97	2.07

leaf SPAD value and carbohydrate accumulation across different months in relation to the flowering response of cacao var UF667. Flower production peaked in June when the leaf SPAD value declined in mature leaves, while carbohydrate contents were high. Although statistical analysis revealed that carbohydrate accumulation did not significantly influence flowering in contrast to leaf SPAD value, flower production increased as the level of carbohydrates started to increase in June with the highest accumulation in July. However, flower production declined thereafter as the number of cherelles and pods increased. Overall, flower production was higher during the rainy than in dry periods.

Several studies have mentioned the important role of carbohydrates in influencing the flowering of fruit trees and reported that it is directly related to flower production. According to Lahive et al. (2018), increased accumulation of carbohydrates on the sink tissues (i.e., stems) also increased flowering, cherelle formation, and pod retention in cacao. Hence, the intensity of flower production is indicative of carbohydrates allocation to the reproductive tissues (Lahive et al. 2018). The increase in flower production in June coincided with the increase of carbohydrate accumulation in cacao var UF667 sustaining flower development. This supports the findings of Owusu et al. (1978) that the peaks of sugar levels in the bark of cacao coincide with peaks in flowering intensity.

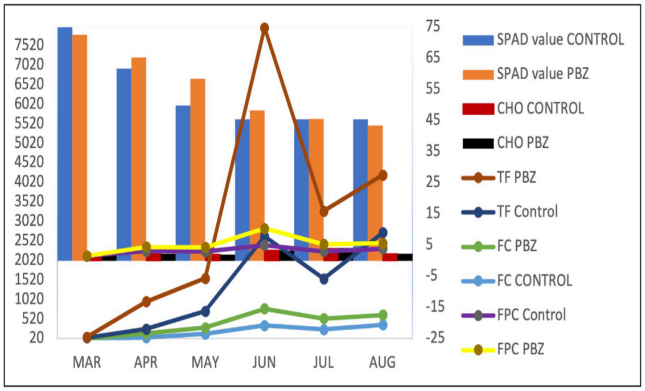

Figure 1. Periodic patterns of flower production: flower cushions (FC), total flowers (TF) and flowers per cushion (FPC) in relation to leaf SPAD value and carbohydrate content (CHO; % starch w/w) in PBZ-treated and untreated trees of cacao (*Theobroma cacao* L.) var UF667

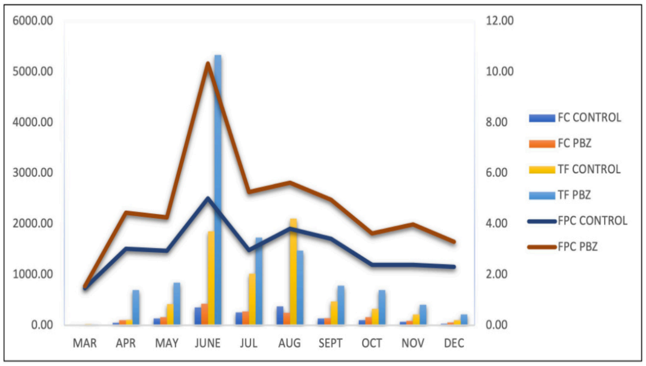

Figure 2. Periodic patterns of flower production: flower clusters (FC), total flowers (TF), and flower per cluster (FPC) as influenced by PBZ application in cacao (*Theobroma cacao* L.) var UF667 at the Plantation Crops Demonstration Area, UPLB, College, Los Baños, Laguna from March to December 2021.

In mango, the competency to flower is enhanced when the level of carbohydrates in stems is achieved above a certain threshold while gibberellin level is below a certain threshold (Protacio et al. 2000). On the other hand, chlorophyll has a direct or indirect role in the induction of flowering. Chlorophyll is an important pigment that absorbs light energy and converts it to chemical energy to produce glucose during photosynthesis. Carbohydrates as the product of photosynthesis, provide energy for the growth and development of plants. Studies have shown that non-structural carbohydrate accumulation in leaves represses photosynthesis or vice versa to maintain equilibrium, which is crucial for plant growth (Stitt and Quick 1989; Krapp and Stitt 1995, Jeannette et al. 2000). From the data, carbohydrate accumulation was inversely related to the leaf SPAD value. Table 3 shows that leaf SPAD value was highest in March and tend to level off starting June and toward August. Whereas carbohydrate accumulation started to increase in June when the flowering intensity was at its highest. However, further study is needed to validate this response.

