# APPLICATION OF BRASSINOSTEROID TO *Tabebuia alba* (BIGNONIACEAE) PLANTS

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**ABSTRACT** - The objective of this study was to observe the effects of brassinosteroid, gibberelin, and auxin application on the development and foliar anatomy of *Tabebuia alba* (Cham.) Sandw. seedlings. *T. alba* seedlings were grown in plastic bags with fertilized soil and treated with the following: 1- water (control); 2- brassinolide ($BR_1$) 0.104 mM; 3- $BR_1$ 0.208 mM; 4- 3-indoleacetic acid (IAA) 0.2854 mM; 5- IAA 0.5708 mM; 6- $GA_3$ (gibberellin $A_3$) 0.1443 mM; 7- $GA_3$ 0.2887 mM; 8- $GA_3$ 0.072 mM + IAA 0.1427 mM; 9- $GA_3$ 0.1443 mM + IAA 0.2854 mM; 10- $GA_3$ 0.072 mM + $BR_1$ 0.052 mM; and 11- $GA_3$ 0.1443 mM + $BR_1$ 0.104 mM. Plant height and petiole length were measured before the treatments and 21 days after application of the growth regulators. These data allowed the calculation of stem and petiole growth rates. The results showed that $GA_3$ + brassinolide produced the highest stem and petiole growth rates and brassinolide application stimulated petiole growth but not stem growth. The anatomical study of leaves showed alterations in blade and petiole thickness, palisade and spongy parenchyma height, and epidermis cells.

**ADITIONAL INDEX TERMS**: *Tabebuia alba* L., brassinosteroids, $GA_3$, auxin, growth.

# APLICAÇÃO DE BRASSINOESTERÓIDE EM PLANTAS DE IPÊ (*Tabebuia alba*)

**RESUMO** – O objetivo deste estudo foi de se verificar o efeito da aplicação de brassinoesteróide, giberelina e auxina no desenvolvimento e anatomia foliar de plantulas jovens de *Tabebuia alba* (Cham.) Sandw. Para tanto, utilizaram-se plântulas de ipê de 104 dias cultivadas em saco plástico com capacidade para 1 litro contendo solo adubado. Os tratamentos aplicados via pulverização foliar foram: 1 – água, 2 – brassinólide ($BR_1$) 0,104 mM, 3 – $BR_1$ 0,208mM, 4 – ácido indolil-3-acético (IAA) 0,285mM, 5 – IAA 0,5708mM, 6 – $GA_3$ (ácido giberélico) 0,1443mM, 7 – $GA_3$ 0,2887mM, 8 – $GA_3$ 0,072mM + IAA 0,1427mM, 9 – $GA_3$ 0,1443mM + IAA 0,285mM, 10 – $GA_3$ 0,072mM + BR1 0,052mM e 11 – $GA_3$ 0,1443nM + BR1 0,208mM. As seguintes observações foram realizadas antes da aplicação dos tratamentos e 21 dias após: altura da planta e comprimento do pecíolo. Com base nesses dados, foi calculada a taxa de crescimento do caule e do pecíolo. Pelos resultados, constatou-se que a aplicação de brassinólide estimula o crescimento do pecíolo, mas não do caule. Os estudos anatômicos das folhas mostraram alterações na espessura do limbo e pecíolo, na altura do parênquima paliçádico e lacunoso e nas células da epiderme.

**TERMOS ADICIONAIS PARA INDEXAÇÃO**: *Tabebuia alba* L., brassinoesteróides, $GA_3$, auxina, crescimento.

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## INTRODUCTION

Brassinosteroids are steroids that occur in many plant species with common biological activities, suggesting that they are a new group of plant growth hormones (Yokota and Takahashi, 1985). These hormones affect several biological processes, including stem elongation, pollen tube growth, leaf bending, root growth, and xylem formation (Yokota, 1997). These developmental activities are mainly associated with ATPase activity (Cerana *et al.*, 1983), synthesis of 1-aminocyclopropane-1-carboxylic acid synthase (Arteca *et al.*, 1983), alteration in microtubule orientation (Mayumi and Shibaoka, 1995), and modification of cell walls (Zurek *et al.*, 1994).

Clouse and Zurek (1991) observed that exogenously supplied brassinolide, the most active brassinosteroid, promoted both tracheary element differentiation and cell division in cultured tuber explants. Iwasaki and Shibaoka (1991) also reported that brassinosteroids produced parenchyma cell differentiation into tracheary elements.

