Brassinolide application as plant growth regulators

Brassinolide (BL) represents one of the plant growth regulators essential in multiple developmental processes in plants including cell division, cell elongation and reproductive development. Over the last ten years, the use of Plant Growth Regulator (PGR) has increased exponentially as main objectives in many studies. This book should be of interest to readers especially for researchers and students in the areas of agronomy, horticulture, plant physiology and pomology. There are many studies about plant growth and physiological changes but very little academic literature exists on how to best utilize the brassinolide and warrants further investigation on fig or other plants.

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PREFACE

First of all the author praises the presence of Allah SWT who has bestowed His mercy and gifts on us together in general and to the author in particular, so that the author can finish writing this book. There is no other hope for the author, hopefully this book can be useful for us together.

This book based on author's experiment at University Putra Malaysia, Malaysia. In the discussion of this book there are still many shortcomings. Therefore, critics and suggestions are highly expected for the perfection of this book in the future.

Pekanbaru, April 2019

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LIST OF ABBREVIATIONS AND SYMBOLS

%	percent
*	significant at 0.05 probability level
**	significant at 0.01 probability level
$\mu mol\ m^{-2}\ s^{-1}$	micro mole per meter square per second
$\mu mol\ mol^{-1}$	micro mole carbon dioxide per mole air
^0C	degree-celcius
ℓ	liter
A	net photosynthesis
ANOVA	analysis of variance
cm^2	centimeter square
C.V	coefficient of variation
C_i	intercellular carbon dioxide concentration
CO_2	carbon dioxide
E	evapotranspiration
e.g	example
g	gram
gs	stomata conductance
ha	hectares
H_2O	water
i.e	that is
$LSD_{0.05}$	least significance difference at 5% level
m	metre
MAT	Month After Treatment
m^3	cubic meter (volume)
$mol\ m^{-2}s^{-1}$	mole per meter square per second
n	number of samples
n.s	not -significant
NAR	net assimilation rate
O.D	optical density
p	probability
SRCBD	split plot randomized complete block design
s	second
SLA	specific leaf area
S/R	shoot to root ratio
Var.	variety
WAT	weeks after start of treatment

I. INTRODUCTION

Brassinolide (BL) is one of the brassinosteroids, which are steroidal plant hormones showing a wide occurrence in the plant kingdom, that have unique biological effects on growth and development (Clouse & Sasse, 1998; Khripach, Zhabinskii, & Groot, 2000). They are a group of naturally occurring polyhydroxy steroids initially isolated from *Brassica napus* pollen in 1979. Research on brassinosteroids has revealed that they elicit a wide spectrum of morphological and physiological responses in plants that include stem elongation and cell division (Grove et al., 1979), leaf bending and epinasty (Sandalio, María, & María, 2016). Besides their role in promoting plant growth activities, they also have physiological effects on the growth and development of plants (Khripach, et al., 2000; and Vardhini, 2012).

Much has been written about BL. Clouse (2011), for example, pointed out that:

> Among plant hormones, BL are structurally the most similar to animal steroid hormones, which have well-known functions in regulating embryonic and postembryonic development and adult homeostasis. Like their animal counterparts, BL regulate the expression of numerous genes, impact the activity of complex metabolic pathways, contribute to the regulation of cell division and differentiation, and help control overall developmental programs leading to morphogenesis. They are also involved in regulating processes more specific to plant growth including flowering and cell expansion in the presence of a potentially growth-limiting cell wall. (p.1).

Fig *(Ficus carica.L)* belongs to the Moraceae family. It is a bush or small tree, moderate in size, deciduous with broad, ovate, three- to five-lobed leaves, contains copious milky latex and introduced to Indonesia and Malaysia from Middle East and Western Asia. There are over 700 named varieties of fig trees, but many of them are not grown in home garden (Carroll, 2015). Because fig seeds are non-viable,

trees must be propagated via cuttings or grafts. Though the propagation of *F. carica* by vegetative cuttings insures uniformity, relatively low multiplication rates are achieved because these materials can be obtained only from upright branches, which results in poor rooting (Kumar, Radha, & Chitta, 1998); hence, brassinolide application was attempted by evaluating plant growth and physiological changes in *Ficus Carica*.

In Malaysia and Indonesia, there are at least 21 known varieties of the fig tree and most of them are from Improved Brown Turkey (IBT) and Masui Dauphine (MD) varieties (Ahmad, 2012). There is limited information on exogenous brassinolide application on these varieties. Thus, the aim of this study was to investigate the effect of different concentrations of exogenous application of BL on growth and physiological changes of fig var. IBT and MD.

II.MATERIALS AND METHODS

II.1. Experimental Site and Time

Field experiment was conducted at Ladang 15, Universiti Putra Malaysia which located at 2° 59' 01" N and 101° 44' 00" E with altitude of 58 m. The laboratory experiment was conducted at Faculty of Agriculture, Universiti Putra Malaysia situated at 2° 58" N and 101° 44' 04" E in Serdang, Selangor, Malaysia. The length of time the study was 8 (eight) months from May until December 2017.

II.2. Materials and Instrumentations

The materials used in this research were fig tree seedling cultivar *Improved Brown Turkey (IBT)*, and *Masui Dauphine (MD),* chicken dung, sand, top soil, water, brassinolide, polybag size 16 x 16 cm, insecticides, fungicides, rapia rope, etc.

The instrumentations used in this study were the leaf area meter (Model LI – 3100A Lincoln Inc, Nebraska, USA), a sensitive electronic weighing scale (Model

CDS 125, Mitutoyo Inc, Japan), a portable photosynthesis system (LICOR–6400, Inc., USA), SPAD meter 502 (Minolta Inc, USA), Light spectrophotometer (Model UV-3101P, Labomed Inc, USA), electric oven, shovel, scoop cement, yells, meter, pH meter, machetes, hoes, saws, hand sprayer, stationery, scales, camera, paint, brushes, etc.

II.3. Experimental Design

Fig planting materials were propagated using cutting methods and transferred into media containing 3:2:1 mixed soil (top soil:organic matters :sand). The experiment was arranged as Split Plot Randomized Complete Block Design (SRCBD) with 4 replications. The main plot was fig cultivars (C) consist of two level treatments: Improved Brown Turkey (IBT)(C1) and Masui Dauiphine (MD) (C2). The sub plot was brassinolide concentrations (B) consist of four level treatments: without Brassinolide (control)(B0), Brassinolide with dosage 50 ml/L (B1), Brassinolide with dosage 100 ml/L (B2), and Brassinolide with dosage 200 ml/L (B3). . The description of treatments was presented in Table 1.

