CrossMark

Exogenous brassinosteroid application delays senescence and promotes hyponasty in *Carica papaya* L. leaves

Mara de Menezes de Assis-Gomes [iD] · **Daniel Teixeira Pinheiro** ·
Ricardo Bressan-Smith · **Eliemar Campostrini**

Abstract The application of brassinosteroids (BR) is associated with increased tolerance to various stresses such as those induced by pathogens, temperature, salinity and drought. Here, we studied the influence of BRs analogues on the leaf senescence of papaya juvenile plants. The objective was to determine whether BRs affect plant growth and leaf senescence by promoting or inhibiting the degradation of chlorophyll and/or leaf abscission. Two-month-old plants were divided into five treatments: C (control without BR application); BRWC (BR applied in the whole canopy); BRL1 (BR applied in the youngest fully expanded leaf, L1); BRL2 (BR applied in L2 which is the secondly more mature leaf in relation to L1) and BRL3 (BR applied in L3 which is the third more mature leaf in relation to L2). We concluded that BRs do not interfere with the senescence of the youngest leaf (L1) but in older leaves (L3) BRWC maintains the leaves greener and attached to the plant for a longer time when compared to the leaves from the other treatments. BR application in whole-canopy increased the plant height, and it caused a delay in leaf senescence and, consequently, in leaf abscission. In addition, hyponastic leaves developed after BR-application in the whole-canopy. The senescence process and leaf abscission in papaya, stimulated by exogenous BRs, were directly related to leaf age.

Keywords Chlorophylls · Leaf abscission · Leaf greenness · Leaf ontogeny · Papaya

1 Introduction

Papaya (*Carica papaya* L.) is cultivated in tropical and subtropical regions and the fruit is consumed in natura or processed as jams and jellies. Papaya is a perennial semi-woody plant (giant herbaceous) that lives from 5 to 10 years (Campostrini and Glenn 2007), and grows like a tree single-stemmed with a crown of large leaves emerging from the stem apex. The papaya plant produces large palmate leaves (~ 0.6 m^2), with 5–9 pinnate lobes of various widths (400–600 mm), arranged in a spiral pattern (Jiménez et al. 2014) and clustered in the upper section of adult individuals (Ming et al. 2008). The longevity of a papaya leaf is about 2.5–8 months, and new leaves are released at the rate of 1.5–4 leaves per week, depending on the climatic conditions and water availability. Under greenhouse conditions, at an average of 27 °C air temperature, 1000 μmol m^{-2} s^{-1} of maximum photosynthetic active radiation (PAR) and 1 kPa vapour pressure deficit (VPD),

M. M. de Assis-Gomes (✉) · D. T. Pinheiro ·
R. Bressan-Smith · E. Campostrini
Plant Physiology Laboratory, Universidade Estadual do
Norte Fluminense, Av. Alberto Lamego, 2000,
Campos Dos Goytacazes, RJ 28013-602, Brazil
e-mail: mara.assis.gomes@gmail.com

Golden papaya plants with adequate soil water availability put out about three leaves per week (Lima et al. 2015). The young leaves at the apex emerge from the apical meristem while older leaves located at the bottom of the plant come into senescence and undergo abscission, so there is a constant renewal of plant leaves.

Senescence is the final stage in the development of a plant organ, usually leading to programmed death of all the cells and represents the final developmental activity of the leaf, during which the leaf cell is dismantled in a coordinated manner to remobilize nutrients and to secure reproductive success (Schippers 2015; Rogers and Munné-Bosch 2016). Considering the investment of plants in nutrient acquisition, the remobilization of macronutrients and micronutrients during senescence is critical for efficient nutrient usage and for plant survival. The onset of senescence is strictly regulated and occurs under optimal conditions in an age-dependent manner (Schippers et al. 2015). Although senescence is often considered as a degenerative process, it is in fact an active stage of development in which the expression of many genes is drastically altered and new biochemical processes are activated (Breeze et al. 2011). Some of the activated mechanisms imply a conflict between initiating cell death and keeping cellular functions active long enough to enable efficient remobilization (Guiboileau et al. 2010).

Senescence and leaf abscission are differentiated processes and physiological and biochemical studies suggest that both senescence and abscission are regulated by a series of endogenous and environmental factors. The environmental cues include abiotic disturbances such as extreme temperatures, drought, ozone, nutrient deficiency, pathogen infection, wounding, and shading, whereas the autonomous factors include age, reproductive development, and phytohormone levels (Can and Amasino 1997). There are several studies showing the involvement of brassinosteroids in senescence and leaf abscission (He et al. 1996; Fariduddin et al. 2004; Sağlam-Çağ 2007). Although various authors suggested the involvement of brassinosteroids (BRs) on leaf senescence process, some of them indicate that BRs can induce senescence while others claim that BRs are related to delayed senescence.

