



Application Paclobutrazol and Duration of Drought Stress to Flowering Induction in Chokun Orange

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Abstract

Induction of flowering is one of the efforts that can extend the production period of Chokun oranges. This study aims to determine the effect of the dose of paclobutrazol (PBZ), duration of drought stress and the combination of treatments that gives the best results on the Chokun orange (*Citrus* sp.). The research design used was randomized complete block design (RCBD), consisting of 3 replications. The treatment in this study was a combination of the dose of PBZ (control, active ingredient 0.75 g plant⁻¹ and active ingredient 1.5 g plant⁻¹) and duration of drought stress (control, 1, 2 and 3 weeks). The results show that the application of PBZ and duration of drought stress can induce the flowering of citrus plants as seen from the generative shoot variables with a quadratic model on the equation $y = -17.778x^2 + 31.556x + 26.667$ at the optimum dose of 0.89 g plant⁻¹ of active ingredient and 1 week of drought. The dry period of 3 weeks gives the best results seen from variables of the number of flowers and number of fruits. In general, the results suggest that the application of PBZ and duration of drought stress can transfer from the vegetative phase to the generative phase which in turn could induce the flowering of citrus plants.

Keywords: bud dormancy; gibberellin; off-season flowering; plant growth regulator; water deficit

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INTRODUCTION

Citrus (*Citrus* sp.) is a perennial plant commodity that has a major contribution to horticultural production in Indonesia. Citrus production in 2020 reached 2.72 million tons, 6.22% (159.46 thousand tons) lower than the production in 2019. Consumption of oranges by the household sector in 2020 reached 887.62 thousand tons, an increase of 25.3% (301 thousand tons) compared to the rate in 2019. The household engagement in citrus consumption is 25.69%. Citrus production is seasonal because citrus plants can bear fruit for only a few months each year. Siamese/tangerine production in Indonesia in 2020 fluctuated. Citrus production

in the second and third quarters reached 795,116 tons and 731,735 tons, but in the first and fourth quarters, the production was only 547,758 tons and 518,775 tons. This is because the second and third quarters are citrus fruit harvesting seasons in Indonesia so citrus production is high. This is not profitable because the supply of citrus fruits is abundant during the harvest season. The low supply of oranges outside the harvesting season causes the stability of local citrus fruit prices in the market to be unmanageable (BPS-Statistics Indonesia, 2021). To overcome these problems, plant cultivation techniques are needed to regulate citrus fruit production so that the supply of citrus fruits is always available and can meet consumer needs at any time.

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One effort that can extend the production period of citrus is to regulate flowering induction. Induction of interest is a complex biological process that integrates internal and external factors (Fan et al., 2016). Flower formation is a transitional phase in the plant life cycle. Flowering is a phase that will affect the quality and quantity of fruit production (Jhade et al., 2018). Citrus plants are known for their very short flowering period but often face obstacles because they require special requirements to be able to induce flowering and fruiting (Arcentales et al., 2017). During the flowering period, not all axillary or shoots on citrus plants can bloom and as a result plants that are not induced do not undergo a transition from the vegetative phase to the generative phase (Ogu and Orjiakor, 2017). The flowering process is influenced by the total sugar content in the leaves and the C/N ratio. In the induction stage, there is an increase in the total sugar content and the leaf C/N ratio compared to before induction (Arcentales et al., 2017).

Broadly speaking, flowering induction can be done in two ways, namely chemical/hormonal and physical (Hendrawan, 2013). Much evidence suggests that flower initiation is strongly influenced by hormones (Ahmed et al., 2014). Chemically/hormonally, the active ingredients of growth regulators can be used. The principle of flowering induction by chemical means is to change plant physiology by inhibiting the vegetative growth phase through the role of hormones or certain chemical compounds, so that flowers and fruit appear in the generative phase (Fitri and Salam, 2017). Paclobutrazol (PBZ) is a triazole group that has the most essential role in inhibiting growth and is commonly used for flower induction in fruit plants. The correlation of the effect of PBZ given during the off-season is hormonal changes. Hormonal changes that occur are an increase in cytokinin hormones while gibberellins and auxins are reduced (Rahim et al., 2011a). According to research by Upreti et al. (2013), the C/N ratio in shoots, leaf water potential and ABA content increased, followed by an increase in the number of shoots in plants treated with PBZ. In contrast, the content of cytokinin-zeatin (Z), zeatin riboside (ZR) and dihydrozeatin riboside (DHZR) in shoots increased consistently from 30 days before bud breakage until flower bud initiation.