Brassinosteroids are known to act synergistically with auxin to stimulate cell elongation (Katsumi, 1985; Sasse, 1990) and ethylene production (Arteca *et al.*, 1983), suggesting that their effects are mediated by auxin (Takeno and Pharis, 1982) or that brassinosteroids enhance tissue sensitivity to auxin (Mandava, 1988). Despite indirect evidence that the mode of action of brassinosteroids is different from that of auxin (Katsumi, 1991), there are indications that brassinosteroids may somehow influence the endogenous auxin content. The increase in auxin content in plant cells both after its exogenous application and biosynthetic gene expression was followed by a significant decrease in cytokinin levels or an increase in cytokinin degradation in transformed tobacco plants (Vanková *et al.*, 1991, 1992). Thus brassinosteroids lead to changes in auxin/cytokinin ratio in plant tissues.

Some studies have been performed on woody plants to evaluate the effect of brassinosteroid application. The objective of this study was to observe the effects of application of brassinosteroid alone or in combination with gibberellin or auxin on the development and foliar anatomy of *Tabebuia alba* (Cham.) Sandw plants.

## MATERIALS AND METHODS

This study was performed at the Department of Botany, Institute of Biosciences, São Paulo State University – UNESP, in a fiberglass-covered greenhouse with automated heating and cooling systems at 25 $^{o}$C.

*Tabebuia alba* (Cham.) Sandw. seedlings (104 days old) were obtained from seeds germinated in laboratory conditions. These were planted in plastic bags containing fertilized soil. The plants received the following treatments: 1- water (control); 2- brassinolide ($BR_1$) 0.104 mM; 3- $BR_1$ 0.208 mM; 4- 3-indoleacetic acid (IAA) 0.2854 mM; 5- IAA 0.5708 mM; 6- $GA_3$ (gibberellin $A_3$) 0.1443 mM; 7- $GA_3$ 0.2887 mM; 8- $GA_3$ 0.072 mM + IAA 0.1427 mM; 9- $GA_3$ 0.1443 mM + IAA 0.2854 mM; 10- $GA_3$ 0.072 mM + $BR_1$ 0.052 mM; and 11- $GA_3$ 0.1443 mM + $BR_1$ 0.104 mM.

Treatments were applied once with a manual sprayer, and 4 plants from each treatment were sprayed with 50 mL of each solution.

The following observations were made before the treatments and 21 days after application in order to evaluate the effects of auxin, gibberellin, and brassinoesteroid on plant development:

a) Stem height was considered as the length from the ground surface up to the shoot apex, measured with a ruler; and

b) Petiole length of the apical mature leaf was measured with a caliper.

These data allowed the estimation of stem and petiole growth.

The results obtained were submitted to analysis of variance (F test). Means were compared by the Tukey test at 5% significance level.

Twenty-one days after the treatments, anatomical analyses were undertaken on the petiole and blade median zones of completely expanded leaves. These samples were fixed with Karnovsky's solution (Karnovsky, 1985) for 24 h,

dehydrated in alcoholic series, and included in glycol-methacrylate (Ruetze and Schmitt, 1986). The 6 mm-thick sections were stained with 0.05% Toluidine blue, pH 4.7 (Feder and O'Brien, 1968) and mounted with Permount.

## RESULTS AND DISCUSSION

Tables 1 and 2 show the effect of IAA, $GA_3$, $BR_1$, $GA_3$ + IAA and $GA_3$ + $BR_1$ application on the petiole (F = 5.89) and stem (F = 6.44) growth of *Tabebuia alba* seedlings. Table 1 shows that treatment of *T. alba* plants with the two concentrations of $BR_1$ did not significantly affect petiole growth. However, $BR_1$ at 100 $mg.L^{-1}$ induced a small increase in petiole growth when compared to the control. Treatment of *T. alba* seedlings with IAA at 0.2854 and 0.5708 mM and $GA_3$ at 0.2887 mM induced similar results to the control. The application of $GA_3$ at 0.1443 mM produced a small increase in *T. alba* petiole growth. When $GA_3$ application was followed by that of IAA at the tested concentrations, there was no positive interaction between these two plant growth regulators in relation to petiole growth, rather, an antagonistic interaction was observed or $GA_3$ and IAA act independently on petiole growth. Application of $GA_3$ followed by $BR_1$ at 0.104 mM showed a positive interaction on petiole growth, whereas $GA_3$ and $BR_1$ had a synergistic effect. These results also suggest that $BR_1$ alone had little effect on petiole growth, but the combination of $BR_1$ with $GA_3$ increased petiole growth.

