



## **Cytokinin-Regulated Physiological Parameters Affected by an Exogenous Dopamine Spray in Brussels Sprout (*Brassica oleracea* var. *gemmifera*)**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Root restriction on the first stage of seedling growth decreases post-transplant biomass accumulation. Several studies in different vegetables and ornamental plants have suggested that this restriction would be overcome by means of a single 6-benzylaminopurine (BAP) spray. Based on this, the aim of this work was to evaluate the effects of both pre-transplant single BAP and dopamine (a cytokinin antagonist) sprays on the growth of Brussels sprout (*Brassica oleracea* var. *gemmifera*) plants grown in 200 plug cells tray<sup>-1</sup>, during different times of the annual cropping period. The responses of dopamine-sprayed plants were not completely similar to those previously reported. The different physiological processes studied in dopamine-sprayed plants through some growth parameters showed that dopamine affected mainly the rate of leaf appearance, which in turn positively affected both individual and total leaf area expansion. Dopamine-sprayed plants showed a higher leaf source, which allowed them to accumulate a higher biomass on both a fresh and dry weight basis during the initial vegetative growth stages. The effects of both BAP and dopamine were partially related to the environmental conditions during the experiments.

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**Keywords:** Brussels sprout; cytokinin inhibitor; fresh and dry weight accumulation; growth parameters.

## 1. INTRODUCTION

Since plug trays started to be commercially offered, plug seeding has thoroughly replaced direct seeding, despite the root restriction syndrome related to the limited plug cell volume. To override this negative effect, several authors working on different vegetables have suggested the use of an exogenous cytokinin spray [1,2].

Cytokinins are a class of plant growth substances (phytohormones) that control cell division and morphogenesis [3], and particularly stimulate protein synthesis [4]. The application of cytokinins to a single plant organ (e.g. leaves) leads this organ to become an active sink for amino acids, which then migrate to the organ from surrounding sites [5].

Dopamine, a substituted phenethylamine [6] and chemical analog of cytokinin RNA bases, has been described as a cytokinin synthesis inhibitor [7] and as a precursor to plant-synthesized benzyl isoquinoline alkaloids with promising antioxidant properties.

Most effects of hormones on plant growth have been studied by using exogenous applications. Our previous studies on *Epipremnum aureum*, for example, have suggested that an exogenous cytokinin or auxin spray would affect its photosynthetic active radiation (PAR). The application of cytokinin can also affect the plastochron length, leaf size, photosynthetic rate, leaf mesophyll anatomy and photo assimilate partitioning [8,9,10]. In contrast, little is known about the effects of an exogenous dopamine spray and data on Brussels sprout (*Brassica oleracea* var. *gemmifera*) are also lacking.

Although Kotov and Kotova [11] indicated that the higher the root system, the higher the amount of cytokinin-ribosides synthesized, not all the numerous zeatin riboside isomers show the same biological activity [12]. In fact, the biological activity of cytokinin-like compounds normally depends on several structural aspects [13]. In this way, to test the effect of a cytokinin synthesis inhibitor such as dopamine is a novelty alternative to quantify cytokinin-like growth regulators effects on plant growth.

Thus, the aim of this work was to evaluate the effect of different BAP and dopamine spray concentrations on the biomass accumulation of

Brussels sprout plants grown in pots at three different times of the year. Our hypotheses were that a BAP application would override the previous plug tray root restriction and that a dopamine spray would be able to inhibit endogenous cytokinin on metabolic control.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Three experiments were conducted under a greenhouse located around Mar del Plata city, Argentina (37° 54' S, 57° 35' W and altitude 130 m). Experiment 1 was conducted from August 16<sup>th</sup> to November 20<sup>th</sup> 2017 (winter-spring), experiment 2 from December 12<sup>th</sup> 2017 to March 25<sup>th</sup> 2018 (summer), and experiment 3 from February 28<sup>th</sup> to June 7<sup>th</sup> 2018 (autumn).

Brussels sprout 'Davlin' seeds (Bejo, Warmenhuizen, The Netherlands) were germinated and grown in 200-cell-plug trays (13.90 cm<sup>3</sup> cell<sup>-1</sup>) in Klasmann 411® medium (Klasmann-Deilmann, GmbH, Germany) (Canadian Sphagnum peat moss-perlite-vermiculite 70/20/10 v/v/v).

Leaves were sprayed at sunset with different BAP or dopamine (0, 5, 50, 100 and 200 mg L<sup>-1</sup>) solutions when the first true leaf pair was developed.

### 2.2 Cultivation and Meteorological Data

When seedlings reached to the transplant stage (between 35 to 45 days from sowing), they were transplanted into 5-L pots filled with a 1:1 (v/v) mix of *Sphagnum maguellanicum* peat and river waste during near 60 days (post-transplant stage).

Plants were irrigated as needed with high quality tap water using intermittent overhead mist. The growing medium was fertilized with 1.0: 0.5: 1.0: 0.5 (v/v/v/v) N: P: K: Ca through the overhead irrigation water (Stage 2: 50 mg L<sup>-1</sup> N; Stage 3-4: 100 mg L<sup>-1</sup> N; pot: 150 mg L<sup>-1</sup> N).

Daily maximum air temperature (20.73, 28.78 and 16.25°C), daily minimum air temperature (9.02, 14.14 and 8.05°C) and global solar radiation (8.81, 10.83 and 7.04 MJ m<sup>-2</sup> day<sup>-1</sup>) for the three experiments were recorded with a HOBO sensor (H08-004-02) (Onset Computer

Corporation, MA, USA) connected to HOBO H8 data logger. The plants arrangement at a density of 6 plants m<sup>-2</sup> avoided mutual shading.

### 2.3 Sampling and Growth Evaluations

For destructive measurements, five plants per treatment and sampling date were randomly chosen at the transplant stage and at 7-day intervals during the experiments. Roots were washed and total fresh weights (FW) were recorded. Dry weights (DW) were recorded after drying roots, stems, petioles and leaves to constant weight at 80°C for 96 hours. The number of leaves was recorded and each leaf area was determined using the ImageJ® (Image Processing and Analysis in Java) software.

The rate of leaf appearance (RLA), the relative growth rate (RGR), the rate of leaf area expansion (RLAE), the mean net assimilation rate (NAR) and the specific leaf area ratio (SLA) were calculated according to Di Benedetto and Tognetti [14].

The allometric coefficients between root and shoot were calculated as the slope ( $\beta$ ) of the straight-line regression of the  $\ln$  of the root DW vs. the  $\ln$  of the shoot DW ( $\ln$  root DW = a +  $\beta$  x  $\ln$  shoot DW).

