Chapter

Impact Brassinolide on Two Fig Varieties

Zulias Mardinata, Mardaleni and Tengku Edy Sabli

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. In this chapter, we present the effect of different concentration of exogenous application of BL on growth and physiological changes of fig. 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 in plant height and total dry biomass.

Keywords: Brassinolide, fig, growth, physiological changes

1. 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 [1, 2]. 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 [3], leaf bending and epinasty [4]. Besides their role in promoting plant growth activities, they also have physiological effects on the growth and development of plants [2, 5].

Much has been written about Clouse [6], 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 post-embryonic 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 [7]. 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 [8]; 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 [9]. There is limited information on exogenous brassinolide application on these varieties.

2. Brassinolide

Brassinolide (BL) or 2,3,22,23-Tetrahydroxy-β-homo-7-oxaergostan-6-one or $C_{28}H_{48}O_6$ with molar mass 480.69 g mol^{-1} is a plant hormone [10]. The first isolated brassinosteroid (BRs), it was discovered when it was shown that pollen from rapeseed (*Brassica napus*) could promote stem elongation and cell division. The biologically active component was isolated and named BL [3].

Common structural characteristics of BL (**Figure 1**) are A/B *cis* combined steroidal skeletons, oxygenated task in rings A, the lateral chain and with very few exceptions the ring B. All the naturally happening BRs apply the same qualitative results as BL. The biological activity magnitude has been found to rely on the location and spatial orientation of the hydroxyl groups in ring A, the oxygenated function nature locate at ring B, the configuration of chiral centers C_{22} and C_{23} and the substitution pattern and configuration of the chiral center C_{24}. Attempts to expand a Quantitative Structure–Activity Relationship (QSAR) system that enable to look the biological action of a given compound have been developed [11].

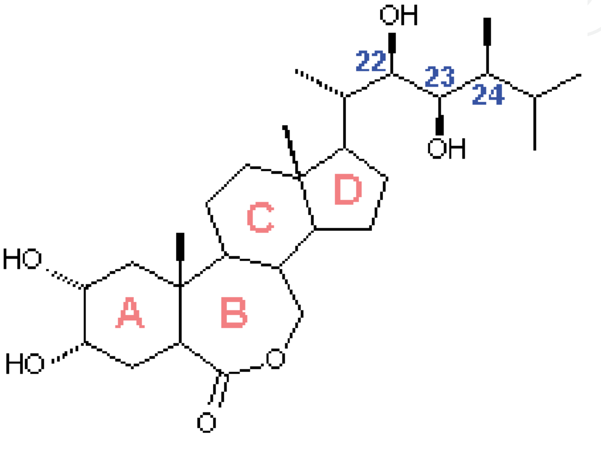

Figure 1.
Chemical structure of brassinolide [10].

3. History of Brassinolide

BL for the first time was found in 1968. To produce a strong plant growth promoting effect, Marumo et al. [12] gained three extracts from an evergreen Japanese plant known as Isonuki (*Distylium rasemosun* Sieb et Zucc.). A couple years later, Mitchell et al. [10] used rape pollen *Brassica napus* L. resulting an oil fraction to use the same effect. They guessed that the compounds that yielded such effects might be a plant hormones new kind, but at that time no structural characterization was possible caused by the limited amount of material availability.

Grove et al. [3] purified 40 kg of collected bee pollen of *Brassica napus* L. to establish its structure, which shows resemblance to animal steroid hormones resulted 4 mg of a crystalline ingredient which was known as (22R,23R,24S)-2a,3a,22,23-tetrahydroxy- 24-methyl-B-homo-7-oxa-5a-cholestan-6-one. In consequence of its low concentration, the BL identification took 10 years of loyal work at U.S. Department of Agriculture at a cost of over 1 million U.S. dollars [13]. This steroidal lactone which stimulates plant development when utilized to plants in concentrations lower than 10^{-4} mg ml^{-1} received the name of BL, product of the combination of Brassica, the Latin name of the plant from which it was isolated and the suffix '*-olide*' characteristic of the lactones. After isolation and identification of BL a number of manufacturally connected steroids have been isolated [14]. Till 2001 year, over 40 naturally happening BRs are identified; these bring an oxygen moiety at the C-3 location in fusion with others at the C-2, C-6, C-22 or C-23 locations [15].

