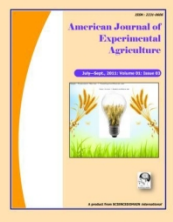

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Lettuce and Celery Responses to Both BAP and PBZ Related to the Plug Cell Volume

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Authors' contributions

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

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ABSTRACT

Aims: Decreasing vegetable transplant costs involves decreasing the plug cell volume, which is known to have negative effects during post-transplant growth. The objective of this work was to evaluate the impact of different plug cell volume and hormonal regulators on both lettuce and celery yield.
Study Design: Two genotypes of lettuce ('Dolly' and 'Shirley') and two of celery ('Golden Boy' and 'Green Fox') were used in the experiments. Plants grown in 288- or 200-cell trays and were sprayed with different solutions of BAP and PBZ.
Place and Duration of Study: Experiments were conducted at the INTA Balcarce Experimental Station, Argentina (37º45′S, 58º18′W) during the 2008-2009 and 2010-2011 growing seasons.
Methodology: Three experiments were performed. In the experiment 1, base temperature from lettuce and celery plants grown in 288- or 200-cell trays were determined through the method of temperature summation. Experiment 2 showed the response to 100 mg L^{-1} BAP of both vegetables grown in two plug cell volume. Experiment 3 showed the combined effect of different BAP and PBZ concentrations related to different plug cell volume.
Results: Results showed that spraying lettuce and celery plants with a single pre-transplant application of BAP or PBZ increased post-transplant fresh weight. On the other hand, the different genotypes of lettuce and celery evaluated showed significant

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differences in the T_b.
Conclusion: We proposed that the use of larger plug cells and growth regulator sprays may allow plants to overcome the root restriction imposed by the cell volume, with a correlative increase in post-transplant productivity of lettuce and celery. A significant decrease in T_b would partially explain the highest growth rate in the largest cell size volume.

Keywords: Growth rate; growth regulators; nursery; root restriction; yield.

1. INTRODUCTION

The use of containerized vegetable transplants has increased yield and uniformity and allowed a more predictable timing of production relative to direct seeding. Vegetable transplants are produced in a number of various sized containers or plug cell trays.

Container size is important to transplant producers as they seek to optimize production space which involves different factors that govern both seedling production and post-transplant plant performance [1,2]. However, a decrease in the container size alters the rooting volume of the plants, which can greatly affect plant growth. Root restriction, i.e. the physical stress imposed on a root system when plants are grown in small containers may lead to a pronounced decrease in both root and shoot growth of the plants after the transplant stage and in the root:shoot ratio as well.

We have previously shown that the exogenous application of the cytokinin 6-benzylaminopurine (BAP) to plants grown in small pots may override the shoot growth limitation due to root restriction [1,2]. We have also previously shown that the pre-transplant application of BAP increases both lettuce and celery yield [3]. On the other hand, an endogenous decrease in cytokinin levels negatively affects the development of the aerial part [4-7]. Paclobutrazol (PBZ), an inhibitor of gibberellins, has been used to decrease shoot elongation [8], since PBZ also seems to decrease root length too (unpublished data).

Growth and development of field crops with optimal water and nutrient supply are largely controlled by the effects of environmental factors, such as temperature [3,9]. The growth of seedlings given plentiful water and nutrients in a wide range of temperature and light conditions and plant densities can be characterized by the base temperature. This parameter, which is defined as the lowest temperature where metabolic processes result in a net substance gain in aboveground biomass, may vary according to the external conditions, the age of plants and the previous treatments [10].

Lettuce (*Lactuca sativa* L.) is one of the most important crops worldwide, because of its taste and nutritional characteristics and celery (*Apium graveolens* L. var. *dulce* Mill. Pers.) is a leafy vegetable which may be eaten fresh or processed.

Due to the need to optimize the growth of lettuce and celery seedlings in plastic traits, the objective of this work were to analyze yield changes by the use of different plug cell volumes and hormonal regulators (BAP and PBZ).

2. MATERIALS AND METHODS

2.1 Plant Material, Treatments and Experiments

Experiments were conducted at the INTA Balcarce Experimental Station, Argentina (37°45′S, 58°18′W) during the 2008-2009 and 2010-2011 growing seasons. Two genotypes of lettuce ('Dolly' and 'Shirley') and two of celery ('Golden Boy' and 'Green Fox') were used in the experiments.

'Dolly' is a large summer butter-head lettuce, whereas 'Shirley' is a cold butter-head lettuce for autumn-winter production. On the other hand, 'Golden Boy' is a self-blanching celery cultivar for year round production whereas 'Green Fox' is a green celery cultivar for summer production.

Three experiments were performed:

Experiment 1: Lettuce ('Dolly' and 'Shirley') and celery ('Golden Boy' and 'Green Fox') seeds were fortnightly sown between March 2008 and February 2009 and grown in 288- ($6.18 cm^3 cell^{-1}$) or 200- ($13.90 cm^3 cell^{-1}$) cell trays using a Fafard Growing Mix 2® substrate (Canadian *Sphagnum* peat moss-perlite-vermiculite 70:20:10v/v/v). Physical properties of the media were, total porosity: 85.72%, air-filled porosity: 20.94%, container capacity: 22.78% and bulk density: $0.14 gcm^{-3}$. Plants were grown at greenhouse facilities and harvested at the transplant stage. Half hourly averages of the air temperature were measured using a HOBO H08-001-02 data logger (Onset Computer Corporation, MA, USA) protected from direct radiation by aluminum foil shades.

