COVERING LETTER

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

Abstract

Induction of flowering is one of the efforts that can extend the production period of chokun oranges. This study aimed to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of treatments that gave 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 paclobutrazol (control, active ingredient 0.75 g/plant and active ingredient 1.5 g/plant) and duration of drought stress (control, 1, 2, and 3 weeks). The results showed that The application of paclobutrazol and the length of the drought period were able to induce flowering of citrus plants as seen from the generative shoot variables with a quadratic model on the equation y = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient/plant and 1 week of drought. The dry period of 3 weeks gave the best results seen from the variable number of flowers and number of fruit.

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

INTRODUCTION

Citrus (Citrus sp.) is an annual fruit commodity that has a major contribution to horticultural production in Indonesia. Citrus production in 2020 reached 2.72 million tons, down 6.22% (159.46 thousand tons) from 2019. Consumption of oranges by the household sector in 2020 reached 887.62 thousand tons, an increase of 25.3% (301 thousand tons) from 2019. The household participation 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, they were 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 out of season causes the stability of local citrus fruit prices in the market is not guaranteed (Badan Pusat Statistik, 2021). To overcome these problems, plant cultivation techniques are needed to regulate citrus fruit production so that it is hoped that the supply of citrus fruits is always available and can meet consumer needs at any time.

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). According to Jhade et al. (2018), 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. 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, as a result not all axillary or shoots can be induced and the transition phase from the vegetative phase to the reproductive phase (Ogu & Orjiakor, 2017). The flowering process was influenced by the total sugar content in the leaves and the C/N ratio. In the induction stage, there was 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 (Sreekumar et al., 2014). Chemically/hormonally, the active ingredients of growth regulators (ZPT) 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 the generative phase, flowers, and fruit appear(Fitri & Salam, 2017). Paclobutrazol (PBZ) is a triazole group that has the most role in inhibiting growth which is commonly used for flower induction in fruit plants. The correlation of the effect of paclobutrazol given during the off-season is hormonal changes in tropical conditions. Hormonal changes that occur are an increase in cytokinin hormones while gibberellins and auxins are reduced (Rahim et al., 2011b). According to research by Upreti et al. (2013a), 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 paclobutrazol. 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 paclobutrazol in inducing flowering into fruit in citrus plants depends on the threshold of each cultivar (Martínez-Fuentes et al., 2013). According to Desta & Amare (2021), the application of paclobutrazol is more effective when applied to growing media because the absorption of the active ingredient is more than foliar spray. According to researchMoreira et al. (2016), paclobutrazol promotes vegetative growth and results in higher flowering and fruiting growth in Ascolana olives. According to Xing et al. (2016), the application of paclobutrazol caused an increase in the number of flowers on apple plants. (Burondkar et al., 2013) mentioned the application of paclobutrazol 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 carbon (C) and nitrogen (N) in the plant body (Hendrawan, 2013). According to Fitri & Salam (2017), drought stress does not directly cause flowering plants but causes flower induction or a transition from the vegetative phase to the generative phase. The environmental factor that affects the induction of flowering in fruit trees in the tropics is drought stress. Drought stress obtained by plants is often not timely, so it is necessary to manipulate or artificially create stress conditions for plants (Sakhidin & Suparto, 2011).

The use of paclobutrazol in excessive concentrations can lead to the accumulation of residues in the soil (Prates et al., 2021). The combination of paclobutrazol treatment with drought stress is expected to reduce the use of high doses of chemicals to minimize environmental damage in supporting sustainable agriculture. The application of paclobutrazol with the right dose and the right dry time can induce flowering thereby prolonging the production period of citrus fruits. Based on this, the purpose of this study was to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of dose of paclobutrazol 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 can produce out of season.

MATERIAL AND METHODS

Research time and place

This research was carried out on land in Rejasari Village, West Purwokerto District, Banyumas Regency, Central Java Province of Indonesia (-7.4204009, 109.2147000), 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 microscopic analysis of shoots was carried out at the Laboratory of Plant Structure and Development, Faculty of Biology, Jenderal Sudirman University. The research has been carried out from August 2020 to January 2021.

Research materials and design

The materials used in the study included 4 years old Chokun variety citrus plant seeds. This study used a pot experiment which was carried out using a factorial experiment in a Randomized Complete Block Design (RCBD). The first factor is the dose of paclobutrazol. The factor consisted of 3 levels, without paclobutrazol as control (P0), 0.75 g active ingredient/plant (P1), and 1,5 g active ingredient/plant (P2). According to Darmawan et al. (2014), trees that were given paclobutrazol as much as 2 g/plant 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 consisted 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 study consisted of 12 treatment combinations with 3 replications resulting in 36 experimental units. One experimental unit contained 2 plants. Thus 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, proceed with the DMRT (Duncan Multiple Range Test) tests at a level of 5% and regression analysis was performed.

Research procedure

Plants were selected as many as 72 plants based on the uniformity of plant age, plant height, and plant conditions. The selected plants are in good health and have a good root system. The composition of the prepared media was incepticol soil, rice husks, and manure with a ratio of 2:1:1. The orange seedlings that have been prepared are 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 about field capacity. On the last day of the period, the growing medium is saturated with water. Paclobutrazol was applied 2 days after media saturation with a dose of 0.75 and 1.5 g active ingredient/plant dissolved in 1 liter of distilled water by pouring it into the planting medium around the base of the stem. Plants that have been treated are covered with media using plastic so that water from outside cannot enter. Duration of drought stress was carried out according to the treatment, namely after 1 week, 2 weeks, and 3 weeks. After the drought period ended, the media was saturated with water.

The parameter observed in this experiment is 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 (Yoshida et al. 1972; in Susanto et al. 2016). Analysis of N content was carried out using the Kjeldahl Semimicro method (William, 1984 in (Susanto et al. 2016). Leaf sampling was carried out after saturated water was given at the end of the drought period. Calculation of the C/N ratio is based on the C and N analysis that has been done. Analysis of gibberellin content was carried out using the method of Linskensen and Jackson (1987). Leaf sampling was carried out after fertilization induction treatment (Sakhidin & Suparto, 2011). Microscopic observation of shoot tissue was carried out using the paraffin method (Puslitbang Biology-LIPI, 1998). Tissue collection was carried out 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 paclobutrazol and duration of drought stress were able to stimulate generative induction as seen from the generative shoot variables, fruit set, and fruit loss. The application of paclobutrazol did not show any difference. The dry period of 3 weeks showed that there were differences in the variables when the first flowers appeared, flower buds bloomed, and the number of fruits. Table 2 and Figure 4 show that this is supported by the low content of gibberellins.

Generative Phase Induction

Table1. The results of the variance of the effect of the dose of paclobutrazol and the length of and duration of drought stress on the induction of the generative phase

Variable observation	Paclobutrazol doze (g of active ingredient/ plant)	Drought stress (week)				Average
		0	1	2	3	
Generative shoots (units)	0	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
	1,5	2.00 Cb	34.00 Aab	12.33 Bb	40.00 Aa	22.08
	Average	10.50	33.67	23.17	43.00	(+)
The first flower appears (units)	0	88.67	84.00	77.00	51.33	75.25 A
	0,75	93.33	77.00	91.00	65.33	81.67 A
	1,5	81.67	30.33	67.67	67.67	61.83 A
	Average	87.89 a	63.78 a	78.56 a	61.44 a	(-)
Number of flowers (unit)	0	9.33	18.67	8.00	24.50	15.13 A
	0,75	1.83	13.67	13.67	26.33	13.88 A
	1,5	1.33	13.50	3.67	21.33	9.96 A
	Average	4.17 c	15.28 b	8.44 c	24.06 a	(-)

Fruit set (%)	0	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
	1,5	100.00 Aa	100.00 Aa	100.00 Aa	50,63 Aa	87,66
	Average	89.07	82.45	94.67	69.97	(+)
Number of fruit (unit)	0	4.67	8.00	5.33	9.00	6.75 A
	0,75	3.00	8.00	5.33	12.17	7.13 A
	1,5	0.00	7.83	3.33	10.00	5.29 A
	Average	2.56 b	7.94 a	4.67 b	10.39 a	(-)
Fruit drop (%)	0	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
	1,5	100.00 Aa	39.81 Ba	23.33 BCb	11.48 Cb	43.66
	Average	49.26	34.83	43.59	30.19	(+)

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

Table 1 shows the best number of generative shoots obtained at a dose of 0.75 g of paclobutrazol active ingredient/plant and a dry period of 1 week. Figure 1 shows that during the 1 week long dry period, increasing the dose of paclobutrazol from 0 to 0.89 g of the active ingredient/plant increased the number of generative shoots, but after that increasing the dose of paclobutrazol would decrease the number of generative shoots. According to the research of Martínez-Fuentes et al. (2013) that paclobutrazol 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 paclobutrazol significantly reduced the vegetative growth rate by decreasing shoot length and decreasing leaf number. The effect of paclobutrazol can occur in the form of increasing the size and number of fruits to increase crop yields.

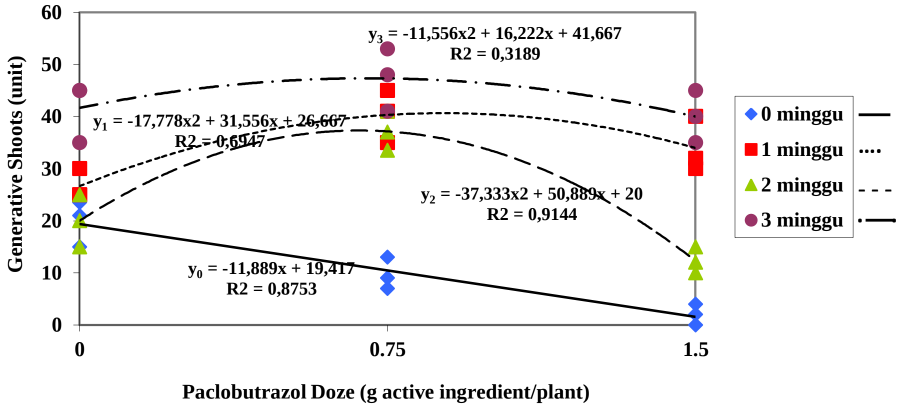

Figure 1. The interaction effect of the dose of paclobutrazol and duration of drought stress on the number of generative shoots

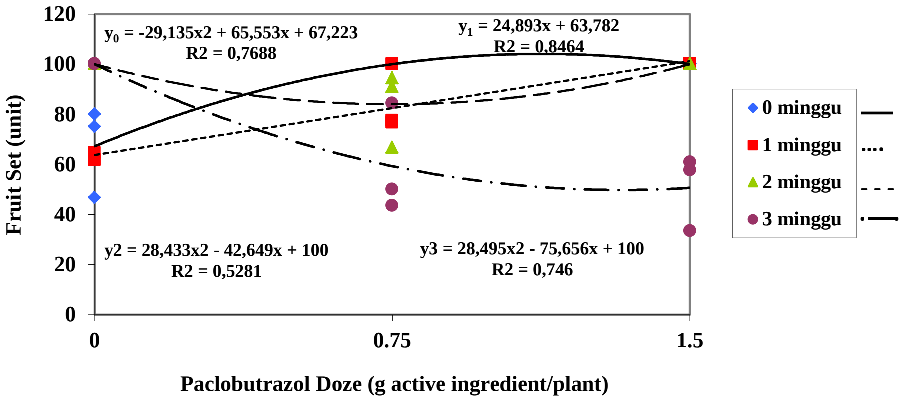

Figure 2. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit set

Table 1 shows that in the administration of 0.75 g of the active ingredient/plant, the best fruit set was shown in the absence of a dry period. Figure 2 shows that in the absence of drought stress, increasing the dose of paclobutrazol from 0 to 1.12 g of the active ingredient/plant increased the fruit set, but after that increasing the dose of paclobutrazol decreased the fruits et. Fruit set is the ratio between the number of fruits formed and the total number of flowers formed (Darmawan et al., 2014). Paclobutrazol acts as a gibberellin inhibitor thereby reducing the level of the vegetative promoter. Thus it 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), paclobutrazol 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 of Kuswandi et al. (2019), initiation of flowering in guava plants was stimulated by the length of the dry period.

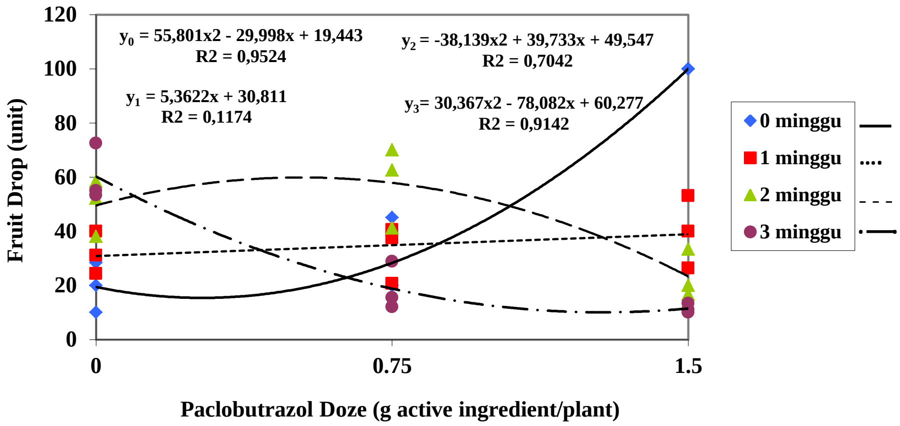

Figure 3. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit drop

Table 1 shows that in the absence of a dry period, the lowest fruit loss was obtained in the absence of paclobutrazol. Figure 3 shows that when without drought stress, increasing the dose of paclobutrazol from 0 to 0.26 g of the active ingredient/plant decreased fruit loss, but after that increasing the dose of paclobutrazol increased fruit loss. The addition of the dose of paclobutrazol causes the gibberellin content in plants to below, this is the cause of high fruit loss. Meanwhile, according to Gollagi et al. (2019), plants treated with paclobutrazol showed an increase in the production of the hormone abscisic acid (ABA). This is by 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 paclobutrazol 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 no drought period. According to Kazan & 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 research Endo et al. (2018), ABA accumulation correlated with CiFT homologous transcript accumulation and flowering intensity of Satsuma mandarin citrus. According to Chica & 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. This is by 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 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. According to research by Panigrahi & Srivastava (2016), treatment of long periods of periodic drought can induce the flowering of tangerines.

