

## SCIENTIFIC ARTICLE

# Analysis of exogenous auxin and cytokinin action in overcoming root restriction in green and variegated Benjamin fig

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## Abstract

Green and variegated Benjamin fig (*Ficus benjamina*) often suffer from root restriction when grown in pots. While exogenous cytokinin applications have proven effective in reversing this stress, the possibility that exogenous auxins, either alone or in combination with cytokinin, may also be helpful has received little attention. In this work, we analyse the response of green and variegated *Ficus benjamina* rooted cuttings growing in small pots to exogenous supply of auxin and cytokinin at different concentrations, either in single or combined applications. Our results show that both benzyl aminopurine (BAP) and indole acetic acid (IAA), at the highest concentration tested (100 mg L<sup>-1</sup>) increased leaf development and plant biomass accumulation in green and variegated *Ficus* genotypes. However, exogenous IAA and BAP appeared to elicit differential plant morpho-physiological responses. While BAP tended to enhance leaf appearance more than IAA did, the latter promoted leaf expansion in a steadier manner than BAP, thus resulting in plants with less, but larger, leaves than those treated with cytokinin. Despite these differences in plant architecture, regression analysis suggests that hormonal-induced growth promotion was solely attributable to enhanced carbon assimilation. Rather unexpectedly, IAA promoted net assimilation and photosynthesis rates at least as effectively as cytokinin. Possible mechanisms involved in growth and development promotion by exogenous application of both hormones are discussed. Auxin treatment may help overcome root restriction in *Ficus* as effectively as cytokinin in terms of growth promotion, although differences in plant architecture may arise as compared with plants sprayed with the latter hormone.

**Keywords:** *Ficus benjamina*, growth regulators, ornamental foliage plants.

## Resumo

*Ficus Benjaminina* (*Ficus benjamina*) verde e variegado geralmente sofre de restrição no crescimento das raízes quando cultivado em vasos. Embora as aplicações de citocinina exógena tenham se mostrado eficazes na reversão desse estresse, a possibilidade de que auxinas exógenas, sozinhas ou em combinação com citocinina, também possam ser benéficas tem recebido pouca atenção. Neste trabalho, foi analisada a resposta de estacas enraizadas de *Ficus benjamina* verdes e variegadas cultivadas em pequenos vasos, em relação ao fornecimento exógeno de auxina e citocinina em diferentes concentrações, em aplicações únicas ou combinadas. Nossos resultados mostram que tanto a benzilaminopurina (BAP) quanto o ácido indol-acético (IAA), na maior concentração testada (100 mg L<sup>-1</sup>), aumentaram o desenvolvimento foliar e o acúmulo de biomassa vegetal nos genótipos de *Ficus* verde e variegado. No entanto, AIA e BAP exógenos parecem provocar respostas morfofisiológicas diferenciadas nas plantas. O uso de BAP teve a tendência de melhorar a aparência das folhas mais do que AIA, sendo que este promoveu a expansão foliar de maneira mais estável do que o BAP, resultando em plantas com menos folhas, porém maiores, do que as tratadas com citocinina. Apesar dessas diferenças na arquitetura da planta, a análise de regressão sugere que a promoção do crescimento induzida por hormônios foi atribuída exclusivamente ao aumento da assimilação de carbono. De forma bastante inesperada, o AIA promoveu assimilação líquida e taxas de fotossíntese pelo menos tão efetivamente quanto a citocinina. Possíveis mecanismos envolvidos na promoção do crescimento e desenvolvimento pela aplicação exógena de ambos os hormônios são discutidos. O tratamento com auxina pode ajudar a superar a restrição radicular em *Ficus* de forma tão eficaz quanto a citocinina em termos de promoção de crescimento, embora possam surgir diferenças na arquitetura da planta em comparação com plantas pulverizadas com o último hormônio.

**Palavras-chave:** *Ficus benjamina*, plantas ornamentais, reguladores de crescimento

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<https://doi.org/10.1590/2447-536X.v29i1.2502>

Received Mar 30, 2022 | Accepted Mar 09, 2023 | Available online Mar 30, 2023

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Area Editor: Ana Maria Mapeli

## Introduction

Benjamin fig (*Ficus benjamina*) is an important foliage plant, comprising green and variegated genotypes. It is usually grown in pots for ornamental purposes (Shih et al., 2019) and may thus suffer from root restriction at early growth stages. In a previous report (Di Benedetto et al., 2020a) we analyzed the effect of spraying the cytokinin benzyl aminopurine (BAP) on growth and development of both *Ficus benjamina* genotypes grown in pots under different light intensities. BAP sprays promoted carbon fixation, leaf appearance and whole-plant growth in all cases, a result that supports the role of exogenous cytokinins in promoting plant development under conditions that impede root growth (Di Benedetto et al., 2020b; Liu et al., 2022). Cytokinins, which are naturally produced by root apices, promote aerial part development through the control of cell cycle in the shoot apical meristem, and stimulate photosynthesis via different physiological mechanisms as well (Cortleven et al., 2019).