The base temperatures (T_b) were determined through the method of temperature summation. To do this, development rates which reflected the fractional amount of development per day were related to the average air temperature (T_S). The T_S-axis intercept of this equation is T_b.

Experiment 2: 'Dolly' lettuce and 'Golden Boy' celery seeds were sown in January 2010 in 288- ($6.18 cm^3 cell^{-1}$) or 200- ($13.90\ cm^3 cell^{-1}$) cell trays as in the experiment 1 and transplanted to a typic argiudol soil with of 5.2% of organic matter the first 25cm depth 35 and 45 days after sowing for lettuce and celery respectively. Seedlings were sprayed with BAP ($100 mgL^{-1}$) (6-benzilaminopurine) (SIGMA EC 214-927-5) when first true leaf pairs were developed.

Experiment 3: 'Dolly' lettuce and 'Golden Boy' celery seeds sown in January 2011 in 288- or 200-cell trays as in the experiment 1 were transplanted as in the experiment 2. The triazol growth retardant Paclobutrazol (PBZ) [(β-[(4-chlorophenyl) methyl]-α-(1, 1- dimethylethyl)-1*H*-1, 2, 4-triazole-1-ethanol)] was added to the media when first true leaves were developed: $2 Lm^{-2}$ of bench area from a solution of 0, 10 and $20 mgL^{-1}$ for lettuce and 0, 5 and $10 mgL^{-1}$ for celery. One week later, seedlings were sprayed to runoff with different BAP solutions (0, 5, 50 and $100 mgL^{-1}$).

Plants were irrigated as needed with high quality tap water (pH: 6.64 and electrical conductivity of $0.486 dSm^{-1}$) using intermittent overhead mist and one weekly fertigation according to Styer and Koranski [11] (Stage 2: $50 mgL^{-1}N$; Stage 3-4: $100 mgL^{-1}N$; post-

transplant: 150mgL^{-1}N) was used for all the experiments. The volume per pot varied according to container volume.

2.2 Growth Evaluations

Plants from experiments 2 and 3 were harvested at both transplant and sale stage (60 and 90 days from transplant for lettuce and celery respectively), separated into roots and shoots and their fresh mass was determined. The relative growth rate (RGR) (g g^{-1} day^{-1}) was calculated as the slope of the straight-line regression of the natural logarithm of whole plant dry mass vs. time in days. The root-shoot ratio was also calculated. The allometric relationships between roots and shoots were estimated using a straight-line regression between the natural logarithm of roots dry weight and the natural logarithm of shoots dry weight during the experiments.

Mean temperatures and global solar radiation during the field development of experiments 2 and 3 were recorded from a meteorological station 500m from the experimental site: 11.3-24.2°C and 5.5-25.3$MJm^{-2}day^{-1}$ (lettuce 2010); 8.5-24.2°C and 4.7-25.3$MJm^{-2}day^{-1}$ (celery 2010); 13.1-28.5°C and 6.1-27.7$MJm^{-2}day^{-1}$ (lettuce 2011); 8.2-28.5°C and 5.8-27.7$MJm^{-2}day^{-1}$ (celery 2011).

2.3 Statistical Analysis

Data from experiment 2 were subjected to a one-way ANOVA for a completely randomized design analysis and means were separated by the Tukey´s test (*P*=0.05). The experimental design for experiment 3 was a randomized factorial with three blocks of 10 single-pot replications of each treatment combination. Data were subjected to a two-way ANOVA and means were separated by Tukey´s test (*P*=0.05). Regression slopes were testing for parallelism (test for equal slope) [12].

3. RESULTS

3.1 Experiment 1: Base Temperature

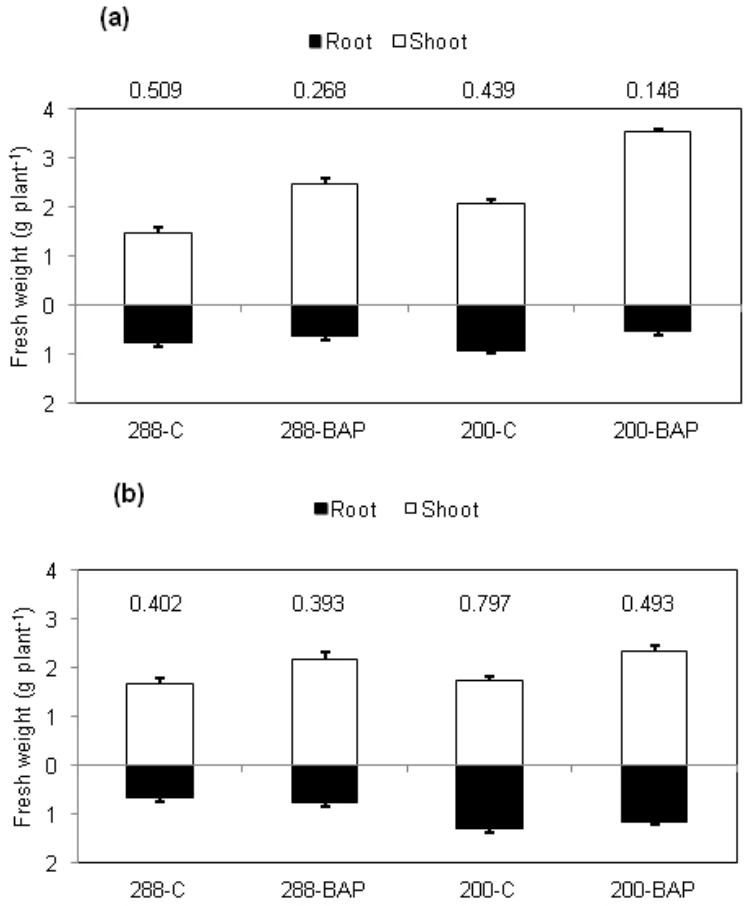
The values of base temperature for the genotypes selected for summer-grown plants were significantly lower than those for the winter-grown plants (*P*=.001). The use of 200-cell trays led to lower base temperature than the use of 288-cell trays for the two species and genotypes analyzed (Table 1).