Table 1. *Treatment Combinations of Cultivars and Brassinolide on Fig Tree*

Cultivars	Brassinolide			
	B0	B1	B2	B3
C0	C0B0	C0B1	C0B2	C0B3
C1	C1B0	C1B1	C1B2	C1B3

From those factors that mentioned above were obtained 8 combined treatment where each treatment activity was repeated 4 times in order to get 32 experimental units. The observed data of each treatment was analyzed statistically, if F count larger than F table 5%, then followed by a further test Least Significant Difference (LSD) 5% using SAS 9. 4 statistic computer program

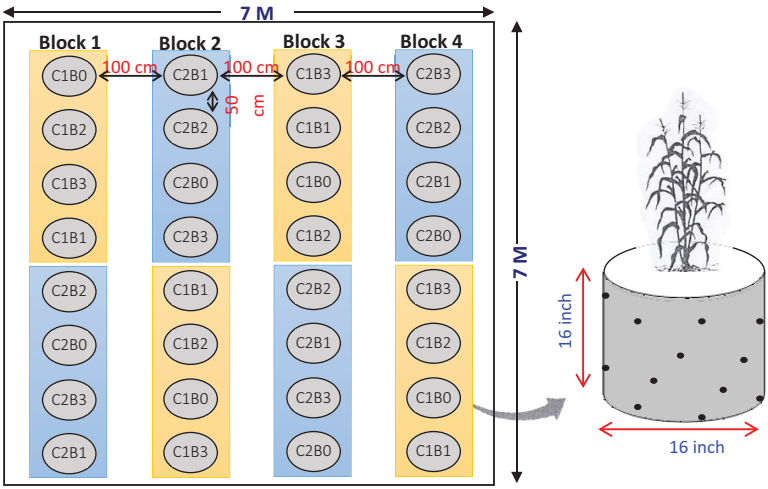


Figure 1. Research layout with Split Plot Randomized Complete Block Design. = Improved Brown Turkey (IBT); = Masui Dauphine (MD); C = fig cultivars (main plot) ; B = brassinolide consentrations (sub plot) ; and Block = replications.

Experiment was conducted in an open field at Ladang 15, Faculty of Agriculture, Universiti Putra Malaysia situated at 2° 58" N and 101° 44' 04" E in Serdang, Selangor, Malaysia from May to December 2017. Data were recorded weekly and monthly.

II.4. Research Procedures

The size of land used 7 m x 7 m. Land had been cleaned from weeds or leftover timber therefore we can put polybags on the land. For main vegetative propagation, stem cuttings were conducted. Choose fig branches of 10-25 mm diameter and 4 to 6 inches long. Stem cuttings had at least single node with a couple of leaves, healthy, disease-free plants, generally consist of the current or past season's growth and avoid material with flower buds in order to get energy that can be used in producing new roots rather than flowers.

Polybags used were big polybag size 16 x 16 inch. Land use was the land of topsoil that has been cleared of weeds and the rest of the timber. During putting the soil in a polybag, the soil must first be crumbled around the roots, and pack it down several times during the filling operation to avoid air. Mix top soil + sand + organic matters (3:2:1), use hoe or shovel. Fill polybags with (1.5 + 1 + 0,5) kg mix soil (Volume polybag = 3.14 x 9.5^2 cm^2 x 29.4 cm = 8,331.52 cm^3 = ± 8.3 Kg mixed soil). After that, make label installation. The goal was to make it easier to carry out observation and treatment for each sample. Labeling was carried out one day before planting to follow lay out of the research.

For maintenance, watering and weeding were conducted. The frequency and the amount of water depends to a large extent on the soil. As a rule of thumb, 1 inch of water per week from rain or irrigation is adequate. Watering was done twice a day in the morning and afternoon. Watering was done by using the hype that splashed across the surface of the plant until the soil reaches field capacity. And if a rainy day watering was not done. Most fig tree roots are close to the soil surface and can easily dry out. For these reasons, apply water to the trees as drying develops. Slight leaf wilting in the afternoon and yellowing and dropping of leaves may indicate drought or of water stress. If that was observed, water more frequently during hot weather. Mulching helps maintain uniform soil moisture and reduces weed competition. Did not overwater in areas of heavy soil with poor drainage. This forces oxygen out of the soil and can cause injury to the tree. Good water management, including regular irrigation and mulching, helps maintain tree health and vigor and reduces fruit drop. After planting, water the tree to settle the soil firmly around the roots.

Weeding were done when there are weeds in the planting area with the aim of reducing the competition of nutrients by plants. Weedings were done by hand of uprooting by hand or by mechanical means using a semi leftovers. The spraying of insecticide or fungicide is only applied when there is evidence of serious attack.

Fungicide containing copper were not be used in this experiment because it could caused scorching to leaves.

II.5. Experimental Treatments

One-month-old fig tree seedlings were sprayed monthly with a solution of brassinollide. The solution was diluted in 50–60^{0}C water to prepare treatment solutions. Brassinollide was diluted with (tetrahydroxy-methyl-B-homo-oxa-cholestan-lactone + Multi Purpose Cultivation [MPC] + water) according to the treatments and applied directly onto leaves at 0900-1100. In specificly, for brassinolide 50 ml/L (diluted with 50 ml A-tetrahydroxy-methyl-B-homo-oxa-cholestan-lactone + 13 ml Multi Purpose Cultivation + 20 L water); for brassinolide 100 ml/L (diluted with 100 ml A-tetrahydroxy-methyl-B-homo-oxa-cholestan-lactone + 26 ml Multi Purpose Cultivation + 20 L water); and for brassinolide 200 ml/L (diluted with 200 ml A-tetrahydroxy-methyl-B-homo-oxa-cholestan-lactone + 52 ml Multi Purpose Cultivation + 20 L water).

II.6. Data Collection

II.6.1.Growth Measurements

II.6.1.1.Determination of plant height (PH).

Plant height was measured using a ruler as the distance between the soil and the shoot apex.

II.6.1.2.Determination of total leaf area per seedling (TLA).

Total leaf area per plant was measured using a leaf area meter (Model LI – 3100A Lincoln Inc., Nebraska, USA). The leaves were passed between an array of light sensors and the total area was estimated from the occlusion of light by the leaf.

The leaves were placed in polythene bags and kept in the refrigerator (6 °C in darkness) for no longer than 12 hours before measuring the leaf areas (Jaafar, 1995). Detached leaves were then passed through the instrument, which was calibrated using a standard calibration plate with an area of about 100 cm^2. The leaves were arranged in the field within view. Overlapping of adjacent leaves was avoided. The mean value of three plant samples were used to represent each experimental unit.

II.6.1.3.Determination of total dry biomass (TDB).

Total dry matter accumulation per plant was taken by calculating the dry weight of the roots, stem and leaves. Prior to drying, the plants were separated into leaves, stem and roots. The plant parts were placed in paper bags and oven-dried at 45 ^{0}C until constant weight (i.e. three days) was reached. Plant total dry weight was taken using a sensitive electronic weighing scale (Model CDS 125, Mitutoyo Inc., Japan).

II.6.1.4.Determination of specific leaf area (SLA).

The SLA measures the leafiness of the plant on dry weight basis (Henson, 1995).

$$SLA = \frac{\text{Seedling leaf area } (cm^2)}{\text{Seedling total weight (g)}} \quad \text{.....(1)}$$

II.6.1.5.Determination of shoot to root ratio (S/R).

S/R of the seedling was determined to know the partitioning of dry matter of the plant. The S/R was determined using the Hunt equation (1990).

$$S/R = \frac{Sw}{Rw} \quad \text{.....(2)}$$

II.6.1.6.Determination of net assimilation rate (NAR).

Values of NAR were measured using the Beadle formula (Beadle, 1998). This formula measures the net gain in dry weight of the plant per unit leaf area per unit time or the rate of dry matter production per unit leaf area.

$$NAR = \frac{(W_2 - W_1)(\ln A_2 - \ln A_1)}{(A_2 - A_1)(t_2 - t_1)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

Where W=Dry weight of whole seedlings (g), A=Leaf area per seedlings (cm^2), and t=time (weeks).