### 2.4 Statistical Analysis

Data were subjected to one-way ANOVA for a completely randomized design and means were separated by Tukey's test ( $P < 0.05$ ). When applicable, Fisher LSD-test ( $P < 0.05$ ) was applied to determine the direction of the differences between treatment mean values. Slopes from straight-line regressions of RLAE, RGR and allometric values were tested using the SMATR package.

## 3. RESULTS

### 3.1 Fresh Weight Accumulation

Control plants showed significant FW differences at the end of the experiments, achieving the highest FW accumulation when grown between December and March (experiment 2). BAP-sprayed plants at relatively high concentrations (50, 100 or 200 mg L<sup>-1</sup>) showed decreased FW in all experiments, whereas dopamine-spray ones showed higher significant FW differences with

both control plants and BAP-sprayed ones (Fig. 1a). BAP led to a decrease in FW of 80% (Figs. 1b and c), whereas dopamine led to an increase in FW between 20% (Fig. 1c) and more than 200% (Fig. 1d).

### 3.2 Dry Weight Accumulation

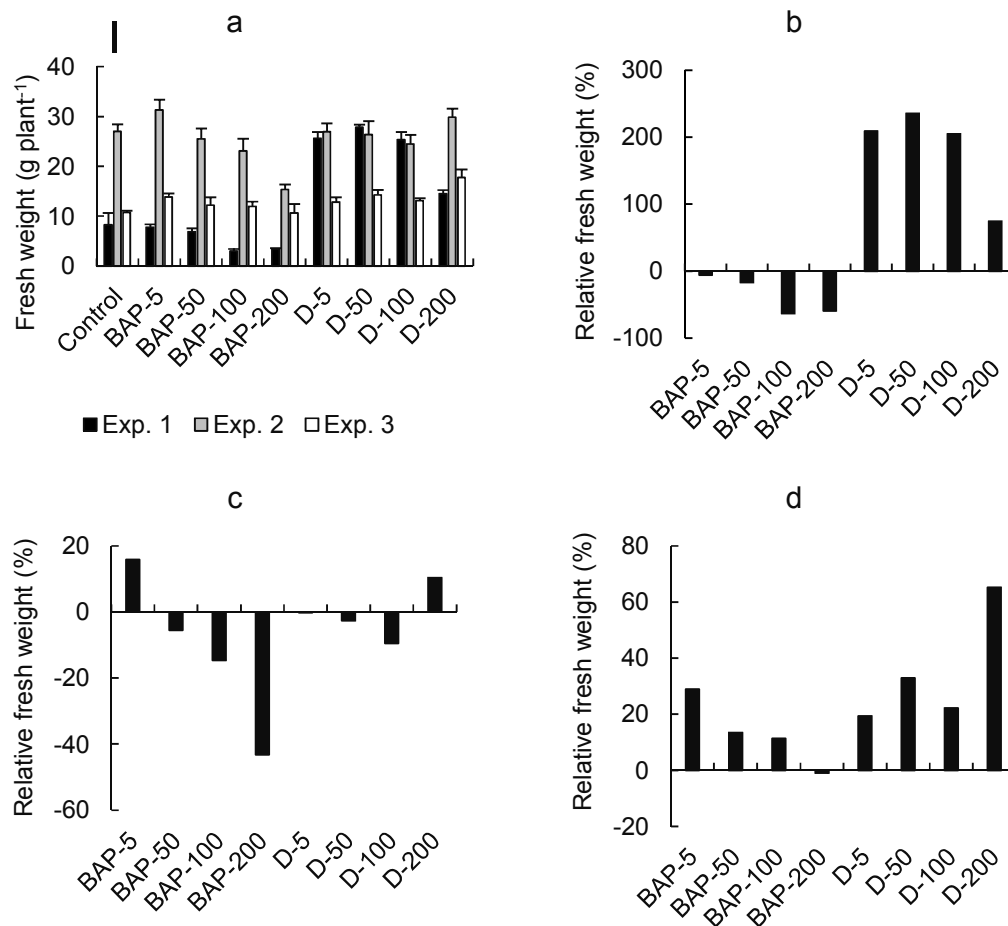
DW showed the same response pattern as total FW, thus indicating that the main differences between the treatments were associated with the different photo assimilate quantities accumulated in leaves. At the end of experiment 1 (August-November), dopamine-sprayed plants showed a significantly higher DW (Fig. 2a). In experiment 2 (December-February), BAP-sprayed plants showed a decrease in DW as the BAP concentration increased, whereas dopamine-sprayed plants showed no significant differences with the control plants (Fig. 2b). In experiment 3 (March-April), both BAP- and dopamine-sprayed plants showed only minor significant differences with control plants (Fig. 2c).

### 3.3 Leaf Area

Total and individual leaf areas were highest in experiment 2. Once again, the lowest values were found in BAP-sprayed plants. While RLAE showed minor differences between treatments, RLAE was higher in BAP-sprayed plants (Table 1). The total leaf area in BAP-sprayed plants decreased between 30 and 50%. The relative effect of the dopamine spray was dependent on the moment of the year when the experiment took place (Figs. 4a, b and c). On the other hand, the lowest BAP doses relatively decreased individual leaf size between 40% and 120%. The same heterogeneous response was found in dopamine-sprayed plants between the different experiments (Figs. 4d, e and f).

### 3.4 Dry Weight Growth Parameters

RGR and NAR decreased as the BAP concentration increased, although with significant differences between experiments. Despite this, the highest RGR values were found in dopamine-sprayed plants. On the other hand, SLA showed an inverse response. The root: shoot allometries showed higher photo assimilate partitioning (lower  $\beta$  coefficient) to shoots in both BAP- or dopamine-sprayed plants (Table 2). The relative effect on RGR (Figs. 5a, b and c) and NAR (Figs. 5d, e and f) showed negative values for BAP-sprayed plants during the three experiments and positive but weak effects on



**Fig. 1.** Changes in total fresh weight at the end of each of the three experiments (a) in Brussels sprout plants sprayed with different BAP or dopamine concentrations (0, 5, 100 or 200 mg L<sup>-1</sup>). Vertical line indicate least significant differences (LSD) and standard errors are indicated. Relative fresh weights (b, c and d respectively) are expressed as the percentage change observed relative to plants sprayed with 0 mg L<sup>-1</sup> BAP

dopamine-sprayed ones. Both BAP- and dopamine-sprayed plants showed relatively decreased SLA (Figs. 5g, h and i) and  $\beta$  coefficient from the root:shoot allometries (Figs. 6a, b and c).