Table 1. Changes in lettuce ('Dolly' and 'Shirley) and celery ('Golden Boy' and 'Green Fox') T_b in plants grown in two different plug cell trays (288- and 200-cells $tray^{-1}$) in the Experiment 1. Lower case letters indicate statistically significant differences (*P*=.001) among plug cell size for each lettuce and celery varieties

T_b (°C)							
Lettuce				Celery			
'Shirley'		'Dolly'		'Golden Boy'		'Green Fox'	
288-cells	200-cells	288-cells	200-cells	288-cells	200-cells	288-cells	200-cells
2.559[a]	0.023[b]	10.245[a]	8.084[b]	1.696[a]	0.692[b]	19.555[a]	17.425[b]

3.2 Experiment 2: Cell Volume and BAP Effects on Biomass Accumulation

'Dolly' lettuce plants sprayed at the pre-transplant stage with a single dose of 100mgL^{-1} BAP showed an increase in shoot fresh weight at the transplant stage in both 288- and 200-cell trays and either no effect -- (288-cell tray^{-1}) or a decrease (200-cell tray^{-1}) in root fresh weight. Both 'Dolly' lettuce and 'Golden Boy' celery BAP-sprayed at the pre-transplant stage showed a decrease in root/shoot ratio (Fig. 1).

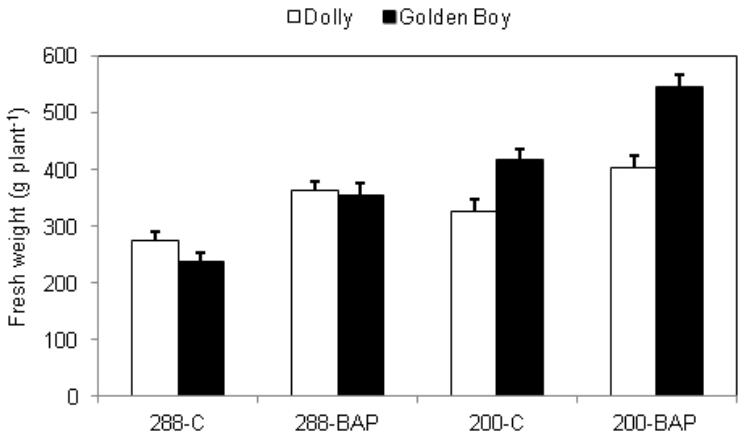


Significantly	'Dolly' lettuce		'Golden Boy' celery	
	Root	Shoot	Root	Shoot
Cell size	*	**	***	ns
BAP	*	***	ns	**
Cell size x BAP	*	***	**	**

*Significance *** .001 ** .01 * .05 'ns' No significant*

Fig. 1. The effect of two plug cell trays (288- and 200-cells tray^{-1}) and a pre-transplant BAP application (100 mg L^{-1}) on the root and shoot fresh weight at the transplant stage of 'Dolly' lettuce (A) and 'Golden Boy' celery (B) in the Experiment 2. Control plants without treatment: -C. Bars are mean of thirty replications and standard errors were indicated; root/shoot ratios were indicated as well (n=24, *P*=.001)

At the sale stage, productivity (expressed as aerial fresh weight) increased significantly when a single BAP spray and 200-cell trays were used for both 'Dolly' lettuce and 'Golden Boy' celer (Fig. 2). Single (Cell size; BAP) and double (Cell sizexBAP) effects for shoot fresh weight accumulation in the ANOVA showed highly significant differences (P<.001).



Significantly	
Cell size	***
BAP	***
Cell size x BAP	***

*Significance *** .001 ** .01 * .05 'ns' No significant*

Fig. 2. The effect of two plug cell trays (288- and 200-cells tray^{-1}) and a pre-transplant BAP application (100mgL^{-1}) on the aerial fresh weight at the sale stage of 'Dolly' lettuce and 'Golden Boy' celery in the Experiment 2. Control plants without treatment: -C. Bars are mean of thirty replications and standard errors were indicated

The relative growth rates (RGR) were higher for plants grown in 200-cell trays and sprayed with 100mgL^{-1} BAP ('Dolly' lettuce as 'Golden Boy' celery) between emergence-transplant and emergence-sale stages, although the values were higher at the emergence-transplant stage (Table 2).

3.3 Experiment 3: Cell Volume, BAP and PBZ Effects on Biomass Accumulation

A single BAP spray at the transplant stage significantly increased lettuce shoot fresh weight in both 288- and 200-cell trays. As root fresh weight did not change in plants-grown in 288-cell trays and significantly decreased in those grown in 200-cell trays, the root/shoot ratio significantly decreased as well. When an early 10mgL^{-1} PBZ solution was applied, plants grown in 288-cell trays showed a slight increase in both shoot and root fresh weight (the root/shoot ratio did not change significantly), whereas those grown in 200-cell trays showed a significant decrease in both shoot and root fresh weight with an increase in the root/shoot ratio (Fig. 3A). There were significant single (Cell size; BAP; PBZ), double and triple effects in the ANOVA test for shoot fresh weight. There was no Cell sizexPBZ double effect for root fresh weight accumulation; the single (Cell size; BAP; PBZ), double (Cell sizexBAP) and triple (Cell sizexBAPxPBZ) effects for roots were significant.

