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Changes in leaf size and in the rate of leaf production contribute to cytokinin-mediated growth promotion in *Epipremnum aureum* L. cuttings.

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SUMMARY
The growth of ornamental foliage plants is often limited by pot size, which exerts a restriction on root growth and, therefore, on the production of root-synthesised cytokinins which play key regulatory roles in the development and growth of the shoot. We studied the effect of exogenous 6-benzylaminopurine (BAP) on plant growth and on the development of leaf area in *Epipremnum aureum* L. plants grown in pots. The hypothesis was that increasing the concentration of shoot cytokinins by foliar spraying of BAP would promote plant growth by overcoming the effects of root restriction on whole plant development. Three glasshouse experiments were conducted using (i) different concentrations of BAP, (ii) different numbers of spray applications, and (iii) different light environments. The results showed that a single spray application of BAP at 5 mg l^{-1} significantly ($P \leq 0.05$) increased leaf area (by 20 – 40%) and biomass [fresh weight (FW)] accumulation (by 30 – 35%), while higher BAP concentrations, or repeated spray applications had less effect. The maximum effect of BAP was observed under intermediate levels of irradiance. The increased development of leaf area in BAP-sprayed plants resulted from increases in both individual average leaf areas (by 100 – 150% *cf*. the controls) and the rate of leaf initiation (by 30 – 120% *cf*. the controls). The latter could be attributed to a shortening of the phyllochron, since no branching was observed under any BAP spray treatment. Alternative physiological explanations, as well as possible commercial applications of these BAP-elicited responses are discussed.

Ornamental foliage plants represent a group of plants in which only the vegetative parts are of commercial interest and which are becoming increasingly important worldwide (Chen *et al*., 2005). *Epipremnum aureum* is an important ornamental foliage plant species (Greig, 2004). There is a need to study plant growth, which may ultimately lead to improved production in commercial facilities. In such species, the success of producing plants rapidly depends on maintaining high growth rates throughout the cultivation period (Di Benedetto *et al*., 2006).

Foliage plants are commonly grown in pots for commercial production, and also at their final location. Root growth restrictions often occur in plants grown in pots. The availability of hormones synthesised at the root apex and transported to the shoots may be reduced when vertical root growth is impeded by the base of the container (Di Benedetto, 2011). It has been claimed that the close coordination between root and shoot growth is controlled by a signalling pathway which is largely hormonal in nature, with the major site of control located in the root system (Kyozuka, 2007). It has been concluded that root cytokinins are a major part of the signalling pathway by which the root:shoot ratio is regulated (Dodd, 2005). Thus, increasing root growth may lead to a corresponding increase in the synthesis of cytokinins (O'Hare and Turnbull, 2004).

In some cases, it has been observed that exogenous applications of cytokinins may overcome limitations in shoot growth due to root restriction (Di Benedetto, 2011; Di Benedetto *et al*., 2010). The application of cytokinins promotes leaf unfolding and expansion in intact plants in a range of species (Doerner, 2007), including ornamental foliage plants (Di Benedetto *et al*., 2010). Cytokinins have also been shown to promote lateral branching in a variety of plants (Ongaro and Leyser, 2008), but this effect has not been observed in *E. aureum*. Ornamental shade plants have both a low total leaf area and a low rate of leaf area expansion (Di Benedetto *et al*., 2006). It has been claimed that the phyllochron (i.e., the time

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interval between the appearance of two successive leaves) may be altered in transgenic plants with reduced cytokinin levels (Zhu *et al*., 2007; Lee *et al*., 2009), but the possibility that exogenous application(s) of a cytokinin may affect the phyllochron has not been explored.

In addition to limitations on root development imposed by the pot, the growth of ornamental foliage plants is usually restricted by being kept in a low light environment. Under shaded conditions, carbon fixation decreased and photoassimilates were preferentially translocated to the shoot rather than to roots (Boonman *et al*., 2009).

The aim of this work was to study the effect of one or more foliar sprays of 6-benzylaminopurine (BAP) on plant growth and the development of leaf area in *E. aureum* L. cuttings grown in pots under different light intensities. The hypothesis was that by increasing the concentration of a shoot cytokinin using foliar spray(s), we might promote plant growth by overcoming the effects of restricted root growth on whole plant development.

MATERIALS AND METHODS

Plant material

Rooted cuttings of *E. aureum* were obtained from two different propagators [The Faculty of Agronomy (UBA) for the 2006 Experiment; and Vivero Kogiso, Buenos Aires, Argentina for the 2007 and 2008 Experiments]. Cuttings (20 per treatment) were transplanted into rigid 1.2 l plastic pots (one cutting per pot) filled with a 1:1 (v/v) mix of *Sphagnum maguellanicum* peat and river waste (Di Benedetto *et al*., 2004). Plants were watered daily with tap water (pH 6.64) with an electrical conductivity of 0.486 dS m^{-1} and fertilised each week with 50 mg l^{-1} N (1.0:0.5:1.0:0.5 N:P:K:Ca) added to the irrigation water.