#### 4. DISCUSSION

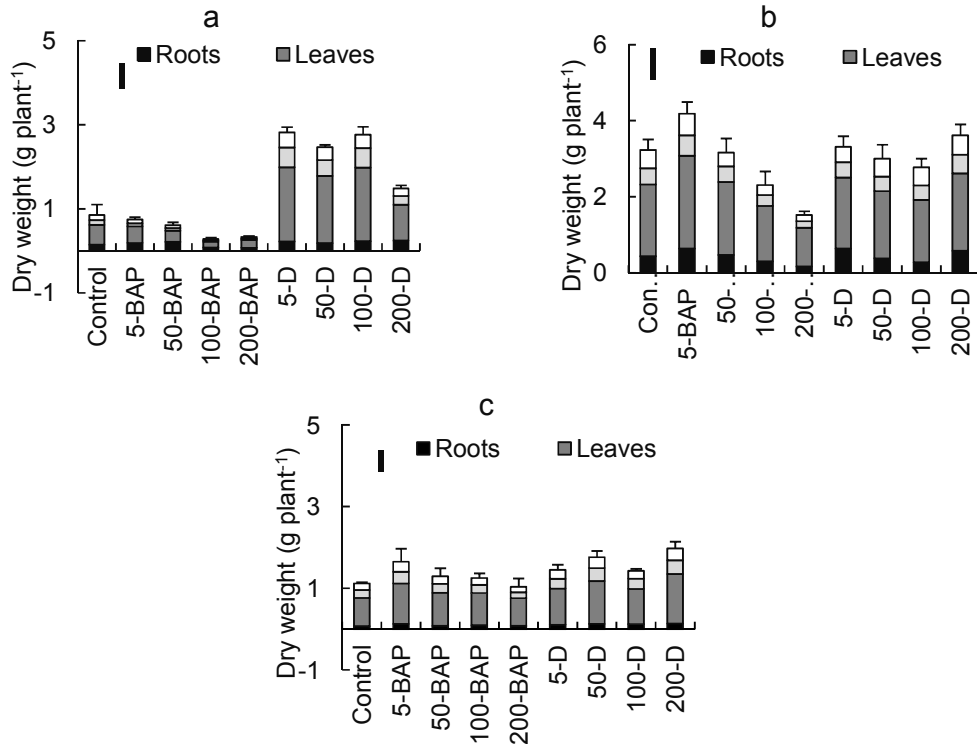
In agreement with the meta-analysis of Poorter et al. [15] on the negative pot effects in plant biology research, previous data published by our laboratory during the last decade strongly showed the negative effects of the root restriction syndrome related to a limited plug cell size. Although we suggested that a single exogenous cytokinin spray early during nursery would override root restriction, most of the experiments included only hormone exogenous applications.

In view of that, in the present study, we decided to test the effect of an exogenous BAP supply on Brussels sprout plants grown in 200-plug cell trays<sup>-1</sup> (a common limited cell size) and then transplanted to 5-liter pots and at the same time test the effect of dopamine, a suggested cytokinin synthesis inhibitor. We expected to find a decrease in biomass accumulation on FW/DW basis, a lower leaf area, a lower photosynthetic rate and a lower photo-assimilate partitioning to roots in controls and dopamine-sprayed plants. However, our results were not completely in agreement with these assumptions.

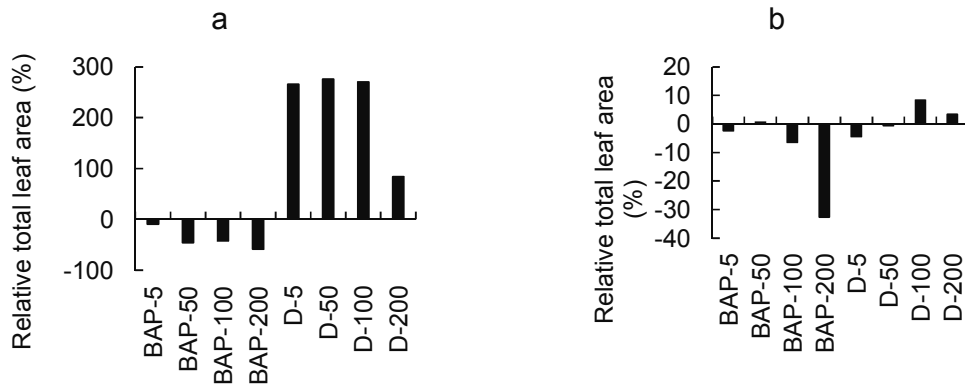
Control plants showed the lowest FW and DW, with a slight or negative increase in BAP-sprayed plants (with significant differences related to the time of the year at which the experiment took

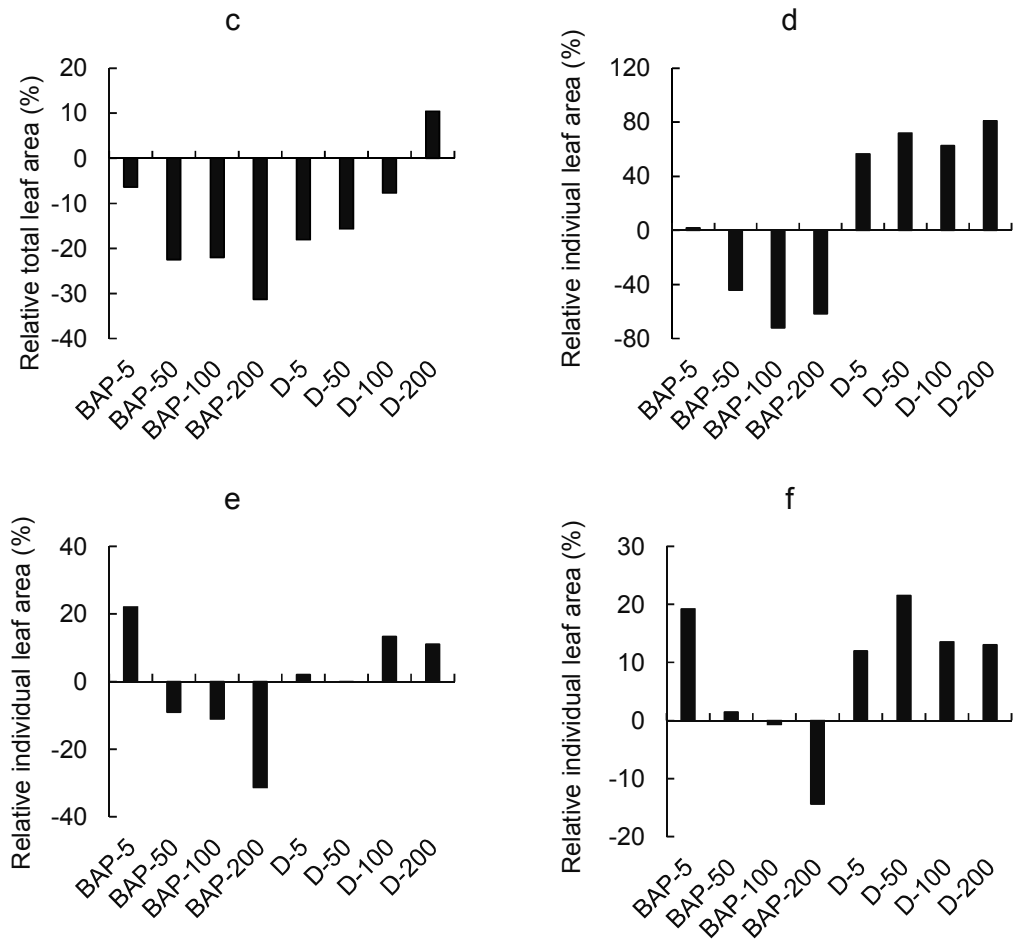
place) (Figs. 1 and 2). These results are not in agreement with our previous reports in other vegetables [2] and ornamental plants [1], but are similar to those in squash [16] and the ornamental fern *Asplenium nidus avis* (unpublished data). Dopamine sprays seem to affect endogenous cytokinin synthesis [7] and the control of total biomass accumulation on either a FW or a DW basis.