Table 2. Changes in the Relative Growth Rate (RGR) (on a fresh weight base) in two vegetables (lettuce and celery) grown at two plug cell trays (288- and 200-cells tray^{-1}) and sprayed or not (-C: control plants) with 100mgL^{-1} BAP pre-transplant solutions in the Experiment 2. Mean values (n=20) in each column followed by a different lower-case letter indicate significantly different slopes according to the test for equal slope (testing for parallelism). The probability of the slope being zero was *P*<.01

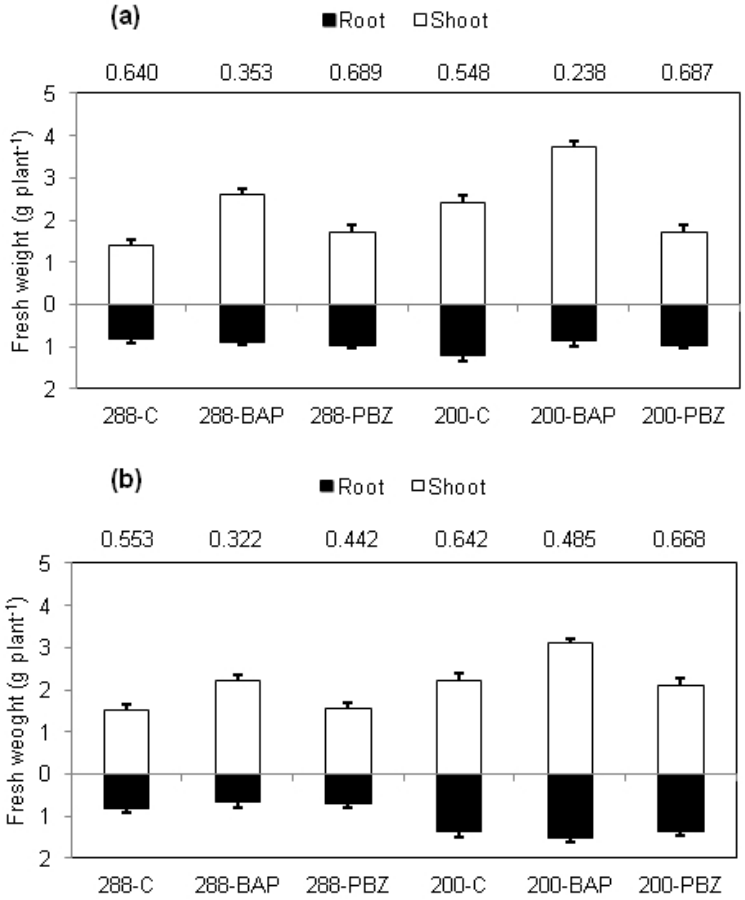
	RGR (g g^{-1} day^{-1})			
	'Dolly' lettuce		'Golden Boy' celery	
	Emergence-Transplant	Emergence-Sale	Emergence-Transplant	Emergence-Sale
288_C	0.0870^c	0.0692^c	0.0688^b	0.0555^b
288_{BAP}	0.0976^b	0.0773^b	0.0745^a	0.0680^a
200_C	0.0963^b	0.0713^b	0.0753^b	0.0693^{ab}
200_{BAP}	0.1054^a	0.0812^a	0.0787^a	0.0710^a

At the transplant stage, 'Golden Boy' celery plants grown in 288- or 200-cell trays showed an increase in shoot fresh weight (in a lesser proportion than in lettuce) when sprayed with 100mgL^{-1} BAP (the root/shoot ratio decreased significantly) and no significant changes when 5mgL^{-1} PBZ was applied; as a result, the root/shoot ratio decreased in the plants grown in 288-cell trays and did not change in those grown in the 200-cell trays (Fig. 3B). Single, double and triple shoot fresh weight accumulation effects were the same as for lettuce, with no significant root fresh weight accumulation effects.

At the sale stage, i.e. 60 days after transplant, 'Dolly' lettuce showed an increase in aerial fresh weight of plants grown in 288-cell trays in all treatments except when 20 mg L^{-1} PBZ and 100mgL^{-1} BAP were applied, and an increase in all plants grown in 200-cell trays all treatments (Fig. 4A). 'Golden Boy' celery showed significant but slight increases in fresh weight in plants grown in 288- and 200-cell trays when sprayed with 100mgL^{-1} BAP or 5mgL^{-1} PBZ was applied at the pre-transplant stage; plants with 10mgL^{-1} PBZ showed no differences as compared to control plants (Fig. 4B). There were highly significant differences (*P*<.001) for the single, double and triple effects in the ANOVA shoot fresh weight for both lettuce and celery.

The rate of leaf appearance in 'Dolly' lettuce and 'Golden Boy' celery was significantly increased over the controls with a single application of 100mgL^{-1} BAP or 10mgL^{-1} PBZ, with higher differences in plants grown in 200-cell trays than in those grown in 288-cell trays (Table 3). There were highly significant differences (*P*<.001) for the single, double and triple effects in the ANOVA RLA for lettuce. On the other hand, there were highly significant differences (*P*<.001) for single (Cell size; BAP; PBZ) and double (Cell sizexPBZ) interactions and significant differences (*P*<.01) for the rest of the effects in celery.