The effectiveness of PBZ in inducing flowering into fruit in citrus plants depends on the threshold of each cultivar (Martínez-Fuentes et al., 2013). According to Desta and Amare (2021), the application of PBZ is more effective when applied to growing media because the absorption of the active ingredient is higher than absorption through the foliar spray. According to Moreira et al. (2016), PBZ promotes vegetative growth and results in higher flowering and fruiting growth in Ascolana olives. According to Xing et al. (2016), the application of PBZ causes an increase in the number of flowers on apple plants. Burondkar et al. (2013) mentioned that due to the application of PBZ to plants for three years the average yield showed a significant difference in flowering (85.4 days) and progress in harvesting (82 days).

Induction of physical flowering can be done by cutting, pruning, wounding, binding and drought stress. The principle of physical induction is to change the ratio of the elements C and N in the plant body (Hendrawan, 2013). Drought stress does not directly affect flowering plants but causes flower induction or a transition from the vegetative phase to the generative phase (Fitri and Salam, 2017). The environmental factor that affects the induction of flowering in fruit trees in the tropics is drought stress. Drought stress is often untimely, so it is necessary to manipulate or artificially create stress conditions for plants (Sakhidin and Suparto, 2011).

The use of PBZ in excessive concentrations can lead to the accumulation of residues in the soil (Prates et al., 2021). The expectancy of this research is that the combination of PBZ treatment with drought stress can reduce residues in the soil to minimize environmental damage in support of environmental sustainability. The application of PBZ with the right dose and the right dry time can induce flowering thereby prolonging the production period of citrus fruits. According to Bithell et al. (2013), mango plants given PBZ treatment and low to moderate pre-flowering irrigation rates increased the number of fruit per tree especially in the off-season. Determining the right irrigation level is important to improve the efficiency-yield relationship water for PBZ-treated trees. Therefore, the purpose of this study was to determine the effect of the dose of PBZ, duration of drought stress and the combination of dose of PBZ and duration of drought stress on flowering induction of Chokun oranges. This

is done to regulate the time of flowering and fruiting on citrus plants so that they could produce out of season.

MATERIALS AND METHOD

Research time and place

This research was carried out on land in Rejasari Village, West Purwokerto Sub-district, Banyumas Regency, Central Java Province of Indonesia at the coordinates point of 7°25'13.4"S 109°12'52.9"E, with an altitude of 100 m above sea level. Analysis of the gibberellin content of leaves was carried out at the Indonesia Agricultural Postharvest Research and Development Bogor and the microscopic analysis of shoots was carried out at the Laboratory of Plant Structure and Development, Faculty of Biology, Universitas Jenderal Soedirman. The research was carried out from August 2020 to January 2021.

Research materials and design

The materials used in the study include 4-year-old Chokun variety citrus plant seeds. This study use a pot experiment with a factorial experiment in a randomized complete block design (RCBD). The first factor is the dose of PBZ, consisting of 3 levels, without PBZ as control (P0), active ingredient 0.75 g plant⁻¹ (P1) and active ingredient 1.5 g plant⁻¹ (P2). This study utilizes a lower dose than the recommended dose because there were 2 treatments with the same goal, namely to induce flowering. Concerning dosage recommendation according to research by Darmawan et al. (2014), trees that were given PBZ as much as 2 g plant⁻¹ increased the number of flowers by 66.28% compared to the control, by increasing the carbohydrate content and C/N ratio in the leaves of tangerine plants. The second factor is the duration of drought stress. These factors consist of 4 types, without drought stress (routine irrigation, K0), 1 week (K1), 2 weeks (K2) and 3 weeks (K3). According to Rahayu et al. (2020), 3 weeks without irrigation with a moisture content of 65.21% of field capacity is the duration without irrigation and the optimum water content for Madura tangerine flower induction.