To understand FW and DW results, it is important to have a good understanding of the physiological processes involved in biomass accumulation. At plant level, biomass accumulation on both FW and DW basis is mainly related to total leaf area expanded and photo-assimilate fixation and partitioning between different sinks, all traits that are positively related to each other [17].

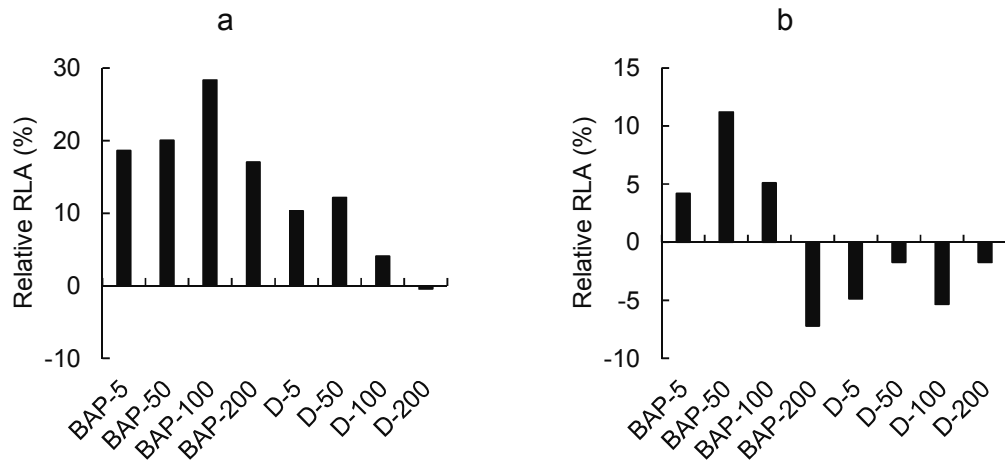


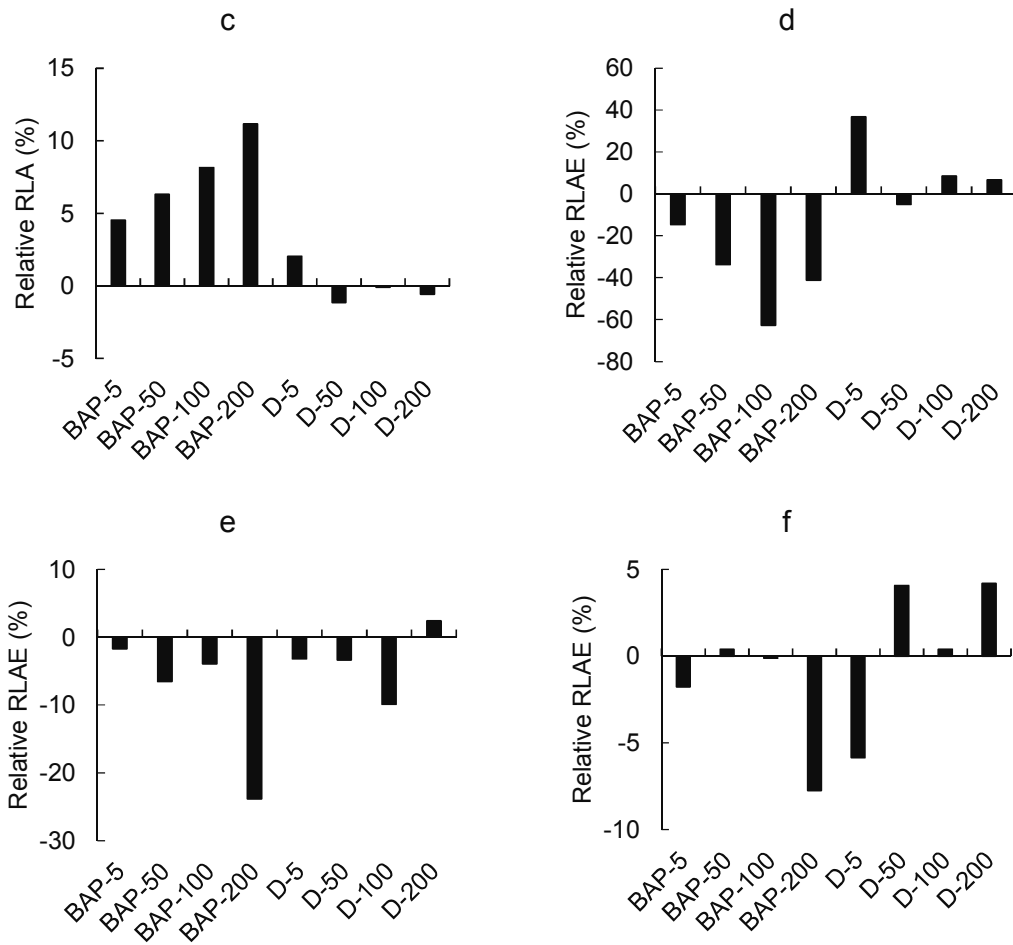
**Fig. 2. Changes in the DW of roots, leaves, petioles and stems at the end of each of the three experiments (a, b and c, respectively) in Brussels sprout plants sprayed with different BAP or dopamine concentrations (0, 5, 100 or 200 mg L<sup>-1</sup>). Vertical lines indicate least significant differences (LSD) and standard error are indicated**