C/N Ratio and Gibberellin Content in Leaves

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

Variable observation	Paclobutrazol doze (g of active ingredient/ plant)	Drought stress (week)				Average
		0	1	2	3	
Ratio C/N (%)	0	5.74	3.93	4.56	5.27	4.87 A
	0,75	6.37	4.82	4.41	4.98	5.14 A

	1,5	4.71	4.36	4.73	4.47	4.57 A
	Average	5.61 a	4.37 a	4.56 a	4.91 a	(-)
Gibberellin content (unit)	0	268.99 Aa	138.31 Ba	60.84 Ca	26.07 D a	123.55
	0,75	206.10 Ab	110.47 Bb	59.57 Ca	17.05 Dab	98.30
	1,5	182.80 Ac	84.48 Bc	31.58 Cb	7.51 Db	76.59
	Average	219.29	111.09	50.67	16.88	(+)

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

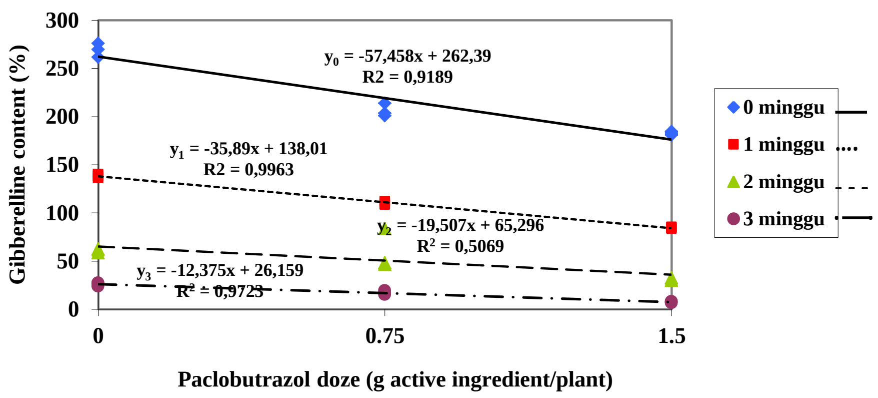

Figure 4. The interaction effect of the dose of paclobutrazol and duration of drought stress on gibberellin content

Table 2 shows the lowest gibberellin content was obtained during the dry period of 3 weeks and the dose of paclobutrazol was 1.5 g of the active ingredient/plant. Figure 4 shows that during the dry period of 3 weeks, increasing the dose of paclobutrazol from 0 to 12.49 g of the active ingredient/plant decreased the gibberellins content. Paclobutrazol and long dry period work by inhibiting gibberellin biosynthesis, thereby inhibiting cell elongation in sub-apical meristems. Jungklang et al., (2015), stated that when gibberellin production is inhibited, cell division still occurs but new cells do not elongate. The paclobutrazol application will reduce the content of GA4, GA3, GA7, and GA1 contained in shoots and leaves (Upreti et al., 2013b). This is also by the research of

Srilatha et al. (2015), the application of paclobutrazol will reduce the content of gibberellins (GA3). According to Rani et al. (2018), paclobutrazol may have acted as an anti-gibberellic 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.

Inhibition of gibberellin biosynthesis by paclobutrazol is at the kaurene stage and has been shown to reduce vegetative growth (Kumar et al. 2019). Paclobutrazol 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.

CONCLUSION

The application of paclobutrazol and the length of the drought period were able to induce flowering of citrus plants as seen from the generative shoot variables with a quadratic model on the equation y = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient/plant and 1 week of drought. The dry period of 3 weeks gave the best results seen from the variable number of flowers and number of fruit.

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COVERING LETTER

Zulfa Rahmadita Nur Azizah[1], Sakhidin*[2], Saparso[3]

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- Please improve the introduction, background to the study, especially a summary of the existing literature, reason why the study was necessary, and the novelty must be explained,

- There are still many words in Indonesian, please check carefully

Application Paclobutrazol and Duration of Drought Stress for Chokun Orange Fertilization

Abstract

This study aimed to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of treatments that gave the best results on the citrus plant fertilization. This research was conducted in Rejasari Village, West Purwokerto District, Banyumas Regency from August 2020 to January 2021. The research design used was Completely Randomized Block Design (RAKL), consisting of 3 replications. The treatment in this study was a combination of the dose of paclobutrazol (control, active ingredient 0.75 g/plant and active ingredient 1.5 g/plant) and duration of drought stress (control, 1, 2, and 3 weeks). The results showed that increasing the dose of paclobutrazol from 0 to 1.12 g of the active ingredient/plant and without a dry period to stimulate fruit set but increasing the dose of paclobutrazol from 0 to 0.26 g of the active ingredient/plant and without a dry period decreased fruit loss. The lowest gibberellin content was obtained through the administration of 1.5 g of the active ingredient/plant paclobutrazol and a dry period of 3 weeks. The combination of these treatments has not been able to induce better flowering and fruiting in citrus plants. A long dry period of 3 weeks increased the number of flowers and fruit number of citrus plants.

Keywords: drought stress duration; chokun tangerine; gibberellin; off-season flowering; paclobutrazol.

Comment [A2]: The title must be revised.
The title must describe the content, keep it concise and informative, and entice the reader

Comment [A3]: Guidelines for abstract:
The abstract should consist of 25% space for the importance and purpose of the research (Introduction); 25% space for what you did (Methods); 35% space for what you found (Results); and 15% space for the implications of the research.

The abstract must be written using 150 until 250 words.

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Comment [A10]: Keywords are the labels of your manuscript and critical to correct indexing and searching. Keywords should not more than 5 words or phrases in alphabetical order which has not been used in the title.

For example: plant growth regulators; water deficit; etc

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INTRODUCTION

Oranges (*Citrus* spp.) are one of the fruit commodities that are well-developed in Indonesia. Citrus fruits are in great demand by the public because of their delicious, fresh taste and high vitamin C content. The market for citrus commodities in Indonesia has wide opportunities due to increased population growth, increased income, and public awareness of the importance of nutrition. Citrus plants in Indonesia each year experience developments in plant cultivation including land area, production volume, and market demand. A technique is needed that can make oranges produce so that consumers can buy oranges throughout the season.

Citrus production is seasonal because citrus plants can bear fruit for only a few months each year. According to Badan Pusat Statistik (2021), the production of Siamese/Keprok oranges 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 only 547,758 tons and 518,775 tons, respectively. 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 during the harvest season the supply of citrus fruits is excessive. The low supply of oranges during the off-season causes the price stability of local citrus fruits in the market to be not guaranteed. To overcome these problems, plant cultivation techniques are needed to regulate citrus fruit production so that the supply of citrus fruits is expected to be available and can meet consumer needs all the time.

One of the efforts that can extend the production period of citrus is to regulate flowering induction. Induction of flowering is one of the efforts that can be done to stimulate citrus plants to flower faster to prolong fruit production. According to Jhade et al. (2018), flower formation is a phase transition in the plant life cycle. Flowering is a phase that will affect the quality and quantity of fruit production. According to Hendrawan (2013), broadly speaking, flowering induction can be done in two ways, namely chemical/hormonal and physical. Chemically/hormonally can use the active ingredients of growth regulators (ZPT).

Paclobutrazol (PBZ) is a triazole group that plays the most role in inhibiting growth which is commonly used for flower induction in fruit plants. The correlation of the effect of paclobutrazol applied during the off-season is hormonal changes in tropical conditions. Hormonal changes that occur are an increase in cytokinin hormones while gibberellins and auxins are reduced (Rahim et al, 2011b). The effectiveness of paclobutrazol in inducing flowering to become fruit in citrus plants depends on the threshold of each cultivar (Martínez-Fuentes et al. 2013). According to research Moreira et al. (2016), paclobutrazol promotes vegetative growth and results in higher flowering and fruiting growth in Ascolana olives. According to Xing et al. (2016), the application of paclobutrazol

Comment [A11]: The introduction is not clear, lack of references, please revise it.
Please explain the background, objectives, methods, and research results clearly and comprehensive so that the state-of-the-art research can be understood.

Please provide a statement to demonstrate this paper in support of sustainable agricultural systems (with references).

Please add more information/existing literature about farmers, traders, and consumers about chili, especially from international journal

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Guidelines for Introduction: Introduction must be written using 750 until 1000 words.

The Introduction section should explain:
a. The background to the study
b. A summary of the existing literature
c. The reason why the study was necessary, and the novelty must be explained
d. The aims

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Comment [A13]: Please every point of information is provided a reference caused an increase in the number of flowers on apple plants. Trees that were applied with paclobutrazol as much as 2 g/plant increased the nu mber of flowers by 66.28% compared to the control, by increasing the carbohydrate content and C/N ratio in the leaves of tangerine plants (Darmawan et al. 2014).

Physical flowering induction can be done by cutting, pruning, wounding, binding and drought stress. The principle of physical induction is to change the ratio of carbon (C) and nitrogen (N) elements in the plant body (Hendrawan, 2013). Environmental factors that affect the induction of flowering in fruit trees in the tropics are drought stress. Drought stress obtained by plants is often not timely, so it is necessary to manipulate or create stress conditions for plants artificially (Sakhidin & Suparto, 2011).

The application of paclobutrazol with the right dose and the appropriate dry period can induce flowering thereby prolonging the production period of citrus fruits. Based on this, this study was carried out to find the effect of the dose of paclobutrazol, duration of drought stress, and the combination of the dose of paclobutrazol and duration of drought stress on the fertilization of chokun oranges. This is done to regulate the time of flowering and fruiting in citrus plants so that they can produce out of season. This study aimed to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of treatments that gave the best results on the citrus plant fertilization.

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MATERIAL AND METHODS

Research time and place

This research was carried out on land in Rejasari Village, West Purwokerto District, Banyumas Regency, Central Java Province of Indonesia, with an altitude of 100 m above sea level. analysis of the gibberellin content of leaves was carried out at the Indonesian Agricultural Postharvest Research and Development - Bogor and microscopic analysis of shoots was carried out at the Laboratory of Plant Structure and Development, Faculty of Biology, Jenderal Sudirman University. The research has been carried out from August 2020 to January 2021.

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Research materials and design

The materials used in the study included 4 years old Chokun variety citrus plant seeds, ~~50 L planter bag, inceptisol soil, straw, goat manure, paclobutrazol (Cultar 250 SC), and plastic. The tools~~

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~~used in the research include digital scales (Ohaus Scout), Soil Plant Analysis Development (SPAD) (Opti-Sciences CCM-200), plastic ruler, microscope, thermohygrometer (HTC-1), Lux Meter (Benetech GM1010), tool watering, measuring cup, calculator, treatment label, stationery, camera, and laptop.~~

This study used a pot experiment which was carried out using a factorial experiment in a Completely Randomized Block Design (RAKL). The first factor is the dose of paclobutrazol. The factor consisted of three levels, without paclobutrazol as control (P0), 0.75 g active ingredient/plant (P1), and 0.75 g active ingredient/plant (P2). The second factor is the duration of drought stress. These factors consisted of four levels, without drought stress (routine irrigation, K0), 1 week (K1), 2 weeks (K2), and 3 weeks (K3). This study consisted of 12 treatment combinations with 3 replications resulting in 36 experimental units. One experimental unit contained 2 plants. Thus 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 difference (significant) then the DMRT test (Duncan Multiple Range Test) was carried out at a level of 5%.

Research procedure

Plants were selected as many as 72 plants based on the uniformity of plant age, plant height, and plant conditions. The selected plants are in good health and have a good root system. The composition of the prepared media was incepticol soil, rice husks, and manure with a ratio of 2:1:1. The orange seedlings that have been prepared are 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 about field capacity. On the last day of the period, the growing medium is saturated with water. Paclobutrazol was applied 2 days after media saturation with a dose of 0.75 and 1.5 g active ingredient/plant dissolved in 1 liter of distilled water by pouring it into the planting medium around the base of the stem. Plants that have been treated are covered with media using plastic so that water from outside cannot enter. Duration of drought stress was carried out according to the treatment, namely after 1 week, 2 weeks, and 3 weeks. After the drought period ended, the media was saturated with water.

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Research design¶

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The parameter observed in this experiment is the variable 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 (Yoshida et al. 1972; in Susanto et al. 2016). Analysis of N content was carried out using the Kjeldahl Semimicro method (William, 1984 in Susanto et al. 2016). Leaf sampling was carried out after saturated water was given at the end of the drought period. Calculation of the C/N ratio is based on the C and N analysis that has been done. Analysis of gibberellin content was carried out using the method of Linskensen and Jackson (1987). Leaf sampling was carried out after fertilization induction treatment (Sakhidin & Suparto, 2011). Microscopic observation of shoot tissue was carried out using the paraffin method (Puslitbang Biology-LIPI, 1998). Tissue collection was carried out after saturating water was given at the end of the drought period.

Data Analysis

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, proceed with the DMRT (Duncan Multiple Range Test) tests at a level of 5% and regression analysis was performed.

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RESULTS AND DISCUSSION

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This section must show the acuity of the analysis and synthesis carried out critically including a description of the findings of the work that discusses incisive, its relation to previous concepts or theories, compares them critically with the work of others, and corroborates or corrects to the previous findings. Unit of measurement used should follow the prevailing international system. All figures and tables should be active and editable by an editor. The discussion should explore the significance of the results of the work. Please highlight differences between your results or findings and the previous publications by other researchers.

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... Table 1 shows that the dose of paclobutrazol affects the gibberellins content. Duration of drought stress affects the number of flowers, fruitset, number of fruits, fruit loss, and gibberellins content. The interaction between the dose of paclobutrazol and the duration of drought stress affected the fruitset, fruit loss, and gibberellin content.

Table 1. The results of the variance of the effect of the dose of paclobutrazol and duration of drought stress on the flowering of citrus plants

No.	Variable	Dose of paclobutrazol	Duration of drought stress	Combination
1.	Number of flowers	ns	**	ns
2.	Fruitset	ns	**	**
3.	Number of fruits	ns	**	ns

4.	Fruit loss	ns	**	**
5.	Leaf C content	ns	ns	ns
6.	Leaf C content	ns	ns	ns
7.	C/N ratio	ns	ns	ns
8.	Gibberellin content	**	**	**

Note : ns = not significant F test $_{0,05}$, ** = very significant F test $_{0,05,}$

Flower and fruit growth

Figure 1 shows that without drought stress, increasing the dose of paclobutrazol from 0 to 1.12 g of the active ingredient/plant increased the fruitset, but after that increasing the dose of paclobutrazol decreased the fruitset. During the dry period of 1 week, increasing the dose of paclobutrazol from 0 to 2,562 g of the active ingredient/plant increased the fruitset. During the dry period of 2 weeks, increasing the dose of paclobutrazol from 0 to 0.75 g of the active ingredient/plant decreased the fruitset, but after that increasing the dose of paclobutrazol increased the fruitset. During the dry period of 3 weeks, increasing the dose of paclobutrazol from 0 to 1.32 g of the active ingredient/plant decreased the fruitset, but after that increasing the dose of paclobutrazol increased the fruitset.

Figure 2 shows that when without drought stress, increasing the dose of paclobutrazol from 0 to 0.26 g of the active ingredient/plant decreased fruit loss, but after that increasing the dose of paclobutrazol increased fruit loss. During the dry period of 1 week, increasing the dose of paclobutrazol from 0 to 5.74 g of the active ingredient/plant increased fruit loss. During the dry period of 2 weeks, increasing the dose of paclobutrazol from 0 to 0.52 g of the active ingredient/plant increased fruit loss, but after that increasing the dose of paclobutrazol decreased fruit loss. During the dry period of 3 weeks, increasing the dose of paclobutrazol from 0 to 1.28 g of the active ingredient/plant decreased fruit loss, but after that increasing the dose of paclobutrazol increased fruit loss.