4. Roles of Brassinolide in plant

According to Tang et al. [16], benefits of BL improves the growth of the germinating seed, improves the plant's ability to deal with stress, such as diseases, drought, salt, and cold, promotes growth of lateral buds, produces deep green leaves, increases the number of flowers and fruit produced, increases the percentage of fruit setting by decreasing the amount of flower and fruit drop, increases sugar content and generally improves the quality of the fruit, promotes fruit enlargement, delays leaf senescence lengthening productivity, and can be used in soil, hydroponics or leaf feeding.

5. *Ficus carica* L.

The genus *Ficus* (**Table 1**) is remarkable for the variation among its 13 species, and is widely distributed throughout the tropics and subtropics of both hemispheres. Several species produce edible figs of varying palatability, whereas many other arborescent *Ficus* sp. are cultivated for shade, as avenue trees and as ornamentals. *Ficus carica* L. (**Figure 2**) is considered to be native to South-Western Asia and to have been brought into cultivation in the Southern Arabian Peninsula by 3000 BC [17]. The fig tree is small or moderate in size, and deciduous with broad, ovate, three- to five-lobed leaves. The leaves are rough above and pubescent below, long stalked and dark green with pronounced venation. The female fig plants have larger leaves and more spreading crowns than male trees [18].

The fig is an accumulation fruit structured by individual small drupes; known as a drupelet. In ovaries, the drupelets develop to a syconium containing amounts of

Rank	Scientific name
Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Alismidae
Order	Rosales
Family	Moraceae
Genus	*Ficus*
Species	*Ficus carica*
Generic Group	*Mulberry*

Source: [21].

Table 1.
Taxonomy of Ficus carica*.*

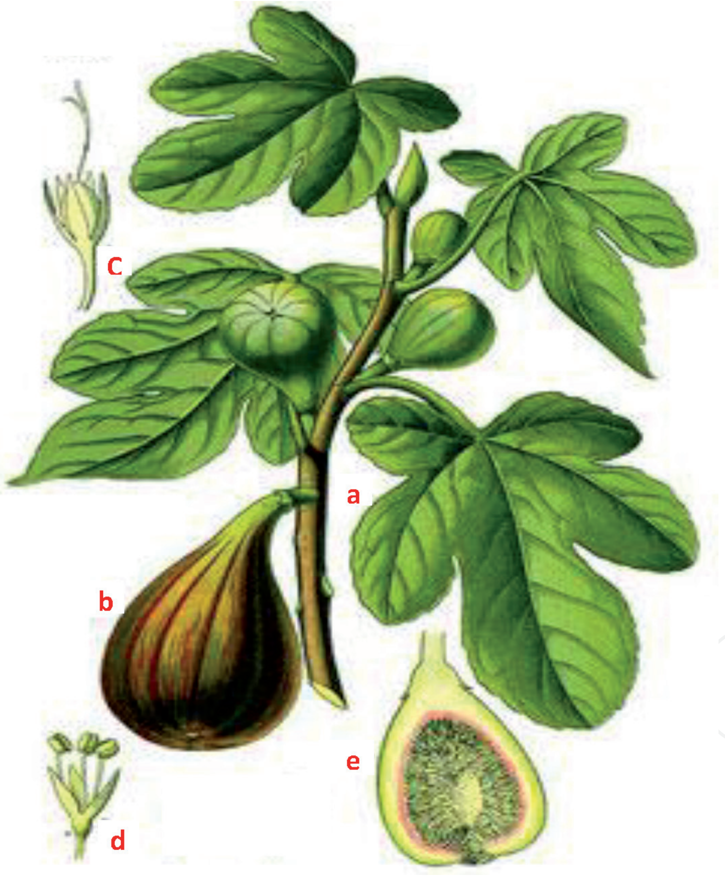

Figure 2.
Part of Ficus carica *L.: (a) five-lobed leaf; (b) fig fruit; (c) female flower; (d) male flower; and (e) inside view of fig fruit [19].*

unisexual flowers and pollinated by wasps via ostiole. The fig may produce multiple crops of annual fruits and need pollen from their pollinator Capri figs in any certain fig types. Breba crop is borne laterally on the growth of the previous season from buds produced in leaf axils and it is not produced in all cultivars. Another fig fruit type is main crop which is produced laterally in the axils of leaves on current season shoots. The leaves fall and the tree enters the dormancy period at the end of the growth period [20].