Brassinosteroids (BR) are structurally similar to steroid hormones of animals and regulate the expression of hundreds of genes, impact the activity of numerous metabolic pathways and help control the development of programs related to morphogenesis (Gomes 2011). Some Arabidopsis mutants that lack brassinosteroids show a significant delay in leaf senescence (Li et al. 1996). Two lines of evidence suggest a leaf senescence-promoting role for brassinosteroids. First, external application of 24-epibrassinolide (24-epiBL) induces leaf senescence in mung bean plants (He et al. 1996). Second, several Arabidopsis brassinosteroid mutants that are deficient in either brassinosteroid biosynthesis or in the brassinosteroid signal transduction pathway display a delayed leaf senescence phenotype (He et al. 2001). Although the molecular mechanism of brassinosteroid action on leaf senescence is unknown, He et al. (2001) demonstrate that external application of 24-epiBL induce the reproducibly of the reporter gene expression in four enhancer trap lines, suggesting that brassinosteroids play a role in activating the senescence process.

Despite the entire mechanisms of BR effects on senescence are still unknown, the influence of the leaf age on senescence process in BR-applied plants has not been observed by researchers. The aim of this study was to determine whether the application of BRs in specific leaves of papaya as well as in the whole-canopy could promote or inhibit the process of chlorophyll degradation and compromise plant development, as well as leaf senescence and abscission.

2 Material and methods

2.1 Plant material and experimental design

Papaya seeds (UENF- Caliman 01) were sown in conic plastic tubes (28 cm^3) with Vivatto$^®$ Slim Plus substrate. The plants were watered daily and maintained in a greenhouse. At 60 days after sowing, five treatments were applied to papaya seedlings. The leaves were marked as L1 (the youngest fully expanded leaf close to the apex, 3rd leaf from the apex), L2 (the mature leaf immediately below the insertion of L1, 4th leaf from the apex), and L3 (the mature leaf immediately below the insertion of L2, 5th leaf from the apex).

The treatments were: C (Control, without BR-application); BRWC (BR application in the whole-

canopy); BRL1 (BR application only on L1); BRL2 (BR application only on L2); BRL3 (BR application only on L3). The brassinosteroid used was a spirostanic analogue of brassinosteroid (BIOBRAS-16) at 1 mg L^{-1} (containing 0.5% Tween 20). BR solution (BR + Tween 20) was sprayed in the whole-canopy or brushed in treated leaves (L1, L2 or L3) at 5:00 pm for three consecutive days. The non-treated leaves were sprayed or brushed only with Tween 20 (0.5%) and water. The experiment was organized in randomized blocks with five replicates, totaling 25 plots, and each plot was constituted of a plant per tube.

2.2 Morphological traits

The height of the papaya plant was measured from the base of the stem until the insertion of the youngest leaf recently launched with a graduated scale. The increment in height was determined for each plant by calculating the difference between the current height value recorded and the height value recorded in the first day of measurements.

The total number of leaves and the number of specific leaves (L1, L2 and L3) were determined by counting these organs throughout the experiment.

The leaf greenness (SPAD readings) was determined using a portable chlorophyll meter model, SPAD-502 (Minolta, Japan). Five readings from each leaf (L1, L2 and L3) were performed during the experiment using five plants per treatment.

2.3 Visual analysis

The photographs were taken with a digital camera (Sony Cybershot 16.1 Mpixels), with intervals of 2 or 3 days after the beginning of experiment.

2.4 Statistical analysis

The data were submitted to an analysis of variance (ANOVA) and the mean results were compared by the Tukey test (5%) using the SAEG System (Ribeiro Junior 2001).

