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MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES OF BANANA (*Musa acuminata* cv. BERANGAN) TO BRASSINOLIDE AT NURSERY STAGE

Md Aiman Takrim Zakaria*, Siti Zaharah Sakimin, Mohd Fauzi Ramlan, Hawa ZE Jaafar, Ali Baghdadi and Siti Norliza Mohd Din

Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor

**Email: aimanupm_91@yahoo.com*

ABSTRACT

Brassinolide (BR) represents one of the plant growth regulators essential in multiple developmental processes in plants including cell division, cell elongation and reproductive development. This study was aimed to investigate the best concentration of BR for optimum growth of young banana (*Musa acuminata* cv. Berangan) tissue culture. The plantlets were foliar sprayed with different concentrations of BR (3, 6 and 12 g/L) at every two weeks intervals. The experiment was arranged as randomised complete block design. The results showed that BR concentration had significant effects on the growth and physiology of *Musa* sp. cv. Berangan. As the BR concentration increased, plant height, pseudo-stem diameter, total leaf numbers, total leaf area, fresh and dry weight of shoot were markedly increased from week 3 to week 8 after transplanting. The BR-induced plantlets had higher chlorophyll content which contributed to the increased rate of photosynthesis. The root size and distributions were, however, not significantly affected by BR. From these findings, it can be concluded that exogenous application of BR at 6.88 g/L was the best concentration for *Musa* sp. cv. Berangan at nursery stage as it was able to increase the plant height and pseudo-stem diameter of the species.

Keywords: Plant growth regulator, brassinolide, banana, pseudo-stem, cell elongation

INTRODUCTION

Bananas which belong to the family Musaceae, have a lot of potential as a sustainable crop with many products, but their growth will be reduced due to the climate change (Ranjitkar et al. 2016). Agrofood Statistic in 2015 recorded that there were 27, 296 ha planted areas of banana in Malaysia with total production of 343, 061 metric tonnes (DOA 2015). Plant growth regulators (PGR) are widely used in modern agriculture at low dosages and typically applied via foliar sprays with water as a carrier that affect developmental or metabolic processes in higher plants such as banana (Rademacher 2015). Brassinolide (BR) is one of the phytohormones essential for plant growth and development as well as important for cell division and expansion which may increase crop yield and stress tolerance (Sasse 2003). Furthermore, BR is also able to alleviate various biotic and abiotic stress effects (Ali et al. 2007; Jager et al. 2008). Krishna (2003) reported that BR acted as anti-stress agent in tomato plants which may help to overcome the problem regarding low and high temperatures, drought condition as well as pathogen infection on the plant. BR stimulates physiological changes, improved water relations and metabolic processes such as photosynthesis in wheat plant under drought stress condition (Sairam 1994). BR treatment also increased the grain weight of rice and translocation of carbon assimilates from leaves into yield organ (Fujii and Saka 2001). Exogenous application with BR hormone at tasseling increases maize yield by enhancing source and sink capacity (Zhen et al. 2017). Therefore, the objective of this study was to identify morphological and physiological responses of *Musa acuminata* cv. Berangan at nursery stage towards different concentrations of BR.

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MATERIALS AND METHODS

Plant materials and experimental site

This research was carried out at Field 15, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor (2°59'30.07"N 101°42'58.64"E). The experiment was conducted under a rain shelter house. Environmental factors are considered uniform throughout the duration of the experiment. *Musa acuminata* cv. Berangan plantlets were obtained from NNS Permata Holdings Sdn. Bhd. located at Kuala Pilah, Negeri Sembilan.