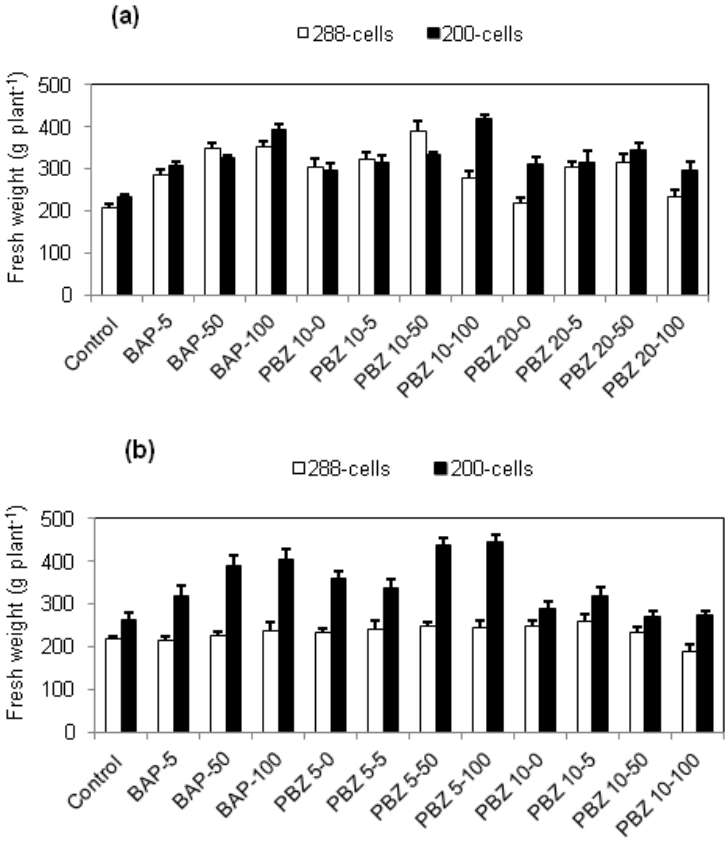
RGR values were significantly higher for the emergence-sale stage in Dolly' lettuce and 'Golden Boy' celery plants-grown in both 288- and 200-cell trays and sprayed with BAP or added PBZ related to controls (Table 4). There were highly significant differences (*P*<.001) for the single, double (Cell sizexPBZ) and triple effects in the ANOVA RGR in lettuce, but only in double (Cell sizexBAP) in celery. The rest of statistical effects showed significant differences (*P*<.01).



Significantly	'Dolly' lettuce		'Golden Boy' celery	
	Root	Shoot	Root	Shoot
Cell size	***	***	ns	***
BAP	**	***	ns	***
PBZ	**	*	ns	*
Cell sizexBAP	**	***	ns	***
Cell sizexPBZ	ns	*	ns	*
Cell sizexBAPxPBZ	**	***	ns	***

*Significance *** .001 ** .01 * .05 'ns' No significant*

Fig. 3. The effect of two plug cell trays (288- and 200-cells tray^{-1}) and a pre-transplant PBZ (10 and 5mgL^{-1} for lettuce and celery respectively) and BAP (100mgL^{-1}) applications on the root and shoot fresh weight at the transplant stage of 'Dolly' lettuce (A) and 'Golden Boy' celery (B) in the Experiment 3. Control plants without treatment: -C. Bars are mean of thirty replications and standard errors were indicated; root/shoot ratios were indicated as well



Significantly	'Dolly' ettuce	'Golden Boy' celery
Cell size	***	***
BAP	***	***
PBZ	***	***
Cell sizexBAP	***	***
Cell sizexPBZ	***	***
Cell sizexBAPxPBZ	***	***

*Significance *** .001 ** .01 * .05 'ns' No significant*

Fig. 4. The effect of two plug cell trays (288- and 200-cells tray^{-1}) and a pre-transplant PBZ (10 and 5mgL^{-1} for lettuce and celery respectively) and BAP (100mgL^{-1}) applications on the aerial fresh weight at the sale stage for 'Dolly' lettuce and 'Golden Boy' celery in the Experiment 3. ---------- Bars are mean of thirty replications and standard errors were indicated

Controls for both lettuce and celery plants grown in 288- and 200-cell trays partitioned a higher photo-assimilate proportion to roots. A single BAP or PBZ application significantly changed photo-assimilate partitioning towards shoots (Table 5). There were highly significant differences (P<.001) for the single, double and triple effects in the ANOVA plant allometries for both lettuce and celery.

Table 3. The Rate of Leaf Appearance (RLA) in two vegetables (lettuce and celery) grown at two cell trays (288- and 200-cell tray^{-1}), and treated with different BAP and PBZ pre-transplant solutions in the Experiment 3. Different lower-case letters indicate significantly different slopes according to the test for equal slope (testing for parallelism) between treatments for each plug cell tray while different capital letters indicate significantly different slopes according to the test for equal slope (testing for parallelism) for each treatment between different plug cell tray. Means (n=20). The probability of the slope being zero was *P*<.001 and *P*<.05 for lettuce and celery respectively