3 Results

The total number of leaves of BRWC treatment was significantly different compared to the other
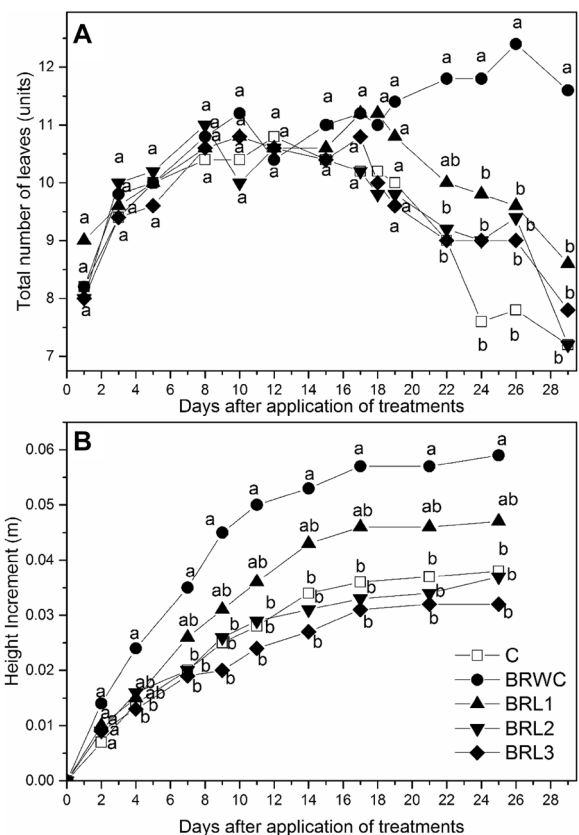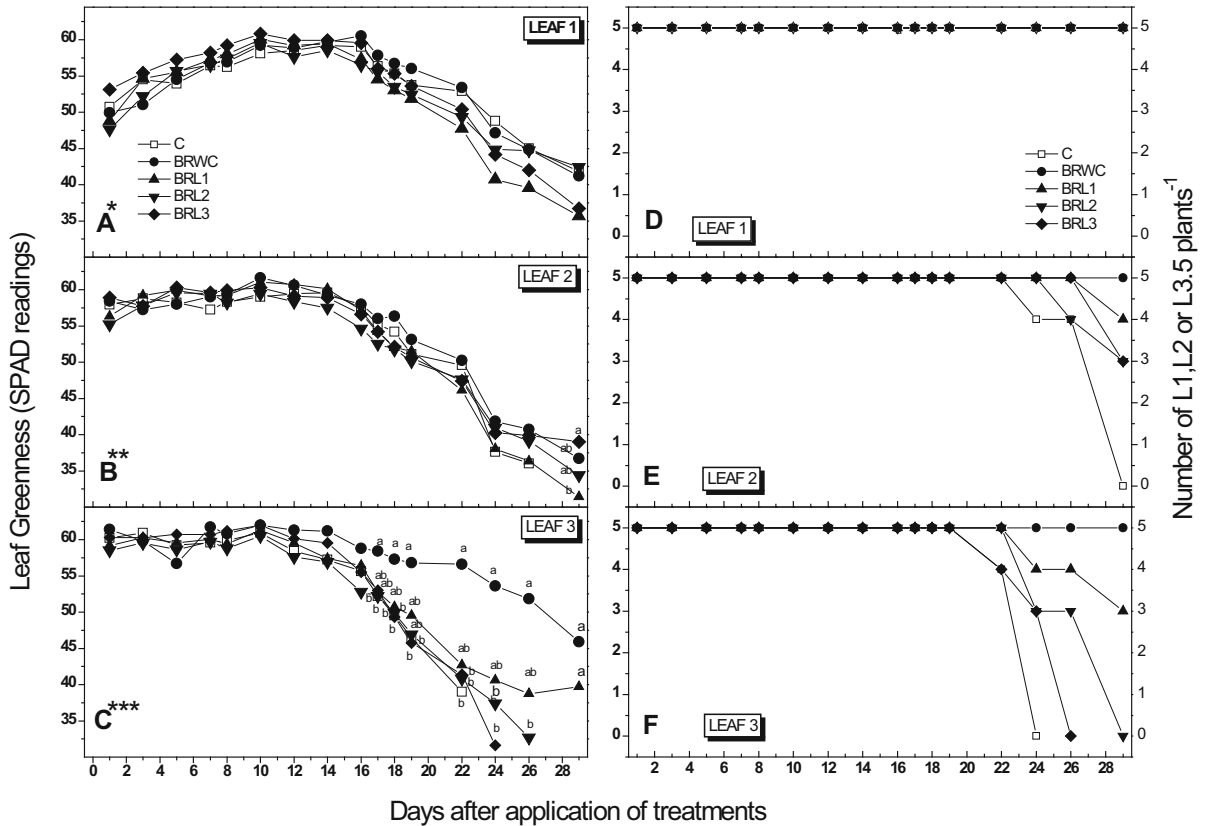

Fig. 1 Number of leaves (**a**) and height increment (**b**) in plants of papaya (*Carica papaya* L.) submitted to brassinosteroids (BR) application: C (Control), BRWC (BR whole-canopy), BRL1 (BR on leaf 1), BRL2 (BR on leaf 2), BRL3 (BR on leaf 3). Means followed by the same letter are not statistically different from each other in relation to the same day of analysis (Tukey test, 5%)

treatments (C, BRL1, BRL2, BRL3) at the end of the experiment (Fig. 1a). BRWC plants had approximately 11.5 leaves while BRL1, BRL3, BRL2 and C showed 9, 8, 7.5 and 7.5 leaves, respectively. This fact could be related to the delay of leaf senescence of plants sprayed with brassinosteroids in the whole canopy. From the 4th day on, BRWC and BRL1 presented the largest increases in height increment compared to the other treatments (Fig. 1b).

In the analysis of leaf greenness, leaves L1 did not show significant differences between the treatments (C, BRWC, BRL1, BRL2 and BRL3) (Fig. 2a). Lower levels of leaf greenness (leaves L1) were detected 25 days after treatment (DAT) (approximately 42 SPAD units), corresponding to the onset of leaf senescence process. In leaves L2 (Fig. 2b) there was

little influence of BRs in leaf greenness but in leaves L3 (Fig. 2c), when BRs were applied in the whole-canopy, this special leaf remained greener than the leaves from the other treatments. L2 and L3 leaves underwent abscission when SPAD readings were nearly 30–45 units.

The adherence of L1, L2 and L3 leaves to the mother plant varied along the experiment. The natural chronological order of leaf abscission was verified first for L3, followed by abscission of L2 and then L1. The leaves L1, the younger ones, were maintained during 29 days of observation (Fig. 2d). All the older leaves L2 and L3 from treatments without BRs were abscissed after 29 and 24 days, respectively, while the majority of leaves from BR-applied plants were still adhered to the mother-plant in these days (Fig. 2e, f).