Figs respond well to heavy mulching with organic materials to conserve moisture, improve soil structure and reduce root knot nematode levels but intolerant in condition of poorly drained and waterlogged. To increase the main crop and maintain size control, fig also responds well to pruning and can be trained or pruned heavily in the dormant season [21]. The fig is fairly salt and drought tolerant. Soils of high lime content produce fruits of better quality suitable for drying. Fig trees can also withstand temperatures as low as −12 to −9.5°C [18].

6. Cultivar improved Brown Turkey (IBT) and Masui dauphine (MD)

In Malaysia and Indonesia, there are at least of 21 known fig varieties being found [9] over 700 named varieties around the world, but many of them are of no

Figure 3.
Cultivars of Ficus carica *L.: (a) Improved Brown Turkey (IBT); (b) Masui Dauphine (MD).*

Item	Improved Brown Turkey (IBT)	Masui Dauphine (MD)
Origin	Turkey	Japan
Skin color	Brownish-purple	Reddish-brown
Flesh color	Pink	Red strawberry
Tree height	4.5–7.5 m	2.5–3 m
Tree width	3.6–4.5 m	3–4 m
Drought tolerance	Good	Good
Annual pruning	Light	Light
Breba crop	Yes	Yes
Fruit weight	50–60 g	110–220 g
Harvesting	Twice/year	Twice/year
Leaf type	Five lobe	Three to five lobe
Pollination	Self-pollinating (common fig)	Self-pollinating (common fig)

Source: [22].

Table 2.
Comparison of two cultivar of Ficus carica.

use to home gardeners [7]. In this study, researcher use two varieties of common fig, they are Improved Brown Turkey (IBT) and Masui Dauphine (MD). Each variety has different key characteristics as shown in **Figure 3** and **Table 2**.

Most people choose to grow fruit trees in containers for easy mobility. Another reason is easy to measure plant quality. Plant quality is divided into three broad categories of attributes including morphological, physiological, and performance.

Morphological attributes are easy to see and measure and does not change readily after plants are harvested and stored [23]. Figs are well-suited to container cultivation. For this purpose, the ideal container size is about 10–15 gallons-substantial enough to support a tree, but small enough to move easily. Container grown figs need regular watering and feeding. Figs will not grow for very long if it does not have adequate drainage. Make sure the container that we use has holes (usually in the bottom and/or sides), so that any excess water can drain, and air can access the soil. This will help us to prevent potentially fatal diseases like root rot [24].

7. 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 $ml.L^{-1}$), had decreased the net assimilation rate.

Application of brassinolide had some effect on plant height, total leaf area, total dry biomass, specific leaf area and net assimilation rate 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.

The effect of exogenous brassinolide application on some growth and physicological traits on two cultivars of fig has been investigated. The main functions of brassinolide are to promote the plant growth especially for cell elongation and division [25] and has the ability to stimulate other physiological processes [26]. Wang et al. [27] 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. [28] for *Leymus chinensis*; Bera et al. [29] for sunflower; and Anjum et al. [30] for maize. The growth stimulation was more pronounced on above ground biomass than below ground biomass, showing a high shoot-to-root ratio [31]. 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 [32].

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 [33]. 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 [34, 35].

The net assimilation rate (NAR) of plants are growth characteristics that best describe plant growth performance under specified conditions [36]. 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. [37], 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.

8. Effect of Brassinolide on physiological changes of fig

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%. As like 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 4** and **5**. 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.

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 [38–40]. For cucumber seedlings, BL treatment has also been found to promote the occurrence of new roots, the formation of lateral roots and nutrient uptake [41].

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 [42] and improved chlorophyll content and higher leaf area in BL-treated plants [43].