	RLA (leaves week^{-1})		
	'Dolly' lettuce		**'Golden Boy' celery**
288 cells tray^{-1}		**288 cells tray^{-1}**	
Control	2.70^{cB}	Control	4.29^{dA}
BAP-5	2.94^{bA}	BAP-5	4.56^{bB}
BAP-50	3.15^{aB}	BAP-50	4.53^{bB}
BAP-100	3.26^{aA}	BAP-100	4.38^{cB}
PBZ 10-0	3.15^{aA}	PBZ 5-0	4.66^{aB}
PBZ 10-5	3.29^{aB}	PBZ 5-5	4.63^{aB}
PBZ 10-50	3.20^{aB}	PBZ 5-50	4.48^{bB}
PBZ 10-100	3.08^{bA}	PBZ 5-100	4.72^{aB}
PBZ 20-0	2.68^{cB}	PBZ 10-0	4.60^{aA}
PBZ 20-5	3.24^{aB}	PBZ 10-5	4.69^{aB}
PBZ 20-50	3.26^{aA}	PBZ 10-50	4.44^{bB}
PBZ 20-100	2.92^{bB}	PBZ 10-100	4.14^{cB}
200 cells tray^{-1}		**200 cells tray^{-1}**	
Control	2.95^{cA}	Control	4.33^{cA}
BAP-5	3.03^{cA}	BAP-5	4.69^{bA}
BAP-50	3.32^{aA}	BAP-50	4.95^{bA}
BAP-100	3.30^{aA}	BAP-100	5.20^{aA}
PBZ 10-0	3.19^{bA}	PBZ 5-0	4.90^{bA}
PBZ 10-5	3.36^{aA}	PBZ 5-5	5.22^{aA}
PBZ 10-50	3.41^{aA}	PBZ 5-50	5.07^{aA}
PBZ 10-100	3.13^{bA}	PBZ 5-100	5.02^{aA}
PBZ 20-0	3.00^{cA}	PBZ 10-0	4.47^{cB}
PBZ 20-5	2.88^{cB}	PBZ 10-5	5.01^{aA}
PBZ 20-50	3.30^{aA}	PBZ 10-50	4.66^{bA}
PBZ 20-100	3.11^{bA}	PBZ 10-100	4.77^{bA}
Significantly			
Cell size	***		**
BAP	***		***
PBZ	***		***
Cell size x BAP	***		**
Cell size x PBZ	***		***
Cell size x BAP x PBZ	***		**

*Significance *** 0.001 ** 0.01 * 0.05 'ns' No significant*

Table 4. The total Relative Growth Rate (RGR) between the emergence-sale stage of two vegetables (lettuce and celery) grown at two cell trays (288 and 200 cell tray^{-1}), and sprayed with BAP or PBZ pre-transplant solutions in the Experiment 3. Different lower-case letters indicate significantly different slopes according to the test for equal slope (testing for parallelism) between treatments for each plug cell tray while different capital letters indicate significantly different slopes according to the test for equal slope (testing for parallelism) for each treatment between different plug cell tray. Means (n=20). The probability of the slope being zero was *P*<.001 and *P*<.05 for lettuce and celery respectively

	RGR (g g^{-1} day^{-1})		
	'Dolly' lettuce		**'Golden Boy' celery**
288 cells tray^{-1}		**288 cells tray^{-1}**	
Control	0.0730^{dB}	Control	0.0508^{bB}
BAP-5	0.0856^{aA}	BAP-5	0.0549^{aA}
BAP-50	0.0832^{aA}	BAP-50	0.0523^{bB}
BAP-100	0.0825^{aA}	BAP-100	0.0532^{bB}
PBZ 10-0	0.0830^{aA}	PBZ 5-0	0.0559^{aB}
PBZ 10-5	0.0857^{aA}	PBZ 5-5	0.0571^{aA}
PBZ 10-50	0.0842^{aA}	PBZ 5-50	0.0555^{aB}
PBZ 10-100	0.0777^{cA}	PBZ 5-100	0.0532^{bB}
PBZ 20-0	0.0822^{bA}	PBZ 10-0	0.0503^{cB}
PBZ 20-5	0.0802^{bB}	PBZ 10-5	0.0507^{cB}
PBZ 20-50	0.0800^{bB}	PBZ 10-50	0.0548^{aB}
PBZ 20-100	0.0844^{aA}	PBZ 10-100	0.0565^{aA}
200 cells tray^{-1}		**200 cells tray^{-1}**	
Control	0.0761^{cA}	Control	0.0532^{cA}
BAP-5	0.0806^{bB}	BAP-5	0.0541^{bA}
BAP-50	0.0790^{bB}	BAP-50	0.0559^{bA}
BAP-100	0.0798^{bB}	BAP-100	0.0547^{bA}
PBZ 10-0	0.0803^{bB}	PBZ 5-0	0.0572^{aA}
PBZ 10-5	0.0802^{bB}	PBZ 5-5	0.0553^{bB}
PBZ 10-50	0.0793^{bA}	PBZ 5-50	0.0583^{aA}
PBZ 10-100	0.0812^{bA}	PBZ 5-100	0.0572^{aA}
PBZ 20-0	0.0840^{aA}	PBZ 10-0	0.0579^{aA}
PBZ 20-5	0.0839^{aA}	PBZ 10-5	0.0589^{aA}
PBZ 20-50	0.0833^{aA}	PBZ 10-50	0.0584^{aA}
PBZ 20-100	0.0836^{aA}	PBZ 10-100	0.0570^{aA}
Significantly			
Cell size	***		**
BAP	***		**
PBZ	***		***
Cell sizexBAP	**		**
Cell sizexPBZ	***		**
Cell sizexBAPxPBZ	***		**

*Significance *** 0.001 ** 0.01 * 0.05 'ns' No significant*

Table 5. Changes in the allometric relationships between the roots and shoots (ln Root dry weight=α+βxln Shoot dry weight) of two vegetables (lettuce and celery) grown at two cell trays (288 and 200 cell trays^{-1}), and sprayed with BAP or PBZ pre-transplant solutions. The coefficients of determination (r^2) of the straight-line regressions in the Experiment 3 were indicated. Different lower-case letters indicate significantly different slopes according to the test for equal slope (testing for parallelism) between treatments for each plug cell tray while different capital letters indicate significantly different slopes according to the test for equal slope (testing for parallelism) for each treatment between different plug cell tray. Means (n=20). The probability of the slope being zero was *P*<.001 for both lettuce and celery