Fig. 3 Papaya (*Carica papaya* L.) plant images submitted ▶ to the application of brassinosteroids in three phases of the study (1, 10 and 21 days after treatments, DAT). Treatments: I (irrigated; **a**, **b**, **c**), BRWC (BR in the whole-canopy, **d**, **e**, **f**), BRL1 (BR on the leaf 1, **g**, **h**, **i**), BRL2 (BR on the leaf 2, **j**, **k**, **l**), BRL3 (BR on the leaf 3, **m**, **n**, **o**). L1 (the youngest fully expanded leaf), L2 (the second more mature in relation to L1) and L3 (the third more mature in relation to L2)

All leaves L2 were present until 22 DAT (Fig. 2e). In relation to L3, all treatments presented five leaves until 19 DAT but only BRWC treatment presented five leaves at the end of the experiment (Fig. 2f). In the 17th DAT, we observed a significant difference between BRWC and the other treatments.


Fig. 2 Leaf Greenness (SPAD readings) of papaya (*Carica papaya* L.) leaves in plants submitted to brassinosteroids (BR) application: C (Control), BRWC (BR whole-canopy), BRL1 (BR on leaf 1), BRL2 (BR on leaf 2), BRL3 (BR on leaf 3). **a** Leaf 1 greenness; **b** Leaf 2 greenness; **c** Leaf 3 greenness; **d** Number of leaves 1 in each treatment; **e** Number of leaves 2 in each treatment **f** Number of leaves 3 in each treatment Leaf 3.

*The means were not significantly different. **The means were not significantly different from the first until the 26th day of measurements. ***The means were not significantly different from the first until the 14th day of measurements. Means followed by the same letter are not statistically different from each other in relation to the same day of analysis (Tukey test, 5%)

1 DAT   10 DAT   21 DAT
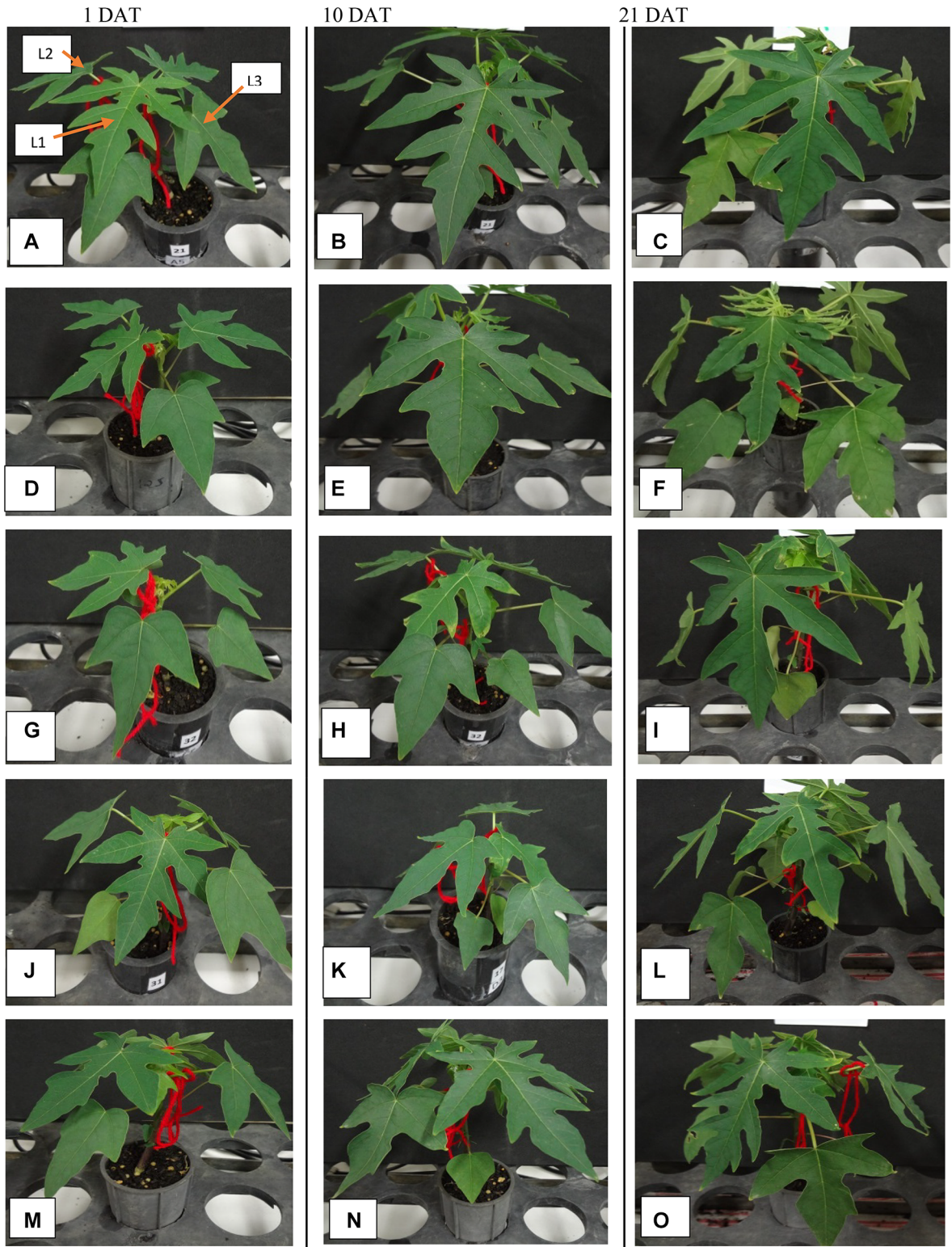
Multiple images captured for the observation of senescence and the leaf abscission process illustrate the treatments in three phases: at the first, the tenth and 21st DAT (Fig. 3a–o). Control plants corresponded to the development of the plants without interference of brassinosteroids (Fig. 3a–c). BRWC showed leaf hyponasty but this treatment showed the greatest increase in height compared to the other treatments (Fig. 3d–f). BRL1, BRL2 and BRL3 did not show any difference related to growth when compared to plants from control treatment (C).