Crop establishment, treatment and experimental design

Uniform and healthy one-month old banana tissue culture plantlets were selected and grown in polybags with the size of 15 × 15 cm. Each polybag consisted of one plantlet and filled with 4 kg of Bungor soil series. Normal watering was done by drip irrigation to maintain soil moisture at field capacity of the soil. Three concentrations of BR 3, 6 and 12 g/L were applied. Each BR treatment at 50 mL per plant was foliar sprayed on the upper and lower surface areas of leaf at two weeks after transplanting (WAT). The plantlets were supplied with chemical fertiliser NPK (15:15:15) about 5 g per polybag at 5 WAT. The treatments were arranged in a randomised complete block design (RCBD) with three replications and each replication consisted of 12 plantlets.

Determination of growth traits

Weekly, plant height and pseudo-stem diameter were measured by using measuring tape and vernier calliper, respectively. Both data were expressed in centimetres (cm). Plant height was taken from the soil surface level until the first internode located at the top of plant shoots. Meanwhile, pseudo-stem diameter was taken at 1 cm height from soil surface level by using vernier calliper. Total leaf number (TLN) was counted manually for each plant. Total leaf area (TLA) was also measured manually by using a ruler for each plant at the end of the experiment. The leaf area was calculated using the formula:

$$LA = [\text{leaf length} \times \text{maximum width} \times 0.755]$$

Where, 0.755 was a fixed correction factor by following Robinson and Nel (1985)

The plantlets were destructively sampled at 8 WAT and separated into root and shoot. The roots were gently washed under running pipe water to remove all the adhering soil particles. Fresh weight of the shoot and root were weighed with an electronic balance (Sartorious A and D FX200Iwp, Germany) before oven-dried. Then, dry weight of the shoot and root were weighed with an electronic balance (Sartorious A and D FX200Iwp, Germany) after drying in an oven at 60ºC for 72 hours until constant weight was achieved. The root to shoot ratio (R:S) was calculated on dry weight basis. Root morphological traits including root length (RL), root diameter (RD), root surface area (RSA) and root volume (RV) were measured at the end of the experiment period at 8 WAT by using a root scanner (EPSON Flatbed Scanner 1680).

Determination of chlorophyll content

Chlorophyll content (mg/cm^2) was determined according to the method of Coombs et al. (1985). Four gnaws were taken from the middle part of young and fully developed leaf by using a cork borer and transferred into a plastic vial (readily covered with aluminium foil) containing 20 mL of 80% acetone. The vials were kept in the dark for 7 days at room temperature until all the pigments were extracted. The absorbance values of the solution of each sample were read at 647 nm and 664 nm using a spectrophotometer (UV-3101PC UV-VIS-NIR, Shimadzu, Japan). Chlorophyll a, chlorophyll b and total chlorophyll contents were calculated as follows:

Chlorophyll a content (mg/cm^2 fresh leaf) = 13.19 (A_{664}) - 2.57 (A_{647})
Chlorophyll b content (mg/cm^2 fresh leaf) = 22.1 (A_{647}) - 5.26 (A_{664})
Total Chlorophyll content (mg/cm^2 fresh leaf) = 3.5 (chl a + chl b)/4

Where, A_{647} and A_{664} represent absorbance of the solution at 647 and 664 nm, respectively, while 13.19, 2.57, 22.1 and 5.26 are the absorption coefficients, 3.5 was the total volume used in the analysis taken from the original solution (mL) and 4 was the total discs area (cm^2).

Measurement of leaf gas exchange

The rate of photosynthesis (Ps), stomatal conductance (Gs), transpiration rate (Ts) and vapour pressure deficit (VPD) were measured on the third fully opened leaves by using a photosynthesis system (L1-6400, Li-COR, USA). The measurement was made in the morning (0800 to 1100). The reading of Ps, Gs, Ts and VPD were expressed as µmol $CO_2/m^2/s^1$, mmol/m^2/s^1, mmol $H_2O/m^2/s^1$ and mol $H_2O/m^2/s^1$.

Statistical analysis

All the data collected were analysed using analysis of variance (ANOVA) by Statistical Analysis System (SAS 9.4) to determine the significance difference between the treatment means. Differences between separated means was made using least significant difference (LSD) at P<0.05 level. A regression analysis was performed to establish relationship between BR concentrations and plant height to determine the best concentration for optimum growth of banana plantlets.