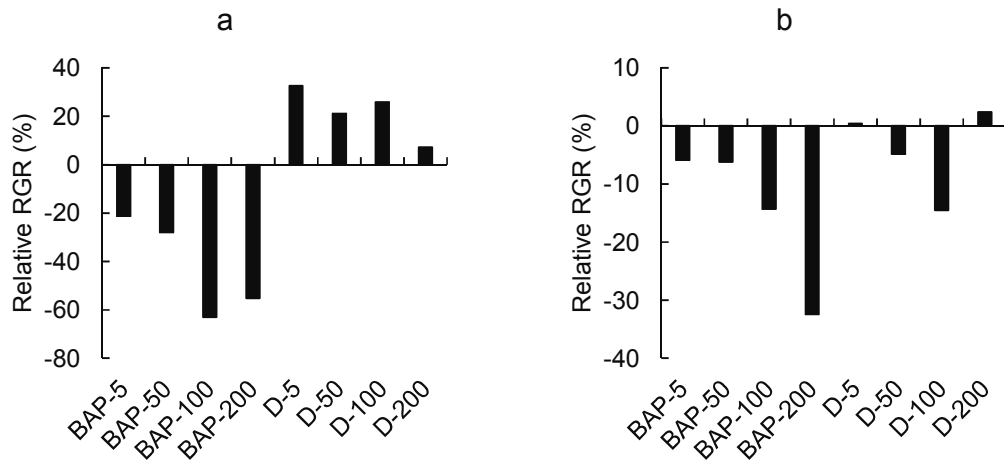


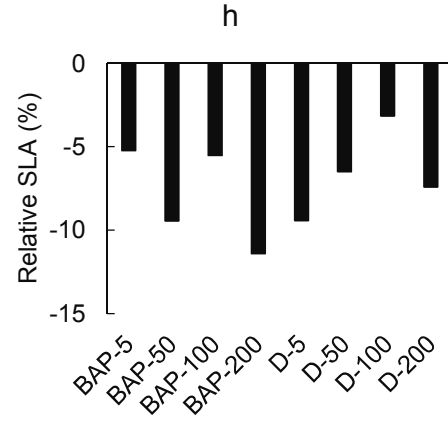
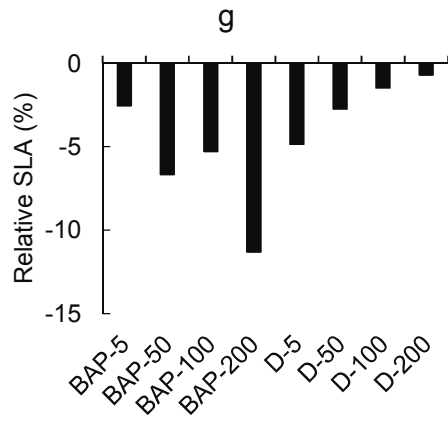
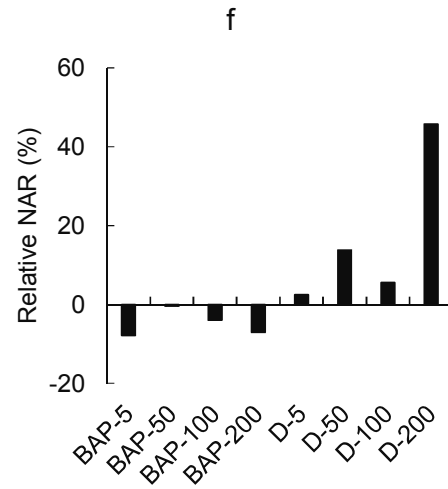
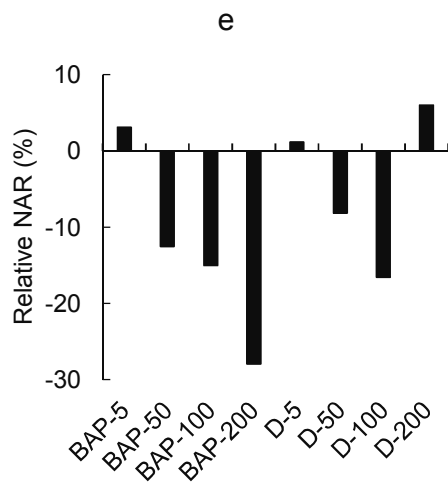
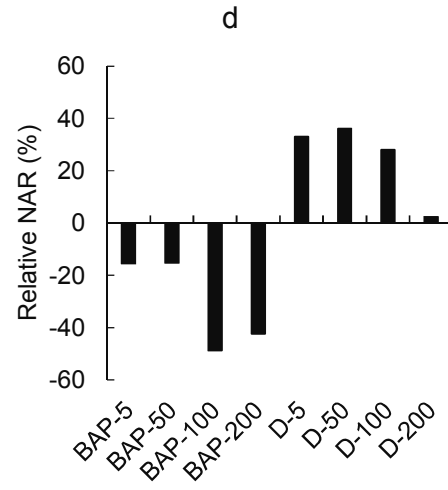
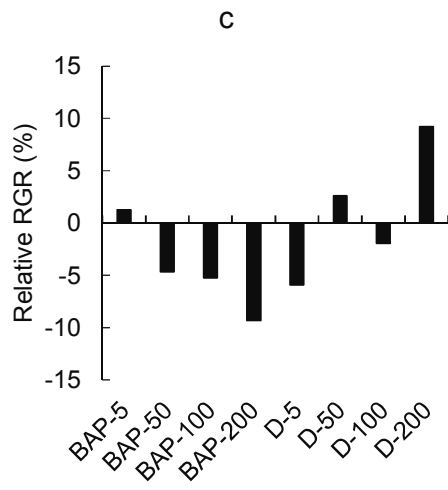
**Fig. 3. Relative total (A, B and C) and individual leaf area (d, e and f) at the end of each of the three experiments in Brussels sprout plants sprayed with different BAP or dopamine concentrations (5, 50, 100 or 200 mg L<sup>-1</sup>). Data are expressed as the percentage change observed relative to plants sprayed with 0 mg L<sup>-1</sup> BAP**



