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# Production and Quality Improvement of Ginger Seed Rhizome by Paclobutrazol Applications

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**Abstract**

Low production and quality of seed rhizomes and voluminous seed rhizomes size are the constraints of ginger seed rhizome production. The aim of the experiment was observing the effect of concentration and time of paclobutrazol (PBZ) application to improve production and quality of ginger seed rhizomes. Experiment was arranged with split plot design with three replications. The main plot was the time of PBZ applications: 1) 4 months after planting (MAP), and 2) 5 MAP, whereas as a sub plot was the five-level concentrations of PBZ: 0, 100, 200, 300 and 400 ppm. The results of research showed that: application 400 ppm of PBZ at 4 MAP was the best treatment for increasing number of tillers, shoots, leaves, yield of the rhizome, and also for producing ginger seed that physiologically meet the quality. Increasing of the rhizome yield after PBZ application also reflected by increasing the content of chlorophyll in leaves and starch in rhizome.**.**

***KeyWords:*** Zingiber officinale; Paclobutrazol; seed rhizome production; seed rhizome quality.

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## 1. Introduction

Ginger (*Zingiber officinale*) is an annual herb plant with its rhizome are not able to be preserved for a long time in natural conditions, due to sensitivity to storage insect and pathogens, sprouting, while the size of ginger rhizome also creates another problem for storage and transporting. Those situations cause the seed rhizome of ginger hard to be provided in sufficient number with appropriate quality at the beginning of planting seasons.

Productivity of plants is influenced strongly by various complex physiological processes happen in the plant body, including the plant growth regulator (PGR) hormone balancing systems. Several plant hormones are well known possess positive effect on plant growth and negative effect for others. Gibberellins hormone production control in the plant tissues is among the most important aspect during preserving planting material, since this hormone able to control stem elongation and cell division [1].

However, in natural condition, the hormones are not always performed in balancing condition that causes the plant growing abnormally. On the other hands, this situation creates a condition that the manipulating PGR content and its combinations in plant tissues would lead to better plant production and quality.

Paclobutrazol (PBZ) that belongs to PGR with triallzole as active compound also able to inhibit biosynthesis of gibberellins. Application of PBZ reduced growth of stems and branches, but increases the abscicic acid (ABA) and chlorophyll content in the leaves [2].

Application of PBZ during fast growing phase of the potato plant increased potato production tubers, because the gibberellins delayed tuberization process [3]. PBZ as much as 4μM induced micro potato tubers formation after exposing to light conditions [4]. The application of PBZ increased potato production by increasing leaf chlorophyll content, photosynthesis rate, improving water usage and modifying dry matter partition to tubers, but reducing shoot growth [5].

Application of PGR for improving production and quality of ginger had been tested. Application of daminozide (gibberellins activity inhibitor) increased rhizome production of ginger, while the application of gibberellins decreased rhizome production. On ginger plants, PBZ application also caused higher starch accumulation than the application triacontanol [6].

Up to present impact of the PBZ application on production and quality of ginger seed rhizomes has not been tested. The study was aimed to observe the effect of time and concentration of PBZ application in improving the production and quality of ginger seed rhizomes.

## 2. Material and Methods

The study was conducted in Bogor (200 m above sea level) (West Java, Indonesia), Seed Technology Laboratory and Green house of Indonesian Spice and Medicinal Crops Research Institute (ISMCRI) and Postharvest Laboratory of Bogor Agriculture Institute, from September 2013 to September 2014.

Experiment was conducted using split plot design, with three replications. The main plot was the time of PBZ application: 1) 4 months after planting (MAP) and 2) 5 MAP, and as the subplot was five PBZ concentration levels: 0, 100, 200, 300 and 400 ppm.

Criteriaof seed rhizomes used: firm, weight 30-40 g, have2-3 buds and free of pest sand seed born diseases. Furthermore, seed rhizomes were soaked in a solution of fungicide and bactericide prior to seeding. Seeding was carried out in a plastic box with cocopit mediafor 1mont to get healthy seed and grow uniformly.