Experimental design

Three experiments were carried out in a greenhouse located at the Faculty of Agronomy, University of Buenos Aires, Argentina (34°28'S) starting on 6 September 2006, 8 September 2007, and 5 September 2008, respectively. The three experiments ended on 11 March 2007, 12 March 2008, and 11 March 2009, respectively. The greenhouse was covered with a black shade-cloth (for 50% full-sunlight) in the 2006-2007 and 2007-2008 experiments. In the 2008-2009 experiment, the greenhouse was divided into three compartments with different neutral shading cloths to obtain irradiance levels equivalent to 70%, 50%, or 30% of full-sunlight. Light quality was not changed significantly by the shade-cloths, as confirmed using a 660/730 sensor (Skye Instruments, Llandrindod Wells, UK). Mean temperatures and light intensities for each experiment were recorded using three HOBO sensors (Onset Computer Corp., Bourne, MA, USA) connected to HOBO H8 data-loggers. Mean temperatures and photosynthetically-active radiation for the 2006-2007, 2007-2008, and 2008-2009 experiments were recorded and are shown in Table I.

Treatments and measurements

The 6-benzylaminopurine (BAP; Sigma-Aldrich Co., St. Louis, MO, USA) solutions were applied by spraying all leaves on each plant to run-off, at sunset. In the 2006-2007 experiment, cuttings were sprayed with 0 (control), 5, 50, 100, or 200 mg l^{-1} BAP solutions 7 d after transplanting. In the 2007-2008 experiment, cuttings were sprayed with 0 (control) or 5 mg l^{-1} BAP solutions 7 d after transplanting, then once a month for a further three applications. In the 2008-2009 experiment, cuttings were sprayed with 0 (control), 2.5, 5, 10, or 50 mg l^{-1} BAP solutions 7 d after transplanting.

Non-destructive measurements of leaf width and leaf length were performed at each harvest, at the end of the Summer season (March) in the 2006-2007 experiment, and at 90, 120, 150, and 180 d after transplanting in the 2007-2008 and 2008-2009 experiments. The numbers of new leaves that had appeared were also recorded on the same dates. When the plants were finally harvested (five plants for each treatment and each harvest date), the fresh weights (FW) of the various aerial parts (i.e., leaf blades, petioles, and stems) were determined separately for each plant. Leaf areas were determined using a LI-COR 3000A automatic leaf area meter (LI-COR Inc, Lincoln, NE, USA).

The relative growth rate on a FW basis (RGR_{FW}) was calculated as the slope of the regression of the natural logarithm (ln) of whole plant FW *vs.* time (in d), while the relative rate of leaf area expansion (RLAE) was calculated as the slope of the regression of ln total leaf area *vs*. time (in d). The rate of leaf appearance (RLA) was calculated as the slope of the number of new (visible) leaves *vs*. time (in weeks). Since no branching was observed under any experimental conditions, or any BAP treatment, changes in RLA only reflected changes in the phyllochron. The specific leaf area on a FW basis (SLA_{FW}) was calculated as the ratio of new individual leaf area:leaf FW. Leaves were oven-dried at 80°C for 48 h, and their percentage dry matter (DM) content (DMC) was assessed as:

$$DMC = (DM/FW) \times 100.$$

TABLE I

Monthly patterms of daily minimum and maximum temperatures and the mean daily photosynthetically active radiation (PAR) integral during the three experimental seasons

	Minimum temperature (°C)			Maximum temperature (°C)			PAR (mol photons m^{-2} d^{-1})		
Month	2006-2007	2007-2008	2008-2009	2006-2007	2007-2008	2008-2009	2006-2007	2007-2008	2008-2009
September	10.28	12.68	10.48	20.94	21.38	18.63	7.61	6.34	5.93
October	14.78	14.37	13.27	24.68	23.75	23.26	8.94	8.15	8.78
November	15.61	12.71	19.49	25.48	24.97	30.00	10.17	10.50	10.56
December	19.57	18.09	19.31	30.03	29.30	29.46	10.71	10.99	10.79
January	20.15	20.69	20.60	29.39	30.62	31.13	9.88	10.46	11.16
February	19.72	20.68	19.39	30.08	29.90	29.90	8.36	8.66	9.74
March	18.03	17.64	19.45	25.97	26.56	28.18	5.85	6.82	7.70

Fresh weight was not assessed in the 2006-2007 experiment, therefore no RGR_{FW}, SLA_{FW}, or DMC (%) data were obtained for this experiment.

The contributions of increased individual leaf area (leaf size) and RLA to the total leaf area per plant, due to each BAP treatment, were calculated in the 2008-2009 experiment by recording the number of leaves that expanded in the untreated controls, as well as the sum of all individual leaf areas. The sum of all individual leaf areas in each BAP treatment, based on the same number of leaves that expanded in the control plants, was then calculated. The difference between these two totals corresponded to the contribution of BAP to the change in individual leaf area and the increase in total leaf area. Finally, the sum of all individual leaf areas for the additional leaves that expanded under each BAP treatment were measured, as the contribution of changes in RLA to the total leaf area change due to each BAP treatment.

Statistical analysis

Data from the 2006-2007 and 2007-2008 experiments were subjected to one-way ANOVA. Means were separated by Tukey's test ($P \leq 0.05$). Data from the 2008-2009 experiment, which had a factorial design (i.e., five BAP concentrations × three irradiance levels), were subjected to two-way ANOVA.