While exogenous cytokinin application may represent the most straightforward manner of overcoming root restriction, the possibility that auxin, another class of plant hormone that plays a key role in shoot and root development (Du et al., 2020) could at least partially fulfill this goal has rarely been investigated. Auxins are mainly synthesized in young leaves (Paque and Weijers, 2016) where they promote cell expansion (Du et al., 2020) and move at long distances through the phloem towards the roots, where they promote branching (Meier et al., 2020). Auxin biosynthesis in the aerial part could be negatively affected in plants subjected to root restriction in which, due to low cytokinin levels, young leaves are scarce and small. There is some evidence suggesting auxins may help promote whole-plant growth in plants grown in small pots (Pal, 2019). Working on the ornamental foliage plant *Epipremnum aureum*, Di Benedetto et al. (2015) observed a promotion of leaf growth and biomass accumulation after spraying plants with auxin, either alone or combined with cytokinin. Similar responses were later reported by Molinari et al. (2020), which worked on pot-grown *Impatiens* “New Guinea”.

The possible contribution of auxins in overcoming root restriction may rely on different mechanisms (Liu and von Wirén, 2022). It may be hypothesized that auxin could either directly promote cell expansion and division in leaves, thus driving whole-plant growth, or they might act in an indirect manner. Because of its well-known effect on root branching, auxin could increase the number of root apices, which are in turn potential sites of cytokinin production (Rivas et al., 2022). In the latter case, auxin-driven growth promotion would resemble that observed by direct application of cytokinin, this is, increased leaf appearance and photosynthesis, as suggested by Di Benedetto et al. (2015). Given the distinct primary roles of both hormones, combined application of them might result in maximum effectiveness in growth promotion, as in several reported synergistic effects (Hurný et al., 2020). Although, due to multiple possible interactions between

these hormones (Schaller et al., 2015), the result of their combined application on pot-grown plants is uncertain.

Exploring the possible use of auxins, either alone or in combination with cytokinin, to overcome root restriction in an important ornamental species (Mallick et al., 2022) including *Ficus benjamina* may be of interest at the crop production level. The aim of this work is to analyse the response of green and variegated *Ficus benjamina* genotypes to auxin both supplied alone or in combination with cytokinin, and compare the response to that obtained solely with cytokinin. Assessing growth responses to the exogenous supply of these hormones besides allowing for improvement of crop management in this species may also provide insights on their inter-relationship at the whole plant level.

## Materials and Methods

The experiment was carried out in a greenhouse located at the Faculty of Agronomy, University of Buenos Aires, Argentina (34° 35' 59" S, 58° 22' 23" W). Rooted cuttings of ‘green’ and ‘variegated’ *F. benjamina* genotypes were obtained from a commercial propagator (Vivero Kogiso, Buenos Aires, Argentina). At the transplant stage, whole-plant fresh biomasses of green and variegated cuttings were not significantly different, having attained 2.06 + 0.10 g (green) or 1.76 + 0.15 g (variegated) per cutting.

Rooted cuttings were transplanted into rigid 1,200-cm<sup>3</sup> plastic pots (one cutting per pot) filled with a 2-2-1 (v/v/v) mix of *Sphagnum maguellanicum* peat: river waste: perlite. Plants were watered daily to saturation and fertilized weekly with N, P, K and Ca fertilizer added to the irrigation water (50 mg L<sup>-1</sup> N) (2:1:2:2 N:P:K:Ca). Seven days after transplant, four groups of plants were sprayed to run-off at sunset with different auxin solutions (0, 5, 50, or 100 mg L<sup>-1</sup> IAA) (Sigma-Aldrich Co., St. Louis, MO, USA). Seven days later, plants under each IAA treatment were sprayed with different cytokinin solutions (0, 5, 50, or 100 mg L<sup>-1</sup> BAP), thus rendering 16 hormone treatments. Both IAA and BAP were first diluted in 80% (v v<sup>-1</sup>) ethanol; no surfactants were used. Plants were arranged into three blocks (10 plants per block per treatment) and distributed at random within each block.

The greenhouse was covered with a black shade cloth to deliver 50% of full-sunlight. Daily mean temperatures (19.73 to 34.90 °C) and daily photosynthetic active radiation (14.94 to 25.71 mol photons m<sup>-2</sup> day<sup>-1</sup>) were recorded with a HOBO sensor (H08-004-02) (Onset Computer Corporation, MA, USA) connected to HOBO H8 data logger.

For destructive measurements, five plants per treatment at 0, 60, 90, and 120 days after transplanting were randomly chosen at each sampling date. At each sampling date, the number of appeared leaves was recorded. Roots were washed and root and shoot fresh weights (FW) were obtained. Dry weights (DW) were assessed after drying roots and shoots to constant weight at 80 °C for 96

hours. The relative leaf area expansion rate (RLAER) was obtained from the lineal regression of the natural logarithm of total leaf area versus time in days, while the rate of leaf appearance (RLA) was obtained from the linear regression of total number of appeared leaves versus time in days. The relative growth rate (RGR), mean net carbon assimilation rate (NAR), specific leaf area (SLA), leaf area ratio (LWR) and root: shoot allometries were calculated according to Di Benedetto and Tognetti (2016).