RESULTS AND DISCUSSION

Effect of BR on growth traits and chlorophyll content

Figure 1 showed that BR at 6 g/L increased plant height of Berangan banana from week 3 to week 8 consistently. Kothule et al. (2003) stated that BR induced synthesis of both indole-3-acetic acid (IAA) and gibberellic acid (GA) in plant and increased in plant height was probably due to their cumulative action. Analysis of variance for plant showed a highly significant difference (P<0.05) at the 8th week. No significant difference was observed by applying BR at 3 g/L and 6 g/L. As for the mean comparison between treatments, 6 g/L BR showed the highest mean value of 18.17 cm, while the control application, without BR has the lowest mean value of 14.67 cm. This indicated that 6 g/L BR significantly increased plant height by 23.56% compared to the control (without BR), while plant height of plant treated with 12 g/L significantly reduced by 8.26% compared to those in 6g/L BR.

These results were in accordance with the study by Sengupta et al. (2011) which found that *Vigna radiata* L. treated with BR were significantly taller than those in control treatment. Jian et al. (2016) stated that the growth of *Leymus chinensis* was improved by BR application in high temperature stressed condition and it was concentration dependent. High concentration about 1.0 mg/L at high temperature (38°C/25°C, day/night), the plant height was lower compared to room temperature (20°C/15°C, day/night).Thus, plant height was improved with increasing BR concentration reaching its maximum at 6 g/L, then decreased with 12 g/L compared to control.

Figure 2 showed the effects of BR on pseudo-stem diameter. The growth of pseudo-stem diameter increased with increasing concentration of BR up to 6 g/L but reduced with higher concentration (12 g/L) after 8 WAT. Analysis of variance for pseudo-stem diameter only showed significant ($P<0.05$) difference at 8 WAT. For mean comparison between treatments, 6 g/L BR treatment showed the highest mean value of 3.37 cm, while the control has the lowest mean value of 2.40 cm. There was an increased by 29.07% in pseudo-stem diameter growth for plants treated with 6 g/L BR compared to control. However, plant exogenously foliar sprayed with 12 g/L BR reduced pseudo-stem diameter by 13.95% compared to 6 g/L BR treatment. These results corresponded to the finding by Futoshi and Naoto (2013) which reported that exogenous application of BR appeared to promote stem growth in water spinach.