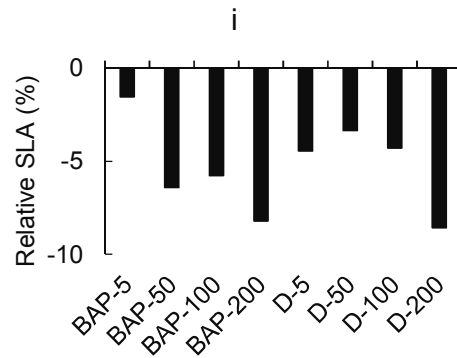


**Fig. 4. Relative RLA (a, b and c) and RLA (d, e and f) for each of the three experiments in Brussels sprout plants sprayed with different BAP or dopamine concentrations (5, 50, 100 or 200 mg L<sup>-1</sup>). Data are expressed as the percentage change observed relative to plants sprayed with 0 mg L<sup>-1</sup> BAP**









**Fig. 5. Relative RGR (a, b and c), NAR (d, e and f) and SLA (g, h and i) for each of the three experiments in Brussels sprout plants sprayed with different BAP or dopamine concentrations (5, 50, 100 or 200 mg L<sup>-1</sup>). Data are expressed as the percentage change observed relative to plants sprayed with 0 mg L<sup>-1</sup> BAP**

Total leaf area is the result of the leaf primordium initiation rate, and the leaf expansion rate (responsible for the individual leaf size of each leaf expanded), which can be estimated through RLA and RLAE respectively. Although our results showed significant differences in total and individual leaf area between experiments, the lower values were found in BAP-sprayed plants (Table 1). This was the most unexpected result because in other previously studied vegetables (except in squash) [16] and ornamental plants (except in *A. nidus avis*), BAP-sprayed plants significantly increased individual and total leaf area. In turn, the dopamine spray had a weak positive effect (Fig. 3). On the other hand, the RLA (an estimate of the plastochron length) of BAP-sprayed plants showed higher absolute values (Table 1) and relative effects (Fig. 4a, b and c). Since Brussels sprout leaves appeared on a single shoot without branches, the increase in RLA would indicate a shorter plastochron. One of the main results of this work was that the exogenous dopamine sprays did not suppress the effects of endogenous cytokinins on RLA.

To understand these results, it must be kept in mind that the primary shoot apical meristem is responsible for generating all above ground organs and is controlled by hormones, which regulate biosynthesis and transport of other hormones, and by hormone interactions. These hormones include auxins, cytokinins and gibberellins, which act both independently and in combination to regulate meristem function [9,10,14,18]. Leaves are formed into an initial group of cells within the meristem; one of the earliest markers for leaf initiation is the down-regulation of the *KNOTTED* and *WUSCHEL*

genes in these cells [19]. Genetic analyses have demonstrated that a high cytokinin: low gibberellin ratio is important for *KNOX* gene function [20]. Cytokinins are synthesized mainly in the roots [21] and move through the stem xylem to the shoot apical meristem, although the effective cytokinin concentration is the result of endogenous and environmental signals [22]. The main function of endogenous cytokinins is to control the cell cycle and the growth of the shoot apical meristem [4,23]. Although a higher individual leaf area would be explained by the common effect of cytokinins on leaf expansion [24], each new leaf needs that the shoot apical meristem increases and a non-limiting photo-assimilate supply to hold vegetative plant growth. Although cytokinin-rich tissues, such as the shoot apical meristem, are photo-assimilate sinks, it is possible to hypothesize that a higher leaf initiation would exceed the photo-assimilate supply from plant sources and restrict leaf expansion. In this way, the negative effect of a dopamine spray on RLA would leave more photo-assimilates available for the growth of each expanded leaf.

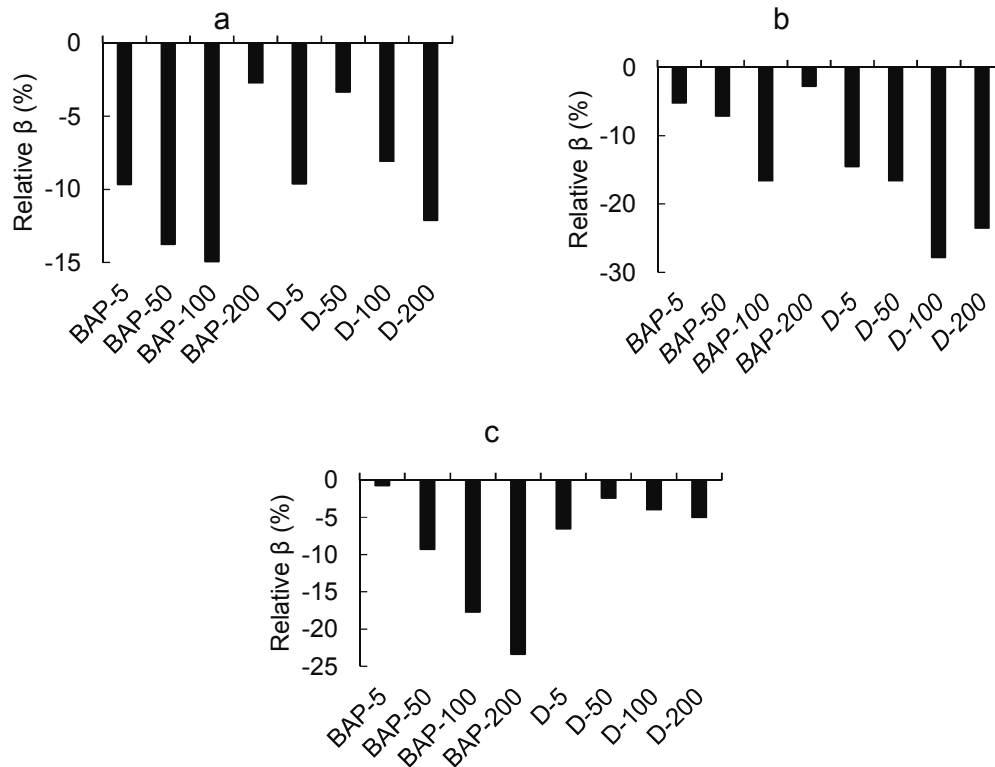
Biomass accumulation can be estimated through RGR. In our experiments, RGR allowed explaining the lower DW in BAP-sprayed plants and the higher DW in dopamine-sprayed ones as the relative response related to controls (Table 2; Fig. 5). On the other hand, NAR (the physiological RGR component) and SLA (an estimator of mesophyll thickness) would indicate that the higher biomass accumulation in control and dopamine-sprayed Brussels sprout plants would be related to a direct higher photosynthetic capacity but not to changes in leaf structure.