The net photosynthetic rate was measured at ambient O<sub>2</sub> and CO<sub>2</sub> concentrations at a saturating photon flux density (>1,700 μmol photons m<sup>-2</sup> s<sup>-1</sup>) between 11.30-13.00 hours on a sunny day just before final harvest. The youngest fully expanded leaf of three plants from each treatment was selected for measurements, which were performed using a portable LICOR LI6200 photosynthetic system (LI-COR Inc., Lincoln, NE, USA).

Samples of young, fully expanded leaves were collected to examine leaf thickness on the final harvest date (120 d from transplanting). Tissue from the middle region of each lamina was fixed in a mixture of 70% (v v<sup>-1</sup>) ethanol, 5% (v v<sup>-1</sup>) formalin, 5% (v v<sup>-1</sup>) glacial acetic acid, and 20% (v v<sup>-1</sup>) distilled water prior to dehydration in an ethanol and tert-butyl alcohol series. Fixed tissues in paraffin were sectioned (10 -12 μm thick) on a rotary microtome and stained with safranin-crystal violet-fast green. The data

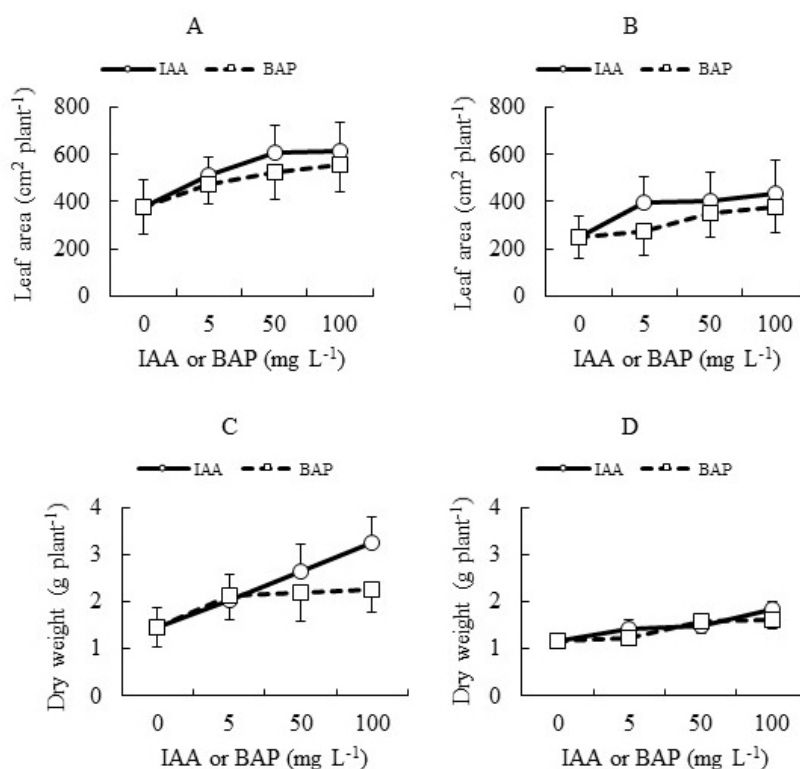
presented are the means of three leaves per treatment, using ten cross-sections per leaf (n = 3). Quantitative anatomical data were obtained using Image Pro Express Version 6.0 (Media Cybernetics, Rockville, MD, USA).

The experiment was arranged in a 16-way factorial design. Data were subjected to two-way analysis of variance (ANOVA) using STATISTICA 8 (Statsoft) software. The assumptions of ANOVA were checked, and means were separated by Tukey's test (p ≤ 0.05). Slopes from straight-line regressions were tested using the SMATR package.

## Results and Discussion

### Dose-response effects of single hormone treatments

Exogenous supply of both IAA and BAP at the highest concentration, significantly increased total leaf area (Figure 1A) and dry weight (Figure 1C) of the green genotype at harvest. In the case of variegated plants (Figure 1B and D), leaf area and dry weight accumulation took place at lower rates and differences between hormone treatments and controls were smaller. Nevertheless, significant differences were again found for the final harvest. Although the participation of auxins and cytokinins in the control of the root system has been previously documented, our results are consistent with what is known about the action of both hormones (Hussain et al., 2021).