This experiment consists of 12 treatment combinations with 3 replications resulting in 36 experimental units and 1 experimental unit contains 2 plants. Therefore, there were 72 plants for the total of all experiments. The data obtained

from the results of the study were analyzed using the F test at a level of 5% and if there was a (significant) difference, analysis proceeded with the DMRT (Duncan Multiple Range Test) at a level of 5% and regression analysis was performed.

Research procedure

Plants used for research were selected based on the uniformity of plant age, plant height and plant conditions. The selected plants were in good health with a good root system. The composition of the prepared media was incepticols, rice husks and manure with a ratio of 2:1:1. The orange seedlings that have been prepared were transplanted by mixing the prepared media using a 50-liter planter bag. After transplanting, the media was watered until it reached field capacity and then acclimatized for 1 month. The planting medium was analyzed for moisture content using the oven method which would later be used for irrigation determination.

Drought treatment was carried out after acclimatization for 1 month. During this period groundwater conditions were maintained at 100 % field capacity. On the last day of the period, the growing medium was saturated with water by continuously adding water until water came out of the polybag. PBZ was applied 2 days after media saturation with a dose of active ingredient 0.75 and 1.5 g plant⁻¹ dissolved in 1 l of distilled water by pouring it into the planting medium around the base of the stem. Plants that had been treated were covered with media using plastic so that water from outside could not enter the planting medium. Duration of drought stress was carried out according to the treatment, namely 1 week, 2 weeks and 3 weeks. After the drought period ended, the media was saturated with water.

The parameters observed in this experiment were the variable generative shoots and number of flowers observed once a week until the 16th week. Variables of fruit set, the number of fruits and fruit loss were observed every two weeks until the 16th week. Analysis of C content was carried out using the Luff Schoorl method. Analysis of N content was carried out using the Semimicro-Kjeldahl method. Leaf sampling was carried out after saturated water was given at the end of the drought period. Calculation of the C/N ratio was based on the C and N analysis.

Analysis of gibberellin content was carried out using the method. Leaf sampling was performed after fertilization induction treatment (Sakhidin and Suparto, 2011). Microscopic observation of shoot tissue was conducted using the paraffin technique. Tissue collection was done after saturating water was given at the end of the drought period.

RESULTS AND DISCUSSION

The results of this study showed that citrus plants with the application of PBZ and duration of drought stress could stimulate generative induction as seen from the generative shoot variables, fruit set and fruit loss. The application of PBZ did not show any difference. The dry period of 3 weeks showed that there were differences in the variables when the first flowers appeared and when flower buds bloomed, as well as in the number of fruits. This is supported by the low content of gibberellins.

Generative phase induction

Table 1 shows the best number of generative shoots obtained at a dose of PBZ active ingredient $0.75 \text{ g plant}^{-1}$ and a dry period of 1 week. Figure 1 shows that during the 1 week long dry period, increasing the dose of PBZ active ingredient from 0 to $0.89 \text{ g plant}^{-1}$ increased the number of generative shoots, but after that increasing the dose of PBZ would decrease the number of generative shoots. According to the research of Martínez-Fuentes et al. (2013),

PBZ will promote flowering in citrus trees by increasing the number of generative shoots that will later experience flowering and reducing the number of vegetative shoots. According to Lolaei et al. (2013), the application of PBZ significantly reduced the vegetative growth rate by decreasing shoot length and decreasing number of leaves. The effect of PBZ can occur in the form of increase in the size and number of fruits to increase crop yields.

Table 1 shows that in the administration of the active ingredient $0.75 \text{ g plant}^{-1}$, the best fruit set was shown in the absence of a dry period. Figure 2 demonstrates that in the absence of drought stress, increasing the dose of PBZ active ingredient from 0 to $1.12 \text{ g plant}^{-1}$ increased the fruit set, but after that increasing the dose of PBZ decreased the fruit set. Fruit set is the ratio between the number of fruits formed and the total number of flowers formed (Darmawan et al., 2014). PBZ acts as a gibberellin inhibitor thereby reducing the level of the vegetative promoter. This will increase the ratio of florigenic promoters in plants so that it will induce flowering (Rahim et al., 2011b). According to Gollagi et al. (2019), PBZ has been shown to reduce growth, initiate flower budding, increase flower number and increase yield and fruit quality. The drought period can trigger flowering induction after shoot initiation (Ramírez et al., 2014). According to the research by Kuswandi et al. (2019), initiation of flowering in guava plants is stimulated by the length of the dry period.