Based on the fact that Boonman et al. [25] indicated that cytokinins stimulate the expression of photosynthetic enzymes, Oguchi et al. [26] suggested a relationship between photosynthetic rates and leaf anatomy. Light-saturated rates of photosynthesis on a leaf area basis depend not only on photosynthetic biochemistry but also on mesophyll structure. Because resistance to CO<sub>2</sub> diffusion from the sub-stomatal cavity to the stroma is substantial, it is likely that the mesophyll structure affects the photosynthetic rate by affecting CO<sub>2</sub> diffusion in the leaf [27]. Our results showed that dopamine sprays did not significantly change the effect of endogenous cytokinins on leaf mesophyll thickness but did affect the leaf photosynthetic capacity (Fig. 5).

The root: Shoot allometries allowed estimating the photo-assimilate partitioning between them. The lower  $\beta$  coefficients in Brussels sprout plants showed a higher photo-assimilate partitioning to shoots in both BAP- and dopamine-sprayed plants (Table 2). Dopamine sprays had a non-

significant effect on the root: shoot allometries (Fig. 6).

The temperature requirements for optimum production of Brussels sprouts ranged between 17° and 21°C during the 3-4 months of early growth [28]. Because the environmental conditions (daily temperatures and photon flux radiation) in the three experiments were quite different, we can hypothesize that endogenous cytokinin synthesis can change in response to environmental variables such as temperature and photon flux radiation. In agreement with this assumption, several studies have found a positive interaction between cytokinins and photosynthetic active radiation [9,10,29] or temperature [30,31]. We may also speculate that some of the dopamine-inhibited physiological processes had different endogenous cytokinin thresholds, which define the final response of plants. Both of these hypotheses would be involved in the responses of BAP- and dopamine-sprayed Brussels sprout plants.



**Fig. 6. Relative partition  $\beta$  coefficient for each of the three experiments (a, b and c respectively) in Brussels sprout plants sprayed with different BAP or dopamine concentrations (5, 50, 100 or 200 mg L<sup>-1</sup>). Data are expressed as the percentage change observed relative to plants sprayed with 0 mg L<sup>-1</sup> BAP**

**Table 1. Changes in total leaf area, individual leaf area, rate of leaf appearance (RLA) and relative leaf expansion rate (RLAE) in Brussels sprout plants sprayed with different BAP or dopamine concentrations (0, 5, 50, 100 or 200 mg L<sup>-1</sup>) for each of the three experiments. Different lower case letters indicate significant differences ( $P \leq 0.05$ ) between control and BAP- or dopamine-sprayed plants. Different capital letters indicate significant differences ( $P \leq 0.05$ ) between experiments**

	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )			Leaf area (cm <sup>2</sup> leaf <sup>-1</sup> )			RLA (leaves week <sup>-1</sup> plant <sup>-1</sup> )			RLAE (cm <sup>2</sup> cm <sup>-2</sup> day <sup>-1</sup> )		
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
Control	279.69 <sup>CC</sup>	1887.71 <sup>AA</sup>	699.99 <sup>BB</sup>	27.06 <sup>BC</sup>	157.31 <sup>BA</sup>	50.00 <sup>BB</sup>	0.160 <sup>DB</sup>	0.219 <sup>CA</sup>	0.173 <sup>CB</sup>	0.077 <sup>BB</sup>	0.121 <sup>AA</sup>	0.079 <sup>AB</sup>
BAP-5	253.17 <sup>CC</sup>	1843.51 <sup>AA</sup>	655.50 <sup>CB</sup>	27.54 <sup>BC</sup>	191.95 <sup>AA</sup>	59.59 <sup>AB</sup>	0.191 <sup>BB</sup>	0.229 <sup>BA</sup>	0.181 <sup>BB</sup>	0.066 <sup>CC</sup>	0.119 <sup>AA</sup>	0.077 <sup>AB</sup>
BAP-50	150.40 <sup>DC</sup>	1899.88 <sup>AA</sup>	542.54 <sup>DB</sup>	15.17 <sup>CC</sup>	143.10 <sup>AA</sup>	50.73 <sup>BC</sup>	0.194 <sup>BB</sup>	0.244 <sup>AA</sup>	0.183 <sup>BB</sup>	0.051 <sup>DC</sup>	0.113 <sup>AA</sup>	0.079 <sup>AB</sup>
BAP-100	159.87 <sup>DC</sup>	1766.38 <sup>BA</sup>	546.15 <sup>DB</sup>	7.55 <sup>DC</sup>	139.95 <sup>AA</sup>	49.65 <sup>BC</sup>	0.206 <sup>AB</sup>	0.231 <sup>BA</sup>	0.187 <sup>BC</sup>	0.029 <sup>EC</sup>	0.116 <sup>AA</sup>	0.079 <sup>AB</sup>
BAP-200	115.57 <sup>EC</sup>	1270.94 <sup>CA</sup>	480.94 <sup>EB</sup>	10.41 <sup>DC</sup>	108.08 <sup>DA</sup>	42.82 <sup>CB</sup>	0.188 <sup>BB</sup>	0.204 <sup>DA</sup>	0.192 <sup>AB</sup>	0.045 <sup>DC</sup>	0.092 <sup>CA</sup>	0.073 <sup>BB</sup>
D-5	1023.30 <sup>AB</sup>	1803.41 <sup>AA</sup>	573.67 <sup>DC</sup>	42.33 <sup>AC</sup>	160.49 <sup>DA</sup>	55.99 <sup>AB</sup>	0.176 <sup>CB</sup>	0.209 <sup>DA</sup>	0.177 <sup>CB</sup>	0.105 <sup>AB</sup>	0.117 <sup>AA</sup>	0.074 <sup>BC</sup>
D-50	1051.15 <sup>AB</sup>	1878.67 <sup>AA</sup>	590.25 <sup>DC</sup>	46.49 <sup>AC</sup>	157.15 <sup>CA</sup>	60.77 <sup>AB</sup>	0.180 <sup>CB</sup>	0.216 <sup>CA</sup>	0.171 <sup>CB</sup>	0.073 <sup>BB</sup>	0.117 <sup>AA</sup>	0.082 <sup>AB</sup>
D-100	1035.55 <sup>AB</sup>	2045.92 <sup>AA</sup>	646.41 <sup>CC</sup>	44.01 <sup>AC</sup>	178.29 <sup>CA</sup>	56.77 <sup>AB</sup>	0.167 <sup>CB</sup>	0.208 <sup>DA</sup>	0.173 <sup>CB</sup>	0.083 <sup>BB</sup>	0.109 <sup>BA</sup>	0.079 <sup>AB</sup>
D-200	514.95 <sup>BC</sup>	1951.10 <sup>AA</sup>	773.15 <sup>AB</sup>	48.96 <sup>AC</sup>	174.64 <sup>DA</sup>	56.51 <sup>AB</sup>	0.160 <sup>DB</sup>	0.216 <sup>CA</sup>	0.172 <sup>CB</sup>	0.082 <sup>BB</sup>	0.124 <sup>AA</sup>	0.082 <sup>AB</sup>