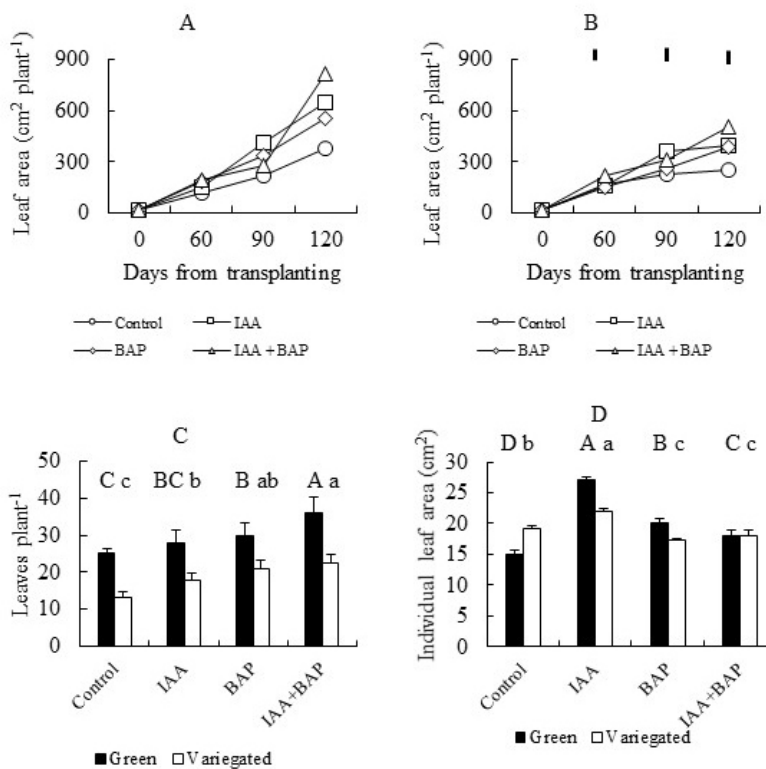


**Figure 1.** Leaf area expanded (Figures A-B) and dry weight accumulated (C-D) at the end of the experiment according to IAA or BAP concentration applied for green (Figures A-C) and variegated (B-D) *Ficus benjamina* genotypes. Vertical lines indicate standard errors.

### Leaf area accumulation

During the experimental period, green *Ficus* plants accumulated leaf area in an exponential fashion. In the green genotype, exogenous supply of both IAA and BAP at the highest concentration, either alone or in combined treatments, significantly increased total leaf area at harvest (Figure 2A). In the case of variegated plants

(Figure 2B), leaf area accumulation took place at lower rates and differences between hormone treatments and controls were smaller, although significant differences were again found for the final harvest. Hormone treatments had an impact on both leaf number (Figure 2C) and individual leaf area (Figure 2D) of both genotypes.



**Figure 2.** Total leaf area of green (A) or variegated (B) and number of leaves per plant at final harvest (C) and mean individual leaf area (D) of *Ficus benjamina* sprayed with either IAA or BAP at 100 mg L<sup>-1</sup> or the combination of both (IAA + BAP), one week after transplant stage. Controls were sprayed with water. Vertical lines indicate least significant differences (LSD). Different capital letters and lower case letters indicate significant differences ( $P < 0.05$ ) between treatments for green and variegated *Ficus* respectively.

The exponential nature of leaf growth during the experimental period allowed for the calculation of mean rates of leaf area accumulation, RLAER (Di Benedetto and Tognetti, 2016). In the green genotype, all hormone treatments significantly increased mean RLAER in comparison with controls, being single IAA sprays the most effective treatment. In the variegated

genotype, again the single IAA spray was the most effective treatment, followed by the combined hormone treatment, while spraying solely with BAP did not significantly promote RLAER (Table 1). Changes in RLAER may be due to variation in leaf number or size. Similar results were observed for the rate of leaf appearance (Table 1).

**Table 1.** Changes in RLAER and RLA in *Ficus benjamina* plants sprayed with either IAA or BAP at 100 mg L<sup>-1</sup>, or the combination of both (IAA + BAP), one week after transplant stage. Controls were sprayed with water. Different lower-case letters indicate significant differences ( $P < 0.05$ ).

| Treatments | RLAER (cm cm <sup>-2</sup> day <sup>-1</sup> ) |            | RLA (leaves day <sup>-1</sup> ) |            |
|------------|--|------------|---------------------------------|------------|
|            | Green  | Variigated | Green                           | Variigated |
| Control    | 0.031c   | 0.024c     | 0.209c                          | 0.107c     |
| IAA        | 0.038a   | 0.035a     | 0.249bc                         | 0.145b     |
| BAP        | 0.034b   | 0.026c     | 0.262b                          | 0.167a     |
| IAA+BAP    | 0.031c   | 0.030b     | 0.289a                          | 0.174a     |

Cytokinins are known to promote leaf appearance due to their effect on the shoot apical meristem, which increases in size and in the rate on cell division, resulting in a shortening of the plastochron in response to higher levels of this hormone (Di Benedetto et al., 2020b) and subsequent breakdown of apical dominance (Gao, 2020). The leaf number increase may be the consequence of a lower phyllochron or an increased branching, or both. No attempt was done to record branching in the present work.

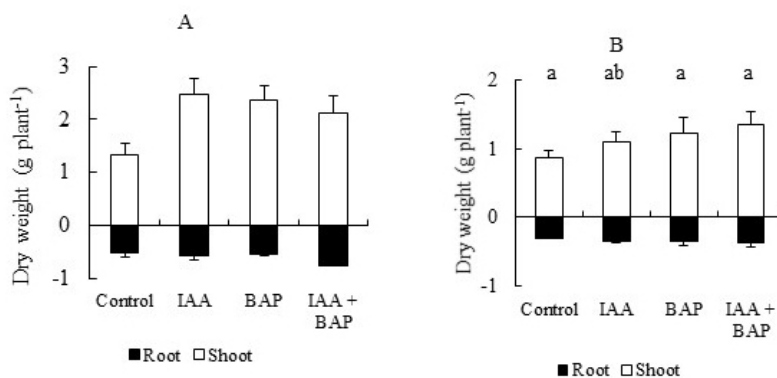
The fact that IAA also promoted leaf number, which is consistent with general auxin effects reported by Xiong and Jiao (2019) could be due to either a direct effect on the meristem (Pernisová and Vernoux, 2021), or an indirect effect, through the promotion of root branching, being root tips a source of cytokinin. In any case, spraying both hormones in combined IAA + BAP applications led to the highest promotion of leaf appearance (Figure 2).