**TABLE 1 -** Petiole growth rate of *Tabebuia alba* seedlings 21 days after application of the growth regulators.

| Treatments | Petiole growth rate ($mm\ day^{-1}$) |
|---|---|
| Control | $5x10^{-2}$ b |
| $BR_1$ 0.104mM | $9x10^{-2}$ b |
| $BR_1$ 0.208mM | $14x10^{-2}$ b |
| IAA 0.2854mM | $4x10^{-2}$ b |
| IAA 0.5708mM | $5x10^{-2}$ b |
| $GA_3$ 0.1443mM | $17x10^{-2}$ ab |
| $GA_3$ 0.2887mM | $4x10^{-2}$ b |
| $GA_3$ 0.072mM + IAA 0.0.1427mM | $8x10^{-2}$ b |
| $GA_3$ 0.1443mM + IAA 0.2854mM | $9x10^{-2}$ b |
| $GA_3$ 0.072mM + $BR_1$ 0.052mM | $24x10^{-2}$ ab |
| $GA_3$ 0.1443mM + $BR_1$ 0.104mM | $39x10^{-2}$ a |

Averages followed by the same letter are not significantly different according to the Tukey test ($p \leq 0.05$).

**TABLE 2 -** Stem growth rate of *Tabebuia alba* seedlings 21 days after application of the growth regulators.

| Treatments | Stem growth rate (mm day $^{-1}$) |
|---|---|
| Control | 0.26 c |
| $BR_1$ 0.104 mM | 0.39 c |
| $BR_1$ 0.208 mM | 0.37 c |
| IAA 0.2854 mM | 0.40 bc |
| IAA 0.5708 mM | 0.25 c |
| $GA_3$ 0.1443 mM | 2.45 abc |
| $GA_3$ 0.2887 mM | 2.25 abc |
| $GA_3$ 0.072 mM + IAA 0.1427 mM | 0.39 c |
| $GA_3$ 0.1443 mM + IAA 0.2854 mM | 2.84 a |
| $GA_3$ 0.072 mM + $BR_1$ 0.052 mM | 2.99 a |
| $GA_3$ 0.1443 mM + $BR_1$ 0.104 mM | 2.75 ab |

Averages followed by the same letter are not significantly different according to the Tukey test ($p \leq 0.05$).

The application of $BR_1$ and IAA at the two concentrations did not cause stem growth in *T. alba* seedlings (Table 2). However the application of $GA_3$ at 0.1443 and 0.2887mM significantly increase stem growth. The use $GA_3$ at 0.1443mM followed by IAA at 0.2854mM caused the highest increase in stem growth, showing a positive or synergistic interaction between IAA and $GA_3$, or a synergistic at a higher concentration. The interaction between $GA_3$ at 0.1443mM and $BR_1$ at 0.052 and 0.104mM was positive. This interaction led to a significant increase in stem growth of *T. alba* seedlings, when compared with each plant growth regulator used alone.

Xu *et al.* (1995), studying the link between plant growth substances and the TCH4 gene expression, found that auxin and brassinosteroid stimulated TCH4 expression, increasing TCH4 MRNA levels that encode an xyloglucan endotransglycosylase (XET). They also observed that $GA_3$, benzyladenine (cytokinin), and 1-aminocyclopropane-1-carboxylic acid (ACC) had no detectable effect on TCH4 expression. However, Potter and Fry (1994) and Smith *et al.* (1996) observed a GA-stimulated growth and XET activity in many tissues. Xu *et al.* (1995) suggested that no relationship was seen between the effects of auxins on cell elongation and XET activity, but endogenous IAA (3-indoleacetic acid) stimulated TCH4 gene expression.

The results of this study showed that $GA_3$ and $BR_1$ stimulated petiole and stem growth in *Tabebuia alba* seedlings, mainly when applied in sequence. Literature reports suggest that $BR_1$ stimulates TCH4 gene expression leading to the production of XET MRNA and promoting growth. Also, $GA_3$ stimulates enzyme activity, suggesting that $GA_3$ and $BR_1$ can be additive on petiole and stem growth, each complementing the effect of the other.

IAA application on *T. alba* seedlings showed a positive interaction with $GA_3$ on stem growth. It can be suggested that the interaction between $GA_3$ and IAA, and $GA_3$ and $BR_1$ is

important for stem growth, since, according to the literature, $GA_3$ stimulates XET activity and IAA and brassinolide stimulates TCH4 gene expression.

