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Effect Brassinolide Aplication on Growth and Physiological Changes in Two Cultivars of Fig (*Ficus carica* L.)

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ABSTRACT

Brassinolide (BL) is a plant hormone showing wide occurrence in the plant kingdom with unique biological effects on growth and physiological traits. The fig varieties, Improved Brown Turkey (IBT) and Masui Dauphine (MD), are commonly found in Indonesia and Malaysia. There is limited information on exogenous brassinolide application on these varieties. Thus, the aim of this study is to investigate the effect of different concentration of exogenous application of BL on growth and physiological changes of fig. Fig planting materials were propagated using stem cutting and then transferred into media containing 3:2:1 mixed soil (top soil: organic matters: sand). Two fig cultivars treated with BL (control, 50, 100 and 200 ml.L^{-1}) were arranged as Split Plot Randomized Complete Block Design (SRCBD) with four replications. Plant growth (Plant Height [PH], Total Leaf Area [TLA], Total Dry Biomass [TDB], Specific Leaf Area [SLA], Shoot to Root Ratio [S/R] and Net Assimilation Rate [NAR]) and physiological changes (Photosynthesis Rate [A], Stomatal Conductance [g_s], Transpiration Rate [E] and Chlorophyll Content [CC] were investigated every three weeks and at monthly intervals, respectively. 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

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except in the parameters of plant height and total dry biomass.

Keywords: Brassinolide, fig, growth, physiological changes

INTRODUCTION

Brassinolide (BL) is one of the brassinosteroids, which are the steroidal plant hormones that show a wide occurrence in the plant kingdom and have unique biological effects on growth and development (Clouse & Sasse, 1998; Khripach et al., 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 et al., 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; 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 et al., 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.

MATERIALS AND METHODS

Fig-planting materials were propagated using cuttings taken from mature two- to three-year-old figs and transferred into media containing 3:2:1 mixed soil (top soil: organic matters: sand). Two different fig (IBT and MD) varieties were subjected to four levels (0, 50, 100 and 200 $ml.L^{-1}$) of BL concentration. One-month-old fig tree seedlings were sprayed monthly with a solution of brassinollide (tetrahydroxy-methyl-B-homo-oxa-cholestan-lactone + Multi Purpose Cultivation [MPC] + water) according to the treatments. Fig varieties were considered as the main treatment and BL concentrations (B) as sub-treatments. The experiment was arranged in Split Plot Randomised Complete Block Design (SRCBD) with four replications. There were four plants as destructive samples observed monthly for each replication. The 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. Data were recorded weekly and monthly.

Growth Measurements

Determination of Plant Height (PH). Plant height was measured using a ruler as the distance between the soil and the shoot apex.

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.

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 ^0C 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).

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

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 (Hunt, 1990).

Determination of Net Assimilation Rate (NAR). Values of NAR were measured using the Beadle formula (Beadle, 1998).

Physiological Measurements

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.

Determination of Chlorophyll Content (CC). Total chlorophyll content was measured using the method of Idso et al. (1996) on fresh weight basis.

Statistical Analysis

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

RESULTS

Effect Brassinolide on Growth of Fig

The growth of the fig plants was affected by the brassinolide levels. Treatment of the fig plants with different concentrations of brassinolide (50, 100 and 200 $ml.L^{-1}$) caused an increase in plant height and total dry biomass compared to control samples. Total leaf area, specific leaf area and shoot-to-root ratio increased with increasing concentrations of brassinolide up to 100 $ml.L^{-1}$, followed by a decline whereas net assimilation rate fluctuated over a period of study. At the first Month After Treatment (MAT), increasing brassinolide concentration (50 and 100 $ml.L^{-1}$) caused an increase in the net assimilation rate when compared to control but there was a decrease when brassinolide concentration was 200 $ml.L^{-1}$. At the second MAT, by increasing the brassinolide concentration (50, 100 and 200 $ml.L^{-1}$), the net assimilation rate had decreased.

Application of brassinolide had some effect on plant height, total leaf area, total dry biomass, specific leaf area and net assimilation rate (Table 1) but it was not significant on the shoot-to-root ratio. Among the varieties, IBT showed higher growth than MD at every five-weekly observation. There was a significant interaction between the brassinolide and the cultivar for total leaf area, specific leaf area, shoot-to-root ratio and net assimilation rate parameters. Additionally, only shoot-to-root ratio parameter showed a significant effect of interaction between the brassinolide and cultivar at 1% level of significance.