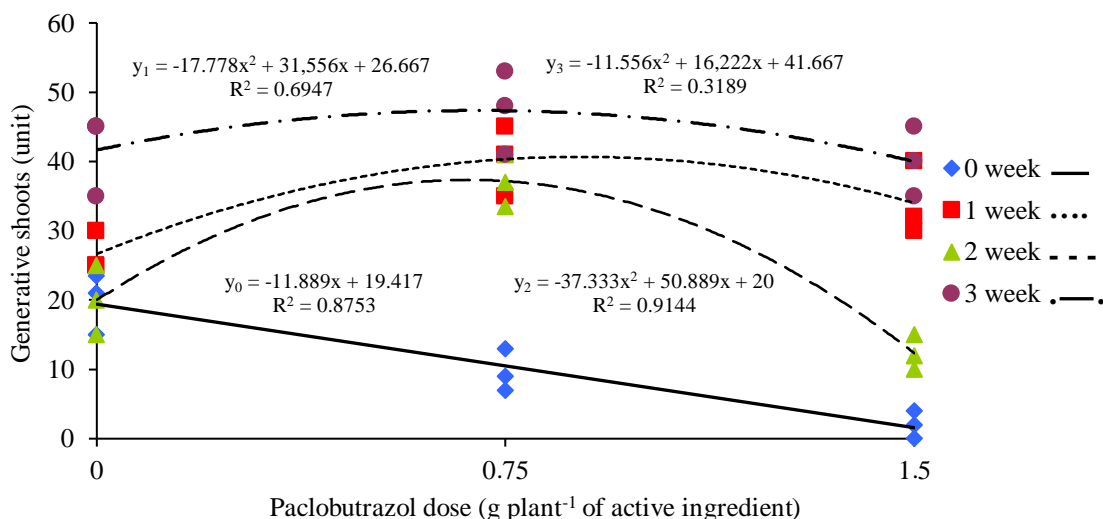


Figure 1. The interaction effect of the dose of PBZ and duration of drought stress on the number of generative shoots of Chokun orange

Table 1. The results of the variance of the effect of the dose of PBZ and duration of drought stress on the induction of the generative phase of Chokun orange

Variable observation	PBZ dose (g plant ⁻¹ of active ingredient)	Drought stress (week)				Mean
		0	1	2	3	
Generative shoots (units)	0.00	19.83 Ba	26.67 Bb	20.00 Bb	41.67 Aa	27.04
	0.75	9.67 Cb	40.33 ABa	37.17 Ba	47.33 Aa	33.63
	1.50	2.00 Cb	34.00 Aab	12.33 Bb	40.00 Aa	22.08
	Mean	10.50	33.67	23.17	43.00	(+)
The first flower appearances (units)	0.00	88.67	84.00	77.00	51.33	75.25 A
	0.75	93.33	77.00	91.00	65.33	81.67 A
	1.50	81.67	30.33	67.67	67.67	61.83 A
	Mean	87.89 a	63.78 a	78.56 a	61.44 a	(-)
Number of flowers (unit)	0.00	9.33	18.67	8.00	24.50	15.13 A
	0.75	1.83	13.67	13.67	26.33	13.88 A
	1.50	1.33	13.50	3.67	21.33	9.96 A
	Mean	4.17 c	15.28 b	8.44 c	24.06 a	(-)
Fruit set (%)	0.00	67.22 Ba	62.66 Ba	100.00 Aa	100.00 Aa	82.47
	0.75	100.00 Aa	84.70 Aa	84.01 Aa	59.28 Ba	82.00
	1.50	100.00 Aa	100.00 Aa	100.00 Aa	50.63 Aa	87.66
	Mean	89.07	82.45	94.67	69.97	(+)
Number of fruits (unit)	0.00	4.67	8.00	5.33	9.00	6.75 A
	0.75	3.00	8.00	5.33	12.17	7.13 A
	1.50	0.00	7.83	3.33	10.00	5.29 A
	Mean	2.56 b	7.94 a	4.67 b	10.39 a	(-)
Fruit drop (%)	0.00	19.44 Bb	31.77 Ba	49.55 Aa	60.28 Aa	40.26
	0.75	28.33 Bb	32.90 Ba	57.89 Aa	18.80 Bb	34.48
	1.50	100.00 Aa	39.81 Ba	23.33 BCb	11.48 Cb	43.66
	Mean	49.26	34.83	43.59	30.19	(+)