Influence of PBZ on flower production of cacao var UF667

The application of PBZ in cacao var UF667 presented that the total flower cushions were not significantly influenced by PBZ treatment ($p > 0.05$; Table 1). However, the total flowers and the number of flowers per cushion had a significant correlation with PBZ treatment ($p = 0.021$ and $p = 0.006$, respectively; Table 1). Figure 2 shows that the total number of cushions, total flowers, and flowers per cushion was higher in PBZ-treated trees than in untreated ones. The difference in values between treatments for the total cushion was negligible, except for the total flowers and number of flowers per cushion. The results substantiated that PBZ significantly enhanced flowering in cacao var UF667 by increasing the total flowers and the number of flowers per cushion**.** While the number of flower cushions did not significantly increase with PBZ treatment, the number of flowers per cushion did, resulting in an overall increase in the total flowers per tree.

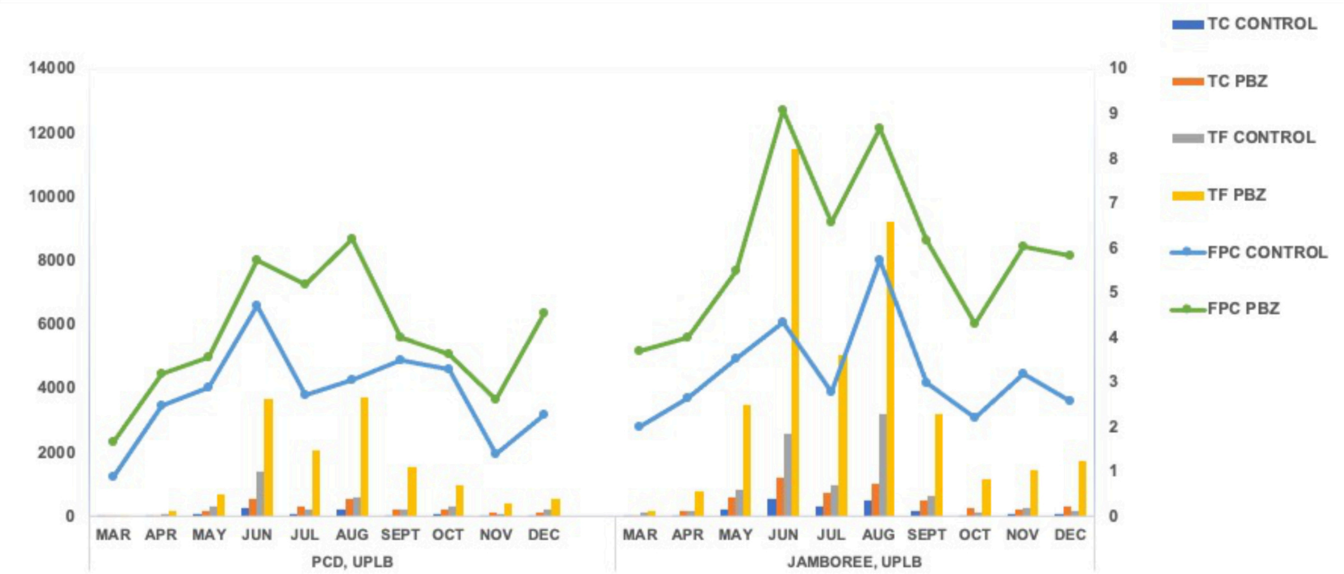

Figure 3. Phenological patterns of flower production: total cluster (TC), total flowers (TF), and number of flowers per cluster (FPC) as influenced by PBZ application in cacao (*Theobroma cacao* L.) var UF18 at the Plantation Crops Demonstration Area (PCD), and Jamboree, UPLB, College, Los Baños, Laguna from March to December 2021.