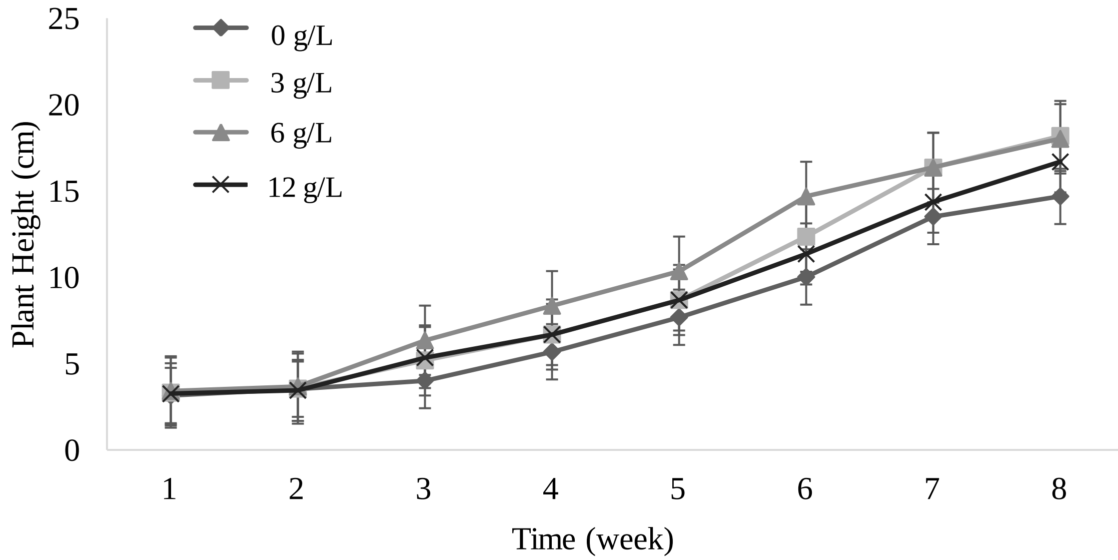

Figure 1. Plant height of Berangan banana over a duration after treatment using different concentrations of BR.

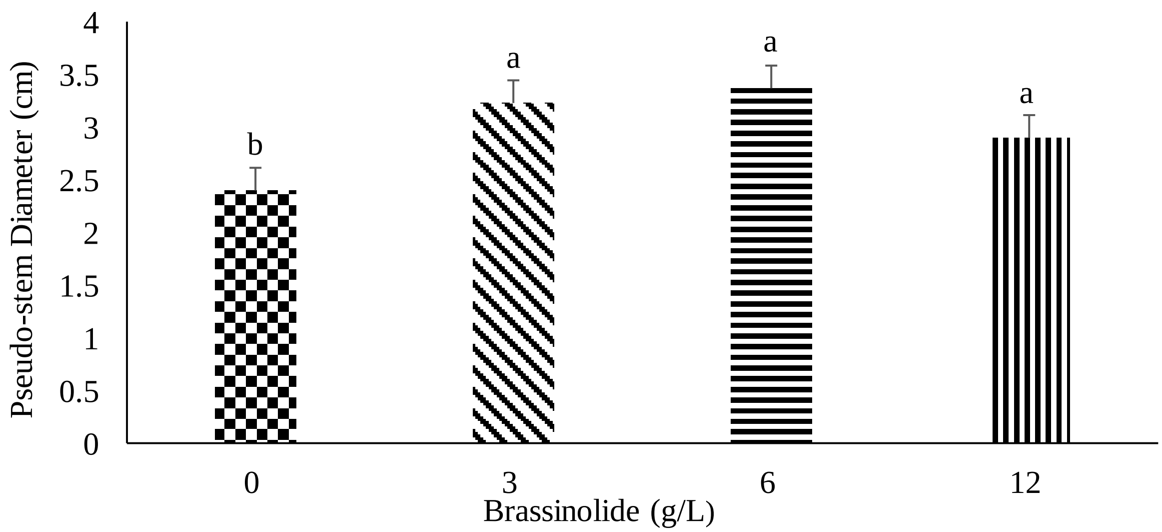

Figure 2. The pseudo-stem diameter after treatment using different concentrations of BR at 8 WAT. Mean values with the same letter are not significantly different at $P>0.05$ by least significant difference (LSD) with n=12.

Results showing various growth traits were presented in Table 1. TLN and TLA of banana plantlets significantly increased by increasing the concentration of BR up to 6 g/L but reduced with higher concentration (12 g/L). Plantlets treated with 12 g/L BR significantly reduced TLN by 16.07% and TLA by 36.79% compared to 6 g/L BR. However, application with 6 g/L BR gained the highest TLN (10.33) and TLA (1790.43 cm^2) compared to other treatments. According to Iwahari et al. (1990) BR-treated plants resulted in higher TLA as well as leaf numbers of citrus plant under well-watered condition.

The highest shoot fresh weight (SFW) and shoot dry weight (SDW) were observed in 6 g/L BR treatment compared to other treatments. Application of 6 g/L BR significantly increased SFW by 77.99% compared to control treatment. Control treatment showed the lowest SDW with mean value about 5.67 g. The present study found that root fresh weight (RFW), root dry weight (RDW) and R:S were not affected by different concentrations of BR. Harris (1992) stated that under well watered and normal condition, the aerial part of a plant may increase in weight more than the roots, the roots of a plant are able to supply water, nutrients and certain growth regulators to aerial part of plant. Elazab et al. (2012) also reported that root weight density and aerial biomass of durum wheat had a negative correlation under well-watered conditions, while this correlation was absent under water-stressed condition.