II.6.2.Physiological Measurements

II.6.2.1.Determination of photosynthesis rate (A), stomatal conductance (g_s) and transpiration rate (E).

Photosynthetic rate, stomatal conductance and transpiration rate of fully expanded leaves were measured using a portable photosynthesis system (LICOR–6400, Inc., USA). Prior to use, the instrument was warmed and calibrated for 30 min on ZERO IRGA mode. The measurements of gas exchange were carried out between 0900 and 1100.

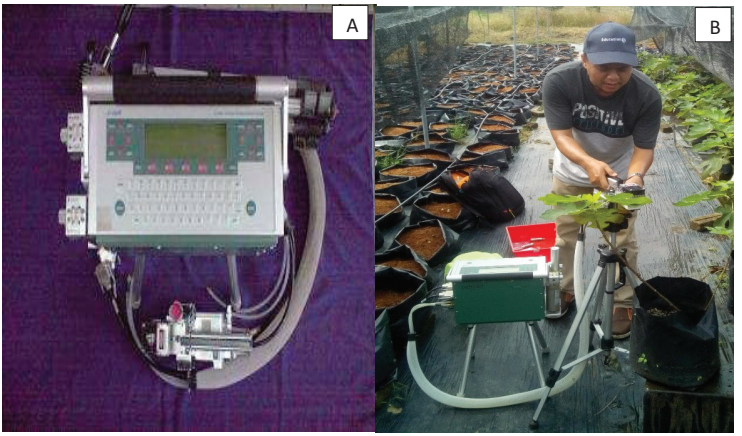


Figure 2. LICOR 6400 portable photosynthesis system used to measure leaf gas exchange parameters (A). The second leaf was choose when the leaf gas exchange measurment was conducted (B)

II.6.2.2.Determination of chlorophyll content (CC).

Total chlorophyll content was measured using the method of Idso, Kimball and Hendrix (1996) on fresh weight basis. Prior to destructive harvest each seedling was analyzed for the leaf chlorophyll relative reading (SPAD meter 502, Minolta Inc, USA). The leaves of fig with different greenness (yellow, light green and dark green) were selected for analysis and total leaf chlorophyll content was analyzed. Leaf disk 3 mm in diameter was obtained from leaf sample using a hole puncher. The leaf disks were immediately immersed in 20 ml of acetone in aluminum foil-covered glass bottle for approximately 72 hours at 0°C until all the green colour had bleached out. Finally, 3.5 ml of the solution was transferred to measure at absorbances of 664 and 647 nm for chlorophyll a and b content respectively, using a spectrometer (UV-3101P, Labomed Inc, USA). The least squares regression was used to develop predictive relation between SPAD meter readings and pigment concentrations (mg g^{-1} fresh weight) obtained from the chlorophyll destructive analysis.

$$CC=\frac{(7.93*OD^1{}_{664}+19.53*OD^1{}_{647})\text{x(total volume of 80\% acetone)}}{2(\text{total volume of 80\% acetone for O.D reading})} x \frac{1}{\text{Leaf fresh weight (mg)}}$$

….(4)

Where OD^1 = Optical Density

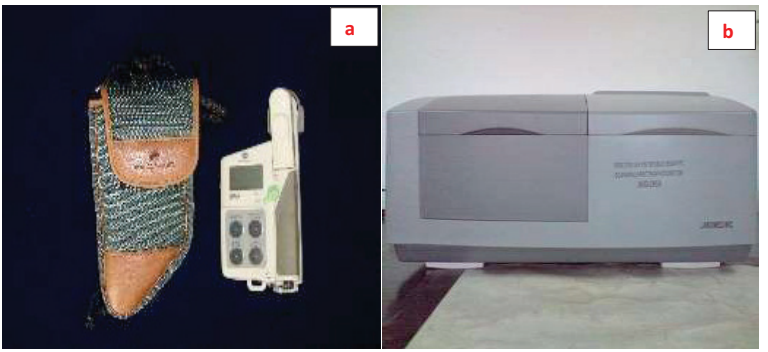


Figure 3. SPAD meter used to measure the relative chlorophyll meter by placing leaf lamina within the SPAD clip and values recorded (a) Light spectrophotometer (Model UV-3101P, Labomed Inc, USA) that determined destructive chlorophyll content value (b)

II.6.3.Statistical Analysis

All the data obtained were analysed using Statistic Analysis System (SAS) version 9.4. Significant difference in mean values were determined and analysed using two-way ANOVA and the mean differences were compared using the Least Significand Different Test (LSD) at 5% and 1% level of significance.

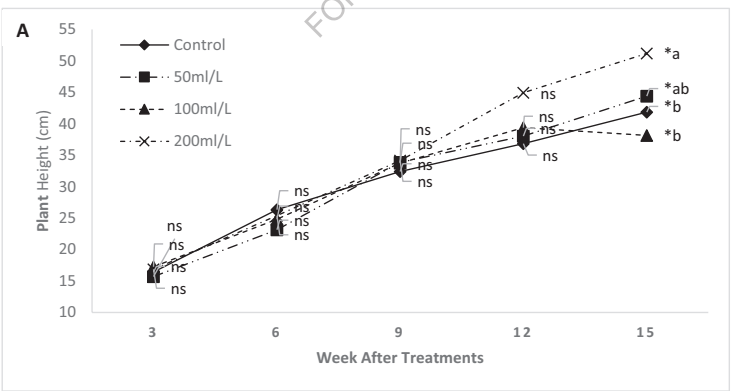
III.RESULTS

III.1.Effect Brassinolide on Growth of Fig

III.1.1. Plant Height (cm /plant)

Plant height of fig (Figure 4; Table 2) were affected by brassinolide levels. The brassinolide 200 ml.L^{-1} gave higher yield of plant height than brassinolide 50 ml.L^{-1} than control than brassinolide 100 ml.L^{-1}. Increasing of brassinolide levels increased plant height every five week observations except at brassinolide 100 ml.L^{-1}. Treatment brassinolide 100 ml.L^{-1} increased plant height at 3, 6, 9, and 12 WAT (Week After Treatment) but it decreased at 15 WAT. There was significant effect brassinolide on plant height only at 15 WAT (Figure 3.4B). Brassinolide 200 ml.L^{-1} gave tallest plant height than the others at 54.00 cm.

There was effect culitivar on plant height at 3, 6 and 9 WAT but resulted not significant at 12 and 15 WAT. In additional, plant height at 6 WAT significant at 1 % level of significance. Variety IBT gave higher plant height than variety MD at every five week observations. There wasn't significant effect of interaction between brassinolide and cultivar among five week observations on plant height.



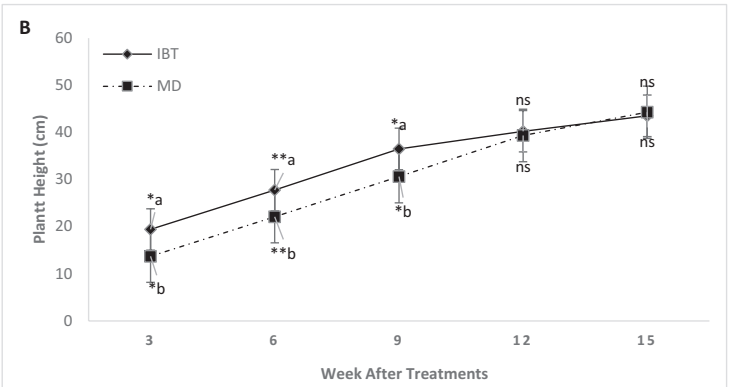


Figure 4. Changes in Plant Height as main effect of : A) Brassinolides, B) Cultivars with time of *Ficus carica L.* during 15 weeks of exposure. Data were standard error of differences between means of 32 replicates. Means followed by the different small letters at same line were significant at : *=5%,**= 1% and ns=not significant.