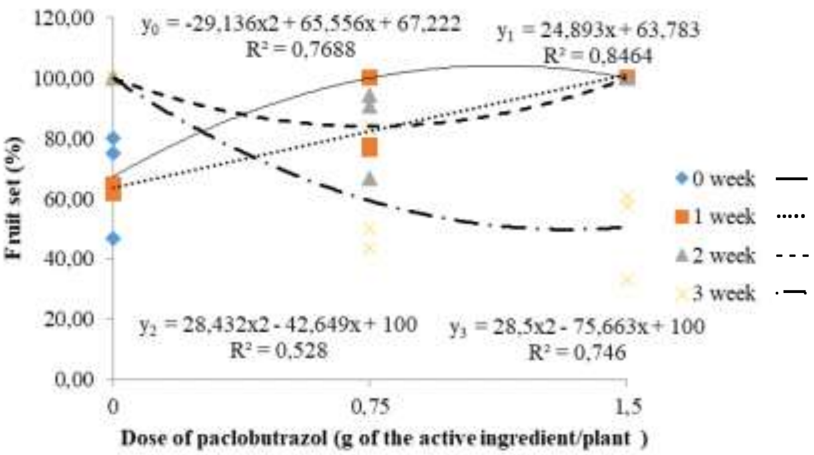

Figure 1. The interaction effect of paclobutrazol dose and duration of drought stress on fruitset

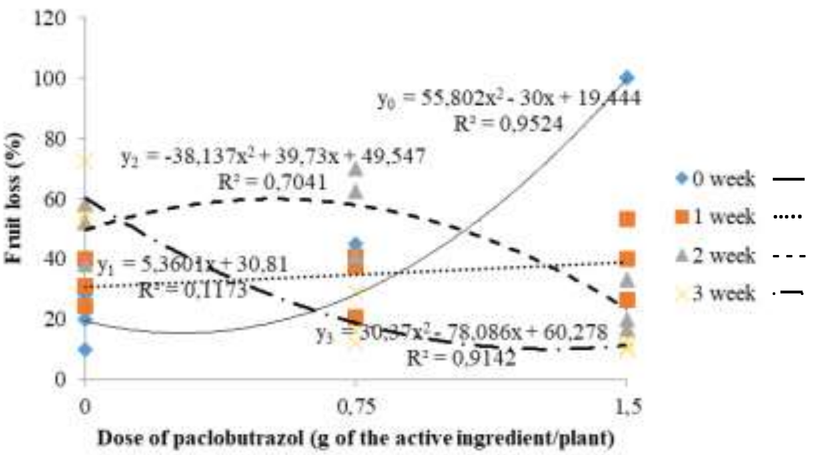

Figure 2. The interaction effect of paclobutrazol dose and duration of drought stress on fruit loss

Table 2 shows that the treatment of paclobutrazol did not show any difference. The dry period of 3 weeks was able to increase the number of flowers by 476.97% and the highest number of fruit by 305.85% compared to without the drought period. The best percentage of fruitset is shown in the

2-week drought period, which is 94.67%, but the best percentage of fruit loss is shown in the 3-week drought period, which is 30.19%.

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Table 2. Effect of paclobutrazol dose and duration of drought stress on the number of flowers, fruitset, number of fruits, and fruit loss.

Treatment	Number of flowers (unit)	Fruitset (%)	Number of fruit (unit)	Fruit loss (%)
Dose of paclobutrazol (g of the active ingredient/plant)				
0	15,13 a	82,47 a	6,75 a	40,26 a
0,75	13,46 a	82,00 a	7,13 a	34,48 a
1,5	9,96 a	87,66 a	4,50 a	43,66 a
Duration of drought stress (week)				
0	4,17 c	89,07 ab	2,56 b	49,26 a
1	14,72 b	82,45 b	7,94 a	34,83 bc
2	8,44 c	94,67 a	4,67 b	43,59 ab
3	24,06 a	69,97 c	10,39 a	30,19 c

Note: Numbers followed by the same letter in the same column show no significant difference in the DMRT test p=0.05

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Content of C, N, C/N ratio, and gibberellin content in leaves

Figure 3. shows that without drought stress, increasing the dose of paclobutrazol from 0 to 4.56 g of the active ingredient/plant decreased the gibberellins content. During the dry period of 1 week, increasing the dose of paclobutrazol from 0 to 3.84 g of the active ingredient/plant decreased the gibberellins content. During a long dry period of 2 weeks, increasing the dose of paclobutrazol from 0 to 3.35 g of the active ingredient/plant decreased the gibberellins content. During the dry period of 3 weeks, increasing the dose of paclobutrazol from 0 to 2.11 g of the active ingredient/plant decreased the gibberellins content.

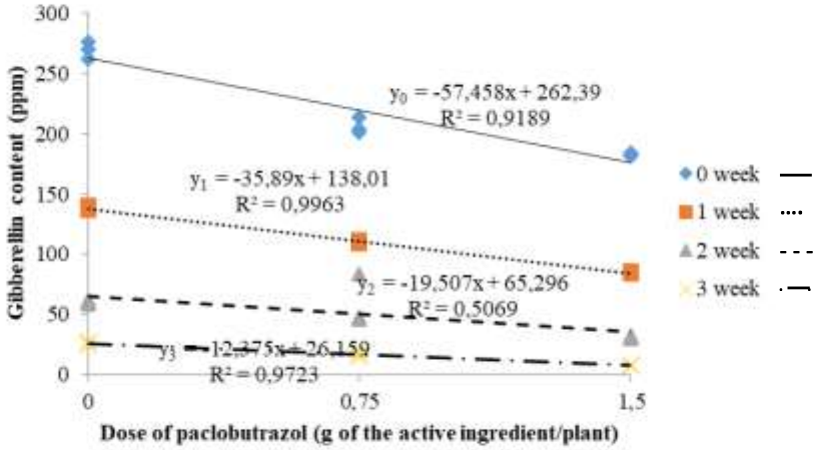


Figure 3. The interaction effect of paclobutrazol dose and duration of drought stress on gibberellin content

Table 3 shows that the treatment of 0.75 g of the active ingredient/plant of paclobutrazol was able to reduce the gibberellin content by 38% compared to plants that were not given paclobutrazol. A long dry period of 3 weeks was able to reduce the content of gibberellins by 92.30% compared to no drought period.

Comment [A30]: It will be better if you add some discussion to this issue. So that, the paragraph will consist at least 3 sentences.

Table 3. Effect of paclobutrazol dose and duration of drought stress on C, N, C/N ratio, and gibberellin content

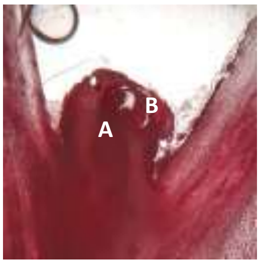
Treatment	C (%)	N (%)	C/N ratio (%)	Giberrelin (ppm)
Dose of paclobutrazol (g of the active ingredient/plant)				
0	4,05 a	0,91 a	4,87 a	123,55 a
0,75	3,96 a	0,80 a	5,14 a	98,30 b
1,5	3,93 a	0,87 a	4,57 a	76,59 c
Duration of drought stress (week)				
0	4,08 a	0,82 a	5,61 a	219,29 a
1	3,84 a	0,89 a	4,37 a	111,09 b

2	4,07 a	0,91 a	4,56 a	50,67 c
3	3,94 a	0,83 a	4,91 a	16,88 d

Note: Numbers followed by the same letter in the same column show no significant difference in the DMRT test p=0.05

Microscopic observation of shoot tissue

Microscopic observation of citrus shoots tissue was carried out 2-3 days after rewatering of drought stress treatment. The results of observations made showed that control plants showed faster vegetative shoot growth compared to plants treated with paclobutrazol and drought stress. In plants treated with paclobutrazol and drought stress, the shoots that grew experienced dormancy and the growth of shoots tended to be slower than the control. The growth of potential flowers has not been seen at the time the observations were made.



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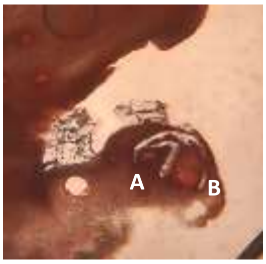

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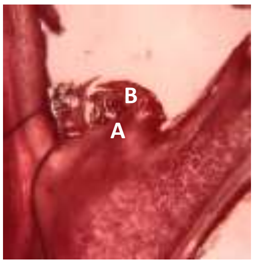

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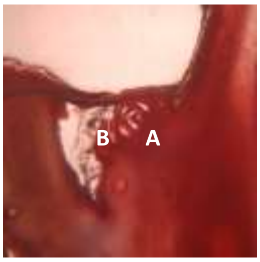

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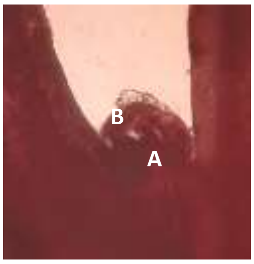

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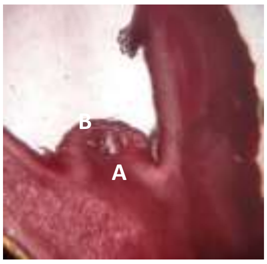

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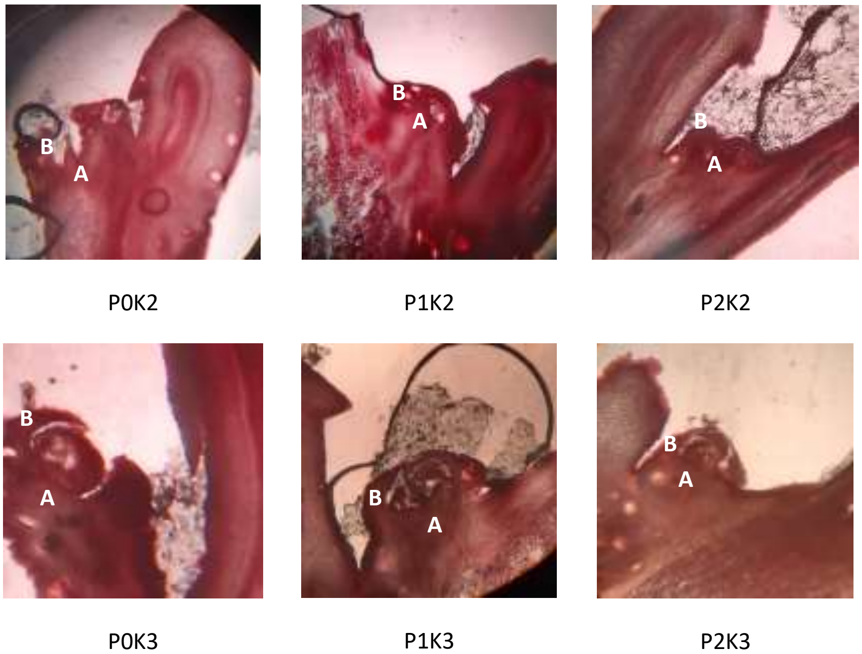

Figure 4. Effect of paclobutrazol dose and duration of drought stress on microscopic observation of shoot tissue. A = shoot base, B = leaf candidate

The results of this study showed that citrus plants with the application of paclobutrazol and duration of drought stress were able to increase the percentage of fruitset and the percentage of fruit loss, but the combination was not able to induce better flowering and fruiting of citrus plants. Table 2 shows the number of flowers and the number of fruits which were higher than the control indicated in the single treatment with a 3-week drought period. Figure 3 shows that this is supported by the low content of gibberellins.

Paclobutrazol and duration of drought stress act by inhibiting gibberellin biosynthesis (Figure 3). Jungklang et al. (2015), stated that when gibberellin production is inhibited, cell division still occurs but new cells do not elongate. According to Rani et al. (2018), paclobutrazol has acted as an anti-gibberellic 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 and fruiting.

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Table 2 shows that in the application of 0.75 g the active ingredient/plant of paclobutrazol, the best fruitset was shown in the no dry period. Fruitset is the ratio between the number of fruits formed and the total number of flowers formed (Darmawan et al. 2014). According to Gollagi et al. (2019), paclobutrazol has been shown to reduce growth, initiate flower budding, increase flower number and increase yield and fruit quality. Table 3 shows that without drought stress, the lowest fruit loss was obtained without paclobutrazol. The combination of these treatments had low fruit loss due to the small amount of fruit produced. High fruit loss can be caused by photosynthetic competition between fruits.

The application of paclobutrazol and the duration of drought stress did not have a significant effect on the flowering and fruiting variables of citrus plants. Plants treated with paclobutrazol showed an increase in the production of the hormone abscisic acid (ABA) (Gollagi et al. 2019). Duration of drought stress can also increase the leaf abscisic acid (ABA) content (Iwasaki et al. 2017). An increase in ABA can induce bud dormancy which causes inhibition of flower differentiation (Rahim et al. 2011a). Therefore, the application of paclobutrazol and the duration of drought stress need to be combined with dormancy breaking agents such as ethephon and KNO3. Substances that break dormancy can function in the breakdown of generative shoots so that flowers and fruit appear. According to research by Sakhidin & Suparto (2011), the combination of treatment with 4 g of paclobutrazol and 800 ppm ethephon per tree showed the lowest content of gibberellins and abscisic acid and produced the highest number of flowers.

CONCLUSION

Increasing the dose of paclobutrazol from 0 to 1.12 g of the active ingredient/plant and without a dry period to stimulate fruitset but increasing the dose of paclobutrazol from 0 to 0.26 g of the active ingredient/plant and without a dry period decreased fruit loss. The lowest gibberellin content was obtained through the application of 1.5 g of the active ingredient/plant paclobutrazol and a dry period of 3 weeks. The combination of these treatments has not been able to induce better flowering and fruiting in citrus plants. A long dry period of 3 weeks increased the number of flowers and fruit number of citrus plants.

Comment [A31]: Conclusion point must answer the goals of the research, and also please give some suggestion to proceed research

Comment [A32]: Please provide recommendations, for example proceed research.

ACKNOWLEDGEMENT

The author would like to express his deepest gratitude to the Institute for Research and Community Service (LPPM) at Jenderal Soedirman University which has funded this research through the BLU Unsoed Schame Risin program in 2020.

Comment [A33]: If any, please provide grant number

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Comment [A34]: Recommended bibliography used **at least 35**, to compare and discuss with previous studies, etc. please see the guidelines of the References

Please use journal last 5-10 years, and it must more than 80% from total references. Add more and more again related references to compare and discuss with previous findings from international journal.

Reference writing must use a reference management tool such as Mendeley/Zotero/Endnote, Style APA 6th edition.