Hyponasty is characterized by the further abaxial leaf cell development in relation to the adaxial part. The hyponastic phenomenon was observed at 10 DAT on the newly leaves formed in BRWC plants (Fig. 4a–d). It is important to note that this phenomenon was only visible in the leaves launched after the application of BRs. Hyponastic characteristics appeared only in the leaves emitted after BR application in the whole-canopy, that is, this phenomenon occurred only in BRWC. The leaves that had these characteristics were too small and plants showed only leaf primordia when BR was applied. When these hyponastic leaves were totally expanded, newest leaves were emitted from the shoot apex but they did not present this hyponastic pattern. The spraying of BR probably caused the absorption of this compound by the shoot apex, including the meristem and leaf primordia. However, it is not possible to assert whether meristematic cells were responsible for originating leaves with hyponastic pattern or pre-existing leaf primordia received stimuli for hyponastic growth. In plants from the other treatments we did not observe this hyponastic phenomenon.

4 Discussion

The application of BRs in the whole-canopy stimulated a greater growth in height of papaya plants. The promoter role of BR on plant growth is well known (Ali 2017; Vardhini 2017). In mung bean (*Vigna radiata*), for example, the application of BR by root inoculation or foliar spraying promoted the increase in height (Sana and Rahman 2014). Liu et al. (2014) have even verified BR-promoting effect in the growth of canola plants subjected to salt stress and the application of BRs analogues (24-epiBL and 28-homoBL) enhanced the leaf number, leaf area, and dry weight of leaves of coleus (Swamy and Rao 2011).

Fig. 4 a, **b** Development of young leaves 10 days after BR application. L1 (leaf 1) **c** Hyponastic growth observed in younger leaves from BRWC treatment (Irrigated/BR in the whole-canopy) 16 days after BR application. **d** Side view of hyponastic growth in papaya leaf BRWC treatment, 16 days after BR application
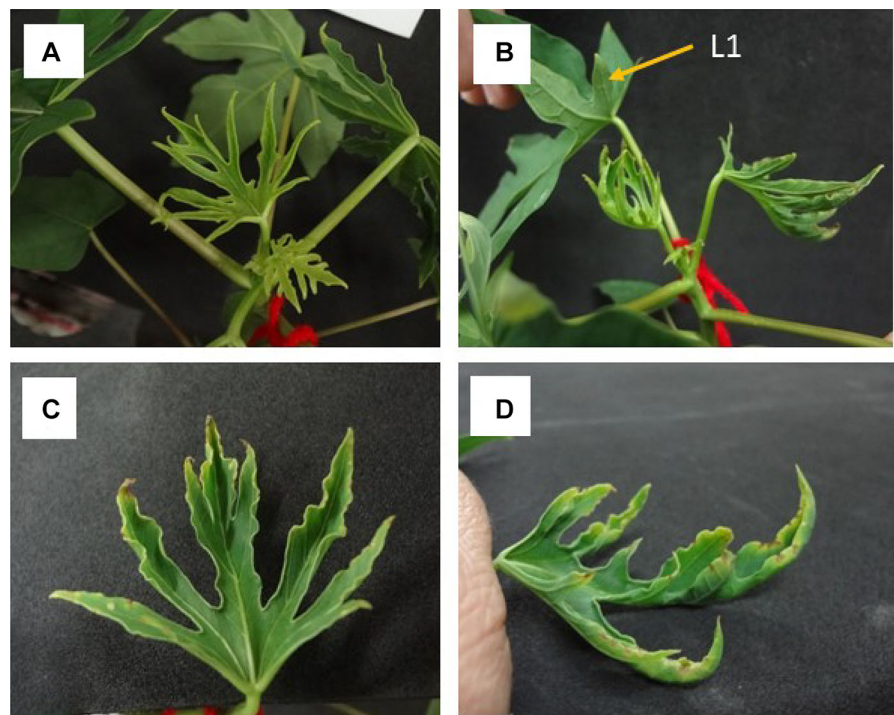
Auxin, GAs and brassinosteroids promote increased cell size as well as proliferation (Hepworth and Lenhard 2014). As shoot organs are derived from different layers of the apical meristem, it seems that there is a tissue type hierarchy in controlling growth and the epidermis both limits and drive growth via local BRs signaling (Hepworth and Lenhard 2014). PSK is an extracellular soluble peptide supposed to participate in cell-to-cell communication and controls root and shoot growth. This peptide in the epidermis promotes cell expansion throughout leaves and roots, acting via BR-signaling (Hepworth and Lenhard 2014). Hartmann et al. (2013) suggested that PSK perception in a subset of epidermal cells is sufficient to drive PSK-dependent elongation growth and PSK receptor proteins show great structural similarity to BRI1 (BR receptor). These results suggest that growth after BR application in papaya should be directly related to this promotive effect in shoot apex cells. The application of BRs in pineapple (*Ananas comosus*) plantlets also caused an increase in the number of leaves and the development of axillary buds and stem sections (Freitas et al. 2012).