III.1.2.Total Leaf Area Per Seedling (cm^2 /plant)

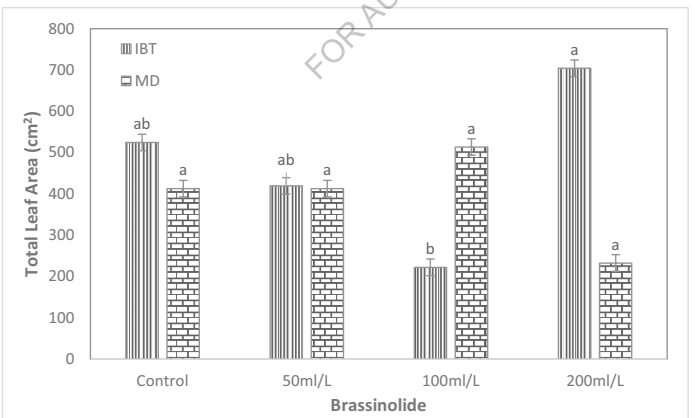
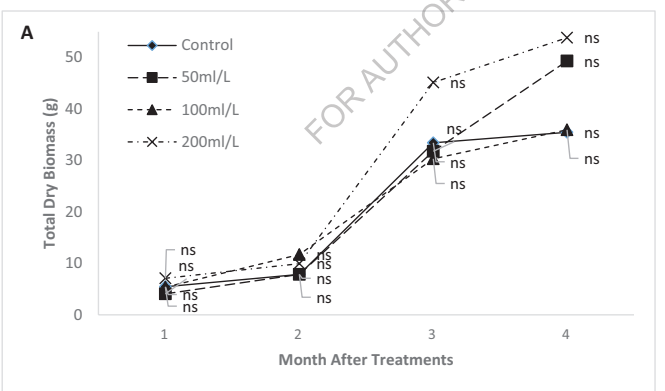


Figure 5. Changes in Total Leaf Area of *Ficus carica L.* at third month after treatment as affected by Cultivars and Brassinolide. Data were standard error of differences between means of 32 replicates. Bars represent means followed by the different small letters at same pattern were significant at 5%.

Total leaf area of fig (Figure 5; Table 2) were affected by interaction between brassinolide levels and cultivars. Treatment interaction between brassinolide levels and cultivars had significant effect on TLA at 3 MAT (Month After Treatment). Increasing concentration of brassinolides caused the increment of SLA value. The highest TLA value of interaction between brassinolide and fig variety was 704.15 cm^2 on treatment of IBT + 200 ml/L and the lowest TLA value of interaction between brassinolide and fig variety was 179.05 cm^2 on treatment of IBT + 100 ml/L.

III.1.3.Total Dry Biomass (g)

Differ to plant height, the total dry biomass was significant only at cultivar treatment. However, there was no statistical significance at elevated brassinolide levels. Treatment cultivar alone was significant on TDB at first to third MAT but there wasn't effect at fourth MAT. Between the varieties, IBT showed higher TDB than MD at every month observation.



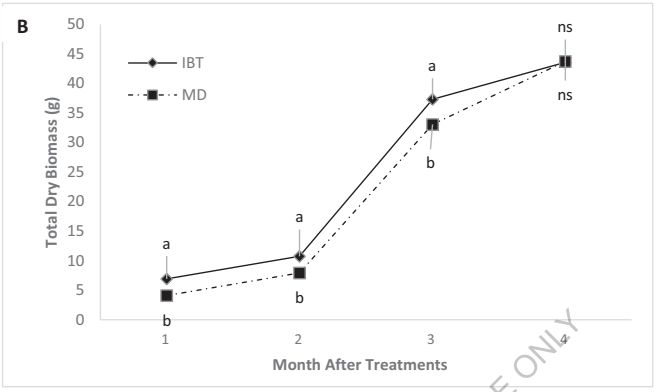


Figure 6. Changes in Total Dry Biomass as main effect of : A) Brassinolides, B) Cultivars with time of *Ficus carica L.* during 4 months of exposure. Data were standard error of differences between means of 32 replicates. Means followed by the different small letters significant at 5% and ns=not significant.

III.1.4.Specific Leaf Area (SLA; cm^2/g)

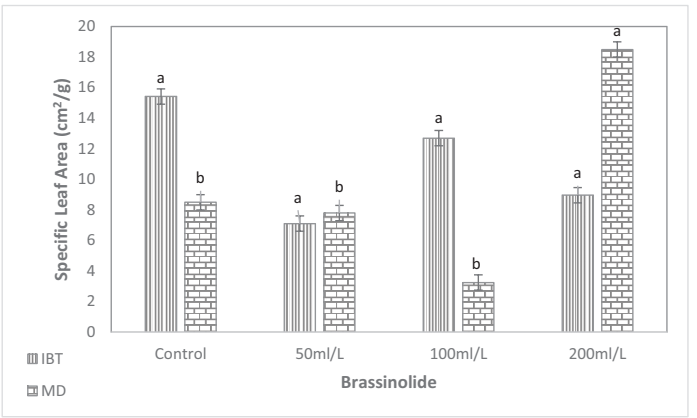


Figure 7. Changes in Specific Leaf Area of *Ficus carica L.* at first month after treatment as affected by Cultivars and Brassinolide. Data were standard error of differences between means of 32 replicates. Bars represent means followed by the different small letters at same pattern was significant at 5%.

Specific Leaf Area of fig were affected by interaction between brassinolide levels and cultivars. In Figure 7, at first MAT SLA of cultivar MD and brassinolide 100 ml/L was the lowest compared to cultivar IBT and brassinolide control, 50 and 200 ml/L treatments. Although there weren't differences between the treatments, treatment of interaction between cultivar and brassinolide just only significant at first MAT, but elevated treatment showed lower SLA throughout the experimental period. The low SLA in the elevated treatment clearly indicated the enhanced thickness of leaves under elevated brassinolide (Table 2).

III.1.5.Shoot to Root ratio (S/R)

The partitioning between shoot and root growth can be derived from shoot to root ratio. This rate depends on the leaf morphology and biomass allocation to specific organs. Analysis of variance showed a significant difference ($P<0.05$) at the fourth MAT treatment of interaction between cultivar and brassinolide. Treatment of brassinolide 100 ml/L and cultivar MD was the highest of S/R value (Figure 8). The shoot to root ratio was between 2.57 and 5.42 throughout the 0 - 4 MAT period. This implies that at brassinolide 100 ml/L of *Ficus carica* seedlings partitioned 2 to 5 fold of the total dry biomass to the production of leaf and stem as compared to 1 – 2.5 fold allocation by the elevated treatments.