Table 1

Effect of different concentrations of brassinolide on growth of two cultivars of fig

Treatments	Plant Height (cm)					Total Leaf Area (cm²)				Total Dry Biomass (g)			
	Week After Treatment					Month After Treatment				Month After Treatment			
	3	6	9	12	15	1	2	3	4	1	2	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
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
100ml/L	17.26	24.82	33.76	39.39	*38.17b	*176.33ab	320.77	367.95	314.47	5.38	11.72	30.33	35.98
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
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
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
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
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
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
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
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
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
LSD V	1.31	1.93	4.25					73.57	90.54	2.17	2.69	4.05	
LSD B					9.28	279.29							
LSD V*B								479.04*504.01					

Treatments	Specific Leaf Area (cm²/g)				Shoot-to-Root Ratio				Net Assimilation Rate (g/cm²/month)			
	Month After Treatment				Month After Treatment				Month After Treatment			
	1	2	3	4	1	2	3	4	1	2	3	4
Control	11.96	8.06	8.43	4.40	*2.95b	9.65	*3.67b	*3.55ab	*0.27b	*0.54a	*0.48b	1.03
50ml/L	7.45	9.64	7.07	5.19	*4.13ab	2.89	*3.10b	*3.01b	*0.41a	*0.24b	*0.58b	0.69
100ml/L	7.97	7.37	6.22	4.76	*2.44b	3.78	*2.62b	*3.99a	*0.42a	*0.39ab	*0.58b	1.09
200ml/L	13.73	9.99	7.83	5.16	*5.83a	7.54	*4.41a	*4.01a	*0.13b	*0.49a	*0.91a	0.84
IBT	11.04	8.36	*6.84b	*4.33b	3.54	5.06	3.49	3.42	0.30	0.55	*0.56b	1.00
MD	9.51	9.17	*7.94a	*5.43a	4.13	6.86	3.41	3.86	0.31	0.27	*0.71a	0.82
IBT + 50ml/L	*7.10a	11.45	5.99	4.42	4.25	2.50	3.29	**3.08bc	*0.55a	*0.15b	0.60	0.81
IBT + 100ml/L	*12.69a	8.42	5.58	3.66	2.76	3.23	2.64	**2.57c	*0.16a	*0.38ab	0.58	1.09
IBT + 200ml/L	*8.96a	9.10	8.18	5.48	4.76	11.10	3.51	**4.10a	*0.17a	*0.73ab	0.50	0.88
MD + 50ml/L	*7.79b	7.82	8.15	5.95	4.00	3.28	2.90	**2.94b	*0.27b	*0.34a	0.57	0.56
MD + 100ml/L	*3.25b	6.33	6.86	5.86	2.13	4.33	2.61	**5.42a	*0.67a	*0.40a	0.57	1.09
MD + 200ml/L	*18.49a	10.88	7.48	4.84	6.89	3.97	5.31	**3.92ab	*0.09b	*0.24a	1.32	0.79
LSD V			0.88	0.62							0.14	
LSD B					2.74		0.85	0.89	0.12	0.11	0.29	
LSD V*B	13.25*9.07							0.91*1.70	0.55*0.41	0.76*0.41		

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

Effect of Brassinolide on Physiological Changes of Fig

Table 2 shows that the physiological changes of fig were affected by the brassinolide levels and the cultivars. Interaction between brassinolide concentrations and fig variety was significant only at 5%. Similar to morphological parameters, physiological traits such as photosynthesis, transpiration rate, and chlorophyll have shown some differences with brassinolide application, but the differences were not consistent and most of the changes happened only in first or second month. Both the brassinolide and the cultivar treatments were effective on the physiological changes of fig except on stomatal conductance.

Varietal performance of brassinolide application was analyzed at specific period of the study and the result is presented in Figures 1 and 2. Increasing concentration of brassinolide (50, 100 and 200 ml.L^{-1}) had decreased the rate of photosynthesis, transpiration and chlorophyll content in IBT than MD.