Leaf size followed a different pattern than leaf number. Single IAA application was the most effective treatment in increasing mean individual leaf area at final harvest in both genotypes. This is consistent with the well-known role of auxins in the promotion of cell expansion (Du et al., 2020). In green *Ficus*, single-BAP application resulted only in a moderate increase in leaf area, while in variegated *Ficus* cytokinin treatment did not increase leaf

area at all. In turn, the combined IAA + BAP treatment was the least effective one regarding changes in leaf size, in both genotypes. The reason of this contrasting response between single IAA and combined IAA + BAP treatments is unknown, but it might be hypothesized that promotion of leaf development by BAP could result in increased local synthesis of auxin, being this hormone mainly synthesized in young leaf tissue (Paque and Weijers 2016). Thus, a supra optimal auxin concentration might be reached in plants subjected to combined IAA + BAP treatment. Clearly, this hypothesis should be addressed in future studies.

#### Dry weight accumulation and plant growth analysis

In general, dry weight accumulation of green *Ficus* presented a similar fashion than that observed for leaf area accumulation. Lower dry weight accumulation values were observed in variegated plants but again, all hormone treatments led to higher values than controls. In both genotypes, hormone treatments were more effective in promoting growth of the aerial part than that of roots (Figure 3A and B). This is consistent with previous reports regarding action of cytokinins (Di Benedetto et al., 2020a, b) and auxins (Di Benedetto et al., 2015; Molinari et al., 2020; Liu et al., 2022; Liu and von Wirén, 2022).



**Figure 3.** Dry weight at final harvest of green (A) and variegated (B) *Ficus benjamina* sprayed with either IAA 100 mg L<sup>-1</sup> or BAP 100 mg L<sup>-1</sup> or the combination of both. Controls were sprayed with water. Vertical lines indicate standard errors. Different lower case letters indicate significant differences ( $P < 0.05$ ) in total dry weight (roots + shoots).

Plant growth analysis revealed that, in general, RGR of green plants was higher than RGR of variegated ones, and that all hormone treatments significantly promoted RGR of both *Ficus* genotypes (Table 2). Within RGR components, LAR was the less affected one by hormone treatments, which is consistent with our previous reports on BAP-treated pot-grown *Ficus benjamina* (Di Benedetto et al., 2020a). Conversely, the increased RGR due to hormone treatment in both genotypes was associated with higher values of NAR a variable associated with plant photosynthetic rate (Di Benedetto and Tognetti, 2016) (Table 2).

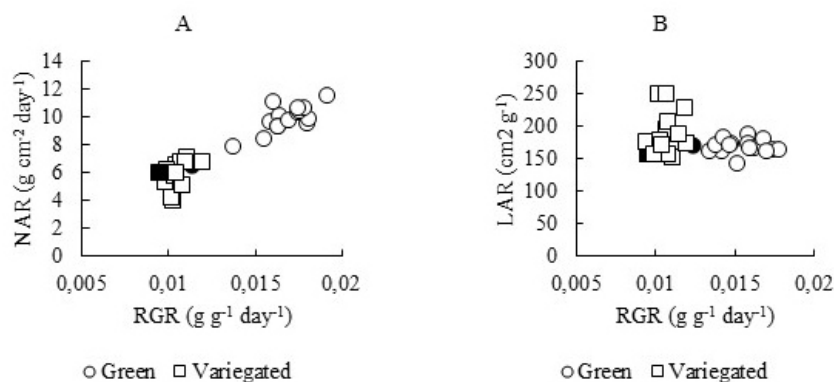
Despite little hormone effects were observed in LAR, the  $\beta$  allometric coefficient showed that partitioning to

roots significantly decreased with hormone treatment (Table 2). This supports the role of exogenous IAA and BAP in promoting growth of the aerial part more than that of roots, which is in agreement with literature (Saravia-Castillo et al., 2022) and with results obtained for LWR (see below).

When data from all treatment combinations (4 IAA- x 4 BAP-concentrations) were included in a regression analysis, it became evident that a single, linear relationship with NAR could explain most variation in RGR ( $r^2 = 0.894$ ) due to hormone sprays in both genotypes (Figure 4A). Conversely, no significant relationship between RGR and LAR was found (Figure 4B).

**Table 2.** Plant growth parameters of *Ficus benjamina* sprayed with either IAA or BAP at 100 mg L<sup>-1</sup>, or the combination of both (IAA + BAP), one week after transplant stage. Controls were sprayed with water. Different lower-case letters indicate significant differences ( $P < 0.05$ ).