These references maybe relevant to the paper:

Drought tolerance of a microcitrangemonia when treated with paclobutrazol and exposed to different water conditions; https://doi.org/10.1016/j.scienta.2018.04.038

Paclobutrazol in flowering of some tropical and subtropical fruits;

Effect of dose and time of application of paclobutrazol on fruit quality parameters of acid lime (Citrus aurantifolia L.);

Plant growth regulator induced mitigation of oxidative burst helps in the management of drought stress in rice (Oryza sativa L.);

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COVERING LETTER

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Manuscript has main author and co-authors. Author names should not contain academic title or rank. Indicate the corresponding author clearly for handling all stages of pre-publication and post-publication. Consist of full name author and co-authors. **Corresponding author** is a person who is willing to handle correspondence at all stages of refereeing and publication, also post publication.

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General comments:

Overall, this research very interesting. However, there are some improvements and corrections needed to get the manuscript to an accepted condition.

Decision: Revisions Required

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

Abstract

Induction of flowering is one of the efforts that can extend the production period of chokun oranges. This study aimed to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of treatments that gave 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 paclobutrazol (control, active ingredient 0.75 g/plant and active ingredient 1.5 g/plant) and duration of drought stress (control, 1, 2, and 3 weeks). The results showed that the application of paclobutrazol and the length of the drought period were able to induce flowering of citrus plants as seen from the generative shoot variables with a quadratic model on the equation y = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient/plant and 1 week of drought. The dry period of 3 weeks gave the best results seen from the variable number of flowers and number of fruits. ...

Comment [A35]: Please add the implications of the research

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

INTRODUCTION

Citrus (*Citrus* sp.) is an annual fruit commodity that has a major contribution to horticultural production in Indonesia. Citrus production in 2020 reached 2.72 million tons, down 6.22% (159.46 thousand tons) from 2019. Consumption of oranges by the household sector in 2020 reached 887.62 thousand tons, an increase of 25.3% (301 thousand tons) from 2019. The household participation 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, they were 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 out of season causes the stability of local citrus fruit prices in the market is not guaranteed (Badan Pusat Statistik, 2021). To overcome these problems, plant cultivation techniques are needed to regulate citrus fruit production so that it is hoped that the supply of citrus fruits is always available and can meet consumer needs at any time.

Comment [A36]: perrennial plant?

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). According to Jhade et al. (2018), flower formation is a transitional phase in the plant life

Comment [A37]: It is better to move to the end of sentences so it does not mix up with Arcentales et al 2017 cycle. Flowering is a phase that will affect the quality and quantity of fruit production. 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, as a result not all axillary or shoots can be induced and the transition phase from the vegetative phase to the reproductive phase (Ogu & Orjiakor, 2017). The flowering process was influenced by the total sugar content in the leaves and the C/N ratio. In the induction stage, there was an increase in the total sugar content and the leaf C/N ratio compared to before induction (Arcentales et al., 2017).

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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 (Sreekumar et al., 2014). Chemically/hormonally, the active ingredients of growth regulators (ZPT) 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 the generative phase, flowers, and fruit appear (Fitri & Salam, 2017). Paclobutrazol (PBZ) is a triazole group that has the most role in inhibiting growth which is commonly used for flower induction in fruit plants. The correlation of the effect of paclobutrazol given during the off-season is hormonal changes in tropical conditions. Hormonal changes that occur are an increase in cytokinin hormones while gibberellins and auxins are reduced (Rahim et al., 2011b). According to research by Upreti et al. (2013a), 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 paclobutrazol. 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.

Comment [A39]: English, please

Comment [A40]: It is hard to understand

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

Comment [A41]: Higher ?

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 carbon (C) and nitrogen (N) in the plant body (Hendrawan, 2013). According to Fitri & Salam (2017), drought stress does not directly cause flowering plants but causes flower induction or a transition from the vegetative phase to the generative phase. The environmental factor that affects the induction of flowering in fruit trees in the tropics is drought stress. Drought stress obtained by plants is often not timely, so it is necessary to manipulate or artificially create stress conditions for plants (Sakhidin & Suparto, 2011).

Comment [A42]: Its better to add the ref at the end of sentence to make it different from the sentence of Sakhidin & Suparto

The use of paclobutrazol in excessive concentrations can lead to the accumulation of residues in the soil (Prates et al., 2021). The combination of paclobutrazol treatment with drought stress is expected to reduce the use of high doses of chemicals to minimize environmental damage in supporting sustainable agriculture. The application of paclobutrazol with the right dose and the right dry time can induce flowering thereby prolonging the production period of citrus fruits. Based on this, the purpose of this study was to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of dose of paclobutrazol 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 can produce out of season.

Comment [A43]: Please add the references here

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Comment [A45]: Could ?

Comment [A46]: Before stating the research objectives, first explain the previous research regarding the research aspects studied (paclobutrazol and drought stress duration) as state of the art of the research

MATERIAL AND METHODS

Research time and place

This research was carried out on land in Rejasari Village, West Purwokerto District, Banyumas Regency, Central Java Province of Indonesia (-7.4204009, 109.2147000), 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 microscopic analysis of shoots was carried out at the Laboratory of Plant Structure and Development, Faculty of Biology, Jenderal Sudirman University. The research has been carried out from August 2020 to January 2021.

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Research materials and design

The materials used in the study included 4 years old Chokun variety citrus plant seeds. This study used a pot experiment which was carried out using a factorial experiment in a Randomized Complete Block Design (RCBD). The first factor is the dose of paclobutrazol. The factor consisted of 3 levels, without paclobutrazol as control (P0), 0.75 g active ingredient/plant (P1), and 1,5 g active ingredient/plant (P2). According to Darmawan et al. (2014), trees that were given paclobutrazol as much as 2 g/plant 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 consisted 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 study consisted of 12 treatment combinations with 3 replications resulting in 36 experimental units. One experimental unit contained 2 plants. Thus 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, proceed with the DMRT (Duncan Multiple Range Test) tests at a level of 5% and regression analysis was performed.

Research procedure

Plants were selected as many as 72 plants based on the uniformity of plant age, plant height, and plant conditions. The selected plants are in good health and have a good root system. The composition of the prepared media was incepticol soil, rice husks, and manure with a ratio of 2:1:1. The orange seedlings that have been prepared are 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 about field capacity. On the last day of the period, the growing medium is saturated with water. Paclobutrazol was applied 2 days after media saturation with a dose of 0.75 and 1.5 g active ingredient/plant dissolved in 1 liter of distilled water by pouring it into the planting medium around the base of the stem. Plants that have been treated are covered with media using plastic so that water from outside cannot enter. Duration of drought stress was

Comment [A49]: why is this explained? isn't the concentration used 0, 0.75 and 1.5 a.i g/plant

Comment [A50]: How about : A number of 72 Chokun orange plants were selected based….. ?

Comment [A51]: What method to use? How much % of field capacity is maintained?

Comment [A52]: Explain how to make the growing medium saturated with water

Comment [A53]: had

Comment [A54]: were ?

Comment [A55]: could not?

Comment [A56]: correct the sentence please 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 parameter observed in this experiment is 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 (Yoshida et al. 1972; in Susanto et al. 2016). Analysis of N content was carried out using the Kjeldahl Semimicro method (William, 1984 in (Susanto et al. 2016). Leaf sampling was carried out after saturated water was given at the end of the drought period. Calculation of the C/N ratio is based on the C and N analysis that has been done. Analysis of gibberellin content was carried out using the method of Linskensen and Jackson (1987). Leaf sampling was carried out after fertilization induction treatment (Sakhidin & Suparto, 2011). Microscopic observation of shoot tissue was carried out using the paraffin method (Puslitbang Biology-LIPI, 1998). Tissue collection was carried out 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 paclobutrazol and duration of drought stress were able to stimulate generative induction as seen from the generative shoot variables, fruit set, and fruit loss. The application of paclobutrazol did not show any difference. The dry period of 3 weeks showed that there were differences in the variables when the first flowers appeared, flower buds bloomed, and the number of fruits. Table 2 and Figure 4 show that this is supported by the low content of gibberellins.

Generative Phase Induction

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

Variable observation	Paclobutrazol doze (g of active ingredient/	Drought stress (week)				Mean
		0	1	2	3	

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Comment [A60]: I think this should be past tense

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Comment [A62]: Why Table 2 and Figure 4 were mentioned before Table 1 and Figure 1?

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Comment [A63]: In my opinion table and/or graphs should not come directly below the heading/sub heading.

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	plant)					
Generative shoots (units)	0	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
	1.5	2.00 Cb	34.00 Aab	12.33 Bb	40.00 Aa	22.08
	Average	10.50	33.67	23.17	43.00	(+)
The first flower appears (units)	0	88.67	84.00	77.00	51.33	75.25 A
	0.75	93.33	77.00	91.00	65.33	81.67 A
	1.5	81.67	30.33	67.67	67.67	61.83 A
	Average	87.89 a	63.78 a	78.56 a	61.44 a	(-)
Number of flowers (unit)	0	9.33	18.67	8.00	24.50	15.13 A
	0.75	1.83	13.67	13.67	26.33	13.88 A
	1.5	1.33	13.50	3.67	21.33	9.96 A
	Average	4.17 c	15.28 b	8.44 c	24.06 a	(-)
Fruit set (%)	0	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
	1.5	100.00 Aa	100.00 Aa	100.00 Aa	50.63 Aa	87.66
	Average	89.07	82.45	94.67	69.97	(+)
Number of fruit (unit)	0	4.67	8.00	5.33	9.00	6.75 A
	0.75	3.00	8.00	5.33	12.17	7.13 A
	1.5	0.00	7.83	3.33	10.00	5.29 A
	Average	2.56 b	7.94 a	4.67 b	10.39 a	(-)
Fruit drop (%)	0	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
	1.5	100.00 Aa	39.81 Ba	23.33 BCb	11.48 Cb	43.66
	Average	49.26	34.83	43.59	30.19	(+)

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

Comment [A64]: It is hard to understand, please revise it

Table 1 shows the best number of generative shoots obtained at a dose of 0.75 g of paclobutrazol active ingredient/plant and a dry period of 1 week. Figure 1 shows that during the 1 week long dry period, increasing the dose of paclobutrazol from 0 to 0.89 g of the active ingredient/plant increased the number of generative shoots, but after that increasing the dose of paclobutrazol would decrease the number of generative shoots. According to the research of Martínez-Fuentes et al. (2013) that paclobutrazol 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 paclobutrazol significantly reduced the vegetative growth rate by decreasing shoot length and decreasing leaf number. The effect of paclobutrazol can occur in the form of increasing the size and number of fruits to increase crop yields.

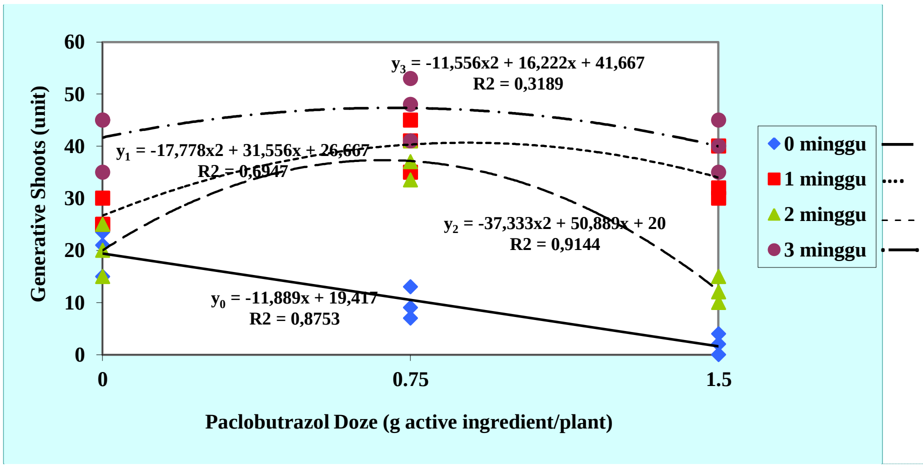

Figure 1. The interaction effect of the dose of paclobutrazol and duration of drought stress on the number of generative shoots of Chokun orange

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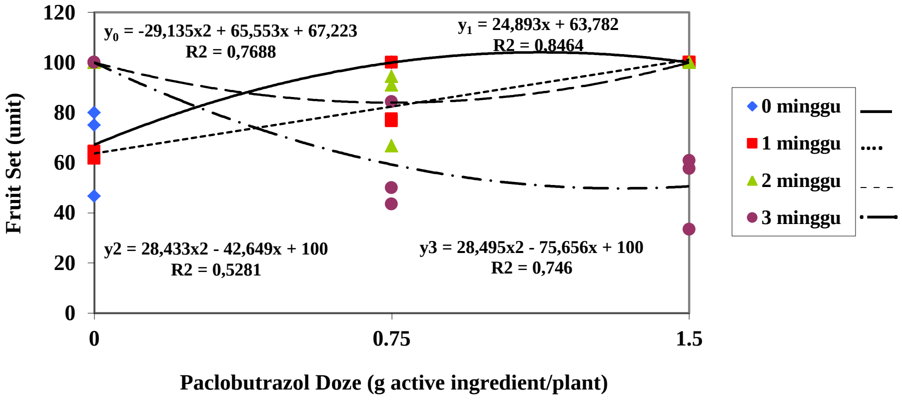

Figure 2. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit set of Chokun orange

Table 1 shows that in the administration of 0.75 g of the active ingredient/plant, the best fruit set was shown in the absence of a dry period. Figure 2 shows that in the absence of drought stress, increasing the dose of paclobutrazol from 0 to 1.12 g of the active ingredient/plant increased the fruit set, but after that increasing the dose of paclobutrazol decreased the fruits et. Fruit set is the ratio between the number of fruits formed and the total number of flowers formed (Darmawan et al., 2014). Paclobutrazol acts as a gibberellin inhibitor thereby reducing the level of the vegetative promoter. Thus it 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), paclobutrazol 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 of Kuswandi et al. (2019), initiation of flowering in guava plants was stimulated by the length of the dry period.

Comment [A66]: set ?

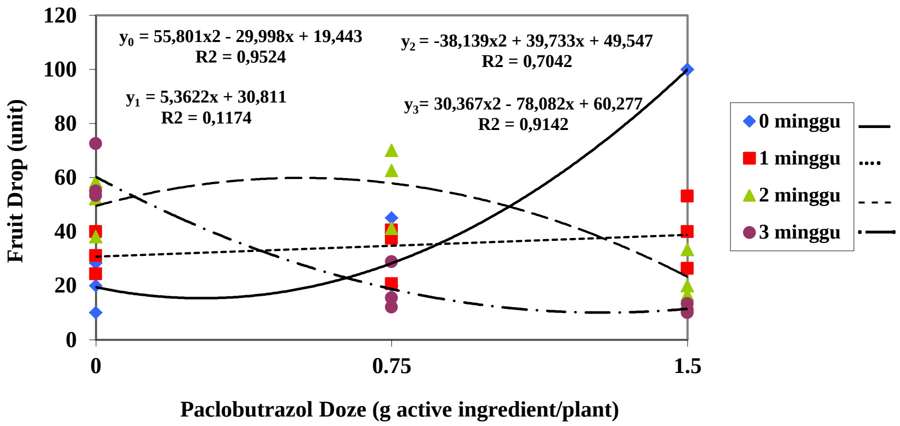

Figure 3. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit drop

Table 1 shows that in the absence of a dry period, the lowest fruit loss was obtained in the absence of paclobutrazol. Figure 3 shows that without drought stress, increasing the dose of paclobutrazol from 0 to 0.26 g of the active ingredient/plant decreased fruit loss, but after that increasing the dose of paclobutrazol increased fruit loss. The addition of the dose of paclobutrazol causes the gibberellin content in plants to below, this is the cause of high fruit loss. Meanwhile, according to Gollagi et al. (2019), plants treated with paclobutrazol showed an increase in the production of the hormone abscisic acid (ABA). This is by 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.