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 [44]. **Figure 6A** and **C** showed a significant positive inter-relation among chlorophyll content, transpiration

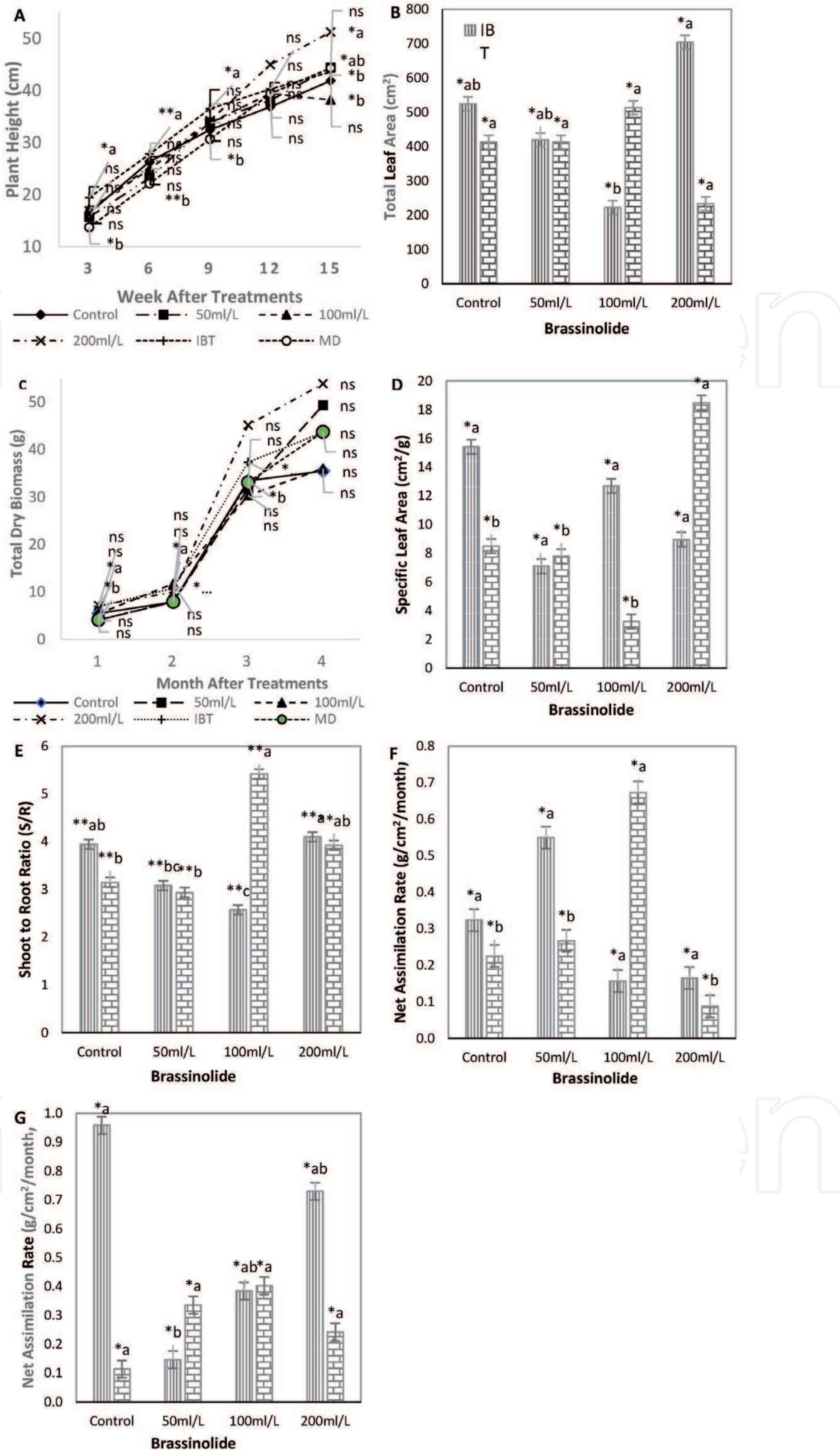

Figure 4.