RESULTS

Leaf area development was promoted by the application of BAP in the 2006-2007 experiment. The rate of relative leaf area expansion (RLAE) increased significantly when *E. aureum* cuttings were sprayed with BAP at any concentration. This response plateaued at approx. 170% of untreated control plants at the lowest BAP concentration applied (5 mg l^{-1}). The BAP-induced increase in RLAE was a consequence of increases in both RLA and in final leaf size. The response of RLA to the application of BAP was concentration-dependent. The greatest effect was obtained at the highest dose of BAP used in this study (200 mg l^{-1}; Table II). On the other hand, the final leaf size response to BAP reached an apparent plateau at 5 mg l^{-1} (Table II). As a consequence of these effects, the total leaf area of plants sprayed with BAP increased by approx. 20 – 40%, depending on BAP concentration. In addition, the leaf length:leaf width ratio tended to decrease with increasing BAP concentration (Table II).

When the effects of multiple applications of BAP (the 2007-2008 experiment) were tested, shoot FW increased significantly with one, two, or three applications of 0.5 mg l^{-1} BAP compared to control plants. The effect of BAP was observed in all aerial parts (leaf blades, petioles and stems; Figure 1). In agreement with this response, higher RGR_{FW} values were found in plants given a single spray of 5 mg l^{-1} BAP solution (Table III). BAP-sprayed plants also had increased RLAE values, and higher RLA values than control plants. The maximum responses for these three parameters were achieved with a single application of BAP. Further BAP sprays had no effect (Table III).

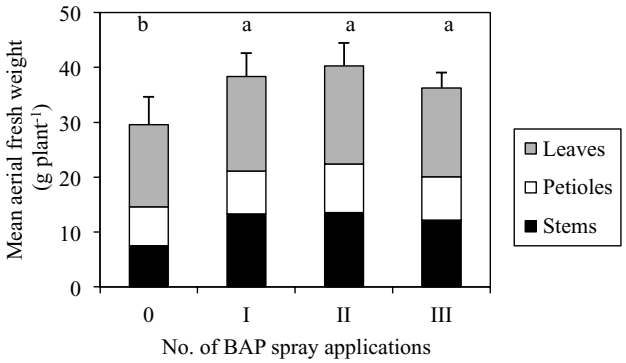

Fig. 1
Mean fresh weights (n = 20) of different aerial plant parts (stems, petioles, and leaves) at the end of the 2007-2008 experiment in *E. aureum* plants subjected to different numbers of applications of 5 mg l^{-1} 6-benzylaminopurine (BAP). Bars indicate standard errors. Different lower-case letters indicate statistical significance ($P \leq 0.05$) based on total aerial biomass.

E. aureum plants sprayed once or twice with 5 mg l^{-1} BAP increased their total leaf area by ≥ 100% compared to control plants (Table IV). Consequently, the mean individual leaf area was increased significantly by one or two applications of 5 mg l^{-1} BAP, while the effect of three applications of BAP was not significant. The leaf length:leaf width ratio decreased significantly with any number of BAP applications. SLA_{FW}, a measure of leaf thickness, showed a slight, but significant increase in all BAP treatments. There were no significant differences in the DMC percentage between controls and BAP-sprayed plants (Table IV).

In the 2008-2009 experiment, increased aerial FWs (Figure 2), as well as higher RGR_{FW} and RLAE values (Table V) were found at the highest irradiance and, in all cases, the applications of BAP significantly increased these values. In general, these effects were greatest at the lowest BAP concentration (i.e., the highest RGR_{FW} or RLAE differences were achieved with 2.5 mg l^{-1} BAP under 30% and 70% full-sunlight, or with 5 mg l^{-1} BAP in plants grown under 50% full-sunlight). An increase in light intensity, or a BAP application, significantly increased RLA values (Table V).

In general, the higher the BAP concentration the greater the increase in both total and mean individual

Table II
Total leaf area, individual leaf area, leaf length:leaf width ratio, rate of leaf appearance (RLA), and relative rate of leaf area expansion (RLAE) in E. aureum *cuttings sprayed with different concentrations of 6-benzylaminopurine (BAP) in the 2006-2007 experiment*

BAP concn. (mg l^{-1})	Total leaf area (cm^2 $plant^{-1}$)	Individual leaf area (cm^2 $leaf^{-1}$)	Leaf length: leaf width ratio	RLA (number of leaves $week^{-1}$)	RLAE (cm^2 cm^{-2} d^{-1})
0	517.07 ± 36.56b[†]	54.88 ± 4.12b	1.475 ± 0.050a	0.3405 ± 0.0140c	0.0136 ± 0.00085
5	606.07 ± 59.73ab	67.31 ± 4.39a	1.429 ± 0.059a	0.3763 ± 0.0160b	0.0168 ± 0.00047
50	667.65 ± 45.90ab	64.93 ± 4.84a	1.417 ± 0.058a	0.3973 ± 0.0123a	0.0167 ± 0.00048
100	764.83 ± 40.12a	71.01 ± 5.07a	1.412 ± 0.057a	0.4081 ± 0.0164a	0.0170 ± 0.00053
200	705.82 ± 49.43ab	70.06 ± 5.53a	1.375 ± 0.060a	0.4216 ± 0.0201a	0.0167 ± 0.00056

[†]Mean values (n = 20) ± SE in each column followed by different lower-case letters were significantly different at $P \leq 0.05$ by Tukey's test for one-way pairwise ANOVA.