Applications of PBZ were implemented at 4 and 5 MAP according to treatment. Applications of PBZ were applied once every 2 weeks (five time applications) for each treatment tested. Applications of PBZ were carried out in the morning by watering into the rhizome, with a volume of 500 ml per plant according to treatment concentrations.

Observations were carried out on plant growth (plant height, pseudostem length, number of tillers, increased number of tillers, number of aerial shoots, number of nodes, internode lengt and number of leaves), total chlorophyll content, production (wet weight, number of rhizome branches) and physiologica quality of seed rhizomes (starch content, growth ability, growth speed and shoots height).

Plant height was measured from the stem base to the tip of the highest leaf. Pseudostem length was measured from the stem base to the tip of the stem (growing point). The number of tillers was determined by counting all tillers with leaves, while the number of shoots was determined by counting all non-leafy shoots. The number of nodes and the number of leaves were counted from the stem tip (growing point) to the stem base, along the 30 cm. Observations were carried out monthly, from one month after treatment until the plants 7 MAP. Chlorophyll content of leaves was determined after PBZ application of each of the three time applications. Measurement of chlorophyll content was conducted using Spectrometry.

Observations on the rhizome production per plant: wet weight, rhizome length, number of rhizome branches, and the ratio of wet weight to rhizome branch, were conducted after the crop was harvested at 9 MAP. Observations of the characteristics of the rhizome carried on: rhizome length, rhizome height, height of the rhizome branch (primary, secondary, and tertiary finger), internode length of rhizome branch (primary, secondary and tertiary finger), and the thickness of the rhizome branch (primary, secondary, and tertiary finger).

## 3. Results

### *3.1. Plant growth of ginger*

Application of PBZ at 4 MAP showed better growth of ginger than 5 months application on a number of tillers and aerial shoots, but neither for shoot height nor plant height (Table 1). Increasing of PBZ concentration also significantly influenced numbers of tillers and aerial shoot formation linearly. High PBZ concentration showed a negative effect on plant height and aerial shoot height.

**Table 1:** The influence of an application time and concentration of PBZ on plant height, number of tillers, shoot height and number of aerial shootsof ginger at the age of 7 MAP

| Treatments | Plant height (cm) | Number of tillers | Aerial shoot height (cm) | Number of aerial shoots |
|---|---|---|---|---|
| Aplication time ( MAP): | | | | |
| 1. 4 | 110.18 b | 16.80 a | 5.64 | 6.72 a |
| 2. 5 | 112.48 a | 15.33 b | 5.38 | 5.91 b |
| Concentration of PBZ | | | | |
| (ppm): | | | | |
| 1. 0 | 118.30 a | 118.30 a | 7.73 a | 4.53 c |
| 2. 100 | 116.07 a | 116.07 a | 6.68 b | 6.07 b |
| 3. 200 | 108.27 bc | 108.27 bc | 5.39 c | 6.13 b |
| 4. 300 | 105.45 c | 105.45 c | 4.31 d | 6.89 b |
| 5. 400 | 108.57 b | 108.57 b | 3.43 e | 7.97 a |

Note: The same letters in each column are not significantly different at 5% level by DMRT

The pseudostem length formation decreased significantly as the PBZ concentration increase and the effect of PBZ application was more obvious at the age of 4 MAP than 5 MAP (Figure 1). Meanwhile, increasing PBZ concentration give reverse effect on tiller formation numbers, and application of highest concentration of PBZ at 4 MAP increased number of tillers formation (Figure 2).

The numbers of nodes and leaves formation per tiller were influenced significantly by the time of application of PBZ and PBZ concentrations amount. The highest number of nodes and leaves were obtained at the time of application of PBZ at 4 MAP and 200-300 ppm of PBZ concentration (Table 2), but the same treatments of PBZ caused internodes length formation shorter than the one of lower concentration of PBZ.

Increasing PBZ concentration 100 ppm, leads the formation nodes number of pseudostem higher compared to without PBZ significantly. The internodes number per tiller rose as long the PBZ concentration increasing. On the other hand internodes length of the stem was reduced by the increasing application of PBZ concentration (Table 2).