Table 1. The total leaf number (TLN), total leaf area (TLA), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW) and root shoot ratio (R:S) after treatment using different concentrations of BR

BR Concentration (g/L)	TLN	TLA (cm^2)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	R:S
0	8.67b	916.66c	72.66b	26.00a	5.67b	1.07a	0.18a
3	9.67ab	1196.64b	85.67b	30.67a	10.67b	1.63a	0.20a
6	10.33a	1790.43a	129.33a	51.00a	20.67a	3.33a	0.16a
12	8.67b	1131.68b	71.00b	33.00a	7.00b	1.83a	0.26a
LSD $_{P<0.05}$	1.15*	182.3***	21.72**	NS	6.45**	NS	NS

Means followed by the same letter within a column are not significantly different at (P>0.05) by least significant difference (LSD) with n=12. *, ** and *** significantly different at P<0.05, 0.01 and 0.001 respectively and NS= not significant.

One of the most important factors influencing the photosynthetic capacity in plant is chlorophyll content in the leaf tissues. The results presented in Table 2 showed that the high concentration of BR (6 and 12 g/L) caused highly significant increase in chlorophyll a, chlorophyll b and total chlorophyll content. Application of 3 g/L BR significantly increased chlorophyll a by 9.21%, and chlorophyll b by 24.73% compared to control. For mean comparison between treatments, total chlorophyll of 6 g/L BR gained the highest mean value of 7.030 mg/cm^2, while without any BR treatment gained the lowest mean value of 4.822 mg/cm^2. Similar result was reported by Sairam (1994) where plants treated with BR treatment resulted in increased chlorophyll content of two wheat varieties under irrigated and moisture-stress conditions.

Table 2. The chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll content (Chl_{a+b}) after treatment using different concentrations of BR

BR Concentration (g/L)	Chl a (mg/cm^2)	Chl b (mg/cm^2)	Chl_{a+b} (mg/cm^2)
0	4.310b	1.201c	4.822b
3	4.707b	1.498bc	5.430b
6	5.935a	2.099a	7.030a
12	5.460a	1.807ab	6.358a
LSD $_{P<0.05}$	0.58**	0.505*	0.744**

Means followed by the same letter within a column are not significantly different at (P>0.05) by least significant difference (LSD) with n=12. * and ** significantly different at P<0.05 and 0.01 respectively and NS= not significant.

Bao et al. (2004) reported that BR treatment has also been found to promote the occurrence of new roots and the formation of lateral roots of cucumber seedlings. Analysis of variance showed there were no significant difference (P>0.05) between the BR treated and control plants on the root part of banana plantlets after two months of planting at nursery (Table 3). The results showed that exogenous foliar sprayed on leaves of plantlets with different BR concentrations did not affect the root growth of RL, RD, RSA and RV. However, the study by Yueqing et al. (2016) showed that BR applied directly on potato root *in vitro* may affect the root growth in a dose-dependent manner. Lower BR concentrations (0.1 and 0.01 μg/L) promoted root elongation and lateral root development, whereas higher BR concentrations (1 to 100 μg/L) inhibited root elongation. Application of BR at 0.1, 1.0 and 10.0 µM promotes hypocotyl elongation of *Arabidopsis* in dark grown, although high concentrations of applied BR result in inhibition of root elongation (Leonid et al. 2015). The exogenous application with too high BR concentration may reduce the growth of banana plant, but did not show any significant difference on root parts. Thus, optimum concentration and balanced BR signaling is required to maintain normal root growth rates through the control of the root meristem size.