TABLE III
Relative growth rate on a FW basis (RGR_{FW}), relative rate of leaf area expansion (RLAE), and rate of leaf appearance (RLA) in E. aureum *plants subjected to a different number of 5 mg l^{-1} BAP spray applications in the 2007-2008 experiment*

No. of BAP sprays	RGR_{FW} (g g^{-1} d^{-1})	RLAE (cm^2 cm^{-2} d^{-1})	RLA (number of leaves $week^{-1}$)
0	0.0134 ± 0.00062†	0.0141 ± 0.00064	0.4086 ± 0.0386c
1	0.0154 ± 0.00059	0.0155 ± 0.00069	0.6054 ± 0.0378a
2	0.0146 ± 0.00062	0.0149 ± 0.00069	0.5902 ± 0.0543a
3	0.0144 ± 0.00076	0.0147 ± 0.00076	0.5221 ± 0.0367ab

†Mean values (n = 20) ± SE in a column followed by a different lower-case letter were significantly different at $P \leq 0.05$ by Tukey's test for one-way pairwise ANOVA.

leaf areas (Table VI). The maximum promotion of individual leaf area was found in plants sprayed with 2.5, 5.0, or 10.0 mg l^{-1} BAP under 70%, 50%, or 30% full-sunlight respectively. In general, both the leaf length:leaf width ratio and SLA_{FW} decreased with increasing irradiance and BAP concentration. There were significant differences in the DMC percentage between plants grown under different light environments, but no effect of BAP spray was found (Table VI).

When the BAP-induced increase in total leaf area was plotted as a percentage of control plant values under each level of irradiance, the highest values were observed in plants grown under 50% full-sunlight. The highest value (an approx. 300% increase) was observed in plants sprayed with 5 mg l^{-1} BAP. At 70% full-sunlight, a slightly lower increase was observed, while at 30% full-sunlight, the promotive effect of BAP decreased markedly (Figure 3).

The relative contribution of both individual leaf area and RLA on the BAP-induced increase in total leaf area was calculated for each light environment in the 2008-2009 experiment. In plants grown either at 30% or 70% full-sunlight, the increase in total leaf area was associated mainly with changes in individual leaf area (Figure 4A,C). In the same way, in plants grown in 50% full-sunlight, the contribution of changes in RLA to the increase in total leaf area was closely related to that of changes in individual leaf area, except at the highest BAP concentration (Figure 4B). The contribution of changes in RLA to the increase in total leaf area, plotted as a function of the percentage of the total leaf area compared to the controls, increased linearly ($r^2 = 0.752$) after a threshold of an approx. 70% increase in leaf area over the controls (Figure 4D).

DISCUSSION

The productivity of ornamental plants is closely associated with an increase in their total leaf area over time. Most ornamental foliage plants are adapted to shade, and show low rates of leaf expansion (Di Benedetto *et al.*, 2006). This is frequently aggravated by reduced pot volumes that lead to root restriction (Di Benedetto *et al.*, 2010). The application of exogenous cytokinins can improve plant growth in ornamental shade plants grown in pots at commercial facilities (Di Benedetto *et al.*, 2010). Cytokinins have a strong influence on many aspects of shoot development and plant metabolism, including branching (Müller and Leyser, 2011) and leaf expansion (Shani *et al.*, 2010). However, the possibility that the rate at which leaves appear in the meristem could be regulated by cytokinins has attracted little attention. This study presents evidence that BAP sprays increase the total leaf area in *E. aureum* (in cm^2 $plant^{-1}$; Table II; Table IV; Table VI) due to increasing both individual leaf areas and an enhanced RLA.

There are few reports on whether more than a single application of cytokinin is needed for a prolonged effect on plant growth. Maene and Debergh (1982) made