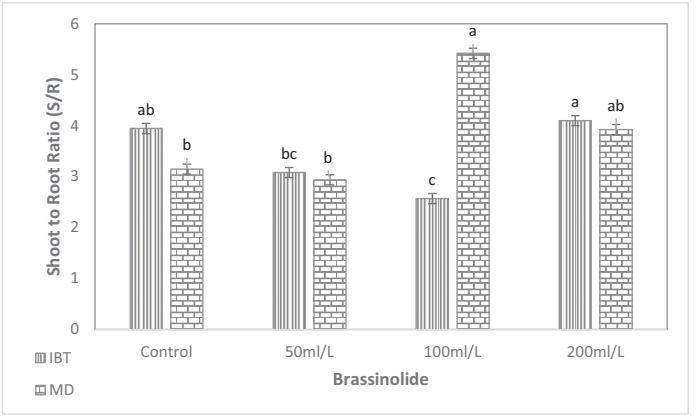


Figure 8. Changes in Shoot to Root Ratio of *Ficus carica L.* at fourth month after treatment as affected by Cultivars and Brassinolide. Data were standard error of differences between means of 32 replicates. Bars represent means followed by the different small letters at same pattern was significant at 5%.

III.1.6.Net Assimilation Rate (NAR; g/cm^2/week)

Figure 9 presented the net assimilation rates (NAR) of fig exposed to control (without brassinolide) to three levels of elevated brassinolide (50, 100 and 200 ml/L) levels. NAR of fig was affected by brassinolide levels alone. NAR showed increased strongly when increasing concentration of brassinolide (control and 50 ml/L) at 1-2 MAT and 2-3 MAT and decreased strongly when increasing concentration of brassinolide (100 and 200 ml/L) at 3-4 MAT (Fig.1b). Based on Table 2, showed that treatment of brassinolide was significant only at 2-3 MAT on NAR and there was no effect of fig variety on NAR.

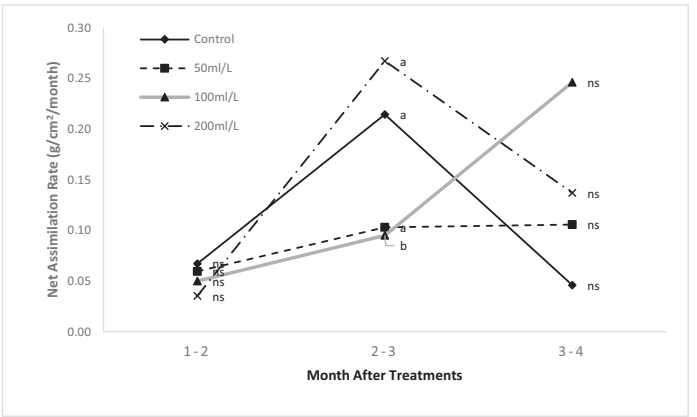


Figure 9. Changes in Net Assimilation Rate of *Ficus carica L.* as main effect of Brassinolide with time of *Ficus carica L.* during 4 months of exposure.. Data were standard error of differences between means of 32 replicates. Curves represent means followed by the different small letters at same line was significant at 5%.

Figure 10. Growth of *Ficus carica L.* at different Brassinolide levels

III.2.Effect Brassinolide on Physiological Changes

III.2.1.Photosynthesis Rate (A; µmol $m^{-2}s^{-1}$)

The leaf gas exchange parameters of fig were affected by interaction between brassinolide levels and cultivars (Figure 11;Table 3). Treatment interaction between brassinolide levels and cultivars had significant effect on A at second MAT. Increasing concentration of brassinolides (50 to 200 ml/L) caused the inconsistent increment of A value. The highest A value of interaction between brassinolide and fig variety was 14.20 µmol m^{-2} s^{-1} on treatment of MD + 200 ml/L and the lowest A value of interaction between brassinolide and fig variety was 7.94 µmol m^{-2} s^{-1} on treatment of MD + 200 ml/L too.

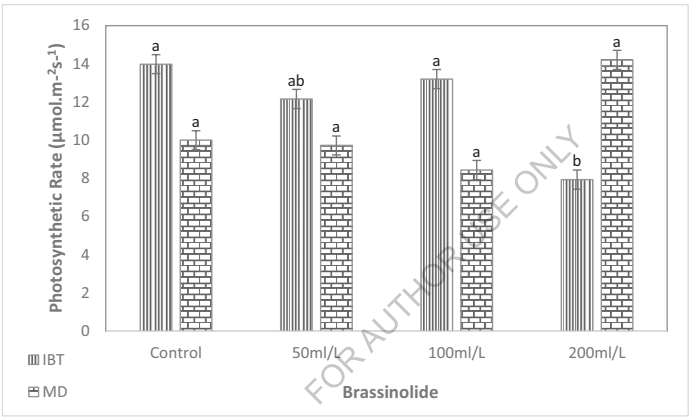


Figure 11. Changes in Photosynthesis Rate of *Ficus carica L.* as affected by Cultivars and Brassinolide at second month after treatment. Data were standard error of differences between means of 32 replicates. Bars represent means followed by the different small letters at same pattern was significant at 5%.

III.2.2.Stomatal Conductance (g_s; mmol m^{-2} s^{-1})

Throughout the experiment, those plants elevated with brassinolide were found to have lower stomatal conductance (g_S) than the control plants in all months of measurement (Figure 12). Stomatal conductance of fig was affected by the brassinolide levels and the cultivars. Interaction between brassinolide concentrations and fig variety was significant only at 5%. Varietal performance of

brassinolide application was analyzed at specific period of the study and the result is presented Table 2. Increasing concentration of brassinolide (50, 100 and 200 ml.L^{-1}) had decreased the stomatal conductance in IBT than MD. The results imply that plant under exposure to higher than control levels demonstrated lower gs value compared to the plant under control condition.

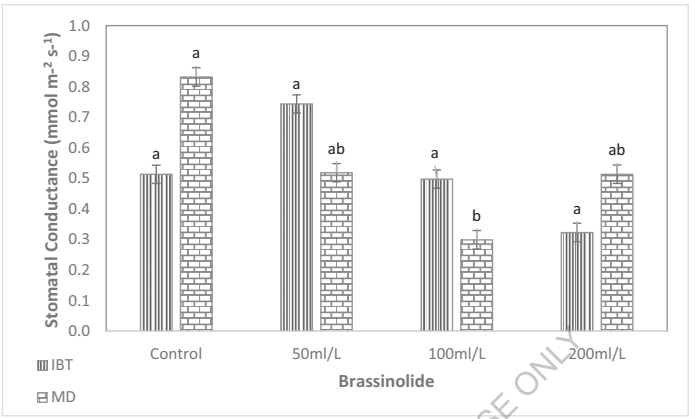


Figure 12. Changes in Stomatal Conductance of *Ficus carica L.* as affected by Cultivars and Brassinolide at first month after treatment. Data were standard error of differences between means of 32 replicates. Bars represent means followed by the different small letters at same pattern was significant at 5%.

III.2.3.Transpiration Rate (E; mol m-^{2} s-^{1})

The transpiration rates of fig were affected by interaction between brassinolide levels and cultivars (Figure 13;Table 3). Treatment interaction between brassinolide levels and cultivars had significant effect on E at second MAT. Increasing concentration of brassinolides (50 to 200 ml/L) caused the inconsistent increment of E value. The highest E value of interaction between brassinolide and fig variety was 3.85 mol m^{-2} s^{-1} on treatment of IBT + 50 ml/L and IBT + control. The lowest E value of interaction between brassinolide and fig variety was 2.42 mol m^{-2} s^{-1} on treatment of IBT + 200 ml/L. The significantly reduction in E at elevated brassinolide could be attributed to low stomatal conductance under high brassinolide levels.