Anatomical analysis transverse sections of the leaf blade showed that treatments with brassinosteroids alone (Figure 1B and 1C) or in combination with $GA_3$ (Figure 1D and 1E) produced a generalized increase in epidermis size and mesophyll cells, when compared to the control plants (Figure 1A). Table 3 shows the effects of the treatments on the thickness of leaf blade and length of constituent cells. A significant increase could be observed in blade length, epidermis cells, and chlorophyll parenchyma in response to the application of 0.208 mM of $BR_1$. Application of a combination of $BR_1$ with $GA_3$ (0.052 and 0.072 mM, respectively) also caused a significant increase in blade thickness. This increase is related to an increase in cell size in response to the application of brassinosteroid alone, or in combination with gibberellin. This is in agreement with one of the physiological effects of these growth regulators on cell elongation (Clouse, 1996; Hooley, 1996; Yokota, 1997).

The application of $GA_3$ (0.2887 mM) caused cell divisions in the cortical parenchyma of the petiole abaxial face (Figure 1G), which became slightly circular in comparison with the dorsiventral petiole of control plants (Figure 1F). Stimulation of cell division by brassinosteroids was also reported by Clouse and Zurek (1991) and Iwasaki and Shibaoka (1991).

**TABLE 3 -** Anatomical parameters of the leaf blade of *Tabebuia alba* seedlings analyzed 21 days after application of the growth regulators.

| Treatments | Thickness of the blade | Height of parenchyma | | Height of epidermis | |
|---|---|---|---|---|---|
| | | p.p. | 1.p. | ad. | ab. |
| Control | 90.9 c | 32.6 b | 28.6 ab | 14.3 b | 14.6 b |
| $BR_1$ 0.104 mM | 106.9 a | 39.3 a | 31.6 a | 19.3 a | 21.3 a |
| $BR_1$ 0.208 mM | 96.9 bc | 35.3 ab | 26.3 ab | 20.6 a | 18.6 ab |
| IAA 0.2854 mM | 94.6 bc | 32.2 b | 27.0 ab | 19.3 a | 15.7 ab |
| IAA 0.5708 mM | 97.9 abc | 39.0 a | 26.3 ab | 17.6 ab | 16.5 ab |
| $GA_3$ 0.1443 mM | 101.5 ab | 37.6 ab | 27.0 ab | 16.6 ab | 17.0 ab |
| $GA_3$ 0.2887 mM | 98.2 abc | 35.0 ab | 30.3 ab | 16.3 ab | 17.0 ab |
| $GA_3$ 0.072 mM + IAA 0.1427 mM | 94.2 bc | 40.0 a | 25.6 ab | 17.0 ab | 14.3 b |
| $GA_3$ 0.1443 mM + IAA 0.2854 mM | 89.9 c | 32.6 b | 22.6 b | 18.0 ab | 17.6 ab |
| $GA_3$ 0.072 mM + $BR_1$ 0.052 mM | 101.2 ab | 37.0 ab | 26.6 ab | 17.6 ab | 21.3 a |
| $GA_3$ 0.1443 mM + $BR_1$ 0.104 mM | 97.6 abc | 37.0 ab | 25.0 ab | 18.3 ab | 17.6 ab |

Averages followed by the same letter are not significantly different according to the Tukey test ($p \leq 0.05$).
palisade parenchyma (p.p.), spongy parenchyma (l.p.), adaxial (ad.) and abaxial (ab.) epidermis.