*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; and (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.*

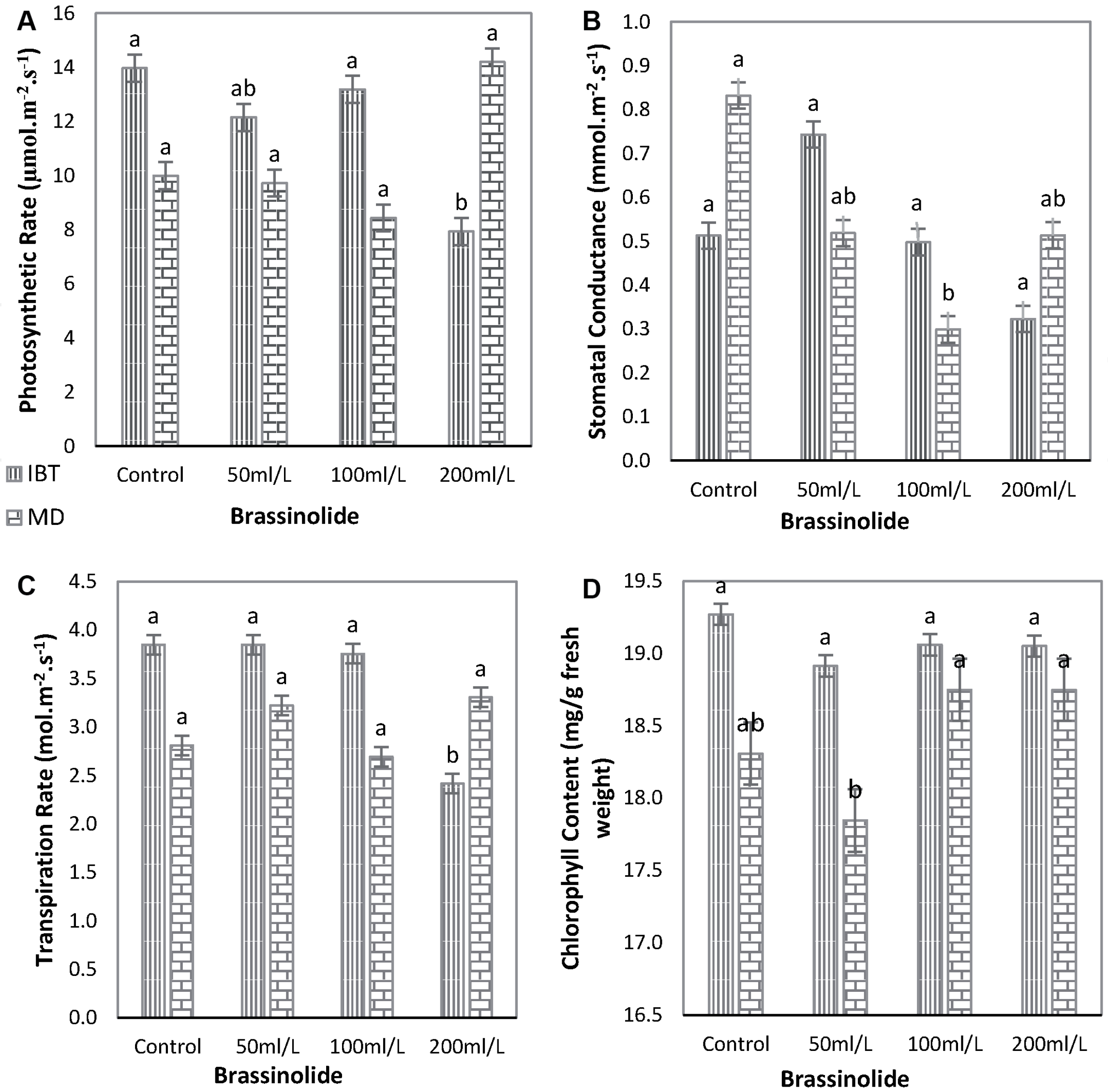

Figure 5.
Significant physiological changes of fig according to parameters: (A) A at second MAT; (B) gs at first MAT; (C) E at second MAT; and (D) CC at first MAT. Bars represent means followed by the different small letters significant at $p < 0.05$.

rate and stomatal conductance, indicating that a decrease in chlorophyll content would associated with same degree of reduction in transpiration rate and stomatal conductance.