Note: Numbers followed by capital letters are the same in the same row not significantly different in the 5% DMRT; numbers followed by lowercase letters are the same in the same column not significantly different in the 5% DMRT

Table 1 shows that in the absence of dry period, the lowest fruit loss was obtained in the absence of PBZ. Figure 3 present that without drought stress, increasing the dose of PBZ active ingredient from 0 to 0.26 g plant⁻¹ decreased fruit loss, but after that increasing the dose of PBZ increased fruit loss. The addition of the dose of PBZ causes the gibberellin content in plants to be low, this is the cause of high fruit loss. Meanwhile, according to Gollagi et al. (2019), plants treated with PBZ show an increase in the production of the hormone abscisic acid (ABA). Confirm in the research of Iglesias et al. (2007), high concentrations of ethylene and ABA and low concentrations of auxin and gibberellins are the causes of fruit loss.

Table 1 shows that the administration of PBZ and the length of the dry period did not show any difference in the variable when the first flowers appeared. The dry period of 3 weeks was able to increase flower buds, blooms and the number of fruits by 516.32%, 476.97% and 30.19% compared to those with no drought period. According to Kazan and Lyons (2016), drought stress is a factor that can affect plants in inducing flowering, thus affecting plant production. Plants accumulate high ABA under water-deprived conditions (Shanker et al., 2014). ABA promotes the transcriptional regulation of FT, TSF and SOC1 leading to plant flowering (Riboni et al., 2013). The flowering of citrus plants is related to the Flowering Locus T (CiFT) gene. This gene

will shorten the juvenile period of citrus plants by promoting flower induction by expressing CiFT (Nishikawa, 2013). According to the research by Endo et al. (2018), ABA accumulation correlates with CiFT homologous transcript accumulation and flowering intensity of Satsuma mandarin citrus. According to Chica and Albrigo (2013), plants that experience a period of drought will increase CiFT expression and flower induction will increase. CiFT expression level can be used to predict flowering potential in citrus plants. Validate in the research of Li et al. (2017), increasing CiFT will result in more flowers forming in plants under water deficit conditions than control plants. The accumulation of CiFT-protein and positive regulatory genes in shoots

will then initiate the shoot transition from the vegetative phase to the generative phase and continues in the differentiation stage until the development of floral organs (Su et al., 2013).

According to research by Li et al. (2017), when the long dry period of treatment is carried out, differentiation occurs quickly and produces sepal primordia. This is supported by the research by Takeno (2016), which shows that flowering induction can be influenced by the dry period of a plant or can be called drought stress. The research by Panigrahi and Srivastava (2016), has concluded that treatment of long periods of periodic drought can induce the flowering of tangerines.