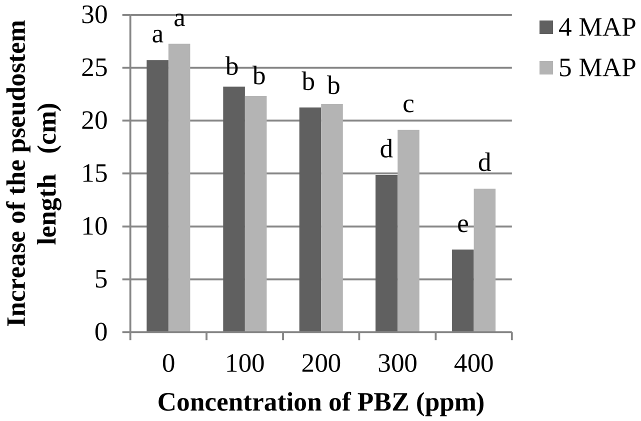


**Figure 1:** The effect of the application time with concentration of PBZ on increase of ginger pseudostem length, from initial application until 7 MAP

Note: The same letters in the bar are not significantly different at 5% level by DMRT

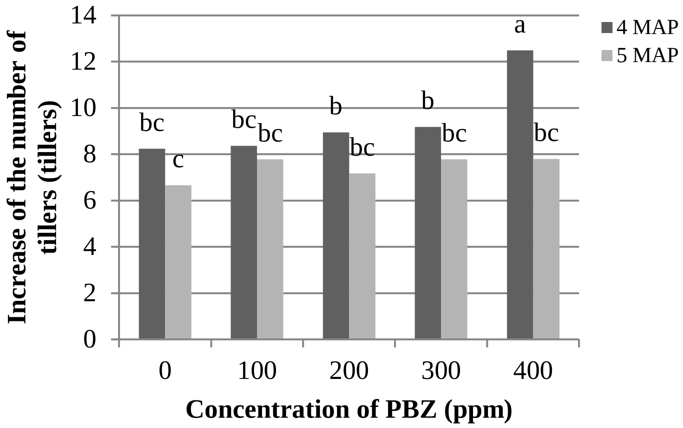


**Figure 2:** The effect of the application time with concentration of PBZ on increase of the ginger number of tillers up to the plant 7 MAP

Note: The same letters in the bar are not significantly different at 5% level by DMRT

### *3.2. Chlorophyll content*

Chlorophyll content of leaves is influenced by the interaction between the times of application and concentration of PBZ. The application time of PBZ at the plant age of 4 MAP with PBZ concentration of 100 ppm, significantly increased leaf chlorophyll content higher compared with without PBZ, while PBZ application on 5 MAP was not different from the control (Figure 3).

**Table 2:** The influence application time and concentration of PBZ on the number of nodes per tillers, length of internode and number of leaves per tiller of ginger at the plant 7 MAP

| Treatments | Number of nodes per tillers | Number of leaves per tiller | Length of internode (cm) |
|---|---|---|---|
| Aplication time ( MAP): | | | |
| 1. 4 | 15.06 a | 18.18 a | 2.18 b |
| 2. 5 | 13.33 b | 16.48 b | 2.35 a |
| Concentration of PBZ | | | |
| (ppm): | | | |
| 1. 0 | 10.55 c | 13.82 d | 3.10 a |
| 2. 100 | 13.01 b | 15.89 c | 2.33 b |
| 3. 200 | 15.42 a | 18.10 b | 2.01 c |
| 4. 300 | 15.87 a | 19.31 a | 1.94 c |
| 5. 400 | 16.13 a | 19.73 a | 1.94 c |

Note: The same letters in each column are not significantly different at 5% level by DMRT