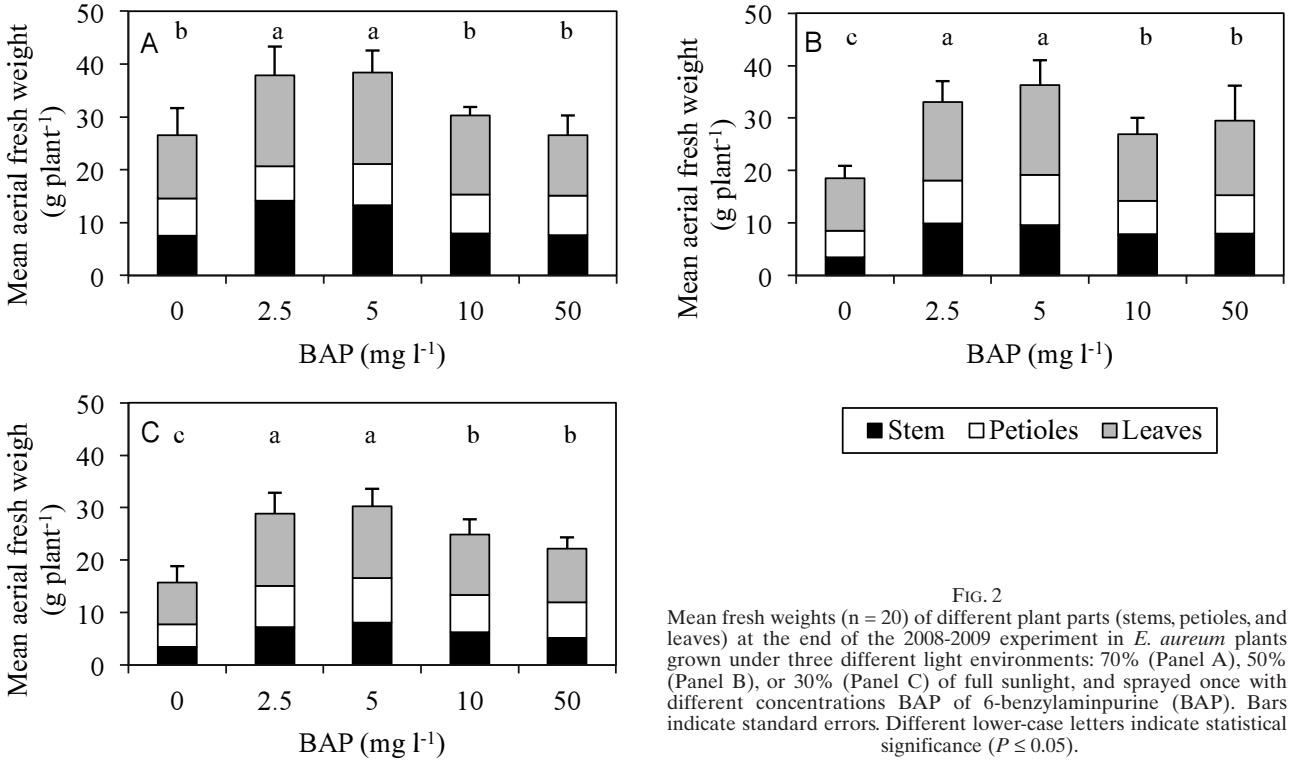

FIG. 2
Mean fresh weights (n = 20) of different plant parts (stems, petioles, and leaves) at the end of the 2008-2009 experiment in *E. aureum* plants grown under three different light environments: 70% (Panel A), 50% (Panel B), or 30% (Panel C) of full sunlight, and sprayed once with different concentrations BAP of 6-benzylaminpurine (BAP). Bars indicate standard errors. Different lower-case letters indicate statistical significance ($P \leq 0.05$).

Table IV

Total leaf area, individual leaf area, leaf length:leaf width ratio, specific leaf area on a FW basis (SLA_{FW}), and leaf dry matter content (DMC) at the end of the 2007-2008 experiment for plants of E. aureum *subjected to a different number of 5 mg l^{-1} BAP spray applications*

No. of BAP sprays	Total leaf area (cm^2 $plant^{-1}$)	Individual leaf area (cm^2 $leaf^{-1}$)	Leaf length: leaf width ratio	SLAFW (cm^2 g^{-1})	DMC (%)
0	309.59 ± 65.86c†	30.96 ± 2.5b	1.598 ± 0.040a	26.23 ± 0.141b	9.21 ± 0.16a
1	630.36 ± 48.64ab	39.98 ± 2.2ab	1.506 ± 0.048b	27.99 ± 0.158a	10.17 ± 0.41a
2	701.19 ± 54.08a	44.95 ± 3.4a	1.478 ± 0.035ab	29.33 ± 0.167a	8.34 ± 0.54a
3	538.13 ± 30.99bc	33.99 ± 3.3ab	1.503 ± 0.033b	28.08 ± 0.094a	9.23 ± 0.19a

†Mean values (n = 20) ± SE in a column followed by different lower-case letters were significantly different at $P \leq 0.05$ by Tukey's test for one-way pairwise ANOVA.

several applications of 100 – 500 mg l^{-1} BAP to stimulate shoot axillary bud branching in *Cordyline terminalis*. Garner *et al*. (1998) reported that a single spray of 3,000 mg l^{-1} BAP increased shoot branching in *Hosta* spp., but additional applications were required for continuous stimulation. Nath and Pal (2007) showed that two sprays of benzyladenine, 45 d apart, were necessary to obtain the optimal commercial forms of *Aglaonema crispum*, *Philodendron erubescens*, and *Dieffenbachia maculata*. In our work, a single spray of 5 mg l^{-1} BAP was sufficient to increase both total and individual leaf areas (Table II), the accumulation of FW (Figure 1), and RGR_{FW}, RLAE and RLA values (Table III).

The increase in individual leaf area as a consequence of BAP treatment agreed with the findings of Nath and Pal (2007) on the three above-mentioned ornamental species. However, the cytokinin concentration used in their study was significantly higher (1,500 mg l^{-1} BAP) than those in our experiments (2.5 – 200 mg l^{-1} BAP). This may be associated with the different plant species and cytokinin formulations used. In the present work, the fact that a single BAP spray at a low concentration (5 mg l^{-1}) was enough to increase and maximise the production of aerial biomass for at least 180 d after BAP application may have commercial impact (Figure 1). The increase in individual leaf area may be a consequence of an increase in cell number and/or in cell size. Cytokinins are known to influence both (Del Pozo *et al*., 2005; Doerner, 2007; Hamada *et al*., 2008).