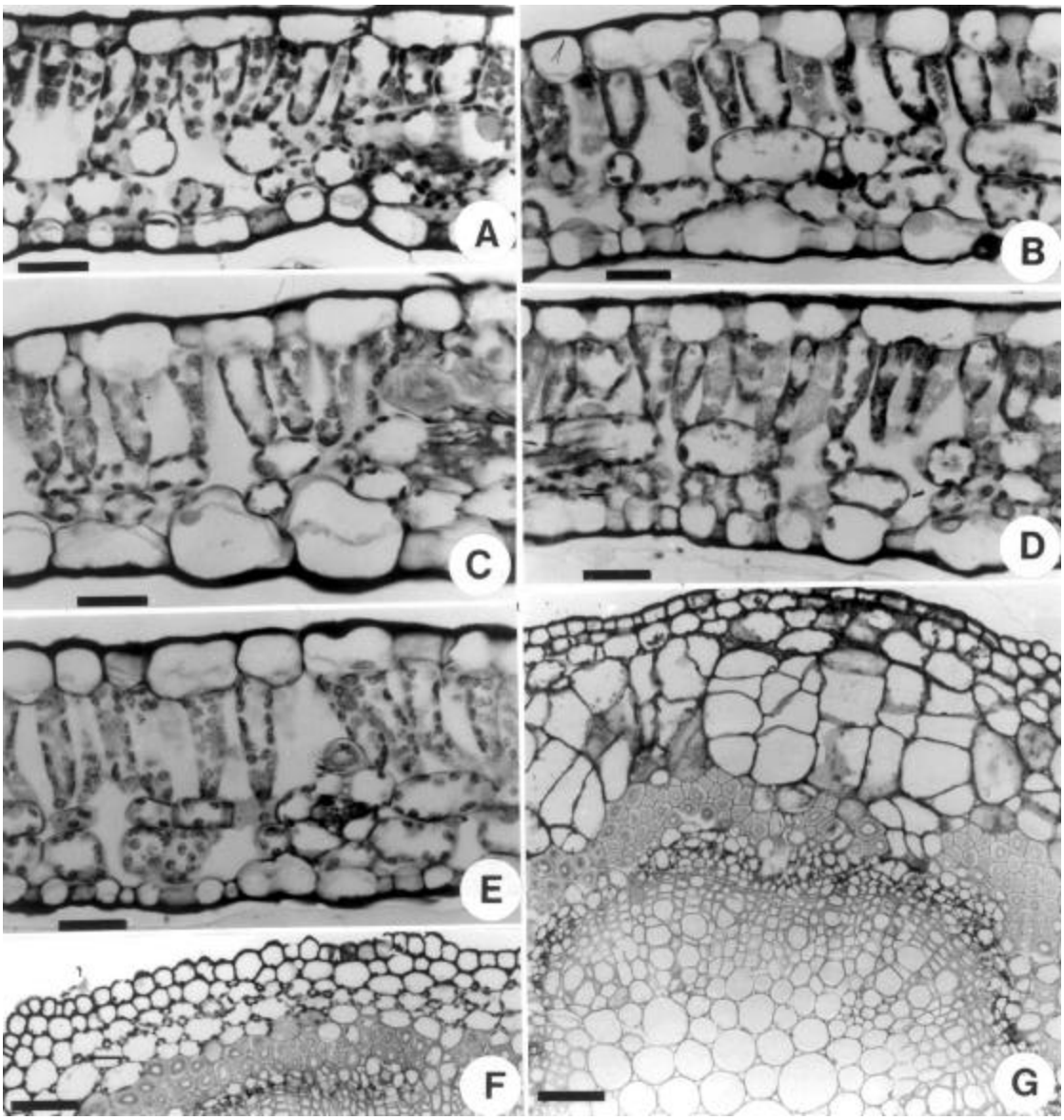


**FIGURE 1A-G**. Transverse sections of the *Tabebuia alba* leaf 21 days after application of the growth regulators. A-E: blade. Bar = 50 mm; F-G: petiole. Bar = 40 mm. A= control; B= $BR_1$ 0.104 mM; C= $BR_1$ 0.208 mM; D= $GA_3$ 0.072 mM + $BR_1$ 0.052 mM; E= $GA_3$ 0.1443 mM + $BR_1$ 0.104 mM; F= control; G= $GA_3$ 0.2887 mM.

Treatment of *Tabebuia alba* plants with $BR_1$ + $GA_3$ produced a significant development of lateral buds. Eun *et al.* (1989) and Fujii *et al.* (1991) observed that $BR_1$ reduced ABA levels in squash cotyledons (*Cucurbita pepo* L.) and rice. Therefore, we suggest that application of $GA_3$ + $BR_1$ reduced ABA level in lateral buds, leading to their development.

The results obtained from petiole and stem growth and anatomical analysis of the leaf blade, hyponasty, and lateral bud development, allowed us to conclude the following:

- There may have been a positive interaction between $GA_3$ and $BR_1$, increasing the $GA_3$ effect,
- There may have been a positive interaction between $GA_3$ and brassinolide, increasing the petiole and stem growth rate of *Tabebuia alba* seedlings,
- $GA_3$ application caused an increase in petiole and stem growth,
- $GA_3$ combined with $BR_1$ stimulated petiole and stem growth,
- Application of brassinolide alone or combined with GA increased leaf blade thickness, and
- $GA_3$ application stimulated cell division in the petiole.

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