Table 4. Summary of t-test results for comparison between the control and paclobutrazol treated trees of cacao (*Theobroma cacao* L.) var UF18 in relation to the morphological and physiological characteristics in two locations.

Parameters	95% Confidence Interval	T	p-value
Total Cushions	(-385.755, -22.979)	-2.27	0.028^{*}
Total Flowers	(-3576.52, -476.03)	-2.65	0.012^{*}
Flower per Cushion	(-2.724, -0.526)	-2.97	0.005^{*}
Cherelle Number	(-11.2690, 3.3524)	-1.17	0.263^{ns}
Cherelle Wilt	(-5.3841, 3.0241)	-0.6	0.56^{ns}
Pod Number	(-9.1994, 4.0594)	-0.82	0.425^{ns}
Total new shoots	(-38.3383, 8.7383)	-1.26	0.213^{ns}
Shoot length (mm)	(-34.1501, 7.8019)	-1.24	0.217^{ns}
Shoot diameter (mm)	(-0.7858, 0.2186)	-1.11	0.267^{ns}
Number of leaves	(-1.3581, 0.0529)	-1.83	0.07^{ns}

Table 5. Summary of correlation results between the fruit production and cherelle wilt in cacao (*Theobroma cacao* L.) var UF18.

Parameters	Correlation Coefficient	p-value	Result	Interpretation
Cherelle number and cherelle wilt	0.422	0.007	*	Moderate
Cherelle number and pod number	0.509	0.001	*	Moderate
Cherelle wilt and pod number	0.624	< 0.0001	**	Strong

In pears (Browning et al. 1992) and castor (Witchard 1997), PBZ is detected in the xylem and phloem, demonstrating stable acropetal and basipetal translocation (Desta and Amare 2021). It alters the source-sink relationship via triazole-1-ethanol, reallocating carbohydrate to other organs rather than shoot apices (Lal et al. 2023). This increases carbohydrate accumulation in source tissues (e.g. stems in mango, trunks, and branches in cacao and durian), directing assimilate partitioning to reproductive organs. Studies have shown that PBZ stimulates early flowering and increases flower production in durian (Tri et al. 2011) and various accessions of acid lime (Tripathi and Dhakal 2005; Kondle et al. 2020). It also stimulates axillary flower initiation in pears (Browning et al. 1992) and in dormant shoot buds arising from trunks in grapevines (Christov et al. 1995). In mango, PBZ enhances flower production by increasing starch accumulation in stems and reducing gibberellin levels below a certain threshold (Protacio et al. 2000; 2006). High gibberellin levels inhibit ADP-glucose pyrophosphorylase (AGPase; Zheng et al. 2012) activity, the key regulatory enzyme for starch biosynthesis and accumulation (Mares et al. 1981; Lloyd and Kossmann, 2019). Low gibberellin levels increase AGPase activity, leading to starch accumulation in stems. Hence, it increases assimilate partitioning to economically important plants parts, like potato tubers, by stimulating low gibberellin levels that enhance sink strength activity (Tekalign and Hammes 2005).

Influence of PBZ on fruit production of cacao var UF667

A t-test comparison between PBZ-treated and untreated trees did not show significant results (p>0.05; Table 1) in increasing cherelle number, while untreated trees significantly (p=0.005) retained more pods. Although PBZ-treated trees had a higher number of cherelles, the difference was not significant than untreated trees. Untreated trees retained more pods, possibly due to lower cherelle wilt. The slight increase in cherelles in PBZ-treated trees could be linked to high flowering response. However, the increase in cherelle number also led to a higher chance of cherelle wilting, as cherelle number was strongly correlated with cherelle wilt (p = 0.004; Table 1). The results suggest that PBZ did not significantly increase pod retention, likely due to insufficient carbohydrate reserves to sustain pod growth amid high flowering

Table 6. Summary of the total number of flowers, cherelles, and pods produced from March to December 2021, and percent conversion of flowers to pods in paclobutrazol-treated and untreated cacao (*Theobroma cacao* L.) var UF18 and UF667.