A significant increase in RLA values was found when plants were sprayed with BAP at different concentrations (Table II; Table V). Since *E. aureum* leaves appeared on a single shoot without branches, the increase in RLA indicated a shorter phyllochron. It has been suggested that cytokinins are involved in the maintenance of vegetative apex and in cellular differentiation (Del Pozo *et al*., 2005; Shani *et al*., 2006) and that they can promote leaf unfolding and expansion in whole plants in a range of species (Doerner, 2007). However, relatively few studies have been reported regarding regulation of the phyllochron (Shani *et al*., 2006; Kyozuka, 2007; Zhu *et al*., 2007; Lee *et al*., 2009). Nevertheless, to our knowledge, there have been no previous reports on the promotional effect of exogenous cytokinins on RLA in intact plants.

How meristem size, and a cytokinin, affect the formation of a leaf primordium is still unclear. A coherent understanding of the regulation of the duration of the phyllochron is lacking. Several genes that regulate the temporal pattern of formation of leaf primordia have been identified (Wang *et al*., 2008). These positively regulate organ size, while extending the length of the phyllochron (i.e., delaying the production of new leaves), thus providing a compensatory mechanism that links the rate at which leaves are produced to the final leaf size. Such a mechanism would mean that the overall rate of production of biomass would remain relatively constant. This would be achieved by producing numerous small organs, or fewer (but larger) organs (Bögre *et al*., 2008). It is noteworthy that such a compensatory mechanism did not apply to BAP-treated *E. aureum* plants in our experiments, since increases in both RLA (Table II;

Table V

Relative growth rate on a FW basis (RGR_{FW}), rate of relative leaf area expansion (RLAE), and rate of leaf appearance (RLA) for E. aureum *plants grown under three light intensities (70%, 50%, or 30% full-sun) and sprayed with different concentrations of BAP (2008-2009 experiment)*

Light intensity (LI)	BAP concn. (mg l^{-1})	RGR_{FW} (g g^{-1} d^{-1})	RLAE (cm^2 cm^{-2} d^{-1})	RLA (No. of leaves $week^{-1}$)
70% Full-sun	0	0.0131 ± 0.00066†	0.0144 ± 0.00065	0.4086 ± 0.021
	2.5	0.0152 ± 0.00058	0.0156 ± 0.00055	0.5978 ± 0.034
	5	0.0154 ± 0.00059	0.0157 ± 0.00069	0.6054 ± 0.021
	10	0.0141 ± 0.00065	0.0146 ± 0.00067	0.4541 ± 0.025
	50	0.0141 ± 0.00072	0.0153 ± 0,00082	0.4389 ± 0.013
50% Full-sun	0	0.0108 ± 0.00047	0.0120 ± 0.00050	0.3103 ± 0.014
	2.5	0.0144 ± 0.00052	0.0147 ± 0.00058	0.4768 ± 0.029
	5	0.0157 ± 0.00075	0.0169 ± 0.00073	0.4843 ± 0.014
	10	0.0129 ± 0.00059	0.0141 ± 0.00065	0.4767 ± 0.011
	50	0.0137 ± 0.00070	0.0148 ± 0.00067	0.4011 ± 0.019
30% Full-sun	0	0.0094 ± 0.00057	0.0109 ± 0.00056	0.2876 ± 0.014
	2.5	0.0132 ± 0.00051	0.0145 ± 0.00050	0.4314 ± 0.025
	5	0.0130 ± 0.00061	0.0146 ± 0.00065	0.5281 ± 0.054
	10	0.0116 ± 0.00062	0.0129 ± 0.00063	0.3708 ± 0.012
	50	0.0115 ± 0.00062	0.0130 ± 0.00060	0.3708 ± 0.023
Probability levels of significance by ANOVA				
Source of variation				
LI	< 0.001			
BAP	< 0.001			
LI × BAP	< 0.001			

†Mean values (n = 20) ± SE.

TABLE VI

Total leaf area, individual leaf area, leaf length:leaf width ratio, specific leaf area on a FW basis (SLA_{FW}), and leaf dry matter content (DMC) at the end of the 2008-2009 experiment for plants of E. aureum *grown under three light intensities (70%, 50%, or 30% full-sun) and sprayed with different concentrations of BAP*