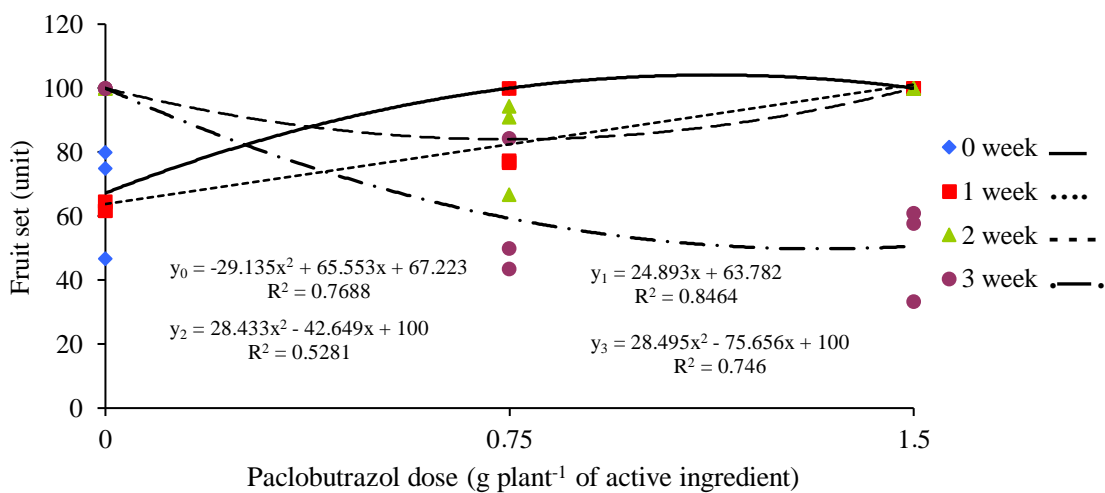


Figure 2. The interaction effect of the dose of PBZ and duration of drought stress on fruit set of Chokun orange

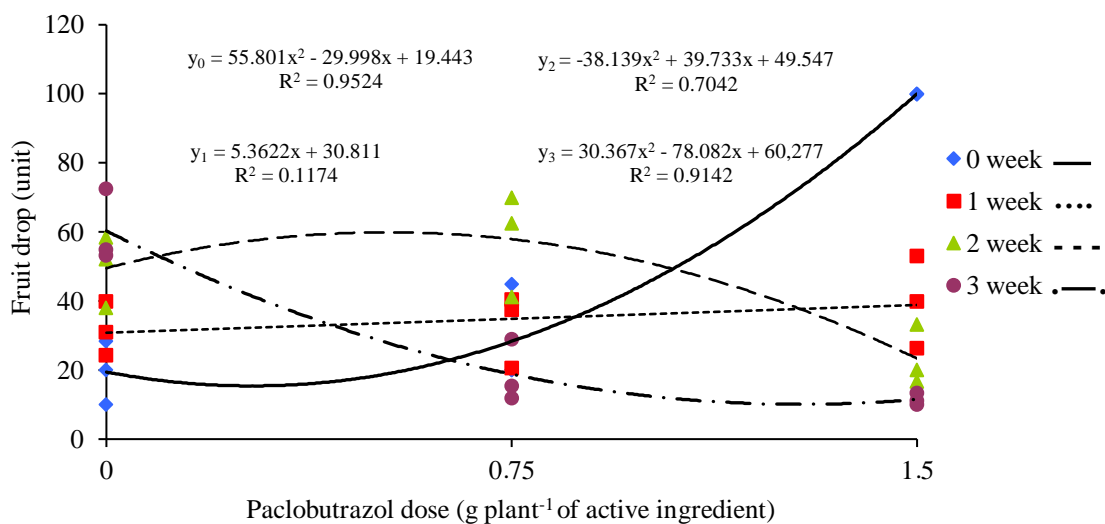


Figure 3. The interaction effect of the dose of PBZ and duration of drought stress on fruit drop of Chokun orange

C/N ratio and gibberellin content in leaves

Table 2 present that the lowest gibberellin content was obtained during the dry period of 3 weeks and the dose of PBZ active ingredient was 1.5 g plant⁻¹. Figure 4 shows that during the dry period of 3 weeks, increasing the dose of PBZ active ingredient from 0 to 2.11 g plant⁻¹ decreased the gibberellins content. PBZ and long dry period work by inhibiting gibberellin biosynthesis, thereby inhibiting cell elongation in sub-apical meristems. Jungklang et al. (2017), stated that when gibberellin production is inhibited, cell division still occurs but new cells do not elongate. The PBZ application will

reduce the content of GA4, GA3, GA7 and GA1 contained in shoots and leaves (Upreti et al., 2013). This is also demonstrated by the research of Srilatha et al. (2015), that the application of PBZ will reduce the content of gibberellins (GA3). According to Rani et al. (2018), PBZ may have acted as an anti-gibberellin compound and inhibited vegetative shoot development, nucleic acid synthesis and protein metabolism. According to Rahayu et al. (2020), a high gibberellin content indicates a response to a long dry period that can inhibit the flowering process, while a low gibberellin content indicates a response to flowering.