Figure 13. Changes in Transpiration Rate of *Ficus carica L.* as affected by Cultivars and Brassinolide at second month after treatment. Data were standard error of differences between means of 32 replicates. Bars represent means followed by the different small letters at same pattern was significant at 5%.

III.2.4.Chlorophyll Content (CC;mg/g FW)

It was observed, that production of chlorophyll content was influenced by interaction between brassinolide levels and cultivars (Figure 14;Table 3). Treatment interaction between brassinolide levels and cultivars had significant effect on CC at first MAT. Increasing concentration of brassinolides (100 to 200 ml/L) caused the increment of CC value but decreased when concentration 50 ml/L. The highest CC value of interaction between brassinolide and fig variety was 19.27 mg/g FW on IBT + control. The lowest CC value of interaction between brassinolide and fig variety was 17.85 mg/g FW on treatment of MD + 50 ml/L.

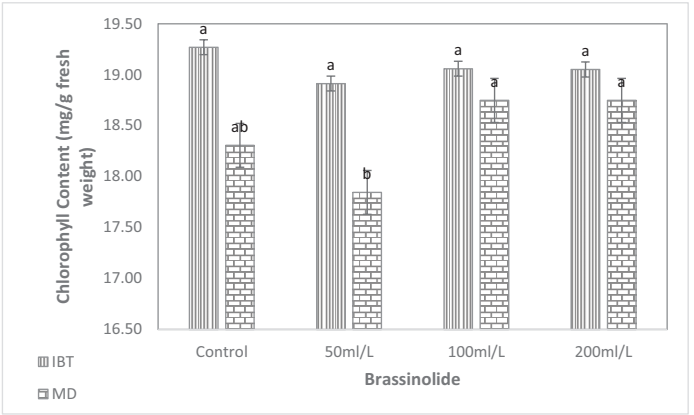


Figure 14. Changes in Chlorophyll Content of *Ficus carica L.* as affected by Cultivars and Brassinolide at first month after treatment. Data were standard error of differences between means of 32 replicates. Bars represent means followed by the different small letters at same pattern was significant at 5%.

Table 2

Effect of Different Concentrations of Brassinolide on Growth of Two Cultivars of Fig

Treatments	Plant Height (cm)					Total Leaf Area (cm^2)				Total Dry Biomass (g)				Specific Leaf Area (cm^2/g)				Shoot-to-Root Ratio				Net Assimilation Rate (g/cm^2/month)		
	Week After Treatment					Month After Treatment				Month After Treatment				Month After Treatment				Month After Treatment				Month After Treatment		
	3	6	9	12	15	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1-2	2-3	3-4
Control	16.44	26.34	32.43	36.83	*41.85b	*295.81ab	298.14	468.71	284.61	5.46	7.85	33.44	35.46	11.96	8.06	8.43	4.40	*2.95b	9.65	*3.67b	*3.55ab	0.27	0.54a	0.48
50ml/L	15.70	23.16	33.87	38.06	*44.39ab	*89.94b	294.89	416.22	468.67	4.07	7.86	31.81	49.35	7.45	9.64	7.07	5.19	*4.13ab	2.89	*3.10b	*3.01b	0.41	0.24b	0.58
100ml/L	17.26	24.82	33.76	39.39	*48.17b	*176.33ab	320.77	367.95	314.47	5.38	11.72	30.33	35.98	7.97	7.37	6.22	4.76	*2.44b	3.78	*2.62b	*3.99a	0.42	0.39b	0.58
200ml/L	16.87	25.39	34.16	44.93	*51.21a	*385.92a	479.70	468.51	430.61	7.12	9.95	45.17	53.86	13.73	9.99	7.83	5.16	*5.83a	7.54	*4.41a	*4.01a	0.13	0.49a	0.91
IBT	*19.41a	**27.74a	*36.50a	40.24	43.52	312.82	397.04	*467.65a	*303.04b	*6.92a	*10.76a	*37.32a	43.60	11.04	8.36	*6.84b	*4.33b	3.54	5.06	3.49	3.42	0.30	0.55	0.56
MD	*13.72b	**22.12b	*30.62b	39.36	44.28	161.19	299.71	*393.04b	*446.14a	*4.09b	*7.92b	*33.05b	43.72	9.51	9.17	*7.94a	*5.43a	4.13	6.86	3.41	3.86	0.31	0.27	0.71
IBT + 50ml/L	17.76	24.88	36.24	37.89	45.57	89.89	421.18	*419.61ab	429.83	3.92	10.33	37.86	56.11	*7.10a	11.45	5.99	4.42	4.25	2.50	3.29	**3.08bc	0.55	0.15	0.60
IBT + 100ml/L	20.71	27.84	36.56	37.40	36.14	322.27	375.60	*222.36b	179.05	7.74	13.99	24.25	32.66	*12.69a	8.42	5.58	3.66	2.76	3.23	2.64	**2.57c	0.16	0.38	0.58
IBT + 200ml/L	17.89	26.70	36.56	44.63	48.42	359.42	450.05	*704.15a	352.39	9.14	9.07	50.12	42.15	*8.96a	9.10	8.18	5.48	4.76	11.10	3.51	**4.10a	0.17	0.73	0.50
MD + 50ml/L	13.63	21.45	31.51	38.23	43.20	90.00	168.61	*412.82a	507.51	4.23	5.38	25.76	42.59	*7.79b	7.82	8.15	5.95	4.00	3.28	2.90	**2.94b	0.27	0.34	0.57
MD + 100ml/L	13.81	21.81	30.96	41.37	40.20	30.40	265.95	*513.55a	449.89	3.01	9.45	36.41	39.31	*3.25b	6.33	6.86	5.86	2.13	4.33	2.61	**5.42a	0.67	0.40	0.57
MD + 200ml/L	15.85	24.09	31.76	45.23	54.00	412.43	509.36	*232.88a	508.83	5.10	10.83	40.22	65.57	*18.49a	10.88	7.48	4.84	6.89	3.97	5.31	**3.92ab	0.09	0.24	1.32
LSD V	1.31	1.93	4.25					73.57	90.54	2.17	2.69	4.05				0.88	0.62							
LSD B					9.28	279.29												2.74		0.85	0.89		0.101	
LSD V*B								4790.4*504.01								12.25*9.07					0.91*1.70			

Means followed by the different small letters are significant at *=p<0.05, **=p<1%.