Light intensity (LI)	BAP concn. (mg l^{-1})	Total leaf area (cm^2 $plant^{-1}$)	Individual leaf area (cm^2 $leaf^{-1}$)	Leaf length: leaf width ratio	SLA_{FW} (cm^2 g^{-1})	DMC (%)
70% Full-sun	0	263.20 ± 65.85[†]	26.86 ± 0.62	1.598 ± 0.040	30.96 ± 0.80	9.21 ± 0.18
	2.5	589.35 ± 57.78	41.50 ± 0.82	1.470 ± 0.069	30.28 ± 0.29	9.12 ± 0.26
	5	559.42 ± 48.64	39.29 ± 1.13	1.505 ± 0.048	30.25 ± 0.28	10.17 ± 0.45
	10	583.60 ± 14.49	44.89 ± 0.93	1.534 ± 0.063	27.86 ± 0.17	9.64 ± 0.28
	50	608.08 ± 60.16	50.67 ± 2.07	1.549 ± 0.057	29.45 ± 0.81	10.54 ± 0.20
50% Full-sun	0	234.01 ± 35.24	24.00 ± 2.99	1.656 ± 0.073	31.59 ± 0.57	8.51 ± 0.23
	2.5	488.70 ± 59.72	32.21 ± 2.12	1.405 ± 0.050	31.17 ± 0.47	9.55 ± 0.29
	5	710.09 ± 54.90	43.48 ± 1.01	1.457 ± 0.052	28.92 ± 0.24	9.13 ± 0.35
	10	640.13 ± 36.28	39.51 ± 0.83	1.510 ± 0.075	31.58 ± 0.36	10.02 ± 0.33
	50	684.88 ± 50.90	54.06 ± 1.62	1.406 ± 0.033	30.91 ± 0.38	8.92 ± 0.32
30% Full-sun	0	237.53 ± 38.62	29.29 ± 0.40	1.646 ± 0.048	29.92 ± 0.92	8.39 ± 0.18
	2.5	488.45 ± 47.16	34.16 ± 0.61	1.566 ± 0.041	31.21 ± 0.49	8.38 ± 0.17
	5	365.12 ± 52.99	37.26 ± 2.21	1.583 ± 0.049	31.97 ± 0.36	8.82 ± 0.21
	10	327.82 ± 33.87	35.98 ± 0.68	1.625 ± 0.076	31.88 ± 0.33	7.80 ± 0.43
	50	322.24 ± 22.45	31.29 ± 1.12	1.602 ± 0.043	29.30 ± 0.27	8.54 ± 0.18
Probability levels of significance by ANOVA						
Source of variation						
LI		< 0.001	< 0.001	< 0.001	< 0.05	< 0.001
BAP		< 0.001	< 0.001	< 0.001	< 0.05	n.s.
LI × BAP		< 0.001	< 0.001	< 0.001	< 0.05	< 0.01

[†]Mean values (n = 20) ± SE.

Table III; Table V) and individual leaf area (Table II; Table IV; Table VI) occurred.

The quantitative relationships shown in Figure 2 and in Figure 4 indicate that, for *E. aureum*, total leaf area was associated mainly with individual leaf area, with lower, but significant, participation of RLA. This was particularly important under 50% full-sun. The contribution of RLA to the increase in total leaf area after BAP spraying appeared to be important only after approx. 70% of the total leaf area increase had been achieved (Figure 4D). This threshold represents those BAP-induced changes in total leaf area that are attributable solely to an increase in leaf size. These results suggest that both the growth of individual leaves, and RLA, might be independent (but coordinated) processes leading to the increase in total leaf area, and are likely to involve the activation of different genes. However, more research will be needed to validate this hypothesis.

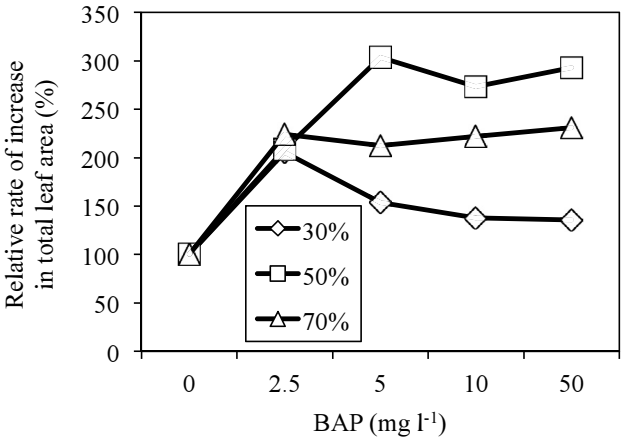

FIG. 3

Relative rate of increase in total leaf area in *E. aureum* plants grown under three different light intensity environments: 70%, 50%, or 30% of full sunlight and sprayed with different concentrations of 6-benzylamino purine (BAP). Untreated control (0 mg l^{-1} BAP) = 100%.

In addition to changing the leaf final area and RLA, BAP appeared to modify leaf shape through a slight decrease in the leaf length:leaf width ratio (Table II; Table IV; Table VI). The leaf length:leaf width ratio tended to be higher in a low light environment (Table VI). An increase in leaf length is usually a response to low light intensity (Ferjani *et al*., 2008) and cytokinins have been shown to mimic this response to light (Carabelli *et al*., 2007).

Light intensity changed the quantitative impact of BAP on individual leaf area and RLA, and on leaf area expansion (Figure 4). At both high and low irradiances (70% and 30% full-sunlight respectively), the responses to BAP were lower than under intermediate irradiance (50% full-sunlight). The reason why BAP failed to elicit similar effects in a low light environment compared to those observed under higher irradiances may be related to the restricted availability of carbon which would become a limiting factor to sustain the promotion of growth by BAP. On the other hand, higher irradiance could be related to greater root development (Nagel *et al*., 2006) which, in turn, would increase endogenous cytokinin concentrations (O'Hare and Turnbull, 2004) to levels which might fulfil the requirements for plant growth. This may also be the reason why plateaux were observed in most of the variables studied in the present work at relatively higher BAP spray concentrations.

The effects of light intensity reported here were most likely a consequence of differences in irradiance, not in light quality. Carabelli *et al*. (2007) reported a decrease in foliar concentrations of cytokinins in shaded environments enriched by red light, suggesting an interaction between light quality and phytochromes. However, the shade cloths used to reduce the light intensity in our experiments had no significant effect on the R:FR ratio of the incident light. Nevertheless, possible involvement of high irradiance response signalling should not be ruled-out.