| Treatments | RGR<br>(g g <sup>-1</sup> day <sup>-1</sup> ) |            | NAR<br>(g cm <sup>-2</sup> day <sup>-1</sup> )<br>x 10 <sup>-5</sup> |            | LAR<br>(cm <sup>2</sup> g <sup>-1</sup> ) |            | Root:Shoot<br>$\beta$ coefficient |            |
|------------|---|------------|--|------------|---|------------|-----------------------------------|------------|
|            | Green   | Variegated | Green  | Variegated | Green                                     | Variegated | Green                             | Variegated |
| Control    | 0.0113c                                       | 0.0095b    | 6.54c  | 5.98b      | 172.83a                                   | 158.89a    | 0.842a                            | 0.971a     |
| IAA        | 0.0163b                                       | 0.0116a    | 10.73a   | 7.39b      | 174.18a                                   | 156.88a    | 0.530b                            | 0.606c     |
| BAP        | 0.0160b                                       | 0.0117a    | 9.36b  | 7.80a      | 165.95b                                   | 150.03b    | 0.535b                            | 0.679b     |
| IAA+BAP    | 0.0178a                                       | 0.0124a    | 11.08a   | 7.76a      | 164.45b                                   | 159.67a    | 0.529b                            | 0.583c     |



**Figure 4.** Relationship between the net assimilation rate (NAR) and the relative growth rate (RGR) (A), the leaf area ratio (LAR) (B) and RGR for *Ficus benjamina* subjected to different hormone treatments. All hormone combinations (4 IAA x 4 BAP concentrations) were included for this analysis. Black symbols indicate control plants. Regression analysis equation:  $NAR = 620.91 RGR - 0.526$  ( $r^2 = 0.894$ ;  $P \leq 0.001$ ). Regression between LAR and RGR was not significant.

#### Net photosynthetic rate, leaf weight ratio and specific leaf area and leaf thickness

Direct measurement of leaf photosynthetic rate showed a pattern quite similar to that displayed by NAR, with some exceptions (Figure 5A). First, the promoting effect of single IAA spray was greater than that of BAP, which solely exhibited a trend to higher photosynthesis values

but was not significant in either genotype. It must be taken into account, however, that the photosynthetic rate was measured on young leaves, which especially in the case of BAP treatment were just recently expanded and thus could not have yet attained maximum photosynthetic capacity. Combining IAA + BAP resulted in the highest promotion of leaf photosynthetic activity in the green genotype, but

not in the variegated one, which appeared to have reached a plateau under any hormone treatment, similarly to what was observed with NAR.

The high promotion of photosynthesis by exogenous BAP supply was rather unexpected, since most studies underline the role of cytokinin in the promotion of photosynthesis of plants under root restriction, including Ficus (Di Benedetto et al. 2020a). The relationship between cytokinins and photosynthesis is relatively well established (Cortleven et al., 2019). Cytokinin may enhance carbon fixation through the development and maintenance of functional chloroplasts and chlorophyll synthesis (Cackett et al., 2021). Cytokinins are also positively associated with nitrogen and photosynthetic protein concentration in leaves, by regulating the expression of genes involved in N uptake and carbon assimilation as well (Gu et al., 2018).

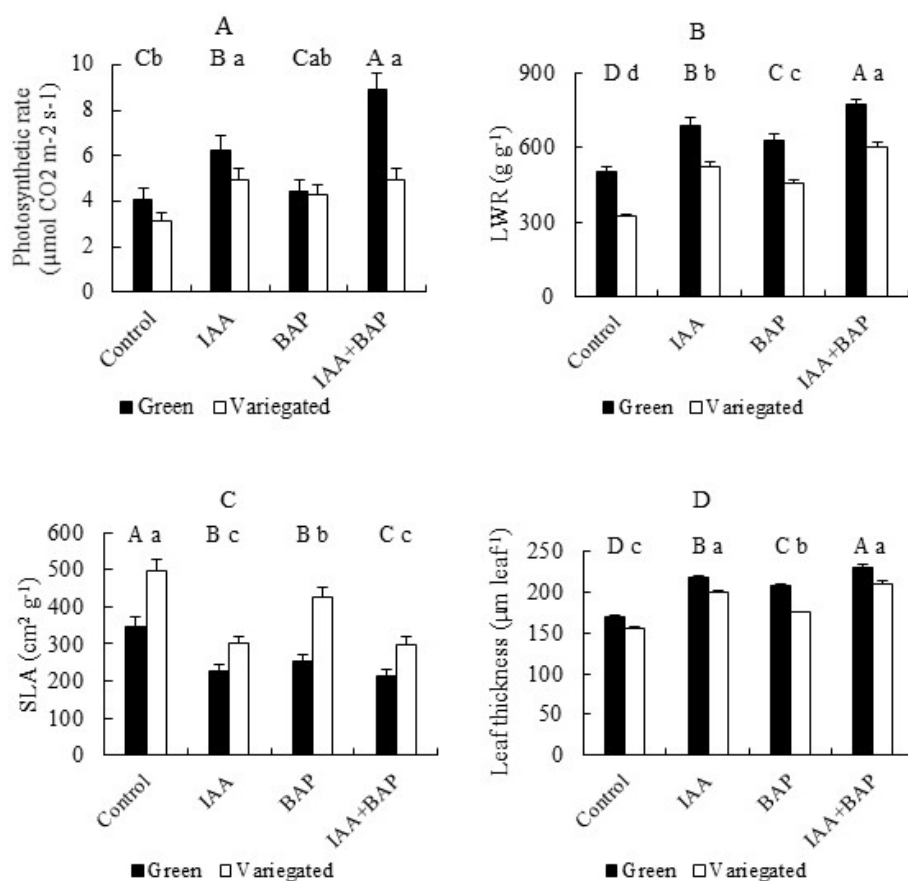
On the other hand, there is much less knowledge about auxin effects on carbon fixation. Nevertheless, recent evidence shows a link between auxins and photosynthesis, mediated both by increased chlorophyll levels and increased leaf venation that helps sustain high carbon fixation levels. Yuan et al. (2019) showed that overexpression of *SIARF6A*, an auxin response factors, increased chlorophyll contents and sizes of chloroplasts in the fruits and leaves of tomato plants, whereas its downregulation decreased chlorophyll contents and number of chloroplasts, compared with those of wild-type plants. In parallel with these effects, overexpression of *SIARF6A* increased the photosynthesis rate. On the other hand, McAdam et al. (2017) reported that mutants at the *Crispoid* (*Crđ*) locus in pea (*Pisum sativum*), which have altered auxin homeostasis and activity in developing leaves, show reduced leaf vein density resulting in a significant reduction in leaf hydraulic conductance and leaf gas exchange. The authors found *Crispoid* to be a member of the *YUCCA* family of auxin biosynthetic genes, and their results linked auxin biosynthesis with maximum photosynthetic rate through leaf venation.