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Comment [A67]: Hard to understand

Table 1 shows that the administration of paclobutrazol 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 no drought period. According to Kazan & 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 research Endo et al. (2018), ABA accumulation correlated with CiFT homologous transcript accumulation and flowering intensity of Satsuma mandarin citrus. According to Chica & 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. This is by 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 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. According to research by Panigrahi & Srivastava (2016), treatment of long periods of periodic drought can induce the flowering of tangerines.

C/N Ratio and Gibberellin Content in Leaves

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

Variable observation	Paclobutrazol doze (g of active ingredient/ plant)	Drought stress (week) 0	1	2	3	Mean
Ratio C/N (%)	0	5.74	3.93	4.56	5.27	4.87 A
	0.75	6.37	4.82	4.41	4.98	5.14 A
	1.5	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	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
	1.5	182.80 Ac	84.48 Bc	31.58 Cb	7.51 Db	76.59
	Average	219.29	111.09	50.67	16.88	(+)

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

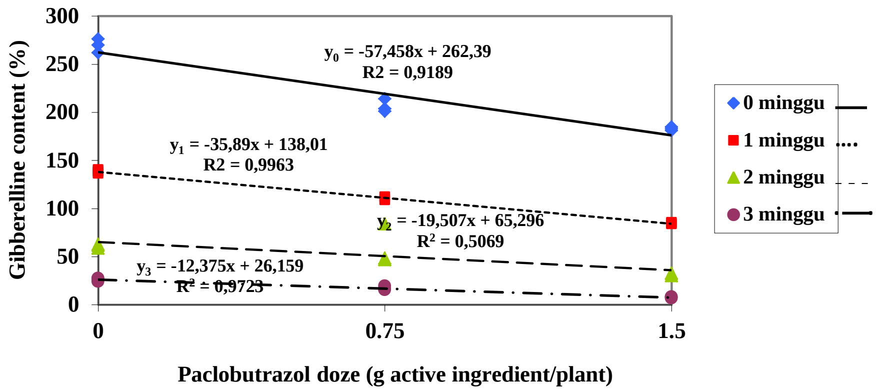

Figure 4. The interaction effect of the dose of paclobutrazol and duration of drought stress on gibberellin content of Chokun orange

Table 2 shows the lowest gibberellin content was obtained during the dry period of 3 weeks and the dose of paclobutrazol was 1.5 g of the active ingredient/plant. Figure 4 shows that during the dry period of 3 weeks, increasing the dose of paclobutrazol from 0 to 12.49 g of the active ingredient/plant decreased the gibberellins content. Paclobutrazol and long dry period work by inhibiting gibberellin biosynthesis, thereby inhibiting cell elongation in sub-apical meristems. Jungklang et al., (2015), stated that when gibberellin production is inhibited, cell division still occurs but new cells do not elongate. The paclobutrazol application will reduce the content of GA4, GA3, GA7, and GA1 contained in shoots and leaves (Upreti et al., 2013b). This is also by the research of Srilatha et al. (2015), the application of paclobutrazol will reduce the content of gibberellins (GA3). According to Rani et al. (2018), paclobutrazol may have acted as an anti-gibberellic 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.

Inhibition of gibberellin biosynthesis by paclobutrazol is at the kaurene stage and has been shown to reduce vegetative growth (Kumar et al. 2019). Paclobutrazol can be absorbed by plants through leaves, stem vessels, or roots, then translocated acropetally through the xylem to other

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Comment [A69]: is there paclobutrazol 12.49 a.i g/plant on the graph?

Comment [A70]: Please add references to support this statement 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.

CONCLUSION

The application of paclobutrazol and the length of the drought period were able to induce flowering of citrus plants as seen from the generative shoot variables with a quadratic model on the equation y = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient/plant 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. ...

Comment [A71]: Please adjust the objective of the research and conclusions

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Comment [A72]: Suggestions for further research?

ACKNOWLEDGEMENT

The author would like to express his deepest gratitude to the Institute for Research and Community Service (LPPM) at Jenderal Soedirman University which has funded this research through the BLU Unsoed Scheme Risin program in 2020.

Comment [A73]: Grant number? If any

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COVERING LETTER

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

Comment [A75]: **General Comments:** This manuscript has excellent revision progress. However, there are some improvements and corrections needed to get the manuscript to an accepted condition. Here are some specific comments [on the manuscript].

Editor Decision: Revision Required

Abstract

Induction of flowering is one of the efforts that can extend the production period of chokun oranges. This study aimed to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of treatments that gave 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 paclobutrazol (control, active ingredient 0.75 g/plant and active ingredient 1.5 g/plant) and duration of drought stress (control, 1, 2, and 3 weeks). The results showed that the application of paclobutrazol and duration of drought stress were able to induce flowering of citrus plants as seen from the generative shoot variables with a quadratic model on the equation y = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient/plant and 1 week of drought. The dry period of 3 weeks gave the best results seen from the variable number of flowers and number of fruits. Suggestions from this research It is necessary to do further research on the use of paclobutrazol with a level different doses to get the best dose in induction flowering.

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

INTRODUCTION

Citrus (*Citrus* sp.) is perennial plant commodity that has a major contribution to horticultural production in Indonesia. Citrus production in 2020 reached 2.72 million tons, down 6.22% (159.46 thousand tons) from 2019. Consumption of oranges by the household sector in 2020 reached 887.62 thousand tons, an increase of 25.3% (301 thousand tons) from 2019. The household participation 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, they were 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 out of season causes the stability of local citrus fruit prices in the market is not guaranteed (Badan Pusat Statistik, 2021). To overcome these problems, plant cultivation techniques are needed to regulate citrus fruit production so that it is hoped that the supply of citrus fruits is always available and can meet consumer needs at any time.

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, as a result plants that are not induced do not transition from the vegetative phase to the generative phase (Ogu & Orjiakor, 2017). The flowering process was influenced by the total sugar content in the leaves and the C/N ratio. In the induction stage, there was 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 (Sreekumar 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 the generative phase, flowers, and fruit appear (Fitri & Salam, 2017). Paclobutrazol (PBZ) is a triazole group that has the most role in inhibiting growth which is commonly used for flower induction in fruit plants. The correlation of the effect of paclobutrazol 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., 2011b). According to research by Upreti et al. (2013a), 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 paclobutrazol. 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 paclobutrazol in inducing flowering into fruit in citrus plants depends on the threshold of each cultivar (Martínez-Fuentes et al., 2013). According to Desta & Amare (2021), the application of paclobutrazol is more effective when applied to growing media because the absorption of the active ingredient is higher more than foliar spray. According to research Moreira et al. (2016), paclobutrazol promotes vegetative growth and results in higher flowering and fruiting growth in Ascolana olives. According to Xing et al. (2016), the application of paclobutrazol caused an increase in the number of flowers on apple plants. (Burondkar et al., 2013) mentioned the application of paclobutrazol 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 carbon (C) and nitrogen (N) in the plant body (Hendrawan, 2013). Drought stress does not directly cause flowering plants but causes flower induction or a transition from the vegetative phase to the generative phase (Fitri & Salam, 2017). The environmental factor that affects the induction of flowering in fruit trees in the tropics is drought stress. Drought stress obtained by plants is often not timely, so it is necessary to manipulate or artificially create stress conditions for plants (Sakhidin & Suparto, 2011).

The use of paclobutrazol in excessive concentrations can lead to the accumulation of residues in the soil (Prates et al., 2021). The hope of this research is that the combination of paclobutrazol treatment with drought stress can reduce residues in the soil so as to minimize environmental damage in support of environmental sustainability. The application of paclobutrazol 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), for mango plants given PBZ treatment and low to moderate preflowering 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. Based on this, the purpose of this study was to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of dose of paclobutrazol and duration of drought stress on flowering induction of chokun oranges. This was done to regulate the time of flowering and fruiting on citrus plants so that they could produce out of season.

MATERIAL AND METHODS

Research time and place

This research was carried out on land in Rejasari Village, West Purwokerto District, Banyumas Regency, Central Java Province of Indonesia at the coordinates point (-7.4204009, 109.2147000), 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 microscopic analysis of shoots was carried out at the Laboratory of Plant Structure and Development, Faculty of Biology, Jenderal Sudirman University. The research was carried out from August 2020 to January 2021.

Research materials and design

The materials used in the study included 4 years old Chokun variety citrus plant seeds. This study used a pot experiment which was carried out using a factorial experiment in a Randomized Complete Block Design (RCBD). The first factor is the dose of paclobutrazol. The factor consisted of 3 levels, without paclobutrazol as control (P0), 0.75 g active ingredient/plant (P1), and 1,5 g active ingredient/plant (P2). This study used a lower dose than the recommended dose because there were two treatments with the same goal, namely to induce flowering. Dosage recommendation according to research Darmawan et al. (2014), trees that were given paclobutrazol as much as 2 g/plant 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 consisted 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 study consisted of 12 treatment combinations with 3 replications resulting in 36 experimental units. One experimental unit contained 2 plants. Thus 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, proceed with the DMRT (Duncan Multiple Range Test) tests 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 are in good health and have a good root system. The composition of the prepared media was incepticol soil, rice husks, and manure with a ratio of 2:1:1. The orange seedlings that have been prepared are 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 is saturated with water by continuously adding water until water came out of the polybag. Paclobutrazol was applied 2 days after media saturation with a dose of 0.75 and 1.5 g active ingredient/plant dissolved in 1 liter 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 parameter 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 (Yoshida et al. 1972). Analysis of N content was carried out using the Kjeldahl Semimicro method (William, 1984). Leaf sampling was carried out after saturated water was given at the end of the drought period. Calculation of the C/N ratio is based on the C and N analysis.

Analysis of gibberellin content was carried out using the method of Linskensen and Jackson (1987). Leaf sampling was carried out after fertilization induction treatment (Sakhidin & Suparto, 2011). Microscopic observation of shoot tissue was carried out using the paraffin method (Puslitbang Biology-LIPI, 1998). Tissue collection was carried out 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 paclobutrazol and duration of drought stress were able to stimulate generative induction as seen from the generative shoot variables, fruit set, and fruit loss. The application of paclobutrazol did not show any difference. The dry period of 3 weeks showed that there were differences in the variables when the first flowers appeared, flower buds bloomed, and 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 0.75 g of paclobutrazol active ingredient/plant and a dry period of 1 week. Figure 1 shows that during the 1 week long dry period, increasing the dose of paclobutrazol from 0 to 0.89 g of the active ingredient/plant increased the number of generative shoots, but after that increasing the dose of paclobutrazol would decrease the number of generative shoots. According to the research of Martínez-Fuentes et al. (2013) that paclobutrazol 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 paclobutrazol significantly reduced the vegetative growth rate by decreasing shoot length and decreasing leaf number. The effect of paclobutrazol can occur in the form of increasing the size and number of fruits to increase crop yields.

Table 1 shows that in the administration of 0.75 g of the active ingredient/plant, the best fruit set was shown in the absence of a dry period. Figure 2 shows that in the absence of drought stress, increasing the dose of paclobutrazol from 0 to 1.12 g of the active ingredient/plant increased the fruit set, but after that increasing the dose of paclobutrazol 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). Paclobutrazol acts as a gibberellin inhibitor thereby reducing the level of the vegetative promoter. This it 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), paclobutrazol 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 of Kuswandi et al. (2019), initiation of flowering in guava plants was stimulated by the length of the dry period.

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

Variable observation	Paclobutrazol doze (g of active ingredient/ plant)	Drought stress (week)				Mean
		0	1	2	3	
Generative shoots (units)	0	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
	1.5	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 appears (units)	0	88.67	84.00	77.00	51.33	75.25 A
	0.75	93.33	77.00	91.00	65.33	81.67 A
	1.5	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	0	9.33	18.67	8.00	24.50	15.13 A
	0.75	1.83	13.67	13.67	26.33	13.88 A

(unit)	1.5	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	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
	1.5	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 fruit (unit)	0	4.67	8.00	5.33	9.00	6.75 A
	0.75	3.00	8.00	5.33	12.17	7.13 A
	1.5	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	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
	1.5	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 test; numbers followed by lowercase letters are the same in the same column not significantly different in the 5% DMRT test

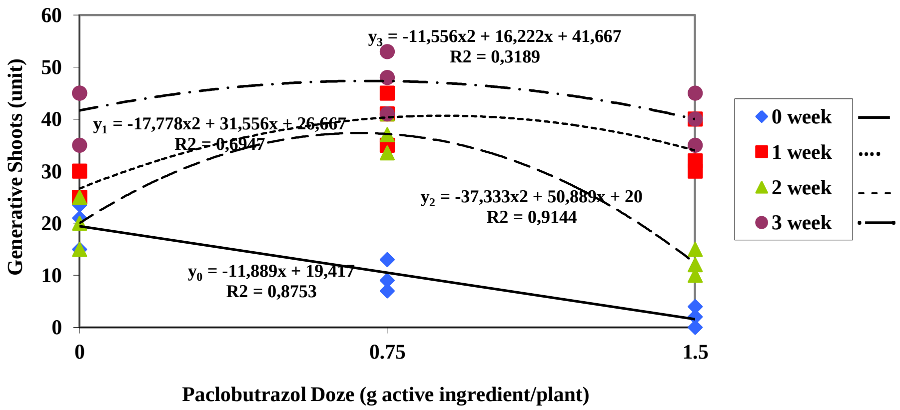

Figure 1. The interaction effect of the dose of paclobutrazol and duration of drought stress on the number of generative shoots of Chokun orange

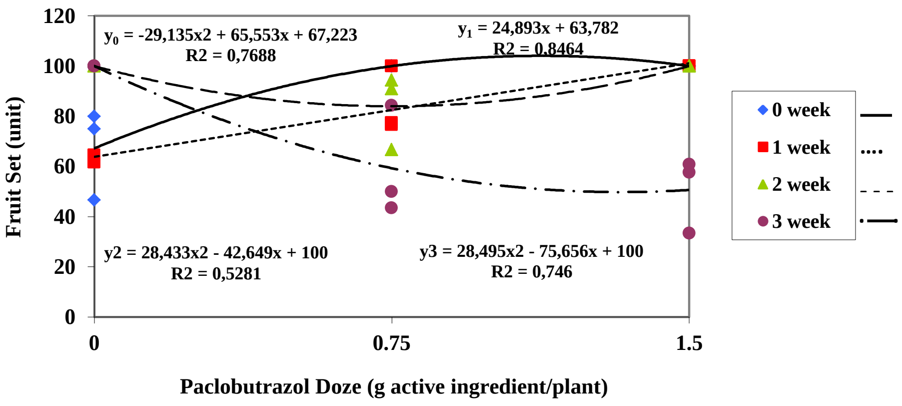

Figure 2. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit set of Chokun orange