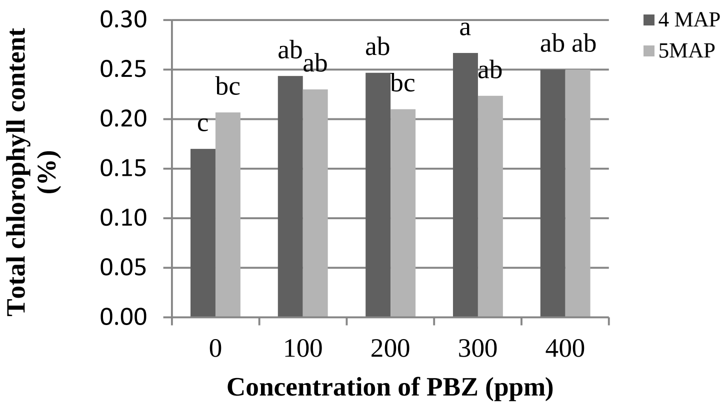


**Figure 3:** The influence of the interaction between the time of PBZ application with various concentrations on total chlorophyll content of leaves

Note: The same letters in the bar are not significantly different at 5% level by DMRT

### *3.3. Production of seed rhizomes*

Production of seed rhizomes (fresh weight, number of rhizome branches and fresh weight ratio to number of rhizome branches), was not affected by time of application of PBZ, but it was influenced by the concentration of applied PBZ. Up to 400 ppm, application of PBZ increased the fresh weight and number of rhizome branches linearly. Increasing of PBZ concentration caused the size of branching rhizome smaller than the one of control without PBZ application. Small rhizome caused by PBZ application was also described by ratio of fresh weight to number of rhizome branches (Table 3).

**Table 3:** The effect of an application time and concentration of PBZ to fresh weight of rhizomes, number of rhizome branches and fresh weight ratio to number of rhizome branches

| Treatments | Fresh weight of rhizomes (g) | Number of rhizome branches (propagule) | Ratio of fresh weight to number of rhizome branches (g) |
|---|---|---|---|
| Aplication time ( MAP): | | | |
| 1. 4 | 1022.90 | 27.29 | 36.65 |
| 2. 5 | 1014.86 | 26.68 | 38.64 |
| Concentration of PBZ (ppm): | | | |
| 1. 0 | 939.06 b | 19.67 d | 47.98 a |
| 2. 100 | 1003.33 ab | 25.03 c | 40.39 ab |
| 3. 200 | 986.67 ab | 27.52 bc | 36.03 b |
| 4. 300 | 1018.67 ab | 29.83 ab | 34.02 b |
| 5. 400 | 1148.00 a | 32.99 a | 34.18 b |

Note: The same letters in each column are not significantly different at 5% level by DMRT

### *3.4. Rhizome characteristics*

Application of PBZ at 4 MAP showed positive results on rhizome length, and height than the one of 5 MAP. The rhizome height of primary, secondary, and tertiary finger, were not influenced by time of application of

PBZ. Application of 100 ppm PBZ inhibited the height of rhizome, HPF, and HSF, but didnot influence the HTF. The length of rhizome was clearly increased by 400 ppm PBZ application (Table 4).

Internode length was not affected by the time of application of PBZ, but it was influenced by the concentration of applied PBZ. The application of PBZ 100 ppm reduced internodes length of primary finger (ILPF), secondary (ILSF) and tertiary (ILTR) compared to without PBZ (Table 5). Similar to the internodes length of the stem pattern, the internodes length of the rhizome was also inhibited by PBZ application. The length of the rhizome and the internodes were shorter compared to without PBZ application.

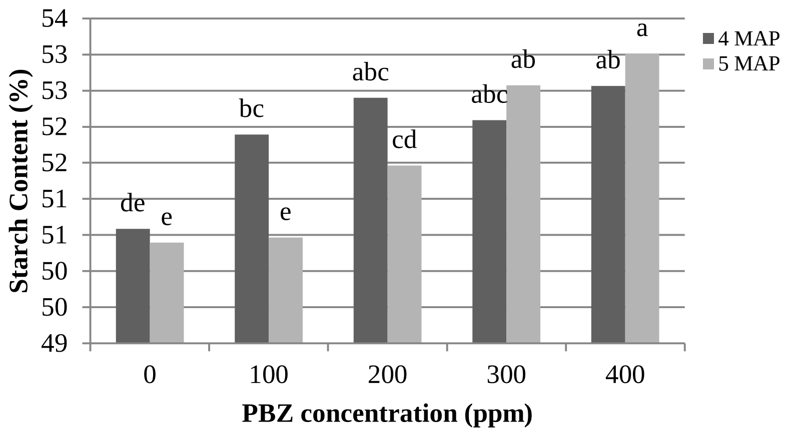


**Figure 4:** The effect of the interaction between the application time with various concentrations of PBZ on starch content of the ginger seed rhizomes