In papaya, new leaves are always being formed while older leaves senesce and undergo abscission. Papaya plants presented approximately an average of 8–10 leaves but we noticed that BR application in the whole-canopy caused the maintenance of older leaves for a longer time so the plants of this treatment had more leaves than the others ones in the same juvenile stage. Although Gomes et al. (2013) have already observed that BR application in papaya plants delayed the senescence, in this work the application made in a single leaf was not sufficient to promote or delay senescence although control plants (without BR application) started to lose older leaves before any other BR-applied plants.

As yellowing is the first sign of leaf senescence, the breakdown of chlorophyll molecules has served as one of the main factor to study this plant developmental process (Rogers and Munné-Bosch 2016). BRs applied in the whole-canopy influenced the leaf greenness of older leaves. Many researchers have identified some genes and transcription factors related to the effect of brassinosteroids in leaf senescence. The brassinosteroids are involved in chlorophyll degradation as they suppress the expression of a large set of senescence-related transcription factor genes (Chung et al. 2014). It is likely that the effect of

brassinosteroids in the maintenance of green colour of older leaves may be related to the interaction of this hormone with other plant hormones such as cytokinins that regulate the content of chlorophyll. Bajguz and Niczyporuk (2014) found that the synergistic action of cytokinins with BRs provided a considerable increase in the number of cells, metabolites accumulation and an increase in chlorophyll content of *Chlorella vulgaris*. Furthermore, Yoshizawa et al. (2014) verified that BRs regulate plant organs and chloroplast development in Arabidopsis. However, these authors noticed that many points on the relationship between BR signaling and chloroplast development remain unknown. Otherwise, these authors have proven that BPG3, which is a protein responsible for regulating the chloroplast photosynthesis, may exert their function by BRs signaling. In our research, the effect of brassinosteroid was more pronounced in leaf longevity than in leaf greenness.

One of the most striking findings of our research was the hyponasty verified in newly developed leaves from plants whose shoot was BR-sprayed. The leaf morphology can change in response to different biotic and abiotic stimuli (salt and flooding for example), ranging from changes in leaf orientation to leaf epinasty (downward curvature) and hyponasty (upward curvature) (Sandalio et al. 2016). Leaf epinasty and hyponasty involve leaf curvature as a result of the effect of differential growth on the adaxial and abaxial surfaces of leaves. Plant hormones, such as ethylene, abscisic acid and brassinosteroids, also regulate epinasty and hyponasty (Sandalio et al. 2016). Changes in microtubule orientation are associated with the hyponasty of petioles and tropisms and have proven to be regulated by IAA and ethylene (Polko et al. 2012). Microtubules appear to guide the cellulose synthase complex involved in the specific deposition of microfibrils on the cell wall. Ethylene is a key regulator of hyponastic growth, which is employed by plants to cope with biotic and abiotic stresses. Some genes like *ROT3* modulates the movement of the petiole induced by ethylene and this function is BR-induced; a mutation in *ROT3* reduces hyponastic growth leading to impairment of local cell expansion and inhibition of BR biosynthesis, indicating that hyponastic growth induced by ethylene is mainly regulated by BR (Polko et al. 2013).

Other hormones like auxins are also involved in hyponasty as changes in growth patterns are controlled

by alterations in auxin accumulation and/or gradients (Enders and Strader 2015). In *Arabidopsis*, hyponasty of leaf blades in the perpendicular direction to the petiole axis can also be caused by auxin, which is responsible for cell elongation (Vandenbussche and Straeten 2004). As BRs act synergistically with auxin, and BRs play a contributory role to the hyponastic phenomenon (Keuskamp et al. 2011; Van Zanten et al. 2009), this leads to the conclusion that BR could be responsible for the hyponastic leaves observed in papaya plants. In addition, BRs were identified to control leaf erectness in monocots (Sun et al. 2015) by inhibiting the proliferation of a specific abaxial cell population in the rice lamina joint regions. If BRs control leaf erectness in rice, which involves specific cyclins, it is possible that leaf hyponasty verified in papaya leaves may be controlled by BRs as well.