**Table 2. Changes in RGR, NAR, SLA and the  $\beta$  coefficient from the root: shoot allometries in Brussels sprout plants sprayed with different BAP or dopamine concentrations (0, 5, 50, 100 or 200 mg L<sup>-1</sup>) for each of the three experiments. The probability of the slope being zero was  $P \leq 0.001$  for RGR, NAR and the  $\beta$  coefficient. Different lower case letters indicate significant differences ( $P \leq 0.05$ ) between control and BAP- or dopamine-sprayed plants. Different capital letters indicate significant differences ( $P \leq 0.05$ ) between experiments**

	RGR (g g <sup>-1</sup> day <sup>-1</sup> )			NAR (g cm <sup>-2</sup> day <sup>-1</sup> ) x 10 <sup>-5</sup>			SLA (cm <sup>2</sup> g <sup>-1</sup> )			$\beta$		
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
Control	0.0691 <sup>EC</sup>	0.1174 <sup>BA</sup>	0.0879 <sup>CB</sup>	21.80 <sup>CA</sup>	16.47 <sup>BB</sup>	11.99 <sup>CC</sup>	73.07 <sup>AB</sup>	136.72 <sup>AA</sup>	78.79 <sup>AB</sup>	0.511 <sup>AC</sup>	0.853 <sup>AA</sup>	0.582 <sup>AB</sup>
BAP-5	0.0544 <sup>FC</sup>	0.1105 <sup>CA</sup>	0.0890 <sup>BB</sup>	18.43 <sup>DA</sup>	16.98 <sup>AB</sup>	11.05 <sup>CC</sup>	71.21 <sup>AC</sup>	129.56 <sup>AA</sup>	77.58 <sup>AB</sup>	0.462 <sup>CC</sup>	0.809 <sup>BA</sup>	0.578 <sup>AB</sup>
BAP-50	0.0497 <sup>GC</sup>	0.1101 <sup>CA</sup>	0.0838 <sup>DB</sup>	18.47 <sup>DA</sup>	14.41 <sup>EB</sup>	11.94 <sup>CC</sup>	68.20 <sup>BB</sup>	123.81 <sup>BA</sup>	73.74 <sup>BC</sup>	0.441 <sup>CC</sup>	0.792 <sup>BA</sup>	0.528 <sup>CB</sup>
BAP-100	0.0256 <sup>HC</sup>	0.1006 <sup>CA</sup>	0.0833 <sup>DB</sup>	11.19 <sup>EB</sup>	14.00 <sup>EA</sup>	11.52 <sup>CB</sup>	69.20 <sup>BB</sup>	129.16 <sup>AA</sup>	74.24 <sup>BB</sup>	0.435 <sup>CB</sup>	0.711 <sup>CA</sup>	0.479 <sup>DB</sup>
BAP-200	0.0310 <sup>IC</sup>	0.0793 <sup>DA</sup>	0.0797 <sup>EB</sup>	12.57 <sup>EA</sup>	11.87 <sup>IA</sup>	11.14 <sup>CA</sup>	64.81 <sup>BC</sup>	121.12 <sup>BA</sup>	72.32 <sup>BB</sup>	0.497 <sup>BB</sup>	0.830 <sup>AA</sup>	0.446 <sup>DB</sup>
D-5	0.0916 <sup>AB</sup>	0.1179 <sup>BA</sup>	0.0827 <sup>DC</sup>	29.00 <sup>AA</sup>	16.66 <sup>CB</sup>	12.29 <sup>CC</sup>	69.52 <sup>BC</sup>	123.84 <sup>BA</sup>	75.29 <sup>BB</sup>	0.462 <sup>CC</sup>	0.729 <sup>CA</sup>	0.544 <sup>CB</sup>
D-50	0.0837 <sup>CC</sup>	0.1117 <sup>CA</sup>	0.0902 <sup>BB</sup>	29.68 <sup>AA</sup>	15.13 <sup>DB</sup>	13.64 <sup>BC</sup>	71.06 <sup>AB</sup>	127.84 <sup>AA</sup>	76.15 <sup>AB</sup>	0.494 <sup>AB</sup>	0.711 <sup>CA</sup>	0.568 <sup>AB</sup>
D-100	0.0870 <sup>BB</sup>	0.1004 <sup>CA</sup>	0.0862 <sup>CB</sup>	27.92 <sup>BA</sup>	13.75 <sup>EB</sup>	12.65 <sup>CB</sup>	71.99 <sup>AB</sup>	132.40 <sup>AA</sup>	75.40 <sup>BB</sup>	0.470 <sup>CC</sup>	0.616 <sup>DA</sup>	0.559 <sup>CB</sup>
D-200	0.0741 <sup>DC</sup>	0.1202 <sup>AA</sup>	0.0960 <sup>AB</sup>	22.32 <sup>CA</sup>	17.46 <sup>AB</sup>	17.47 <sup>AB</sup>	72.55 <sup>AB</sup>	126.58 <sup>AA</sup>	72.04 <sup>BB</sup>	0.449 <sup>BC</sup>	0.653 <sup>DA</sup>	0.553 <sup>CB</sup>

## 5. CONCLUSION

The lower effect of dopamine was that observed on RLA, which positively affected both individual and total leaf area expansion during the first growth stages. Because dopamine-sprayed plants had more photo-assimilates available, they were able to accumulate a higher biomass on both a FW and DW basis. This indicates that dopamine affects the photosynthetic capacity of the plants, estimated through NAR, but not leaf thickness or photo-assimilate partitioning, all cytokinin-regulated processes. The use of hormone synthesis inhibitors is a tool to understand how hormones affect plant responses. Although dopamine has been indicated as a cytokinin synthesis inhibitor, our results showed that this is not totally true, which is a novelty report.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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