Table 3

Effect of Different Concentrations of Brassinolide on Physiological Changes of Two Cultivars of Fig

Treatments	Photosynthesis Rate (µmol.m^{-2}s^{-1})				Stomatal Conductance (mmol m^{-2} s^{-1})				Transpiration Rate (mol m^{-2} s^{-1})				Chlorophyll Content (mg/g fresh weight)			
	Month After Treatment				Month After Treatment				Month After Treatment				Month After Treatment			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Control	*13.31a	11.99	16.96	20.29	0.67	0.22	0.56	0.34	*5.00a	3.33	*3.59a	3.22	18.79	4.73	*14.22b	4.20
50ml/L	*10.66b	10.94	17.35	23.45	0.63	0.26	0.41	0.36	*4.51ab	3.54	*3.07b	2.80	18.38	4.92	*14.27b	4.39
100ml/L	*9.69b	10.81	16.19	23.40	0.40	0.23	0.31	0.22	*3.50b	3.22	*2.82b	2.54	18.90	4.76	*14.24b	4.28
200ml/L	*10.65b	11.07	17.63	23.16	0.42	0.20	0.53	0.32	*3.58b	2.86	*3.69a	3.24	18.90	5.09	*14.35a	4.89
IBT	11.30	*11.82a	17.28	22.00	0.52	*0.26a	*0.54a	0.32	3.92	*3.47a	*3.57a	2.99	**19.07a	*4.96a	14.26	4.57
MD	10.86	*10.59b	16.79	23.15	0.54	*0.20b	*0.37b	0.30	4.38	*3.01b	*3.01b	2.90	**18.41b	*4.80b	14.28	4.31
IBT + 50ml/L	10.57	*12.16ab	18.19	23.06	*0.74a	0.31	0.41	0.52	4.47	*3.85a	3.48	3.25	*18.91a	5.03	14.24	4.44
IBT + 100ml/L	11.64	*13.19a	13.18	23.54	*0.50a	0.28	0.29	0.24	3.76	*3.76a	3.04	2.69	*19.06a	4.65	14.23	4.36
IBT + 200ml/L	9.55	*7.94b	20.36	22.22	*0.32a	0.18	0.71	0.33	3.05	*2.42b	3.78	3.25	*19.05a	5.06	14.38	5.20
MD + 50ml/L	10.75	*9.73a	16.51	23.84	*0.52ab	0.21	0.40	0.20	4.55	*3.22a	2.66	2.35	*17.85b	4.81	14.29	4.35
MD + 100ml/L	7.73	*8.44a	19.19	23.27	*0.30b	0.18	0.33	0.19	3.24	*2.69a	2.59	2.39	*18.75a	4.87	14.25	4.20
MD + 200ml/L	11.75	*14.20a	14.90	24.10	*0.51ab	0.23	0.35	0.31	4.11	*3.31a	3.60	3.23	*18.75a	5.12	14.31	4.57
LSD V		1.22				0.04	0.29			0.45	0.53		0.41	0.13		
LSD B	2.45								1.03		0.47				0.07	
LSD V*B		4.72*8.22			0.63*0.46					1.33*2.16			0.80*0.82			

Means followed by the different small letters are significant at *=p<0.05, **=p<1%.

III.3.Correlation Analysis

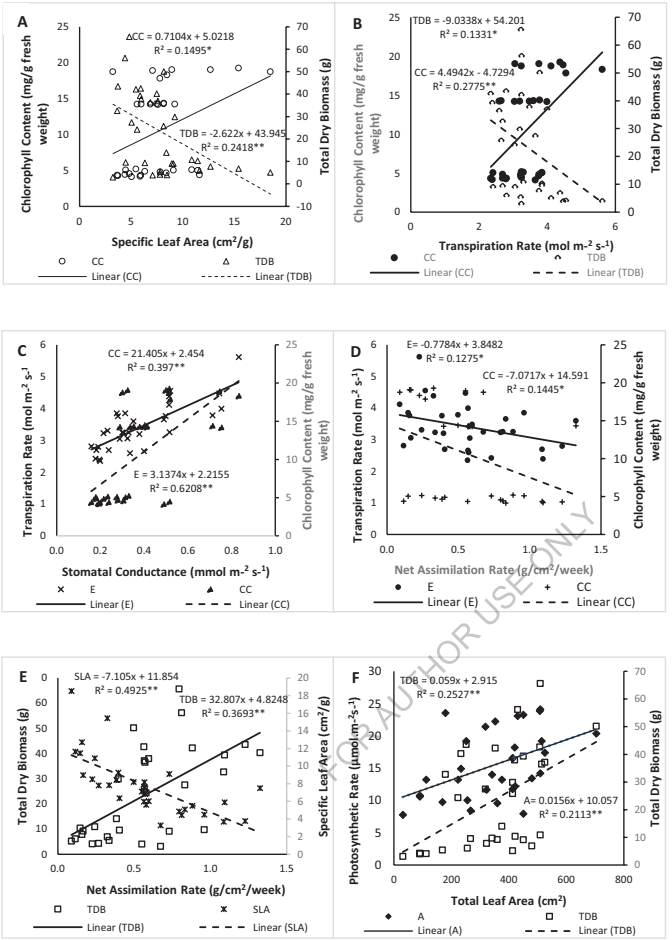


Figure 15. Correlation coefficient between CC and TDB with (a) SLA; (b) E; E and CC with (c) g_s; (d) NAR; TDB and SLA with (e) NAR; (f) TLA. *= p≤ 0.05, **=p ≤0.01, n=128.

Correlation analysis was carried out to establish the relationship between the parameters. Figure 3 shows that a significant positive inter-correlation among parameters such as chlorophyll content, specific leaf area, transpiration rate and stomatal conductance. Increase in chlorophyll content, transpiration rate, total dry biomass, photosynthetic rate, and total dry biomass was associated with an increase in

specific leaf area, transpiration rate, stomatal conductance, net assimilation rate and total leaf area with an r value of 14.95%, 27.75%, 3.97%, 62.08%, 36.93%, 25.27% and 21.13%, respectively.

Significant negative correlation was noted between total dry biomass with specific leaf area; total dry biomass with transpiration rate; transpiration rate with net assimilation rate; chlorophyll content with net assimilation rate; and specific leaf area with net assimilation rate. Increase in total dry biomass, transpiration rate, chlorophyll content and specific leaf area was associated with a decrease in specific leaf area, transpiration rate and net assimilation rate with an r value of 24.18%, 13.31%, 12.75%, 14,45%, and 49.25%, respectively.

IV.DISCUSSION

We studied the effect of exogenous brassinolide application on some growth and physiological traits on two cultivars of fig. The main functions of brassinolide are to promote the plant growth especially for cell elongation and division (Mayumi & Shibaoka, 1995) and has the ability to stimulate other physiological processes (Prusakova et al., 1999).Wang, Cosgrove & Arteca (1993) had found that brassinollide appeared to cause elongation by affecting wall extensibility and increasing wall relaxation properties.

As levels of brassinolide increased (50, 100 and 200 ml.L^{-1}), plant height, leaf area, total dry biomass and net assimilation rate parameters also linearly improved at 28%, 25%, 6% and 66%, respectively, higher than recorded for the control treatment. Similar results were reported by other researchers for other plants i.e. Hu, Shi, Sun and Guo (2013) for *Leymus chinensis*; Bera, Pramanik and Mandal (2014) for sunflower; and Anjum et al. (2011) for maize. The growth stimulation was more pronounced on above ground biomass than below ground biomass, showing a high shoot-to-root ratio (Zaharah, Razi, Zainuddin, & Ghani, 2006). The increase in growth in this study might have been due to increased carboxylation rate after using the BL treatment, which enhanced carbon assimilation, channelling it to stimulate increase in plant height, leaf area and total biomass (Henson, 1992).