Although we found no evidence of hyponastic leaves in our previous work (Gomes et al. 2013), it is important to report that the plants of the present work were in the early juvenile stage of plant development, thereby having only primary growth. In our previous work the plants were already developed, with secondary growth, probably interfering with BR-absorption in the shoot apex. Besides, the total amount of BRs used was also different. As there are studies showing dynamic interactions between BR and gibberellins related to growth regulation and its dependence on the developmental stage of the seedling in Arabidopsis (Stewart Lilley et al. 2013), it should occur in all plants and it could explain why the newly developed leaves of juvenile plants of the present study presented hyponastic growth. Anyway, Zhiponova et al. (2013) showed a complex impact of BR on organ growth in leaves, where the ratio between the number of cells and their size varied when BR signaling and BR biosynthesis were stimulated, with the former promoting cell proliferation and the latter cell differentiation. These authors concluded that the balance between cell proliferation and differentiation in a temporal and spatial manner depends on BR levels and signaling.

We concluded that brassinosteroids do not interfere with the senescence of the youngest leaves. However, in older leaves, the application of BRs in the whole-canopy resulted in the delay of senescence as well leaf abscission. So, the delay on senescence process and leaf abscission in papaya plants, stimulated by exogenous application of brassinosteroids, are

directly related to leaf age. However, BR-application in specific single leaves was not successful to promote or inhibit leaf senescence. In newly developed leaves, brassinosteroids promoted hyponastic growth. This study was the first report on the effect of brassinosteroids in hyponastic growth of papaya leaves.

References

Ali B (2017) Practical applications of brassinosteroids in horticulture—some field perspectives. Sci Hort 225:15–21

Bajguz A, Niczyporuk AP (2014) Interactive effect of brassinosteroids and cytokinins on growth, chlorophyll, monosaccharide and protein content in the green alga *Chlorella vulgaris* (Trebouxiophyceae). Plant Physiol Biochem 80:176–183

Breeze E, Harrison E, McHattie S, Hughes L, Hickman R, Hill C, Kiddle S, Kim YS, Penfold CA, Jenkins D et al (2011) High-resolution temporal profiling of transcripts during Arabidopsis leaf senescence reveals a distinct chronology of processes and regulation. Plant Cell 23:873–894

Campostrini E, Glenn DM (2007) Ecophysiology of papaya: a review. Braz J Plant Physiol 19:413–424

Can S, Amasino RM (1997) Making sense of senescence-molecular genetic regulation and manipulation of leaf senescence. Plant Physiol 113:313–319

Chung Y, Kwon SI, Choe S (2014) Antagonistic regulation of Arabidopsis growth by brassinosteroids and abiotic stresses. Mol Cells 37(11):795–803

Enders TA, Strader LC (2015) Auxin activity: past, present, and future. Am J Bot 102:1–17

Fariduddin Q, Ahmad A, Hayat S (2004) Responses of *Vigna radiata* to foliar application of 28-homobrassinolide and kinetin. Biol Plant 48:465–468

Freitas SJ, Santos PC, Carvalho AJC, Berilli SS, Gomes MMA (2012) Brassinosteroid and nitrogen fertilization on growth and nutritional status of seedlings from pineapple sectioning stem. Rev Bras Frutic 34:612–618

Gomes MMA (2011) Physiological effects related to brassinosteroid application in plants. In: Hayat S, Ahmad A (eds) Brassinosteroids: a class of plant hormone. Springer, Dordrecht, pp 193–242

Gomes MMA, Netto AT, Campostrini E, Bressan-Smith R, Zullo MAT, Ferraz TM, Siqueira LN, Leal NR, Núñez-Vázquez M (2013) Brassinosteroid analogue affects the senescence in two papaya genotypes submitted to drought stress. Theor Exp Plant Phys 25:186–195

Guiboileau A, Sormani R, Meyer C, Masclaux-Daubresse C (2010) Senescence and death of plant organs: nutrient recycling and developmental regulation. C R Biol 333:382–391

Hartmann J, Stührwhldt N, Dahlke RI, Sauter M (2013) Phytosulfokine control of growth occurs in the epidermis, is

likely to be non-cell autonomous and is dependent on brassinosteroids. Plant J 73:579–590

He YJ, Xu RJ, Zhao YJ (1996) Enhancement of senescence by epibrassinolide in leaves of mung bean seedlings. Acta Phytophysiol Sin 22:58–62

He Y, Tang W, Swain JD, Green AL, Jack TP, Gan S (2001) Networking senescence-regulating pathways by using Arabidopsis enhancer trap lines. Plant Physiol 126:707–716

Hepworth J, Lenhard M (2014) Regulation of plant lateral-organ growth by modulating cell number and size. Curr Opin Plant Biol 17:36–42

Jiménez VM, Mora-Newcomer E, Gutiérrez-Souto MV (2014) Biology of the papaya plant. In: Ming R, Moore PH (eds) Plant genetics and genomics: crops and models. Springer, New York, pp 17–33