Parameters	Cacao var UF18			Cacao var UF667		
	Control	PBZ	Percent Increase	Control	PBZ	Percent Increase
Total cushions	2814.33	3818.50	35.68	1531.80	1685.40	10.02
Total flowers	12042.50	25703.00	113.44	6663.19	12222.48	83.43
Flowers per cushion	49.00	48.97	-0.06	29.76	47.37	59.17
Total cherelles	40.25	102.00	153.42	38.80	48.50	25.00
Total pods	23.66	56.88	140.40	38.20	18.50	-51.57
% Flowers to cherelle	0.33	0.40	21.21	0.58	0.40	-31.03
% cherelles to pods	58.78	55.76	-5.13	98.45	38.14	-61.26
% Flowers to pods	0.19	0.22	15.79	0.057	0.15	163.16

Figure 4. Cherelle wilting in cacao (*Theobroma cacao* L.) observed during the early stage of cherelle growth

density and cherelle formation. In addition, a high degree of fungal infection was observed at the peak of the rainy season, affecting cherelle and pod development, which limits the response of cacao to PBZ treatment. Therefore, this study needs further clarification.

Influence of PBZ on leaf flushing of cacao var UF667

The t-test analysis showed that PBZ treatment did not significantly impact leaf flushing (p>0.05; Table 1). However, PBZ application significantly reduced shoot length in cacao trees (p<0.0001; Table 1), as evidenced by the production of new flushes with shorter internodes. It also significantly decreased the number of leaves per new shoot (p<0.001; Table 1) with averages of 5.0 for untreated and 4.2 for PBZ-treated trees. Stem diameter was not significantly affected (p>0.05). Thus, PBZ did not effectively regulate leaf flushing but reduced internode extension and leaf production per new shoot. Leaf flushing occurred monthly, beginning in March, one month after fertilizer application. It drastically decreased from August onwards, with very few flushes (0 to 5). The decrease in shoot length of new flushes in PBZ-treated trees confirms its role as a gibberellin biosynthesis inhibitor, which inhibits cell elongation and internode extension, thereby reducing plant growth (Desta and Amare 2021).

Table 7. Dried bean yield (kg tree^{-1} year^{-1}) of cacao (*Theobroma cacao* L.) based on pod production.

Variety	Number of pods tree^{-1}year^{-1}		Kilogram dry bean tree^{-1}year^{-1}	
	Control	PBZ	Control	PBZ
Cacao var UF18	23.66	56.88	1.18	2.84
Cacao var UF667	38.20	18.50	1.91	0.94

Effect of PBZ application on flower production of cacao var UF18

A t-test comparison revealed that the phenological pattern of flower production in cacao var UF18 was significantly correlated to PBZ treatment. There was a high correlation between flowering intensity (total cushions, total flowers, and the number of flowers per cushion) and the treatments (p = 0.028, p = 0.012, and p = 00.50, respectively; Table 4). Figure 3 showed the trend of flowering response, with high flowering intensity from June to September. Flowering peaked in June and August producing more flowers in PBZ-treated trees than in control trees. The highest flowering was recorded in June with 1222 total cushions, 11492 total flowers, and 9 flowers per cushion per tree for PBZ treated trees, and 579 total cushions, 2582 total flowers, and 4 flowers per cushion per tree for control trees. Flowering intensity decreased in July but increased again in August, with PBZ-treated trees producing more flowers than the control trees. The results suggested that PBZ application in cacao var UF18 enhanced flowering intensity by increasing the total cushions per tree and the number of flowers per cushion, thereby increasing the total flowers per tree.