Specific leaf area (SLA) is one growth parameter that characterized the thickness of the leaves. Usually plant with high SLA had the thinnest leaves. Specific leaf area was found to be lower than the control ($p \leq 0.05$) under brassinolide concentrations of 50 and 100 ml.L^{-1}. The result implies that plants have thicker leaves. The thicker leaf might have been due to increase in the mesophyll layer after receiving brassinolide (Haniff, 2006). The increase in leaf thickness could also have been due to higher leaf weight ratio in fourth MAT compared with first to third MAT. The leaf area was maintained at lowest SLA. That indicated that leaves of fig were thickest at brassinolide 100 ml.L^{-1}. This indicated that increase in SLA was due to increase in leaf

weight compared with increase in leaf area (Lambers & Poorter, 1992; Hayat, Alyemeni, & Hasan, 2012).

The net assimilation rate (NAR) of plants are growth characteristics that best describe plant growth performance under specified conditions (Gardner, Pearce, & Mitchell, 1994). It is evident that plants under elevated BL have high NAR. Increase in plant growth grown under different planting geometries and depths in SRI has also been reported by Rajput, Rajput and Jha (2017), who reported that increase in total biomass by 30% in rice had increased NAR by 4% compared with the control. The reduction in NAR was due to the ontogenical development of fig.

BL had profound impact on leaf photosynthesis and plant performance. BL improved leaf carbon assimilation rate, which is the light harvesting machine of plant photosynthesis. BL treatment also enhanced photosynthetic performance of cotton seedlings under NaCl stress (Xiao et al., 2007; Chen et al., 2007; Shu, Guo, Shen, & Ni, 2011). For cucumber seedlings, BL treatment has also been found to promote the occurrence of new roots, the formation of lateral roots and nutrient uptake (Bao et al., 2004).

BL-treatment enhanced photosynthesis (17.06 %) and chlorophyll content (18.36%). In contrast, BL-treatment decreased stomatal conductance (11.94 %) and transpiration rate (17.83 %). The BL-induced increase in photosynthesis could have been due to improvements in leaf-water balance as indicated by increased water potential (Sairam, 1994) and improved chlorophyll content and higher leaf area in BL-treated plants (Iwahari, Tominaga & Higuchi, 1990).

Stomata are the windows that admit water and CO_2 in and out of the plant. Chlorophyll content and transpiration rate were found to have declined. This could be attributed to the enhanced growth of seedlings under elevated BL treatment that diluted the nitrogen content in the plant tissue (Ibrahim, Jaafar, Rahmat, & Zaharah, 2011). Figure 3A and C showed a significant positive inter-relation among chlorophyll content, transpiration rate and stomatal conductance, indicating that a decrease in chlorophyll content would associated with same degree of reduction in transpiration rate and stomatal conductance.

V.CONCLUSION

Brassinolide application had brought notable changes in growth and physiology among fig varieties. Though increasing BL concentration (50, 100 and 200 $ml.L^{-1}$) caused some differences in growth and physiological changes of fig, but the differences were not consistent and most of the changes happened only in first or second month. Cultivar IBT showed higher growth and physiological changes than cultivar MD after receiving brassinolide treatment. There was significant effect of interaction between brassinolide and variety on growth and physiological changes of fig except for plant height and total dry biomass. In the future, the experiment would be repeated in a greenhouse under controlled environment to verify the effect of brassinolide on fig varieties.

VII.ACKNOWLEDGEMENT

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Education :

1985-1986 : Nursery School of Seruni (TK Seruni Marpoyan)
1986-1992 : Elementary School (SDN 018) Pekanbaru City
1992-1995 : Junior High School (MTsN) Pekanbaru City
1995-1998 : Senior High School (SMUN 05) Pekanbaru City
1998-2003 : Bachelor Degree of Gadjah Mada University, Faculty of Agricultural Technology, Department of Agricultural Engineering, Power Agricultural Machinery Interest, Yogyakarta City.
2006-2008 : Master Agronomy of Riau Islamic University.
2016-2019 : Ph.D of University Putra Malaysia

Research and Publication :

The titles of my work (in Indonesia) as follows:

Mardinata,Z.,Rozaq,A., and Handoyo,R.,2003. Draw Bar Forces For No Tillage Soybean. *J. Agritech*, XXIII (3).

Mardinata,Z. 2009. Power Explants Grown Oil Palm in Tissue Culture By Using Different Sources of Explants and Coconut Water. *Dinamika Journal*, XXIV(2).

Nurhasnin and Mardinata,Z.2012.Plastic lid Use in Plant And Bokashi Caisin. *RAT Journal*.I(1). Pp.47-58.

Dewi,M., Zahrah,S., Sabli,E., and Mardinata,A.2013. Plant Response of Okra *(Abelmoschus esculantus L Moench)* in Various Soil and Amazing Bio-Growth Fertilizer. *RAT Journal*. 2(1).pp.240-250.

Marlinda and Mardinata,Z.2014 The Use of Palm Oil Sludge and Composts on Palm Oil Seed *(Elaeis Guineensis Jacq)* Grown in Main Nursery. *RAT Journal*.3(2). pp.358-368.

Mardinata,Z., and Zulkifli. 2014. The Analysis of Working Capasity and Fuel Consumption of Hand Tractor in Various Tillage Pattern, Plowing Depth and Working Speed. *J.Agritech*.34(3). pp.354-358. DOI: https://doi.org/10.22146/agritech.9465

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The titles of my work (in English) as follows:

Zulkarnaini,Z.M., Sakimin,S.Z., Mahmud,M.T.M., and Jaafar,H.Z.E.2019.Effect brassinolide aplication on growth and physiological changes in two cultivars of fig (*Ficus carica L.) J.Trop.Agr. Sci.* Vol 42(1).

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Zulkarnaini,Z.M., Sakimin,S.Z., Mahmud,M.T.M., and Jaafar,H.Z.E.2019. Relationship Between Extractable Chlorophyll Content and SPAD Values in Two Cultivars of Fig *(Ficus Carica L.)* as Brassinolide Effect at Open Field. In Earth and Environmental Science (Eds.) *Proceedings of The International Conference on Sustainable Agriculture For Rural Development (ICSARD) 2018*, 23-24 October 2018 Java Heritage Hotel, Purwokerto, Indonesia. https://iopscience.iop.org/article/10.1088/1755-1315/250/1/012025/pdf

Zulkarnaini,Z.M., Sakimin,S.Z., Mahmud,M.T.M., and Jaafar,H.Z.E.2019. Responses of Two Fig *(Ficus Carica L.)* Varieties After Receiving Brassinolide on Leaf, Shoot and Root Segment. *[Accepted in Journal of Agronomy]*.

Zulkarnaini,Z.M., Sakimin,S.Z., Mahmud,M.T.M., and Jaafar,H.Z.E.2019. Growth and Physiological Responses of Improved Brown Turkey (IBT) of Fig *(Ficus*

Carica L.) As Influenced By CO_2 Enhancer and Brassinolide. *[Under reviewed in Australian Journal of Crop Science]*.

Book publications as follows :

Mardinata,Z.2011. *Library Automation Using the Senayan3-Stable 14.* Pekanbaru: UIR Press

Mardinata,Z.2013. *Processing Research Data Using the SAS Program*. *For the Department of Agrotechnology, Agronomy, Fisheries, Medicine and Forestry*. Jakarta: Rajawali Pers.

International Training :

- Data Analysis and Experimental Design (Held on 7-11 January 2019 at SEARCA headquarters, Los Banos, Laguna, Philippines)