	'Dolly' lettuce				'Golden Boy' celery		
	α	β	r^2		α	β	r^2
288 cells tray^{-1}				**288 cells tray^{-1}**			
Control	0.55	1.149^{aA}	0.957	Control	0.71	1.156^{aA}	0.946
BAP-5	0.94	0.926^{cA}	0.975	BAP-5	0.65	0.855^{eA}	0.953
BAP-50	1.33	0.944^{cB}	0.963	BAP-50	1.00	0.922^{dA}	0.962
BAP-100	0.94	0.886^{dB}	0.967	BAP-100	1.22	0.972^{cA}	0.962
PBZ 10-0	0.98	0.940^{cA}	0.932	PBZ 10-0	1.08	0.947^{cA}	0.946
PBZ 10-5	0.52	0.813^{eB}	0.962	PBZ 10-5	1.25	0.986^{cA}	0.946
PBZ 10-50	1.64	1.006^{bA}	0.944	PBZ 10-50	1.23	0.954^{cA}	0.965
PBZ 10-100	1.65	1.011^{bA}	0.952	PBZ 10-100	1.41	0.972^{cA}	0.926
PBZ 20-0	0.63	0.839^{eB}	0.976	PBZ 20-0	1.46	1.038^{aA}	0.969
PBZ 20-5	0.88	0.903^{eA}	0.983	PBZ 20-5	1.45	1.019^{bA}	0.965
PBZ 20-50	0.58	0.823^{eA}	0.964	PBZ 20-50	1.78	1.100^{bA}	0.975
PBZ 20-10	0.67	0.847^{eB}	0.937	PBZ 20-100	1.46	1.007^{bA}	0.922
200 cells tray^{-1}				**200 cells tray^{-1}**			
Control	0.50	1.141^{aA}	0.957	Control	0.37	1.105^{aB}	0.967
BAP-5	0.67	0.912^{dA}	0.955	BAP-5	0.46	0.822^{bA}	0.980
BAP-50	1.23	0.982^{cA}	0.976	BAP-50	0.47	0.794^{cB}	0.988
BAP-100	1.74	1.056^{bA}	0.973	BAP-100	0.53	0.784^{cB}	0.969
PBZ 10-0	0.34	0.823^{eB}	0.979	PBZ 10-0	0.29	0.760^{cB}	0.983
PBZ 10-5	0.71	0.890^{cA}	0.988	PBZ 10-5	0.51	0.818^{bB}	0.973
PBZ 10-50	0.56	0.826^{eB}	0.969	PBZ 10-50	0.50	0.785^{cB}	0.981
PBZ 10-100	0.74	0.892^{cB}	0.987	PBZ 10-100	0.49	0.785^{cB}	0.983
PBZ 20-0	0.72	0.881^{cA}	0.921	PBZ 20-0	0.10	0.694^{dB}	0.934
PBZ 20-5	0.81	0.888^{cA}	0.966	PBZ 20-5	0.35	0.773^{cB}	0.975
PBZ 20-50	0.72	0.856^{cA}	0.957	PBZ 20-50	1.35	1.003^{aB}	0.962
PBZ 20-100	1.91	1.040^{bA}	0.924	PBZ 20-100	1.36	1.004^{aA}	0.951
Significantly							
Cell size		***				***	
BAP		***				***	
PBZ		***				***	
Cell sizexBAP		***				***	
Cell sizexPBZ		***				***	
Cell sizexBAPxPBZ		***				***	

*Significance *** .001 ** .01 * .05 'ns' No significant*

4. DISCUSSION

The yield of leafy vegetables is closely associated with an increase in fresh weight over time. Most typical field grown leafy vegetables such as lettuce and celery are initiated with a number of different batches throughout the season, timed to produce a continuous supply; the crops usually take between 40 and 80 days to mature and retailers normally specify a narrow range of acceptable plant weights between 400 and 700g [13]. Shoot growth is associated with the container size from sowing to transplant in lettuce and celery [3]. Our results in control plants are in agreement with this previous information (Figs.1,2 and 4, Table 2).

4.1 Temperature Base Significance

Plant growers have progressively adopted cell trays of reduced size. This leads to a limited substrate volume available for the root system, which imposes a physical stress that leads to a pronounced decrease in root and shoot growth [14]. The concept of base temperature can be described either physiologically or statistically. Physiologically, it is assumed that below a certain temperature level, crop growth and development will cease. However, it is difficult to determine the physiological base temperature. In physiology, the base temperature should be similar for a given crop developmental stage in any growing season. Statistically, the base temperature is that resulting in the lowest variation in growing degree day accumulations. However, the base temperature may sometimes be calculated to be below zero, which is difficult to explain in biology. Values of base temperature much lower than 0°C seem unrealistic; base temperatures around 4-6°C appear common for vegetable crops. The base temperature values calculated and used for lettuce are -4.5°C [15], 0.0°C [9], 3.5°C [16,17], 4.4°C [18,19], 6.0°C [20] and 10.0°C [21]; whereas the base temperature values for celery range from -7.3°C [18] to -1.0°C [16]. In the present study, data of development rate (days^{-1}) *vs*. air temperature (°C) were plotted and fitted through a straight-line regression; the values of the x-interception for this equation, named base temperature, are indicated in (Table 1). The base temperatures in Experiment 1 were all above 0°C for cold lettuce ('Shirley') and year-round celery ('Golden Boy') and were significantly higher for the summer lettuce ('Dolly') and green celery ('Green Fox'). On the other hand, the base temperature was higher for control plants grown in 200-cell trays than for those grown in 288-cell trays, which would explain the higher fresh weight accumulation from sowing to transplant (Fig. 3) and from transplant to sale (Fig. 4). In all cases, as rooting volume decreased (smaller container sizes), less leaf area was produced probably due to fewer leaves per plant (Table 3). In the most of the experiments, plants were monitored frequently to prevent drought stress, since the different flats dried at different rates and there was usually no visual evidence of nutrient deficiency in any of the containers. Water and nutrient stress were not considered to be determinants of plant size under commercial environmental facilities.