Table 3. The root length (RL), root diameter (RD), root surface area (RSA) and root volume (RV) after treatment using different concentrations of BR

BR Concentration (g/L)	RL (cm)	RD (mm)	RSA (cm^2)	RV (cm^3)
0	782.90a	1.66a	397.89a	16.26a
3	505.70a	2.99a	486.57a	40.08a
6	599.40a	2.32a	434.86a	25.32a
12	649.10a	1.84a	383.07a	18.38a
LSD $_{P<0.05}$	NS	NS	NS	NS

Means followed by the same letter within a column are not significantly different at (P>0.05) by least significant difference (LSD) with n=12 and NS= not significant.

Effect of BR on leaf gas exchange parameters

The effects of different BR concentrations on leaf gas exchange of *M. acuminata* were evaluated based on some of the parameters such as Ps, Gs, Ts and VPD of the third leaves from the shoot of the plants. The results stated in Table 4 revealed that treatment with BR significantly increased the photosynthesis rate of banana plantlets. For mean comparison between treatments, Ps of 6 g/L BR gained the highest mean value of 27.599 µmol $CO_2/m^2/s^1$, while the control gained the lowest mean value of 21.916 µmol $CO_2/m^2/s^1$. Application of 6 g/L BR on banana plantlet significantly increased Ps by 25.93% compared to control treatment. These increases might be due to higher leaf area and increases in chlorophyll content in leaves of banana. Iwahari (1990) stated that the increase in Ps could be attributed to the larger leaf area and higher chlorophyll content in BR-treated plants. These beneficial effects resulted in higher biomass production and yield-related parameters in the treated plants. However, there are variations in stomata aperture that control both the influx of CO_2 and water loss via transpiration to the atmosphere (von Caemmerer et al. 2004). However, there was no significant difference for Gs, Ts and VPD between the treatments.

Table 4. The net photosynthesis (Ps), stomata conductance (Gs), transpiration rate (Ts) and vapour pressure deficit (VPD) as affected by four different concentrations of BR

BR Concentration (g/L)	Ps (µmol CO_2 /m^2/s)	Gs (mmol/m^2/s)	Ts (mmol H_2O /m^2/s)	VPD (mol H_2O /m^2/s)
0	21.916c	0.623a	4.202a	0.765a
3	25.232b	0.633a	3.796a	0.722a
6	27.599a	0.713a	4.106a	0.714a
12	25.349b	0.660a	4.089a	0.741a
LSD $_{P<0.05}$	1.91**	NS	NS	NS

Means followed by the same letter within a column are not significantly different at (P>0.05) by least significant difference (LSD) with n=12 and NS=not significant. ** significantly different at P<0.01 respectively and NS= not significant.

Regression analysis

The main function of BR is to promote the plant growth especially for cell elongation and division in excised stem segments. It often shows synergism with auxin (Mandava 1988). Since the major effect of BR is toward stem elongation, the parameter for plant height was chosen for regression analysis. Regression analysis showed a polynomial trend between plant height and the treatments at 8 WAT (Figure 3). Application of BR up to 6.88 g/L increased the plant height within 8 WAT with maximum value at 18.6 cm, but application greater than 12 g/L reduced the plant height to 16.58 cm. Based on the regression analysis result, it seemed that application at 6.88 g/L was the optimum amount to be applied to the banana plantlets. Potential applications of optimum concentration of 6.88 g/L BR in agriculture can reduce dependency on irrigation to promote plant growth during drought season due to climate change, thus reducing the cost of irrigation system. Application of BR has the ability to stimulate other physiological processes under stressful condition such as high salinity, drought or insufficient nutrients (Prusakova et al. 1999). In addition, exogenous BR at low concentration during drought season is able to enhance resistance to stress and promote xylem differentiation in the plant (Sakurai and Fujioka 1993). The results of this study proved a close relationship between concentration of BR applied and the improvement of plant height, thus it has been classified as a plant hormone that has a role in regulating the plant cell elongation for the plant height increment (Hu et al. 2017). Wang et al. (1993) had found that BR appeared to cause elongation by affecting wall extensibility and increasing wall relaxation properties in *Brassica chinensis.* Improvement in plant height by application of BR will result in the increase in leaf number. Increasing leaf number will concurrently enhance total leaf area and thus allow more light penetration into the canopy and have productive regions on the periphery of the canopy. Greater light interception will instantly increase photosynthesis efficiency and improve plant growth performance. BR has the ability to improve the photosynthetic efficiency of plants by exogenous application of BR on *Leymus chinensis* mainly through enhanced biosynthesis of photosynthetic pigments. BR also enhanced the plant height, leaf area, dry mass accumulation and stimulated the accumulation of osmolytes as well as increased the activity of antioxidant enzymes under both normal and high temperature stress conditions (Niu et al. 2016).