Increased FWs of the aerial parts of BAP-sprayed plants were observed, in parallel with higher individual

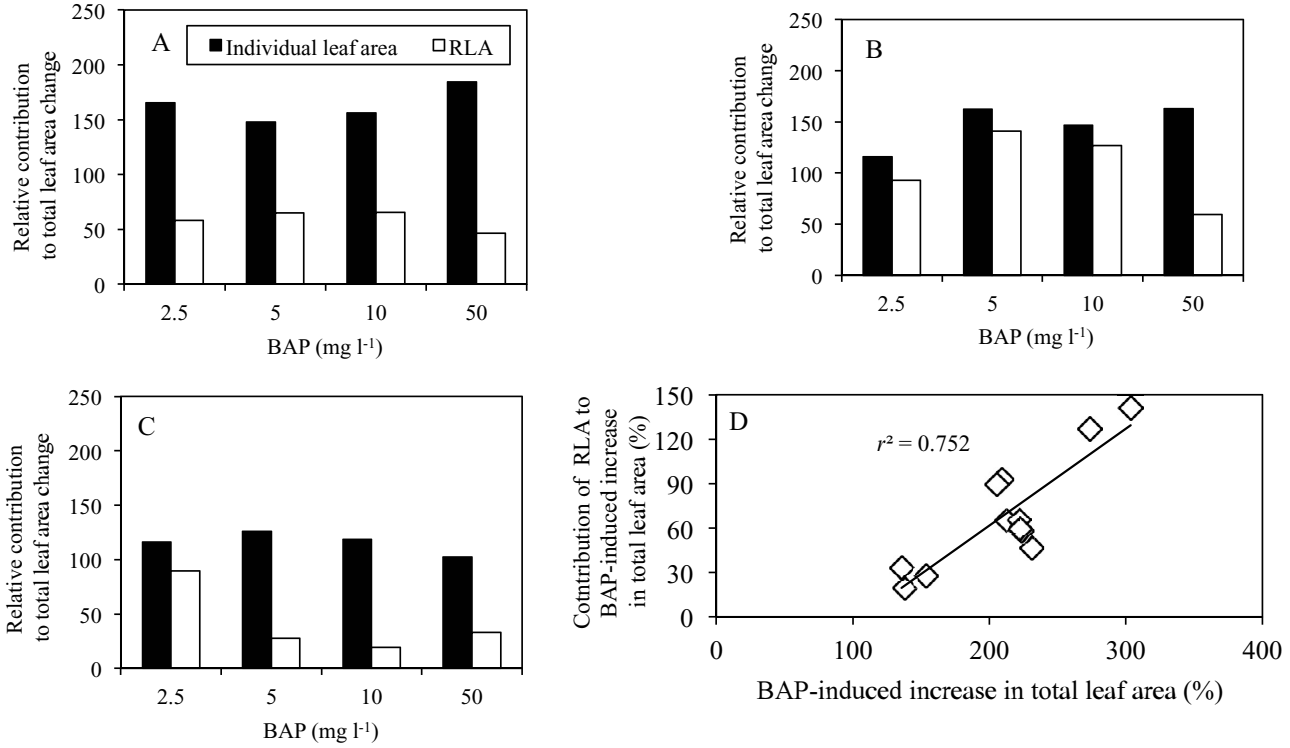

Fig. 4
Relative contribution of individual leaf area and RLA to the total leaf area changes due to a single spray application of 2.5, 5.0, 10.0 or 50.0 mg l^{-1} 6-benzylaminopurine (BAP) to *E. aureum* plants grown under different light intensities: 70% (Panel A), 50% (Panel B), or 30% (Panel C) of full sunlight in the 2008-2009 experiment. Panel D, contribution of increased RLA to the BAP-induced increase in total leaf area compared to untreated controls (= 0%).

leaf areas and RGR_{FW} values (Table III; Table V). The sequence of biomass (FW) accumulation and initiation of primordia depends on photo-assimilate partitioning through an endogenous signal, hormonal in nature (Bolouri-Moghaddam *et al*., 2010), and substrates for cell division and cell expansion (Fleming, 2006). Thus, the BAP-induced accumulation of aerial FW might be a consequence of increased partitioning of carbon to the shoot apical meristem, resulting in a higher leaf area which, in turn, would drive growth through increased carbon assimilation. Alternatively, BAP might enhance carbon fixation directly through an increase in the content of photosynthetic pigments, or changes in the activity of the photosynthetic apparatus (Boonman *et al*., 2009; Cortleven and Valcke, 2012).

In conclusion, an exogenous supply of BAP increased the total leaf area, and FW accumulation, in *E. aureum* plants grown in pots. However, the magnitude of this response depended on both BAP concentration and light intensity. The increase in total leaf area accumulation occurred through an increase in individual leaf area and an accelerated rate of leaf production. From a grower's perspective, this response may be useful to increase the commercial production of foliage plants grown in greenhouse facilities. Since the DMC percentage was not affected by any of the BAP treatments, the changes observed in FW accumulation corresponded to effects on DM accumulation and thus, ultimately, on carbon fixation. The reason why BAP stimulated DM accumulation is unclear, but two possibilities appear likely: (i) higher photo-assimilate partitioning towards the shoot; or (ii) an increase in the photosynthetic efficiency of leaves. Further research is needed to ascertain whether one of these possibilities, or a combination or both, can account for the observed responses of *E. aureum* to a single exogenous spray application of BAP.

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