The components of LAR, namely leaf weight ratio (LWR) and specific leaf area (SLA), showed a contrasting response to hormone supply, as shown for final harvest in Figure 5B. While LWR increased when plants of both genotypes were sprayed with either IAA or BAP or the combination of both hormones (Figure 5B), SLA decreased at equivalent proportions (Figure 5C). This explains why LAR values were almost unaffected by hormone treatments. LWR is a measure of biomass partitioning, and its increase after hormone application is consistent with changes

observed in the  $\beta$  allometric coefficient (Table 2) and also with the fact that cytokinin and auxin differentially promote growth of the aerial part, relative to roots (Molinari et al., 2020a).

SLA, in turn, is associated with leaf thickness and with leaf dry matter content (Poorter et al., 2019), and present results are in agreement with earlier work on Ficus and other ornamental foliage species sprayed with BAP and auxins (Di Benedetto et al., 2015; 2018; 2020b; Molinari et al., 2020). Furthermore, the combined hormone treatment appeared as the most effective one for increasing LWR in both genotypes, and for decreasing SLA in the green one, thus suggesting an additive effect of both hormones on this response. It should also be noticed that variegated plants had lower LWR and higher SLA than their green counterparts, which agrees with earlier results from our laboratory (Di Benedetto et al., 2020a), and also that in that genotype IAA was more effective than BAP in increasing LWR, and in decreasing SLA (or in increasing leaf thickness, see below). The higher effectiveness of IAA, as compared with BAP, might be associated with the effect of auxin in promoting cell expansion in young leaves.

In both genotypes, leaf thickness tended to increase in response to exogenous hormone supply (Figure 5D). This is consistent with lower SLA values under the same treatments (Figure 5C). Besides, leaves of the green Ficus ecotype were thicker (and SLA values lower) than those of their variegated counterpart. The promotion of leaf thickness by cytokinins has been previously reported in several ornamental plants (Di Benedetto et al., 2020a, b; Molinari et al., 2020). The increase in size of the meristems by the application of synthetic cytokinins increases the final foliar thickness of the leaves (Fouracre and Poethig, 2019). These changes may also explain in part the promotion of carbon assimilation prompted by cytokinin because of an increased  $\text{CO}_2$  diffusion (Di Benedetto et al., 2018; Gago et al., 2020; Carnelos et al. 2022). Much less information is available about the relationship between auxin and leaf thickness. Antisense mutants of the *AUXIN RESPONSE FACTOR 4* in tomato resulted in a significant reduction of leaf thickness (Bouzroud et al., 2019). Also, previous results from our laboratory showed an increased thickness in IAA-sprayed *Epipremnum aureum* (Di Benedetto et al., 2015) and *Impatiens* "New Guinea" (Molinari et al., 2020), but it is not clear whether this may be considered as a direct effect of the hormone or, alternatively, due to auxin indirectly promoting cytokinin levels through increased



**Figure 5.** Net photosynthetic rate (A), LWR (B), SLA (C) and leaf thickness (D) of *Ficus benjamina* sprayed with either IAA or BAP at 100 mg L<sup>-1</sup> or the combination of both (IAA + BAP), one week after transplant stage. Controls were sprayed with water. Vertical lines indicate standard errors. Different capital letters and lower case letters indicate significant differences ( $P < 0.05$ ) between treatments for the green and variegated *Ficus* respectively. Vertical lines indicate standard errors.

root branching.