Note: The same letters in the bar are not significantly different at 5% level by DMRT

**Table 4:** The effect of an application time and concentration of PBZ on rhizome length, rhizome height, the height of primary finger (HPF), the height of secondary (HSF) and the height of tertiary (HTF) of ginger

| Treatments | Rhizome lenght (cm) | Rhizome height (cm) | HPF (cm) | HSF (cm) | HTF (cm) |
|---|---|---|---|---|---|
| Aplication time ( MAP): | | | | | |
| 1. 4 | 38.21 a | 17.20 a | 6.06 | 5.72 | 4.98 |
| 2. 5 | 36.84 b | 16.19 b | 5.71 | 5.57 | 5.03 |
| Concentration of PBZ (ppm): | | | | | |
| 1. 0 | 36.17 b | 18.42 a | 8.42 a | 7.50 a | 6.53 |

| | | | | | |
|---|---|---|---|---|---|
| 2. 100 | 36.89 b | 17.00 b | 5.08 b | 5.41 b | 4.81 |
| 3. 200 | 36.30 b | 16.17 c | 5.11 b | 5.14 b | 4.67 |
| 4. 300 | 37.50 b | 16.28 bc | 5.63 b | 5.03 b | 4.59 |
| 5. 400 | 40.97 a | 15.78 c | 5.27 b | 5.18 b | 4.45 |

Note: The same letters in each column are not significantly different at 5% level by DMRT

**Table 5:** The effect of an application time and concentration of PBZ on internodes length of primary finger (ILPF), internodes length of secondary finger (ILSF) and internode length of tertiary finger (ILTF) of ginger

| Treatments | ILPF (cm) | ILSF (cm) | ILTF (cm) |
|---|---|---|---|
| Aplication time ( MAP): | | | |
| 1. 4 | 0.90 | 0.87 | 0.79 |
| 2. 5 | 0.81 | 0.80 | 0.79 |
| Concentration of PBZ | | | |
| (ppm): | | | |
| 1. 0 | 1.31 a | 1.09 a | 0.98 a |
| 2. 100 | 0.78 b | 0.82 b | 0.78 b |
| 3. 200 | 0.81 b | 0.78 b | 0.80 b |
| 4. 300 | 0.71 b | 0.73 b | 0.72 b |
| 5. 400 | 0.67 b | 0.76 b | 0.72 b |

Note: The same letters in each column are not significantly different at 5% level by DMRT

The thickness of the primary finger (TPF), secondary (TSF) and tertiary (TTR) was not affected by the time of PBZ application. The concentration levels of PBZ only affected the thickness of the primary finger (TPF), but did not affect on the thickness of the secondary finger (TSF) and tertiary finger (TTF). The thickness of the primary finger (TPR) increased significantly after application of 200 ppm PBZ compared to without PBZ (Table 6).

**Table 6:** The effect of an application time and concentration of PBZ on thickness of primary fingers (TPF), the thickness of secondary finger (TSF) and the thickness of tertiary finger (TTF) of ginger

| Treatments | TPF (cm) | TSF (cm) | TTF (cm) |
|---|---|---|---|
| Aplication time ( MAP): | | | |
| 1. 4 | 3.11 | 3.44 | 3.18 |
| 2. 5 | 3.06 | 3.50 | 3.16 |
| Concentration of PBZ | | | |
| (ppm): | | | |
| 1. 0 | 2.84 c | 3.38 | 3.25 |
| 2. 100 | 2.98 bc | 3.50 | 3.18 |
| 3. 200 | 3.11 ab | 3.42 | 3.11 |
| 4. 300 | 3.24 a | 3.57 | 3.18 |
| 5. 400 | 3.26 a | 3.49 | 3.15 |

Note: The same letters in each column are not significantly different at 5% level by DMRT

### *3.5. Physiological quality of seed rhizomes*

PBZ application also increased starch content of seed rhizomes, and application of PBZ at 4 MAP showed faster increased significantly at 100 ppm PBZ application and not significantly increased on higher concentration. Whereas, application of PBZ at 5 MAP also increased but start at 200 ppm and still increased at 300-400 ppm (Figure 4).