Correlation analysis was carried out to establish the relationship between the parameters. Figure 3 shows 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.

DISCUSSION

We studied the effect of exogenous brassinolide application on some growth and physiological traits on two cultivars of fig. The main functions of brassinollide 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 et al. (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 et al. (2013) for

Table 2

Effect of different concentrations of brassinolide on physiological changes of two cultivars of fig

Treatments	Photosynthesis Rate ($\mu mol.m^{-2}s^{-1}$)				Stomatal Conductance ($mmol\ m^{-2}\ s^{-1}$)			
	Month After Treatment				Month After Treatment			
	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
50ml/L	*10.66b	10.94	17.35	23.45	0.63	0.26	0.41	0.36
100ml/L	*9.69b	10.81	16.19	23.40	0.40	0.23	0.31	0.22
200ml/L	*10.65b	11.07	17.63	23.16	0.42	0.20	0.53	0.32
IBT	11.30	*11.82a	17.28	22.00	0.52	*0.26a	*0.54a	0.32
MD	10.86	*10.59b	16.79	23.15	0.54	*0.20b	*0.37b	0.30
IBT + 50ml/L	10.57	*12.16ab	18.19	23.06	*0.74a	0.31	0.41	0.52
IBT + 100ml/L	11.64	*13.19a	13.18	23.54	*0.50a	0.28	0.29	0.24
IBT + 200ml/L	9.55	*7.94b	20.36	22.22	*0.32a	0.18	0.71	0.33
MD + 50ml/L	10.75	*9.73a	16.51	23.84	*0.52ab	0.21	0.40	0.20
MD + 100ml/L	7.73	*8.44a	19.19	23.27	*0.30b	0.18	0.33	0.19
MD + 200ml/L	11.75	*14.20a	14.90	24.10	*0.51ab	0.23	0.35	0.31
LSD V		1.22				0.04	0.29	
LSD B	2.45							
LSD V*B		4.72*8.22			0.63*0.46			

Treatments	Transpiration Rate ($mol\ m^{-2}\ s^{-1}$)				Chlorophyll Content (mg/g fresh weight)			
	Month After Treatment				Month After Treatment			
	1	2	3	4	1	2	3	4
Control	*5.00a	3.33	*3.59a	3.22	18.79	4.73	*14.22b	4.20
50ml/L	*4.51ab	3.54	*3.07b	2.80	18.38	4.92	*14.27b	4.39
100ml/L	*3.50b	3.22	*2.82b	2.54	18.90	4.76	*14.24b	4.28
200ml/L	*3.58b	2.86	*3.69a	3.24	18.90	5.09	*14.35a	4.89
IBT	3.92	*3.47a	*3.57a	2.99	**19.07a	*4.96a	14.26	4.57
MD	4.38	*3.01b	*3.01b	2.90	**18.41b	*4.80b	14.28	4.31
IBT + 50ml/L	4.47	*3.85a	3.48	3.25	*18.91a	5.03	14.24	4.44
IBT + 100ml/L	3.76	*3.76a	3.04	2.69	*19.06a	4.65	14.23	4.36
IBT + 200ml/L	3.05	*2.42b	3.78	3.25	*19.05a	5.06	14.38	5.20
MD + 50ml/L	4.55	*3.22a	2.66	2.35	*17.85b	4.81	14.29	4.35
MD + 100ml/L	3.24	*2.69a	2.59	2.39	*18.75a	4.87	14.25	4.20
MD + 200ml/L	4.11	*3.31a	3.60	3.23	*18.75a	5.12	14.31	4.57
LSD V		0.45	0.53		0.41	0.13		
LSD B	1.03		0.47				0.07	
LSD V*B		1.33*2.16			0.80*0.82			