Effect of PBZ application on fruit production, leaf flushing and the regulation of cherelle wilt in cacao var UF18

The t-test analysis revealed no significant difference in fruit production (cherelle and pod number) between PBZ-treated and untreated cacao varUF18 trees (p > 0.05; Table 4). Cherelle wilt showed no significant correlation (p > 0.05) between treatments. However, there was a moderate positive association (p = 0.007; Table 5) between cherelle wilt and cherelle number, and a positively strong correlation (p<0.0001; Table 5) between wilt and pod number. Increase cherelle wilting with more fruits indicated competition among cherelles and pods for photoassimilates. Differences between cherelle and pod numbers were moderately significant ($p = 0.001$; Table 5) between treatments. Increased cherelle numbers led to reduced pod retention due to cherelle wilting. Similarly, leaf flushing showed no correlation between treatments. PBZ had no influence on leaf flushing behavior in cacao, which is seen as a periodic physiological response to replace abscised leaves. Moreover, PBZ did not significantly reduce shoot elongation, stem diameter, or the number of leaves per new shoot ($p > 0.05$; Table 4). Figure 4 shows cherelle wilting in cacao.

Total flower, fruit production, and dried bean yield in UF667 and UF18

From March to December 2021, flower and fruit production were assessed, and the percent conversion of flowers to pods in PBZ-treated and untreated trees was computed. Table 6 shows that PBZ application improved the production of flowers, cherelles, and pods in cacao var UF18. Similarly, flower and cherelle production was enhanced in cacao var UF667, but pod retention was lower in PBZ-treated trees than in untreated trees. PBZ-treated trees of cacao var UF18 recorded higher total cushions per tree over nine months than the control (3818.5 and 2814.33, respectively). Flowering intensity increased by more than 100% in both varieties. Cacao var UF18 was highly responsive to PBZ treatment in terms of flowers, cherelles, and pods. Cherelle formation slightly increased in cacao var UF667, but pod number decreased by 48.05%. The percentage conversion of flowers to cherelle was less than 1%, and flowers to pods ranged from 0.05 to 0.2%. PBZ application doubled the dry bean yield in cacao var UF18 (1.18 to 2.84 kg $\text{tree}^{-1}\ \text{yr}^{-1}$), higher than the estimated local production (0.5 to 1.0 kg $\text{tree}^{-1}\ \text{yr}^{-1}$). However, dry bean yield significantly reduced in cacao var UF667 due to low pod retention. Table 7 shows the dried bean yield based on the number of pods produced, derived from pod index of 20 pods per kilogram of dried beans. The findings indicate that PBZ effectively enhanced the production of flowers, cherelles, and pods in cacao var UF18 and UF667. However, environmental factors (e.g., shading) and health status may have interfered with the response to PBZ treatment, particularly in cacao var UF667, suggesting the need for an appropriate cultural management system (e.g., pruning and pest management) to prove PBZ's efficacy.

CONCLUSION AND RECOMMENDATION

Understanding the source-sink relationships in cacao is crucial for addressing issues such as cherelle wilt and enhancing flowering and pod retention, ultimately leading to increased bean yield. The application of the GA-inhibitor compound PBZ has been shown to increase starch accumulation in sink tissues, thereby supporting the growth of developing cherelles, minimizing cherelle wilt, and enhancing pod retention.

PBZ was effective in increasing leaf SPAD value, although it only slightly enhanced carbohydrate accumulation in cacao leaves. The increase in leaf SPAD value also contributed to increased flowering and cherelle formation. Overall, PBZ significantly boosted flower production, increasing the total number of flowers and flower cushions for UF667, as well as the total number of flowers and flowers per cushion per tree for cacao UF18.

However, fruit production was not influenced by PBZ application, which may be attributed to some other factors and health conditions affecting cherelle formation and pod retention. While flower production increased by more than 100%, the conversion rate to cherelles and pods remained very low. Despite this, the dried bean yield increased significantly by 1.67 kg per tree per year, surpassing local cacao production averages.

Observations also indicated that cherelle wilt increased with the rise in cherelles and pods, suggesting competition among reproductive organs for photoassimilates. The occurrence of fungal infections during the peak of fruit production, especially during the rainy season, further limited this study. Therefore, integrating proper cultural management practices such as pruning and pest management, along with PBZ application, is crucial for increasing yield in cacao trees.

The results of this study lay a foundation for future research aimed at understanding the physiology of cacao, which could lead to improved bean yield.

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