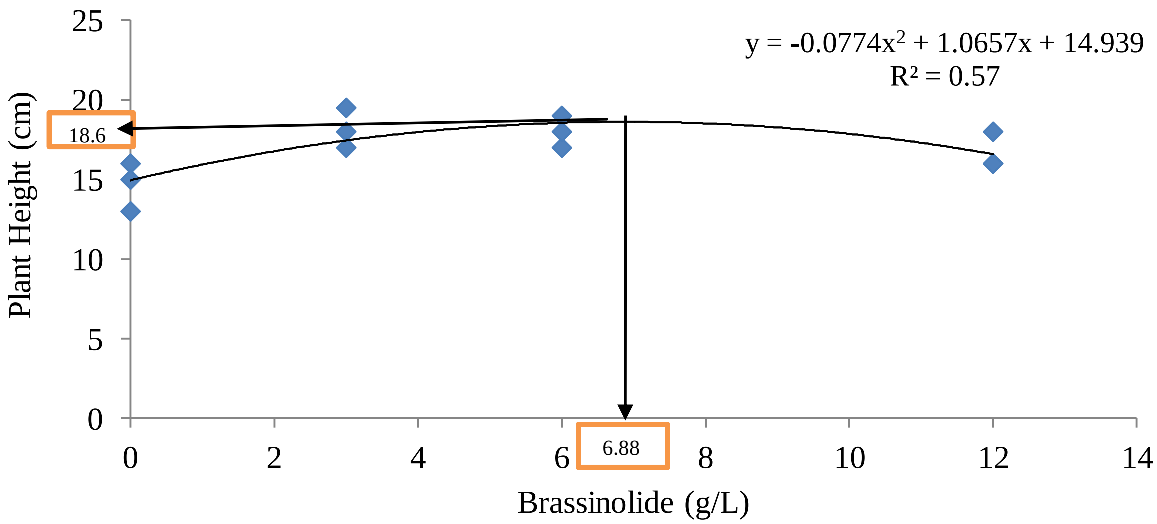

Figure 3. A regression analysis between BR concentration and plant height of banana plantlets 8WAT.

CONCLUSION

One of the most popular approaches to regulate tolerance of plant towards abiotic stress is by induction with PGR. The results presented showed that the application of BR increased banana growth with increasing concentration from 3 to 6 g/L, but it decreased at higher concentration (12 g/L). Application of 6 g/L BR increased plant height as well as major morphological development (pseudo-stem diameter) of Berangan banana at week 3 to week 8 compared to other treatments. In conclusion, the exogenous application of 6.88 g/L of BR is recommended to be used by farmers to enhance vegetative growth of Berangan banana at nursery stage. Strong vegetative growth of Berangan banana can increase the ability of the plant to be more resistant to disease infection, thus will produce better yield. However, it is recommended that further studies should be undertaken to determine the toxicity level and field trial experiment should be carried out to determine the growth performance and yield of the treated banana.

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