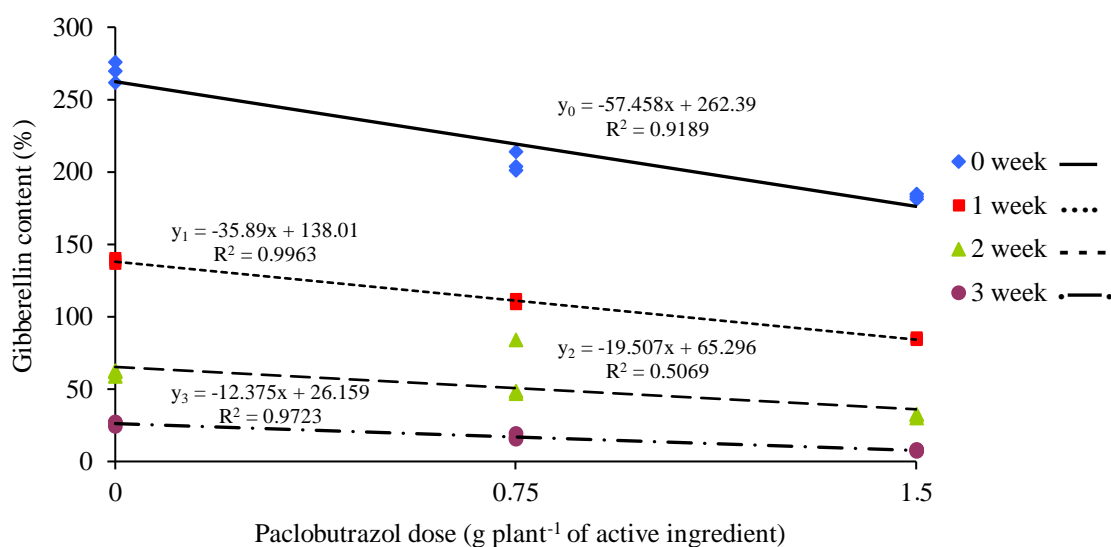


Figure 4. The interaction effect of the dose of PBZ and duration of drought stress on gibberellin content of Chokun orange

Table 2. The results of the variance of the effect of the dose of PBZ and duration of drought stress on the C/N ratio and gibberellin content

Variable observation	PBZ dose (g plant ⁻¹ of active ingredient)	Drought stress (week)				Mean
		0	1	2	3	
C/N ratio (%)	0.00	5.74	3.93	4.56	5.27	4.87 A
	0.75	6.37	4.82	4.41	4.98	5.14 A
	1.50	4.71	4.36	4.73	4.47	4.57 A
	Mean	5.61 a	4.37 a	4.56 a	4.91 a	(-)
Gibberellin content (unit)	0.00	268.99 Aa	138.31 Ba	60.84 Ca	26.07 Da	123.55
	0.75	206.10 Ab	110.47 Bb	59.57 Ca	17.05 Dab	98.30
	1.50	182.80 Ac	84.48 Bc	31.58 Cb	7.51 Db	76.59
	Mean	219.29	111.09	50.67	16.88	(+)

Note: Numbers followed by capital letters are the same in the same row not significantly different in the 5% DMRT; numbers followed by lowercase letters are the same in the same column not significantly different in the 5% DMRT

Inhibition of gibberellin biosynthesis by PBZ is at the kaurene stage and has been shown to reduce vegetative growth (Kumar et al., 2019). PBZ can be absorbed by plants through leaves, stem vessels, or roots, then translocated acropetally through the xylem to other plant parts. This compound will inhibit the biosynthesis of gibberellins by inhibiting the oxidation of ent-kaurene to kaurenoic acid. According to Gollagi et al. (2019), inhibition of gibberellin production causes cell division to still occur, but new cells do not elongate which results in the initiation of vegetative shoots and shorter internodes.

CONCLUSIONS

In conclusion, the application of PBZ and duration of drought stress can induce flowering of citrus plants as seen from the generative shoot variables with a quadratic model on the equation $y = -17.778x^2 + 31.556x + 26.667$ at the optimum dose active ingredient of $0.89 \text{ g plant}^{-1}$ and 1 week of drought. The dry period of 3 weeks gave the best results seen from the number of flowers and number of fruits. In general, the results suggest that the application of PBZ and duration of drought stress can transfer from the vegetative phase to the generative phase which in turn can induce flowering of citrus plants.

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