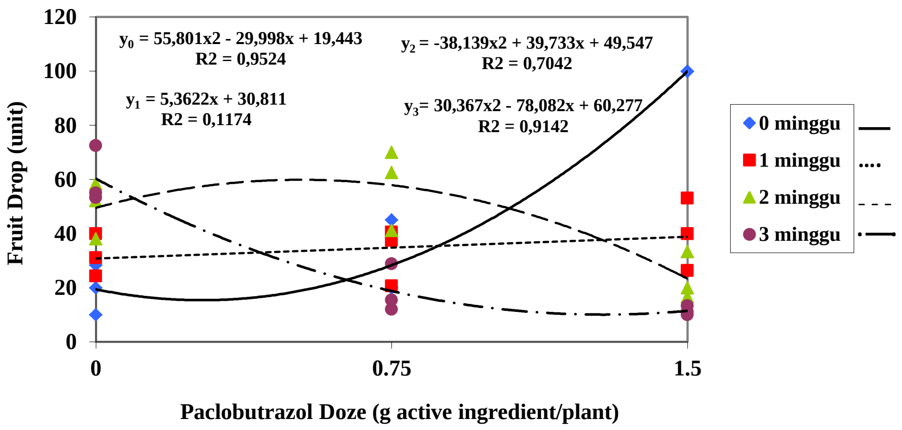

Figure 3. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit drop of Chokun orange

Table 1 shows that in the absence of a dry period, the lowest fruit loss was obtained in the absence of paclobutrazol. Figure 3 shows that without drought stress, increasing the dose of paclobutrazol from 0 to 0.26 g of the active ingredient/plant decreased fruit loss, but after that increasing the dose of paclobutrazol increased fruit loss. The addition of the dose of paclobutrazol 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 paclobutrazol showed an increase in the production of the hormone abscisic acid (ABA). This is by 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 paclobutrazol 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 no drought period. According to Kazan & 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 research Endo et al. (2018), ABA accumulation correlated with CiFT homologous transcript accumulation and flowering intensity of Satsuma mandarin citrus. According to Chica & 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. This is by 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 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. According to research by Panigrahi & Srivastava (2016), treatment of long periods of periodic drought can induce the flowering of tangerines.

C/N Ratio and Gibberellin Content in Leaves

Table 2 shows the lowest gibberellin content was obtained during the dry period of 3 weeks and the dose of paclobutrazol was 1.5 g of the active ingredient/plant. Figure 4 shows that during the dry period of 3 weeks, increasing the dose of paclobutrazol from 0 to 2.11 g of the active ingredient/plant decreased the gibberellins content. Paclobutrazol and long dry period work by inhibiting gibberellin biosynthesis, thereby inhibiting cell elongation in sub-apical meristems. According to Jungklang et al., (2015), stated that when gibberellin production is inhibited, cell division still occurs but new cells do not elongate. The paclobutrazol application will reduce the content of GA4, GA3, GA7, and GA1 contained in shoots and leaves (Upreti et al., 2013b). This is also by the research of Srilatha et al. (2015), the application of paclobutrazol will reduce the content of gibberellins (GA3). According to Rani et al. (2018), paclobutrazol may have acted as an anti-gibberellic 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.

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

Variable observation	Paclobutrazol doze (g of active ingredient/ plant)	Drought stress (week) 0	1	2	3	Mean
Ratio C/N (%)	0	5.74	3.93	4.56	5.27	4.87 A
	0.75	6.37	4.82	4.41	4.98	5.14 A
	1.5	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	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
	1.5	182.80 Ac	84.48 Bc	31.58 Cb	7.51 Db	76.59
	Mean	219.29	111.09	50.67	16.88	(+)

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

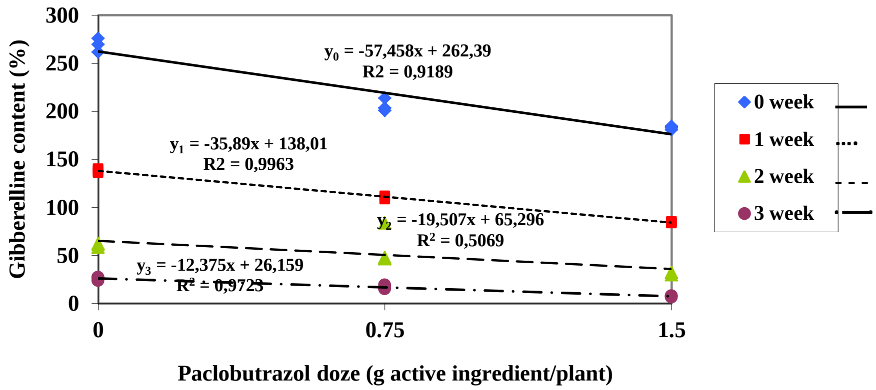

Figure 4. The interaction effect of the dose of paclobutrazol and duration of drought stress on gibberellin content of Chokun orange

Inhibition of gibberellin biosynthesis by paclobutrazol is at the kaurene stage and has been shown to reduce vegetative growth (Kumar et al. 2019). Paclobutrazol 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.

CONCLUSION

~~This study aimed to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of treatments that gave the best results on the Chokun Orange (Citrus sp.).~~ In conclusion, the application of paclobutrazol and duration of drought stress were able to induce flowering of citrus plants as seen from the generative shoot variables with a quadratic model on the equation y = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active

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ingredient/plant 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. Suggestions from this research It is necessary to do further research on the use of paclobutrazol with a level different doses to get the best dose in induction flowering.

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COVERING LETTER

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

Abstract

Induction of flowering is one of the efforts that can extend the production period of chokun oranges. This study aimed to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of treatments that gave 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 paclobutrazol (control, active ingredient 0.75 g/plant and active ingredient 1.5 g/plant) and duration of drought stress (control, 1, 2, and 3 weeks). The results showed that the application of paclobutrazol and duration of drought stress were able to induce flowering of citrus plants as seen from the generative shoot variables with a quadratic model on the equation y = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient/plant and 1 week of drought. The dry period of 3 weeks gave the best results seen from the variable number of flowers and number of fruits. In general, the results suggest that the application of paclobutrazol and duration of drought stress were able to transfer from the vegetative phase to the generative phase which in turn could induce flowering of citrus plants.

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

INTRODUCTION

Citrus (*Citrus* sp.) is perennial plant commodity that has a major contribution to horticultural production in Indonesia. Citrus production in 2020 reached 2.72 million tons, down 6.22% (159.46 thousand tons) from 2019. Consumption of oranges by the household sector in 2020 reached 887.62 thousand tons, an increase of 25.3% (301 thousand tons) from 2019. The household participation 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, they were 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 out of season causes the stability of local citrus fruit prices in the market is not guaranteed (Badan Pusat Statistik, 2021). To overcome these problems, plant cultivation techniques are needed to regulate citrus fruit production so that it is hoped that the supply of citrus fruits is always available and can meet consumer needs at any time.

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, as a result plants that are not induced do not transition from the vegetative phase to the generative phase (Ogu & Orjiakor, 2017). The flowering process was influenced by the total sugar content in the leaves and the C/N ratio. In the induction stage, there was 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 (Sreekumar 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 the generative phase, flowers, and fruit appear (Fitri & Salam, 2017). Paclobutrazol (PBZ) is a triazole group that has the most role in inhibiting growth which is commonly used for flower induction in fruit plants. The correlation of the effect of paclobutrazol 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., 2011b). According to research by Upreti et al. (2013a), 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 paclobutrazol. 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 paclobutrazol in inducing flowering into fruit in citrus plants depends on the threshold of each cultivar (Martínez-Fuentes et al., 2013). According to Desta & Amare (2021), the application of paclobutrazol is more effective when applied to growing media because the absorption of the active ingredient is higher more than foliar spray. According to research Moreira et al. (2016), paclobutrazol promotes vegetative growth and results in higher flowering and fruiting growth in Ascolana olives. According to Xing et al. (2016), the application of paclobutrazol caused an increase in the number of flowers on apple plants. (Burondkar et al., 2013) mentioned the application of paclobutrazol 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 carbon (C) and nitrogen (N) in the plant body (Hendrawan, 2013). Drought stress does not directly cause flowering plants but causes flower induction or a transition from the vegetative phase to the generative phase (Fitri & Salam, 2017). The environmental factor that affects the induction of flowering in fruit trees in the tropics is drought stress. Drought stress obtained by plants is often not timely, so it is necessary to manipulate or artificially create stress conditions for plants (Sakhidin & Suparto, 2011).

The use of paclobutrazol in excessive concentrations can lead to the accumulation of residues in the soil (Prates et al., 2021). The hope of this research is that the combination of paclobutrazol treatment with drought stress can reduce residues in the soil so as to minimize environmental damage in support of environmental sustainability. The application of paclobutrazol 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), for mango plants given PBZ treatment and low to moderate preflowering 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. Based on this, the purpose of this study was to determine the effect of the dose of paclobutrazol, duration of drought stress, and the combination of dose of paclobutrazol and duration of drought stress on flowering induction of chokun oranges. This was done to regulate the time of flowering and fruiting on citrus plants so that they could produce out of season.

MATERIAL AND METHODS

Research time and place

This research was carried out on land in Rejasari Village, West Purwokerto District, Banyumas Regency, Central Java Province of Indonesia at the coordinates point (-7.4204009, 109.2147000), 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 microscopic analysis of shoots was carried out at the Laboratory of Plant Structure and Development, Faculty of Biology, Jenderal Sudirman University. The research was carried out from August 2020 to January 2021.

Research materials and design

The materials used in the study included 4 years old Chokun variety citrus plant seeds. This study used a pot experiment which was carried out using a factorial experiment in a Randomized Complete Block Design (RCBD). The first factor is the dose of paclobutrazol. The factor consisted of 3 levels, without paclobutrazol as control (P0), 0.75 g active ingredient/plant (P1), and 1,5 g active ingredient/plant (P2). This study used a lower dose than the recommended dose because there were two treatments with the same goal, namely to induce flowering. Dosage recommendation according to research Darmawan et al. (2014), trees that were given paclobutrazol as much as 2 g/plant 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 consisted 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 study consisted of 12 treatment combinations with 3 replications resulting in 36 experimental units. One experimental unit contained 2 plants. Thus 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, proceed with the DMRT (Duncan Multiple Range Test) tests 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 are in good health and have a good root system. The composition of the prepared media was incepticol soil, rice husks, and manure with a ratio of 2:1:1. The orange seedlings that have been prepared are 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 is saturated with water by continuously adding water until water came out of the polybag. Paclobutrazol was applied 2 days after media saturation with a dose of 0.75 and 1.5 g active ingredient/plant dissolved in 1 liter 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 parameter 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 (Yoshida et al. 1972). Analysis of N content was carried out using the Kjeldahl Semimicro method (William, 1984). Leaf sampling was carried out after saturated water was given at the end of the drought period. Calculation of the C/N ratio is based on the C and N analysis. Analysis of gibberellin content was carried out using the method of Linskensen and Jackson (1987). Leaf sampling was carried out after fertilization induction treatment (Sakhidin & Suparto, 2011). Microscopic observation of shoot tissue was carried out using the paraffin method (Puslitbang

Biology-LIPI, 1998). Tissue collection was carried out 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 paclobutrazol and duration of drought stress were able to stimulate generative induction as seen from the generative shoot variables, fruit set, and fruit loss. The application of paclobutrazol did not show any difference. The dry period of 3 weeks showed that there were differences in the variables when the first flowers appeared, flower buds bloomed, and 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 0.75 g of paclobutrazol active ingredient/plant and a dry period of 1 week. Figure 1 shows that during the 1 week long dry period, increasing the dose of paclobutrazol from 0 to 0.89 g of the active ingredient/plant increased the number of generative shoots, but after that increasing the dose of paclobutrazol would decrease the number of generative shoots. According to the research of Martínez-Fuentes et al. (2013) that paclobutrazol 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 paclobutrazol significantly reduced the vegetative growth rate by decreasing shoot length and decreasing leaf number. The effect of paclobutrazol can occur in the form of increasing the size and number of fruits to increase crop yields.

Table 1 shows that in the administration of 0.75 g of the active ingredient/plant, the best fruit set was shown in the absence of a dry period. Figure 2 shows that in the absence of drought stress, increasing the dose of paclobutrazol from 0 to 1.12 g of the active ingredient/plant increased the fruit set, but after that increasing the dose of paclobutrazol 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). Paclobutrazol acts as a gibberellin inhibitor thereby reducing the level of the vegetative promoter. This it 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), paclobutrazol 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 of Kuswandi et al. (2019), initiation of flowering in guava plants was stimulated by the length of the dry period.