Viability of seed rhizomes of ginger (growth ability, growth speed and shoot height) seedling was not affected by time of PBZ applications, but it was influenced by the concentration of PBZ applied. The application of PBZ 400 ppm increased the growth ability, speed of growth and shoots height of seed rhizomes compared to without PBZ application (Table 7).

**Table 7:** The effect of application time and concentration of PBZ on the growth ability, growth speed, and shoot height of ginger seedling

| Treatments | Growth ability (%) | Growth speed (%/day) | Shoot height of seedling (cm) |
|---|---|---|---|
| Aplication time ( MAP): | | | |
| 1. 4 | 85.10 | 1.99 | 27.27 |
| 2. 5 | 81.99 | 1.82 | 27.18 |
| Concentration of PBZ (ppm): | | | |
| 1. 0 | 73.33 c | 1.68 b | 23.66 b |
| 2. 100 | 78.89 bc | 1.61 b | 26.14 b |
| 3. 200 | 84.44 abc | 1.85 ab | 24.03 b |
| 4. 300 | 86.11 ab | 1.98 ab | 28.01 ab |
| 5. 400 | 94.99 a | 2.42 a | 34.28 a |

Note: The same letters in each column are not significantly different at 5% level by DMRT

**4. Discussion**

PBZ is a hormone artificially that influencing plant growth physiologically by inhibiting plant growth or promoting for other conditions. Application of PBZ had been tested on *Solanum tuberosum* for increasing tuberlet production [7]; assimilate production and allocation [5], for growth and pigment variation of *Solanum trilobatum* [8], and yield potential of *Jatropha curcas* [9].

Application of PBZ promoted ginger to produce more tiller and produced longer shoots. The PBZ application effect on ginger growth is not only depending on PBZ concentration, but also influenced by age of ginger at application time. Application in earlier time will inhibit strongly plant elongation, but promoting plant multiplication as shown in the numbers of tiller formation, node numbers and numbers of leaf parameters (Table 2). This experiment revealed that the time of application and concentration of PBZ applied physiologically increasing starch content and chlorophyll formation that increasing linearly as PBZ concentration increasing up to 400 ppm.

PBZ is growth inhibiting substance that able to retard plant vegetative growth, particularly of plant stems partially. Fungicide with triazole as active ingredient also having PBZ effect as plant growth regulator by the disturbing hormones balancing system in plants including gibberellin, ABA, and cytokinin production [10]. PBZ mechanisms involving blocking three terpenoid path systems of gibberellin production of plant by blocking the oxidation process of ent kaurene, ent kaurenol and ent kaurenal to be ent kaurenoic [1] and [2]. Gibberellin is growth hormone with its main role in cell division and elongation especially on the plant tissue of the stem [2]. PBZ also reported has an important role in the inhibition of pseudostem elongation that reduced plant height [11]. Therefore, the disturbance of this enzyme synthesis causes inhibition of cell elongation without cell division disruption that leads the formation of dwarf plant with short internodes. This phenomenon also happened in ginger treated with PBZ (Table 1 and 2). The effect of PBZ on plant growth is relatively similar among the plants, but the amount of PBZ concentration varies to have significant results, depend on the plant species, and the size of the plant. Application of 500 ppm PBZ on galangal (*Kaempferia galanga*) increased number of tillers, and leaves, but reduced plant height [12]. Application of 3 ppm of PBZ was enough to increase the numbers of tillers and leaves, but is a cause to begonia plant dwarf [13]. In *in vitro* study, 2 mg $l^{-1}$ of PBZ increased number of tiller and leaves of *Zingiber purpureum* more than the one of control [14]. On the other hands, 5 mg $l^{-1}$ of PBZ reduced the shoot elongation speed and the number of tillers of *Curcuma xanthorriza in vitro* [15].