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

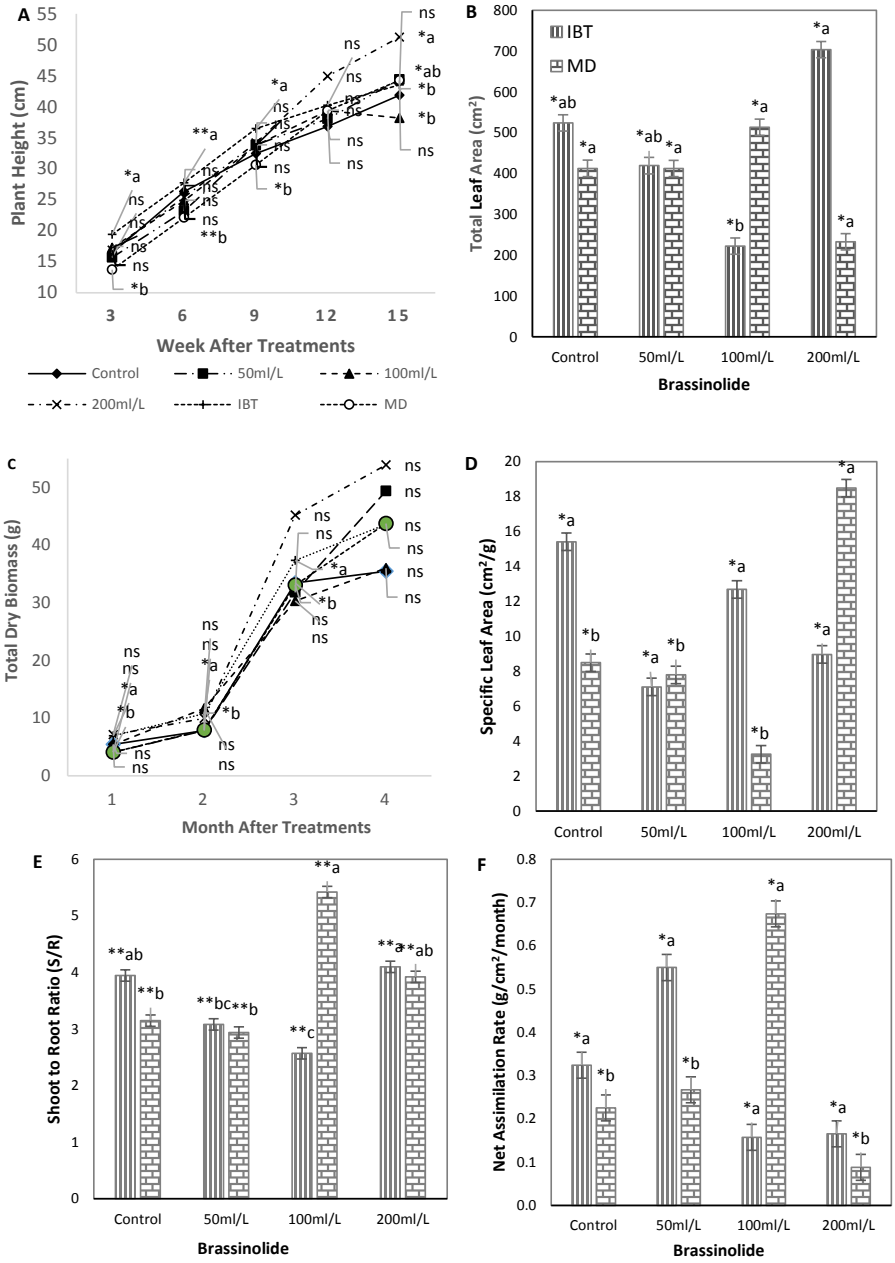

Figure 1. Significant growth of fig according to parameters: (A) Plant height as main effect of brassinolides on the cultivars; (B) TLA at third MAT as interaction between cultivars and brassinolide; (C) TDB as main effect of brassinolides and cultivars; (D) SLA at first MAT as interaction between cultivars and brassinolides.E) S/R at fourth MAT as interaction between cultivars and brassinolides; NAR as interaction between cultivars and brassinolides at: (F) First MAT. Bars and curves represent means followed by the different small letters are significant at *=p<0.05, **=p<1%, and ns=not significant

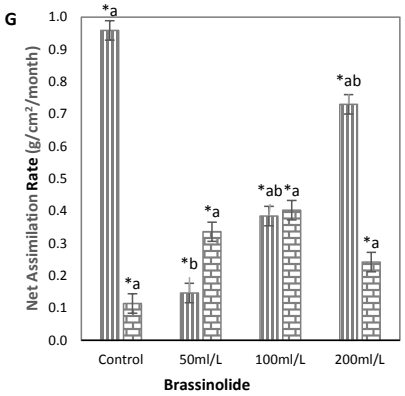

Figure 1. Significant growth of fig according to parameters: (G) Second MAT. Bars and curves represent means followed by the different small letters are significant at *=p<0.05, **=p<1%, and ns=not significant