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

Variable observation	Paclobutrazol doze (g of active ingredient/ plant)	Drought stress (week)				Mean
		0	1	2	3	
Generative shoots (units)	0	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
	1.5	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 appears (units)	0	88.67	84.00	77.00	51.33	75.25 A
	0.75	93.33	77.00	91.00	65.33	81.67 A
	1.5	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	9.33	18.67	8.00	24.50	15.13 A
	0.75	1.83	13.67	13.67	26.33	13.88 A
	1.5	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	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
	1.5	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 fruit (unit)	0	4.67	8.00	5.33	9.00	6.75 A
	0.75	3.00	8.00	5.33	12.17	7.13 A
	1.5	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	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
	1.5	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 test; numbers followed by lowercase letters are the same in the same column not significantly different in the 5% DMRT test

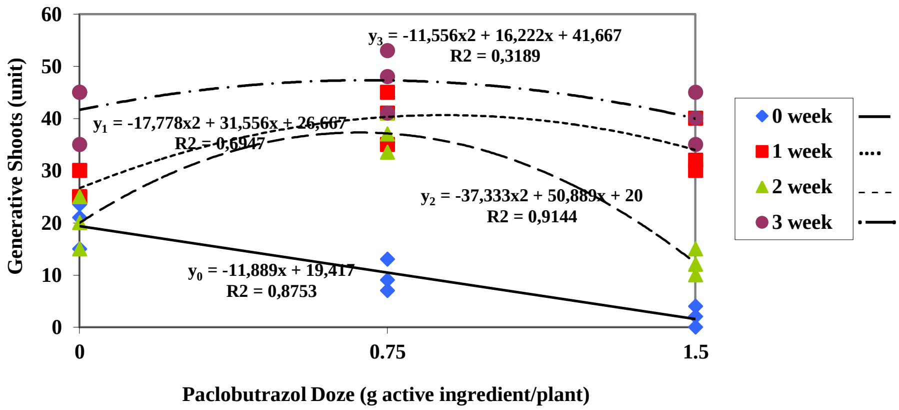

Figure 1. The interaction effect of the dose of paclobutrazol and duration of drought stress on the number of generative shoots of Chokun orange

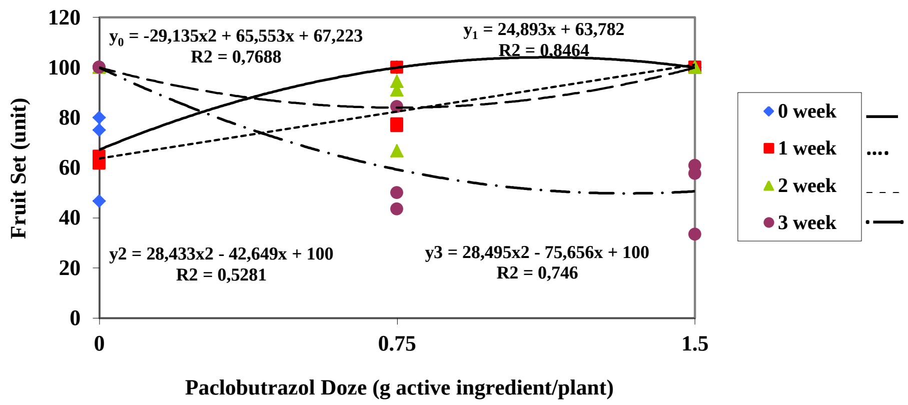

Figure 2. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit set of Chokun orange

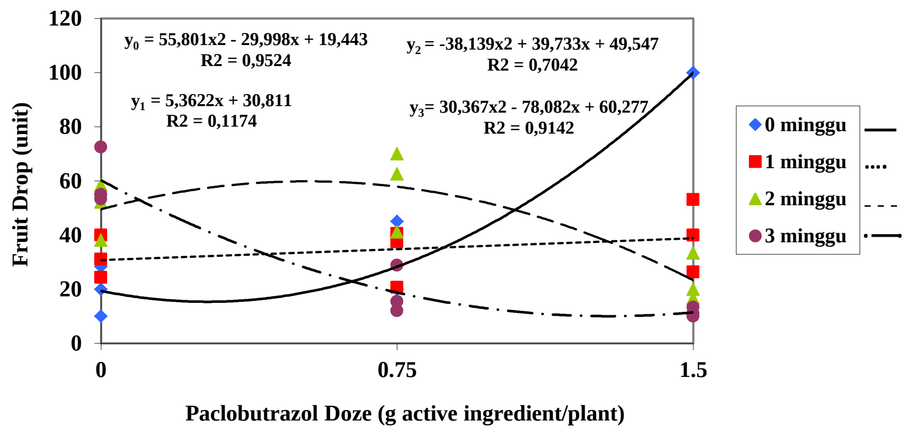

Figure 3. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit drop of Chokun orange

Table 1 shows that in the absence of a dry period, the lowest fruit loss was obtained in the absence of paclobutrazol. Figure 3 shows that without drought stress, increasing the dose of paclobutrazol from 0 to 0.26 g of the active ingredient/plant decreased fruit loss, but after that increasing the dose of paclobutrazol increased fruit loss. The addition of the dose of paclobutrazol 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 paclobutrazol showed an increase in the production of the hormone abscisic acid (ABA). This is by 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 paclobutrazol 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 no drought period. According to Kazan & 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 research Endo et al. (2018), ABA accumulation correlated with CiFT homologous transcript accumulation and flowering intensity of Satsuma mandarin citrus. According to Chica & 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. This is by 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 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. According to research by Panigrahi & Srivastava (2016), treatment of long periods of periodic drought can induce the flowering of tangerines.

C/N Ratio and Gibberellin Content in Leaves

Table 2 shows the lowest gibberellin content was obtained during the dry period of 3 weeks and the dose of paclobutrazol was 1.5 g of the active ingredient/plant. Figure 4 shows that during the dry period of 3 weeks, increasing the dose of paclobutrazol from 0 to 2.11 g of the active ingredient/plant decreased the gibberellins content. Paclobutrazol and long dry period work by inhibiting gibberellin biosynthesis, thereby inhibiting cell elongation in sub-apical meristems. According to Jungklang et al., (2015), stated that when gibberellin production is inhibited, cell division still occurs but new cells do not elongate. The paclobutrazol application will reduce the content of GA4, GA3, GA7, and GA1 contained in shoots and leaves (Upreti et al., 2013b). This is also by the research of Srilatha et al. (2015), the application of paclobutrazol will reduce the content of gibberellins (GA3). According to Rani et al. (2018), paclobutrazol may have acted as an anti-gibberellic 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.

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

Variable observation	Paclobutrazol doze (g of active ingredient/ plant)	Drought stress (week)				Mean
		0	1	2	3	
Ratio C/N (%)	0	5.74	3.93	4.56	5.27	4.87 A
	0.75	6.37	4.82	4.41	4.98	5.14 A
	1.5	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	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
	1.5	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 test; numbers followed by lowercase letters are the same in the same column not significantly different in the 5% DMRT test

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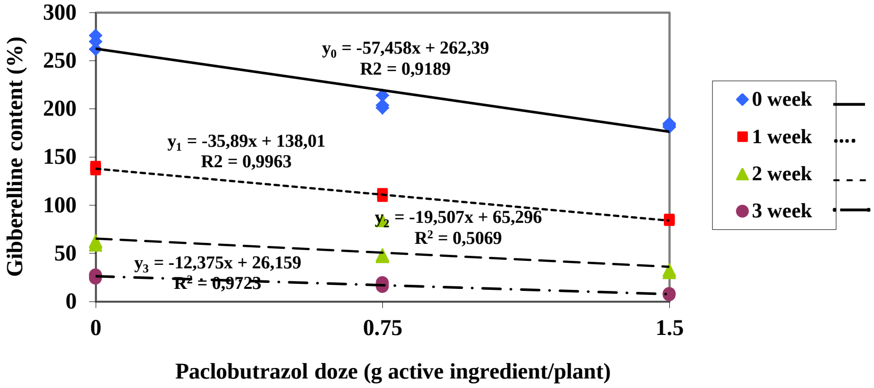

Figure 4. The interaction effect of the dose of paclobutrazol and duration of drought stress on gibberellin content of Chokun orange

Inhibition of gibberellin biosynthesis by paclobutrazol is at the kaurene stage and has been shown to reduce vegetative growth (Kumar et al. 2019). Paclobutrazol 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.

CONCLUSION

In conclution, the application of paclobutrazol and duration of drought stress were able to induce flowering of citrus plants as seen from the generative shoot variables with a quadratic model on the equation y = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient/plant 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 paclobutrazol and duration of drought stress were able to transfer from the vegetative phase to the generative phase which in turn could induce flowering of citrus plants.

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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, 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 paclobutrazol (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 paclobutrazol 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 = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient plant^{-1} and 1 week of drought. The dry period of 3 weeks gives the best results seen from the variables of the number of flowers and number of fruits. In general, the results

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suggest that the application of paclobutrazol 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

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 (Badan Pusat Statistik, 2021). To overcome these problems, plant cultivation techniques are needed to regulate citrus fruit production so that the supply of citrus fruits is always constant and can meet consumer needs at any time.

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).

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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 (Sreekumar 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 paclobutrazol 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., 2011b). According to research by Upreti et al. (2013a), 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 paclobutrazol. 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.

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The effectiveness of paclobutrazol 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 paclobutrazol 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), paclobutrazol promotes vegetative growth and results in higher flowering and fruiting growth in Ascolana olives. According to Xing et al. (2016), the application of paclobutrazol causes an increase in the number of flowers on apple plants. Burondkar et al. (2013) mentioned that due to the application of paclobutrazol to plants for three years, the average yield showed a significant difference in flowering (85.4 days) and progress in harvesting (82 days).

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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 carbon (C) and nitrogen (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).

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The use of paclobutrazol 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 paclobutrazol treatment with drought stress can reduce residues in the soil to minimize environmental damage in support of environmental sustainability. The application of paclobutrazol 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 is to determine the effect of the dose of paclobutrazol, duration of drought stress and the combination of dose of paclobutrazol 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.

MATERIAL AND METHODS

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 -7.4204009, 109.2147000, 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 Sudirman. 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 used a pot experiment with a factorial experiment in a Randomized Complete Block Design (RCBD). The first factor is the dose of paclobutrazol, consisting of 3 levels, without paclobutrazol as control (P0), 0.75 g active ingredient plant^{-1} (P1) and 1.5 g active ingredient plant^{-1} (P2). This study utilized a lower dose than the recommended dose because there were two treatments with the same goal, namely to induce flowering. Concerning dosage recommendation according to research by Darmawan et al. (2014), trees that were given paclobutrazol as much as 2 g plant^{-1} increased the

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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.

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This experiment consists of 12 treatment combinations with 3 replications resulting in 36 experimental units and one 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) tests at a level of 5% and regression analysis was performed.

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Research procedure

Plants used for this 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 incepticol soil, 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.

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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. Paclobutrazol was applied 2 days after media saturation with a dose of 0.75 and 1.5 g active ingredient plant^{-1} dissolved in 1 liter 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.

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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

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using the Luff Schoorl method (Yoshida et al., 1972). Analysis of N content was carried out using the Kjeldahl Semimicro method (William, 1984). 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 of Linskensen and Jackson (1987). Leaf sampling was performed after fertilization induction treatment (Sakhidin and Suparto, 2011). Microscopic observation of shoot tissue was conducted using the paraffin method (Puslitbang Biology-LIPI, 1998). Tissue collection was done after saturating water was given at the end of the drought period.

RESULTS AND DISCUSSION

The results of this study show that citrus plants with the application of paclobutrazol and duration of drought stress could stimulate generative induction as seen from the generative shoot variables, fruit set and fruit loss. The application of paclobutrazol 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 0.75 g of paclobutrazol active ingredient plant^{-1} and a dry period of 1 week. Figure 1 shows that during the 1 week long dry period, increasing the dose of paclobutrazol from 0 to 0.89 g of the active ingredient plant^{-1} increased the number of generative shoots, but after that increasing the dose of paclobutrazol would decrease the number of generative shoots. According to the research of Martínez-Fuentes et al. (2013), paclobutrazol 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 paclobutrazol significantly reduces the vegetative growth rate by decreasing shoot length and decreasing number of leaves. The effect of paclobutrazol 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 0.75 g of the active ingredient 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 paclobutrazol from 0 to 1.12 g of the active ingredient/plant increased

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the fruit set, but after that increasing the dose of paclobutrazol 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). Paclobutrazol 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), paclobutrazol 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.

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

Variable observation	Paclobutrazol doze (g of active ingredient plant^{-1})	Drought stress (week)				Mean
		0	1	2	3	
Generative shoots (units)	0	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
	1.5	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 appearing (units)	0	88.67	84.00	77.00	51.33	75.25 A
	0.75	93.33	77.00	91.00	65.33	81.67 A
	1.5	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	9.33	18.67	8.00	24.50	15.13 A
	0.75	1.83	13.67	13.67	26.33	13.88 A
	1.5	1.33	13.50	3.67	21.33	9.96 A
	Mean	4.17 c	15.28 b	8.44 c	24.06 a	(-)

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Fruit set (%)	0	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
	1.5	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 fruit (unit)	0	4.67	8.00	5.33	9.00	6.75 A
	0.75	3.00	8.00	5.33	12.17	7.13 A
	1.5	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	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
	1.5	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 test; numbers followed by lowercase letters are the same in the same column not significantly different in the 5% DMRT test

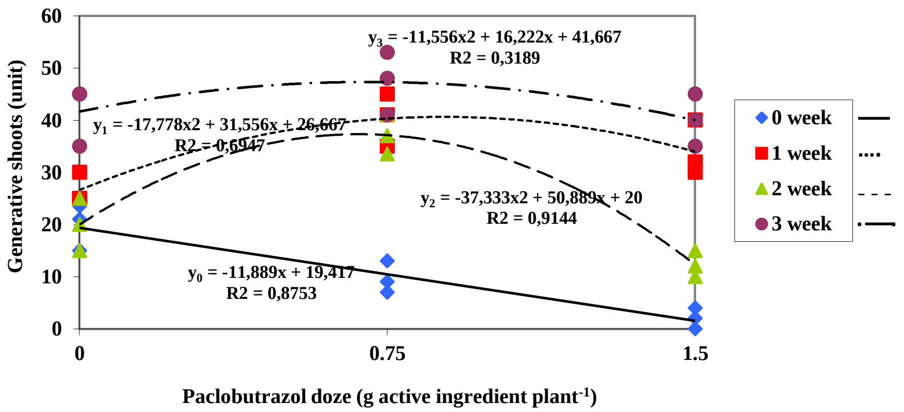

Figure 1. The interaction effect of the dose of paclobutrazol and duration of drought stress on the number of generative shoots of Chokun orange

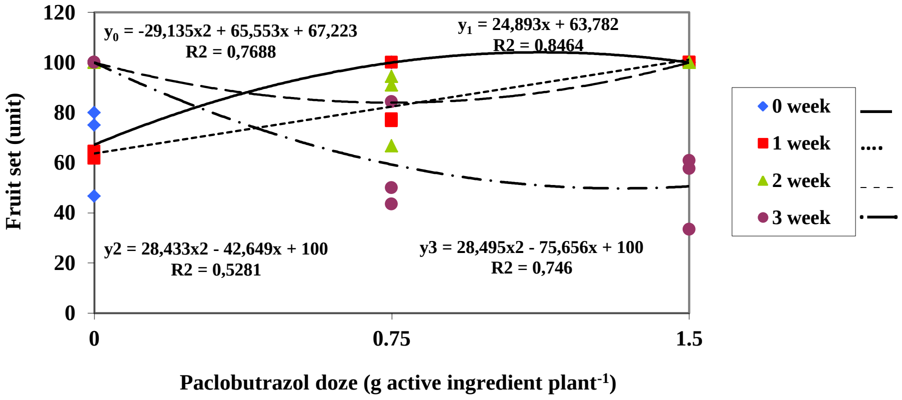

Figure 2. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit set of Chokun orange

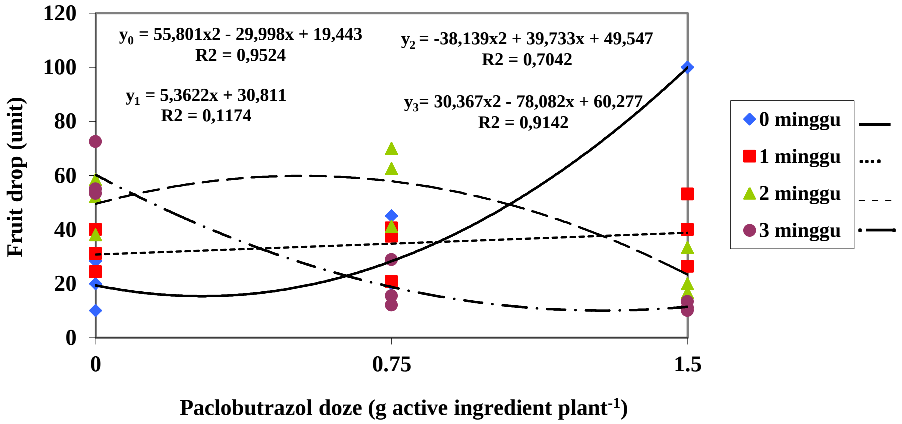

Figure 3. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit drop of Chokun orange

Table 1 shows that in the absence of dry period, the lowest fruit loss was obtained in the absence of paclobutrazol. Figure 3 presents that without drought stress, increasing the dose of paclobutrazol from 0 to 0.26 g of the active ingredient plant^{-1} decreased fruit loss, but after that increasing the dose of paclobutrazol increased fruit loss. The addition of the dose of paclobutrazol causes the gibberellin content in plants to be low; this is the cause of high fruit loss. Meanwhile,

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according to Gollagi et al. (2019), plants treated with paclobutrazol show an increase in the production of the hormone abscisic acid (ABA). Confirmed in the research by 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 paclobutrazol 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. Validated 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.