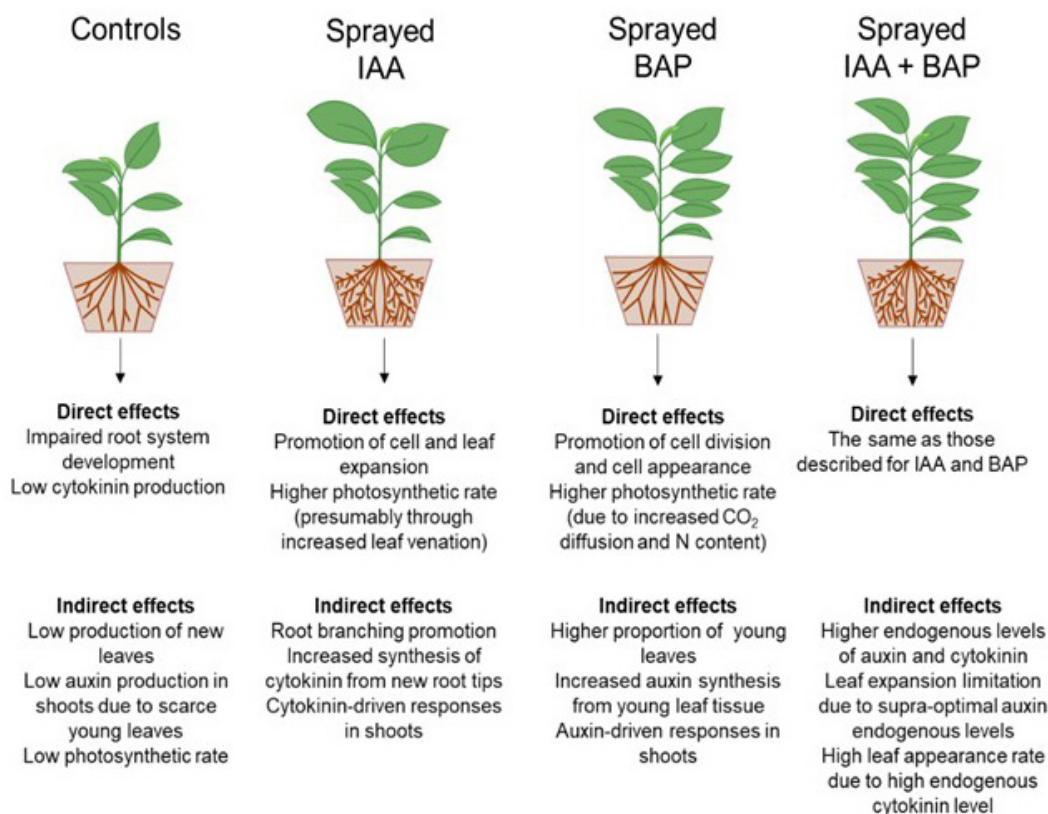
#### An integrative analysis of *Ficus* response to exogenous applications of IAA and BAP

The interpretation of the results presented here, according to which root-restricted *Ficus* responses to exogenous IAA and BAP needs to consider the close inter-relationship of auxin and cytokinin action on plant growth and development. Each of these hormones promotes development of the site of biosynthesis of the other, and therefore they may indirectly favor an increase of the endogenous level of the second hormone. The fact that in the present work auxin mimics many plant responses earlier observed for exogenous cytokinin supply (Di Benedetto et al., 2020a) might be explained in this way. Furthermore, since the promoting action of auxins is known to occur within a relative narrow range of concentrations, supra optimal levels could be reached in plants sprayed with IAA and additionally with BAP, a

direct promoter of development of young leaves, in turn an endogenous source for auxins (Paque and Weijers, 2016; Liu and von Wirén, 2022). This might be the reason of lower leaf area of individual leaves under combined IAA + BAP application, as compared with only IAA spray, in both genotypes (Figure 2B).

This hypothetical auxin-cytokinin interplay, which may explain why for many variables green and variegated *Ficus* responded in a similar manner to exogenous hormone supply, is summarized in Figure 6. To test these hypotheses accurately, experiments should be carried out in which endogenous production, transport or action of auxins or cytokinins is inhibited. In the absence of *F. benjamina* mutants for auxin or cytokinin action, inhibitors of the endogenous synthesis or transport of both hormones could be used, such as naphthylphthalamic acid (NPA) and tri-iodobenzoic acid (TIBA) for auxin. Regarding cytokinin, less specific inhibitors are available,





**Figure 6.** A proposed model for the action of exogenously supplied auxin and cytokinin in overcoming *Ficus benjamina* root restriction.

such as lovastatin and dopamine.

From the point of view of growth analysis, exogenous supply of both hormones may seem to cause similar effects, being IAA as effective as BAP in promoting RGR. This conclusion might also be obtained from that fact that this promotion was the result of increased NAR and that a single linear relationship between NAR and RGR could be established for all hormone combinations, including both genotypes. However, it should also be noted that in an ornamental species such as *F. benjamina*, differences in plant architecture and foliage appearance may be of commercial importance.

## Conclusions

Auxin treatment may help overcome root restriction in *Ficus benjamina* as effectively as cytokinin in terms of promotion of biomass accumulation, although plant architecture, mainly leaf number and size, may differ according to the hormone supplied. Considering that, unlike cytokinins, auxins are inexpensive and easily available in stores, our present results may provide a useful tool for growers. However, future studies are needed to ascertain whether these results can be extended to other pot-grown ornamental species.

## Author Contribution

**ADB:** provided the structure and conditions to develop the experiments and conducted them. **ADB, CG** and **JT:** wrote the manuscript, carried out the statistical analysis and contributed to the discussion of results. All authors read and approved the final version of the article.

## Acknowledgements

This work was supported by the University of Buenos Aires Science Program 2018-2022 (Grant N° 145BA). The authors gratefully acknowledge to Mr. Dario Ganem for preparing figure 9 of this work.

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