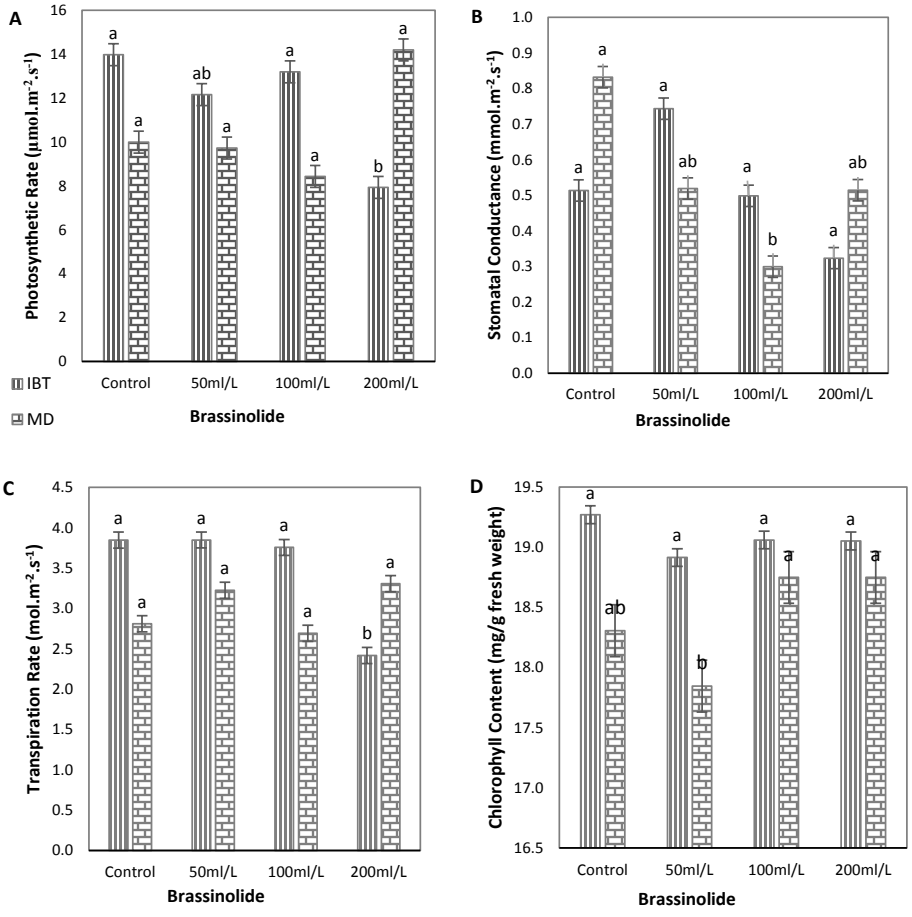

Figure 2. Significant physiological changes of fig according to parameters: (A) A at second MAT; (B) g_s at first MAT; (C) E at second MAT; and (D) CC at first MAT. Bars represent means followed by the different small letters significant at p<0.05