C/N ratio and gibberellin content in leaves

Table 2 presents that the lowest gibberellin content was obtained during the dry period of 3 weeks and the dose of paclobutrazol was 1.5 g of the active ingredient plant^{-1}. Figure 4 shows that during the dry period of 3 weeks, increasing the dose of paclobutrazol from 0 to 2.11 g of the active

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ingredient plant^{-1} decreased the gibberellins content. Paclobutrazol and long dry period work by inhibiting gibberellin biosynthesis, thereby inhibiting cell elongation in sub-apical meristems. Jungklang et al. (2015) stated that when gibberellin production is inhibited, cell division still occurs but new cells do not elongate. The paclobutrazol application will reduce the content of GA4, GA3, GA7 and GA1 contained in shoots and leaves (Upreti et al., 2013b). This is also demonstrated by the research of Srilatha et al. (2015) that the application of paclobutrazol will reduce the content of gibberellins (GA3). According to Rani et al. (2018), paclobutrazol may have acted as an anti-gibberellic 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.

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

Variable observation	Paclobutrazol doze (g of active ingredient plant^{-1})	Drought stress (week) 0	1	2	3	Mean
Ratio C/N (%)	0	5.74	3.93	4.56	5.27	4.87 A
	0.75	6.37	4.82	4.41	4.98	5.14 A
	1.5	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	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
	1.5	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 test; numbers followed by lowercase letters are the same in the same column not significantly different in the 5% DMRT test

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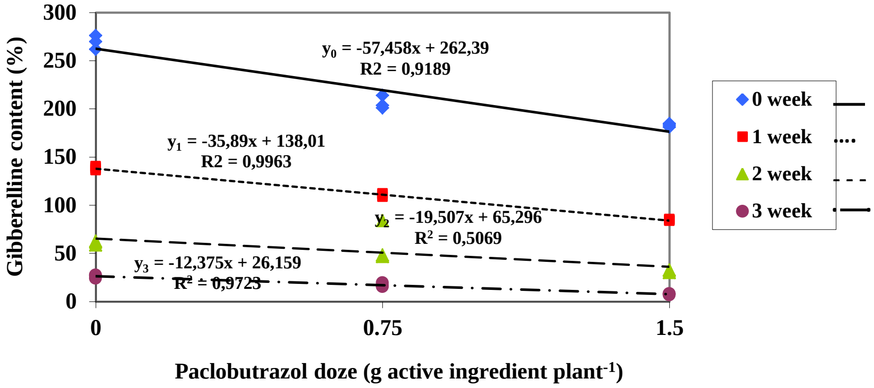

Figure 4. The interaction effect of the dose of paclobutrazol and duration of drought stress on gibberellin content of Chokun orange

Inhibition of gibberellin biosynthesis by paclobutrazol is at the kaurene stage and has been shown to reduce vegetative growth (Kumar et al., 2019). Paclobutrazol 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.

CONCLUSION

In conclusion, the application of paclobutrazol 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 = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient 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 paclobutrazol 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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COVERING LETTER

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

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, 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 paclobutrazol (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 paclobutrazol 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 = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient plant^{-1} 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 paclobutrazol 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

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 (Badan Pusat Statistik, 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.

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 (Sreekumar 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 paclobutrazol 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., 2011b). According to research by Upreti et al. (2013a), 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 paclobutrazol. 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 paclobutrazol 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 paclobutrazol 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), paclobutrazol promotes vegetative growth and results in higher flowering and fruiting growth in Ascolana olives. According to Xing et al. (2016), the application of paclobutrazol causes an increase in the number of flowers on apple plants. (Burondkar et al., 2013) mentioned that due to the application of paclobutrazol 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 carbon (C) and nitrogen (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 paclobutrazol in excessive concentrations can lead to the accumulation of residues in the soil (Prates et al., 2021). The expectacy of this research is that the combination of paclobutrazol treatment with drought stress can reduce residues in the soil to minimize environmental damage in support of environmental sustainability. The application of paclobutrazol 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 paclobutrazol, duration of drought stress, and the combination of dose of paclobutrazol 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.

MATERIAL AND METHODS

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 (-7.4204009, 109.2147000), 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 paclobutrazol, consisting. of 3 levels, without paclobutrazol as control (P0), 0.75 g active ingredient plant^{-1} (P1), and 1,5 g active ingredient plant^{-1} (P2). This study utilizes a lower dose than the recommended dose because there were two treatments with the same goal, namely to induce flowering. Concering dosage recommendation according to research by Darmawan et al. (2014), trees that were given paclobutrazol 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 consist of 12 treatment combinations with 3 replications resulting in 36 experimental units and one 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) tests at a level of 5% and regression analysis was performed.

Research procedure

Plants used for used 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 incepticol soil, 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. Paclobutrazol was applied 2 days after media saturation with a dose of 0.75 and 1.5 g active ingredient plant^{-1} dissolved in 1 liter 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 Kjeldahl Semimicro 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 method. 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 paclobutrazol and duration of drought stress could stimulate generative induction as seen from the generative shoot variables, fruit set, and fruit loss. The application of paclobutrazol 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 0.75 g of paclobutrazol active ingredient plant^{-1} and a dry period of 1 week. Figure 1 shows that during the 1 week long dry period, increasing the dose of paclobutrazol from 0 to 0.89 g of the active ingredient plant^{-1} increased the number of generative shoots, but after that increasing the dose of paclobutrazol would decrease the number of generative shoots. According to the research of Martínez-Fuentes et al. (2013), paclobutrazol 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 paclobutrazol significantly reduced the vegetative growth rate by decreasing shoot length and decreasing number of leaves. The effect of paclobutrazol 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 0.75 g of the active ingredient/plant, 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 paclobutrazol from 0 to 1.12 g of the active ingredient/plant increased the fruit set, but after that increasing the dose of paclobutrazol 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). Paclobutrazol 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), paclobutrazol 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.

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

Variable observation	Paclobutrazol doze (g of active ingredient plant^{-1})	Drought stress (week)				Mean
		0	1	2	3	
Generative shoots (units)	0	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
	1.5	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 appearings (units)	0	88.67	84.00	77.00	51.33	75.25 A
	0.75	93.33	77.00	91.00	65.33	81.67 A
	1.5	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	9.33	18.67	8.00	24.50	15.13 A
	0.75	1.83	13.67	13.67	26.33	13.88 A
	1.5	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	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
	1.5	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 fruit (unit)	0	4.67	8.00	5.33	9.00	6.75 A
	0.75	3.00	8.00	5.33	12.17	7.13 A
	1.5	0.00	7.83	3.33	10.00	5.29 A

	Mean	2.56 b	7.94 a	4.67 b	10.39 a	(-)
	0	19.44 Bb	31.77 Ba	49.55 Aa	60.28 Aa	40.26
Fruit drop	0.75	28.33 Bb	32.90 Ba	57.89 Aa	18.80 Bb	34.48
(%)	1.5	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 test; numbers followed by lowercase letters are the same in the same column not significantly different in the 5% DMRT test

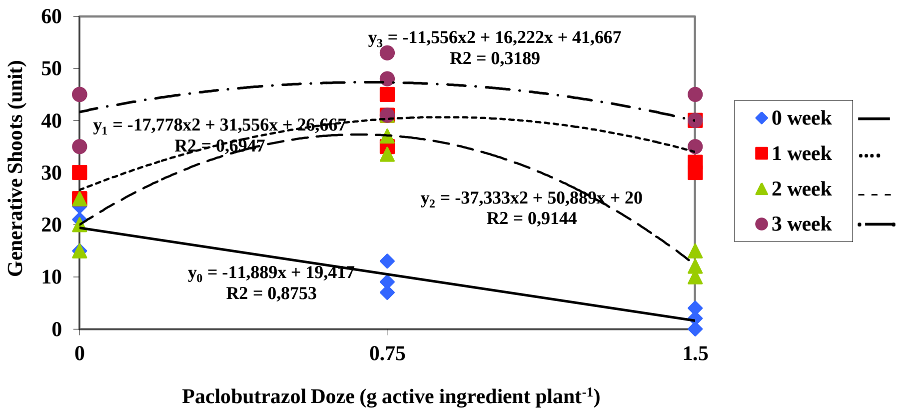

Figure 1. The interaction effect of the dose of paclobutrazol and duration of drought stress on the number of generative shoots of Chokun orange

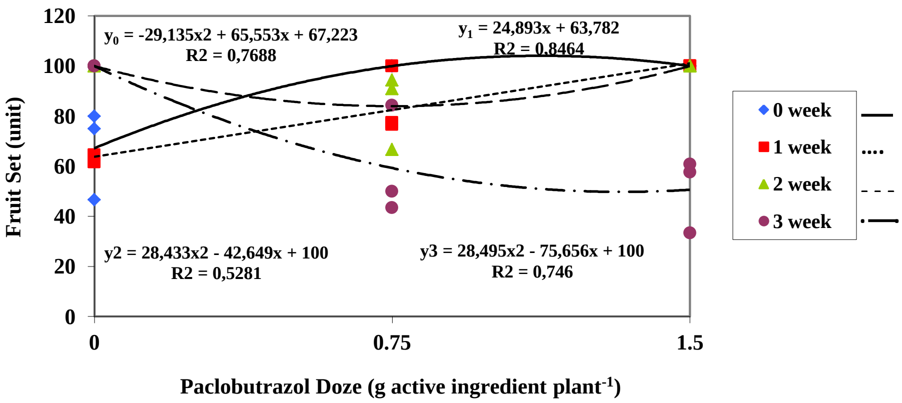

Figure 2. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit set of Chokun orange

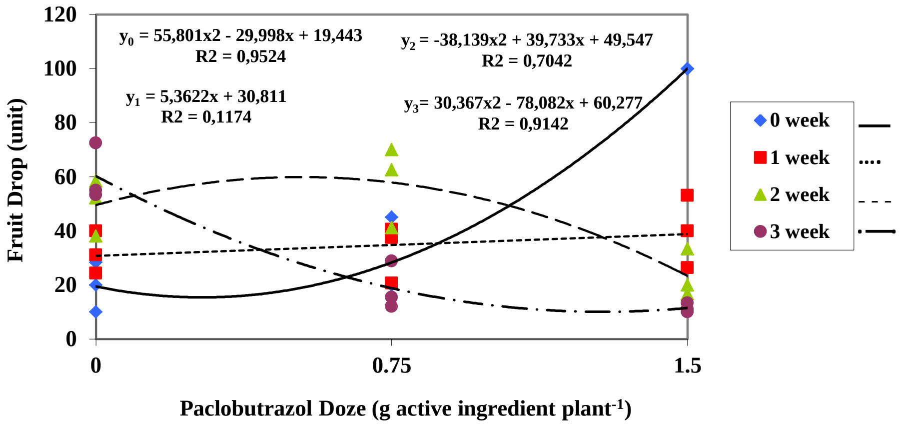

Figure 3. The interaction effect of the dose of paclobutrazol and duration of drought stress on fruit drop of Chokun orange

Table 1 shows that in the absence of dry period, the lowest fruit loss was obtained in the absence of paclobutrazol. Figure 3 present that without drought stress, increasing the dose of paclobutrazol from 0 to 0.26 g of the active ingredient plant^{-1} decreased fruit loss, but after that increasing the dose of paclobutrazol increased fruit loss. The addition of the dose of paclobutrazol 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 paclobutrazol 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 paclobutrazol 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.

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 paclobutrazol was 1.5 g of the active ingredient plant^{-1}. Figure 4 shows that during the dry period of 3 weeks, increasing the dose of paclobutrazol from 0 to 2.11 g of the active ingredient plant^{-1} decreased the gibberellins content. Paclobutrazol and long dry period work by inhibiting gibberellin biosynthesis, thereby inhibiting cell elongation in sub-apical meristems. Jungklang et al. (2015), stated that when gibberellin production is inhibited, cell division still occurs but new cells do not elongate. The paclobutrazol application will reduce the content of GA4, GA3, GA7, and GA1 contained in shoots and leaves (Upreti et al., 2013b). This is also demonstrated by the research of Srilatha et al. (2015), that the application of paclobutrazol will reduce the content of gibberellins (GA3). According to Rani et al. (2018), paclobutrazol may have acted as an anti-gibberellic 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.

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

Variable observation	Paclobutrazol doze (g of active ingredient plant^{-1})	Drought stress (week)				Mean
		0	1	2	3	
Ratio C/N (%)	0	5.74	3.93	4.56	5.27	4.87 A
	0.75	6.37	4.82	4.41	4.98	5.14 A
	1.5	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	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
	1.5	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 test; numbers followed by lowercase letters are the same in the same column not significantly different in the 5% DMRT test

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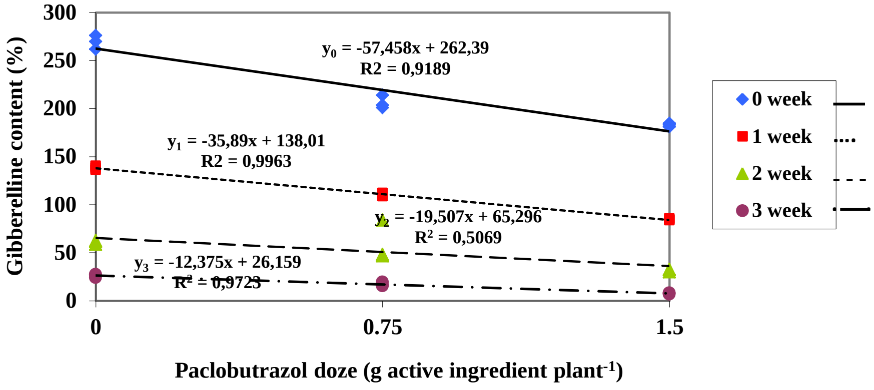

Figure 4. The interaction effect of the dose of paclobutrazol and duration of drought stress on gibberellin content of Chokun orange

Inhibition of gibberellin biosynthesis by paclobutrazol is at the kaurene stage and has been shown to reduce vegetative growth (Kumar et al. 2019). Paclobutrazol 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.

CONCLUSION

In conclusion, the application of paclobutrazol 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 = -17,778x2 + 31,556x + 26,667 at the optimum dose of 0.89 g of active ingredient 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 paclobutrazol 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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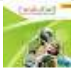

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