9. Correlation analysis

Correlation analysis was carried out to establish the relationship between the parameters. **Figure 6** 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

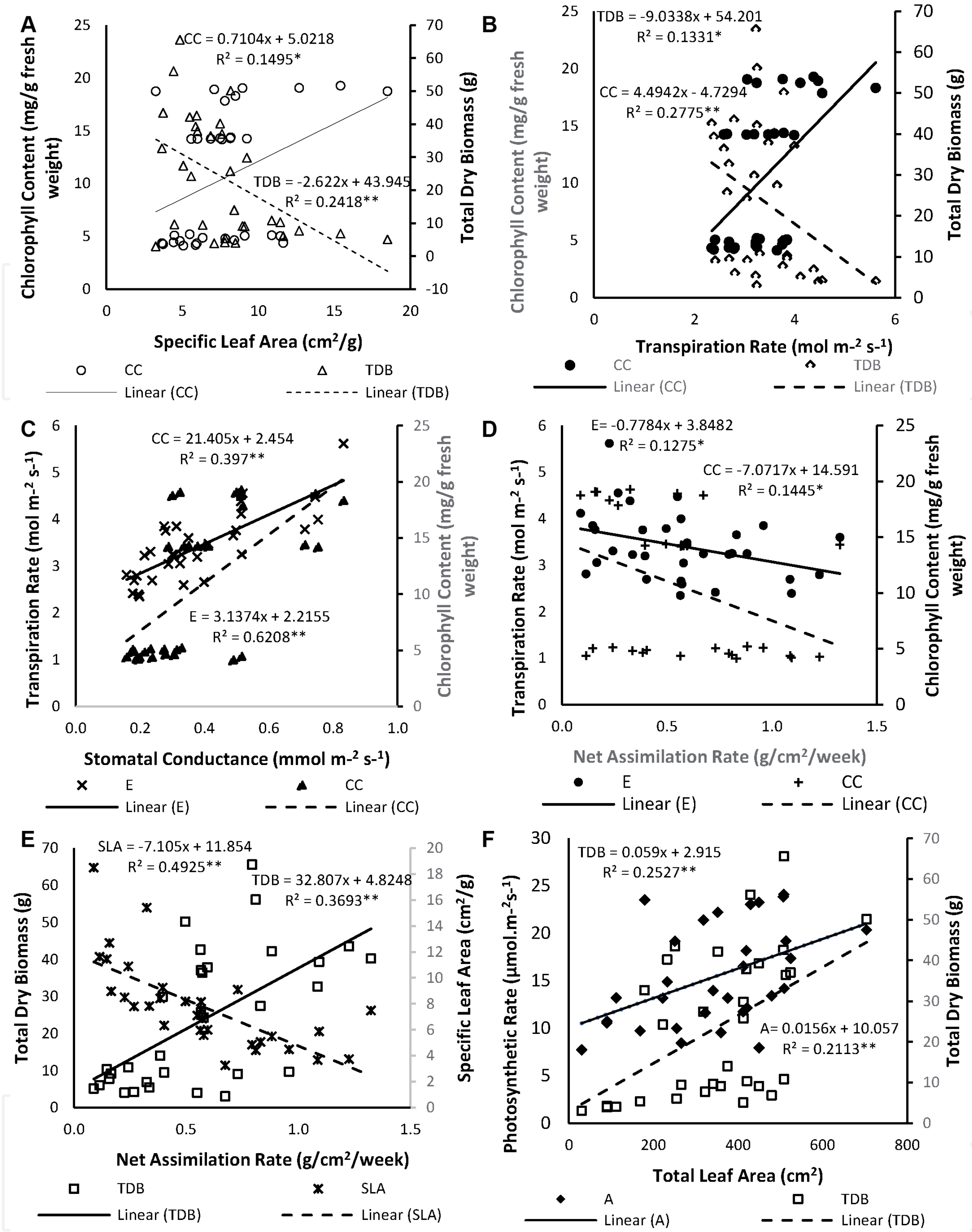

Figure 6.
Correlation coefficient between CC and TDB with (A) SLA; (B) E; E and CC with (C) gs; (D) NAR; TDB and SLA with (E) NAR; (F) TLA. $^{*} = p \leq 0.05$, $^{**} = p \leq 0.01$, $n = 128$.

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.

10. Conclusions

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.

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