Increasing number of tillers, aerial shoots and leaves on ginger correlated positively with plant yield, namely fresh weight of rhizome and tillers parameters. Shifting of transversal growth direction to longitudinal one is considered one of the important mechanism that involving in increasing fresh weight and tiller numbers of ginger, as well as improving seed physiological quality that represented as starch content, seed viability, growth speed and shoot height. Disturbing of gibberellin synthesis in plant after PBZ application is suggested tocreates an imbalance condition of plant hormones by elevating cytokinin endogen hormone concentration in plant tissue. Such kind of situation were reported occurring on potato plant [16, 17, 3]. Gibberellin hormone has important role in initiating the potato tuber formation. Application of exogenous gibberellin will delay tuber formation, while additional of a gibberellin biosynthesis inhibitor such as tetcyclacis, chlorocholine chloride, PBZ or ancymidol caused increasing of tuber formation [16, 17, 3].

Increasing of cytokinin concentration in plant tissue after PBZ application suggested accelerating rhizome formation process due to activate the division process of cell of rhizome that act as sink along preserving photosyntate in the rhizome. [17] reported that tuber formation process of potato could be accelerated by application of cytokinin hormone exogenously.

On ginger PBZ application increased chlorophyl content in leaf that caused the leaves became darker green in color than control. The similar effects were reported also on potato [7] and [5], *Jatropa curcas* [9] and on *Solanum trilobatum* [8]. Fitol is important substance for chlorophyl production. Blocking of gibberellins biosinthesis leads fitol substance concentration increasing that stimulate chlorophyll production [2]. Increasing of chlorophyl content after PBZ application because of triazole able to enhance cytokinin biosynthesis, which the enzime has important role in chloroplas differentiate, chlorophyl biosynthesis and it also preventing chlorophyl from degradating [18]. Application of other groups of triazole, namely uniconazole increased IAA and zaetin (cytokinin) of soybean [19].

While the reverse effect of PBZ application was reported on begonia by [13], that PBZ application caused carotenoid content increasing but reduced chlorophyll content. PBZ application also increased ginger rhizome yield (Table 3), as well as numbers of tillers, aerial shoots, leaves and chlorophyll contents. The same results reported by [12] that foliar application of 250 ppm PBZ on six month old galangal increased number of tillers and dry weight of galangal rhizome more than untreated one. PBZ application stimulated tuber formation, raised chlorophyll content, photosynthetic rate, and water absorption, but minimizing shoot growing on potato [5,20].

On ginger, PBZ application also increased number and size of tillers, therefore ratio of weight of rhizome against number of rhizome branches was lower than control (Table 3). These results indicated that PBZ application promoted small size of rhizome formation, but with normal seed viablity. This circumstance is promoting in using planting material efficiently. [21] stated that two to three tons ginger rhizome was needed for a hectare. This experiment revealed that increasing number of tillers with short, thick and small in size would reducing of rhizome needed for planting 30% lower than control.

The present study also revealed that PBZ application also able improve physiologically ginger seeds quality as reflecting by starch content, growth ability and growing speed parameters. The starch content was increased by 100 ppm PBZ application at four month olds ginger; growth speed at 200 ppm of PBZ; growth ability at 300 ppm of PBZ; while plant height at 400 ppm of PBZ. Increasing of starch content was suggested, as increasing of chlorophyll content and number of leaves that increasing photosyntate results and preserving in rhizome that act as sink and used as source of energy for growing. [12] reported the similar results on curcuma after applied with 250 ppm PBZ at four month olds, which produced the most weight dry rhizome and starch content. While on the same plant, the application of PBZ at six month olds increased the starch content [22]. The higher starch content at application PBZ 300 and 400 ppm increased the viability of seed rhizomes (growth ability and speed of growth) of ginger (Table 7).

## 5. Conclusion

Application 400 ppm of PBZ at 4 MAP was the best treatment for increasing number of tillers, shoots, leaves, yield of the rhizome, and also for producing ginger seed that physiologically meet the quality. Increasing the rhizome yield after PBZ application also reflected by increasing the content of chlorophyll in leaves and starch in rhizome**.**

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