Keuskamp DH, Sasidharan R, Vos I, Peeters AJM, Voesenek LACJ, Pierik R (2011) Blue-light-mediated shade avoidance requires combined auxin and brassinosteroid action in Arabidopsis seedlings. Plant J 67:208–217

Li JM, Nagpal P, Vitart V, McMorris TC, Chory J (1996) A role for brassinosteroids in light dependent development of Arabidopsis. Science 272:398–401

Lima RSN, Figueiredo FAMMA, Martins AO, Deus BCS, Ferraz TM, Gomes MMA, Sousa EF, Glenn DM, Campostrini E (2015) Partial rootzone drying (PRD) and regulated deficit irrigation (RDI) effects on stomatal conductance, growth, photosynthetic capacity, and water-use efficiency of papaya. Sci Hortic 183:13–22

Liu J, Gao H, Wang X, Zheng Q, Wang C, Wang X, Wang Q (2014) Effects of 24-epibrassinolide on plant growth, osmotic regulation and ion homeostasis of salt-stressed canola. Plant Biol 16:440–450

Ming R, Yu Q, Blas A, Chen C, Na JK, Moore PH (2008) Genomics of papaya, a common source of vitamins in the tropics. In: Moore PH, Ming R (eds) Genomics of tropical crop plants. Springer, New York, pp 405–420

Polko JK, van Zanten M, van Rooij JA, Marée AFM, Voesenek LACJ, Tarkowská D, Strnad M, Voesenek LACJ, Peeters AJM, Pierik R (2012) Ethylene-induced differential petiole growth in Arabidopsis thaliana involves local microtubule reorientation and cell expansion. New Phytol 193:339–348

Polko JK, Pierik R, van Zanten M, Tarkowská D, Strnad M, Voesenek LACJ, Peeters AJM (2013) Ethylene promotes hyponastic growth through interaction with ROTUNDIFOLIA3/CYP90C1 in Arabidopsis. J Exp Bot 64:613–624

Ribeiro Junior JI (2001) Análises estatísticas no SAEG. Universidade Federal de Viçosa, Viçosa, MG

Rogers H, Munné-Bosch S (2016) Production and scavenging of reactive oxygen species and redox signaling during leaf and flower senescence: similar but different. Plant Physiol 171:1560–1568

Sağlam-Çağ S (2007) The effects of epi-brassinolide on senescence in wheat leaves. Biotechnol Biotechnol Equip 21:63–65

Sana NH, Rahman A (2014) Comparative changes in metabolism of Vigna radiata by foliar and root application of brassinolide at different concentrations. Int J Plant Physiol Biochem 6:56–65

Sandalio LM, Rodríguz-Serrano M, Romero-Puertas MC (2016) Leaf epinasty and auxin: a biochemical and molecular overview. Plant Sci 253:187–193

Schippers JHM (2015) Transcriptional networks in leaf senescence. Curr Opin Plant Biol 27:77–83

Schippers JHM, Schmidt R, Wagstaff C, Jing HC (2015) Living to die and dying to live: the survival strategy behind leaf senescence. Plant Physiol 169:915–930

Stewart Lilley JL, Gan Y, Graham IA, Nemhauser JL (2013) The effects of DELLAs on growth change with developmental stage and brassinosteroid levels. Plant J 76:165–173

Sun S, Chen D, Li X, Qiao S, Shi C, Li C, Shen H, Wang X (2015) Brassinosteroid signaling regulates leaf erectness in Oryza sativa via the control of a specific u-type cyclin and cell proliferation. Dev Cell 34:220–228

Swamy KN, Rao SSR (2011) Effect of brassinosteroids on the performance of coleus (Coleus forskohlii). J Herbs Spices Med Plants 17:12–20

Van Zanten M, Millenaar FF, Cox MCH, Pierik R, Voesenek LACJ, Peeters AJM (2009) Auxin perception and polar auxin transport are not always a prerequisite for differential growth. Plant Signal Behav 4:899–901

Vandenbussche F, Straeten DVD (2004) Shaping the shoot: a circuitry that integrates multiple signals. Trends Plant Sci 9:500–505

Vardhini BV (2017) Modifications of morphological and anatomical characteristics of plants by application of brassinosteroids under various abiotic stress conditions—a review. Plant Gene 11:70–89

Yoshizawa E, Kaizuka M, Yamagami A, Takeuchi MH, Matsui M, Kakei Y, Shimada Y, Sakuta M, Osada H, Asami T, Nakano T (2014) BPG3 is a novel chloroplast protein that involves the greening of leaves and related to brassinosteroid signaling. Biosci Biotechnol Biochem 78:420–442

Zhiponova MK, Vanhoutte I, Boudolf V, Betti C, Dhondt S, Coppens F, Mille E, Maes S, González-García MP, Cano-Delgado AI, Inzé D, Beemster GTS, Veylder L, Russinova E (2013) Brassinosteroid production and signaling differentially control cell division and expansion in the leaf. New Phytol 197:490–502