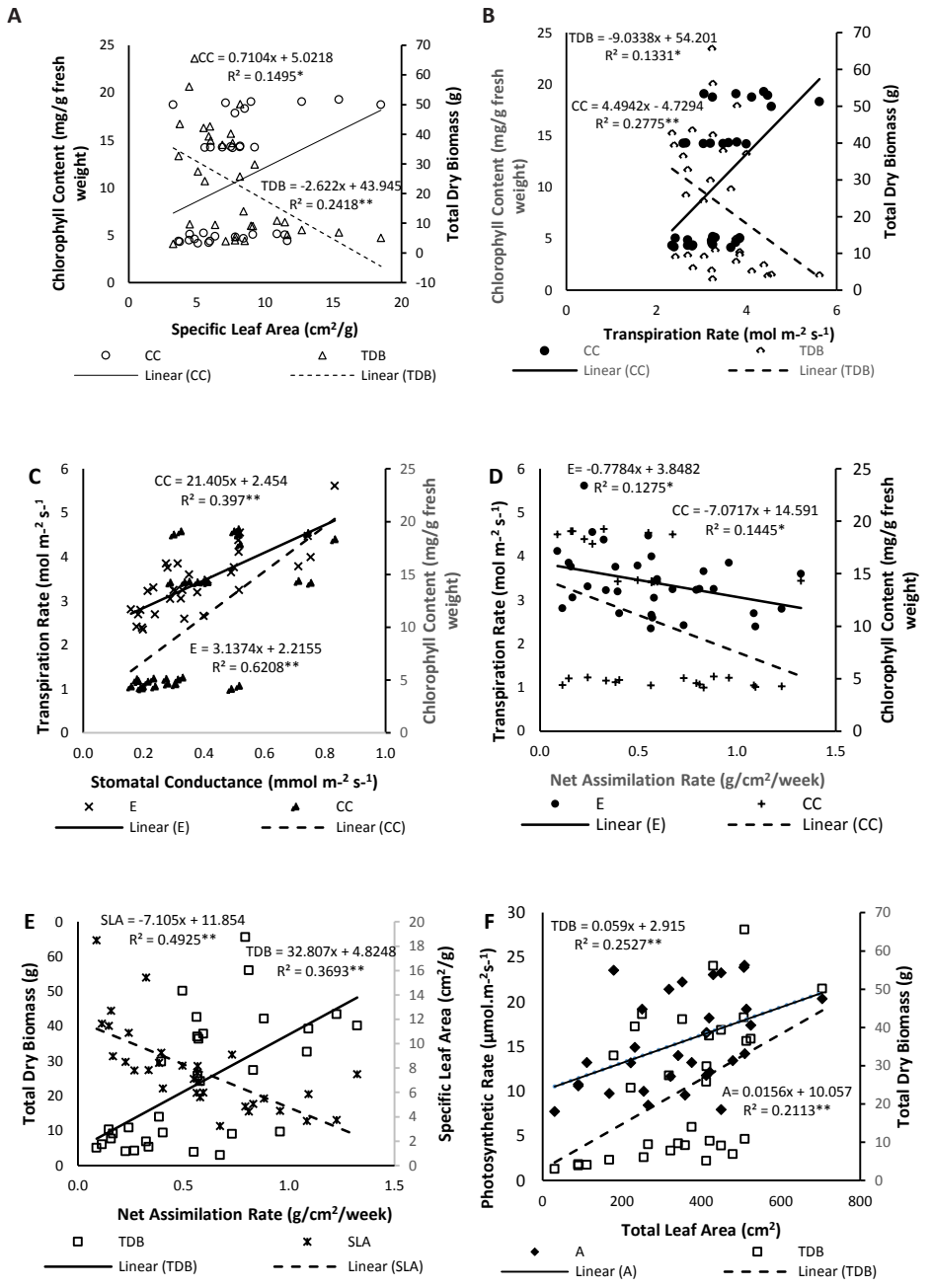

Figure 3. Correlation coefficient between CC and TDB with (A) SLA; (B) E; E and CC with (C) g_s; (D) NAR; TDB and SLA with (E) NAR; (F) TLA. *= p≤ 0.05, **=p ≤0.01, n=128

Leymus chinensis; Bera et al. (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 et al., 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, channeling 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 (Hayat et al., 2012; Lambers & Poorter, 1992).

The net assimilation rate (NAR) of plants are growth characteristics that best describe plant growth performance under specified conditions (Gardner et al., 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 et al. (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.

Brassinolide (BL) had profound impact on leaf photosynthesis and plant performance. Brassinolide (BL) improved leaf carbon assimilation rate, which is the light harvesting machine of plant photosynthesis. Brassinolide (BL) treatment also enhanced photosynthetic performance of cotton seedlings under NaCl stress (Chen et al., 2007; Shu et al., 2011; Xiao et al., 2007). 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).

Brassinolide (BL) treatment enhanced photosynthesis (17.06%) and chlorophyll content (18.36%). In contrast, BL-treatment decreased stomatal conductance (11.94.50%) 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 et al., 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 et al., 2011). Figures 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.

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.

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