4.2 Plug Cell Volume and Both BAP and PBZ Effects

There are strong indications that cytokinins are root factors which are transported via the xylem to the shoot where they exert a major regulatory influence on growth [22]. We have previously reported that the availability of hormones synthesized in the root apex and reallocated to shoots would be reduced when the vertical root growth is impeded by the container base [23,24]. Although total fresh weights are higher in larger container sizes (200-cell tray^{-1}) for control plants, plant productivity can be increased with a BAP spray (Figs.1, 2 and 4). Cytokinins can stimulate shoot growth and decrease root growth [25]. Incorporation

of cytokinin-producing bacteria into the root zone of lettuce plants can double the speed of accumulation of shoot biomass at the normal level of water supply. Roots of bacteria-treated plants are shorter than those from untreated ones [26]. The use of PBZ would reduce root elongation and would delay the root physical restriction.

Although the physiological processes involved in root restriction are largely unknown, our results showed that the responses to a BAP or PBZ application were significantly associated with the cell volume. The RGR was higher for the plants grown in 200-cell trays than for those grown in 288-cell trays both between the emergence-transplant and emergence-sale stages (Tables 2 and 4). We have recently shown a close coordination between roots and shoots growth [1], controlled presumably by a cytokinin signaling pathway which is largely hormonal in nature with a major site of control located in the root system [27]. It has been concluded that root cytokinins are a major part of the signalling pathway by which the root/shoot ratio is regulated [28]. Thus, increasing root growth may lead to a corresponding increase in the synthesis of cytokinins [29]. This would be true for control plants and would explain the higher fresh weight accumulation in plants grown in 200-cell tray^{-1} (Figs. 1-4). On the other hand, a single pre-transplant PBZ application did not reduce root elongation and its effect would not be associated with a delay in root physical restriction (Fig. 3).

Although the net cytokinin concentration which reaches apical meristem is the result of both the synthesis and degradation rates, a larger root size leads to a greater cytokinin synthesis. When plants are transplanted to larger pots of a cell tray, they quickly develop an adventitious root system [23] and it would be possible that the net cytokinin concentration at the shoot apex increase as well. We have previously shown that exogenous application of BAP to plants grown in small pots may override shoot growth limitation due to root restriction [1,2,14]. In agreement with these results, Haver and Schuch [30] showed that isopentenyladenosine and zeatin riboside decreased on a whole petunia shoot basis as rooting volume decreased from 162 to 58cm^3. On the other hand, Di Benedetto et al. [2,24] showed that spraying exogenous BAP solutions increased both fresh and dry weight for *E. aureum* and *F. benjamina* 'green' and 'variegated' ornamental plants.

4.3 Physiological Mechanism Involved

Photo-assimilates from leaves are used for root and stem growth, --- leaf initiation and leaf growth; on the other hand, the size of the different plant sinks determines the photo-assimilate partitioning among the plant organs. Biomass distribution has been shown to differ with container size for some species. Near 46% of assimilates were partitioned into the main stem in root restricted euonymus compared to 21% for the control group [31]. Krizek et al. [32] found that root restricted tomato assimilates were mainly partitioned to the roots. Although our results would show that the smaller the cell volume the higher the percentage of dry matter allocated to roots (Table 3), complementary information about sugar contents in different organs of the plant are needed. On the other hand, a single BAP or PBZ application at the pre-transplant stage changed photo-assimilate partitioning towards shoots (Table 5).

It has been claimed that the phyllochron (i.e. the time interval between the appearance of two successive leaves) may be altered in transgenic plants with reduced cytokinin levels [33,34], but the possibility that exogenous applications of a cytokinin may affect the phyllochron has been explored only recently [2]. The application of cytokinins can promote leaf unfolding and expansion in intact plants in several species [35]. Cytokinins have a strong influence on many aspects of shoot development and metabolism and leaf expansion

[36]. However, the possibility that the rate at which leaves appear in the meristem is regulated by cytokinins or other growth regulators has attracted little attention. A significant increase in the rate of leaf appearance was found when plants were treated with BAP or PBZ (Table 3). Since lettuce and celery leaves appear in a single shoot without branches, the increase in the rate of leaf appearance indicates a shorter phyllochron. It has been suggested that cytokinins are involved in the maintenance of the vegetative apex and in cellular differentiation [37,5] and that they can promote leaf unfolding and expansion in whole plants in several species [35]. However, relatively few studies have explored the regulation of the phyllochron [2,5,7,33,34]. Nevertheless, to our knowledge, there are been no previous reports on the promoting effect of exogenous BAP or PBZ on the rate of leaf appearance in intact vegetable plants.

Plants are complex systems that require monitoring of multiple environmental signals and, in response to those signals, coordination of differentiation and development of an extensive array of cell types at multiple locations. This coordination must rely on the integration of long-distance signals that provide a means of communication among different plant parts. Although a BAP spray or a PBZ soil application gave similar results and affected similar processes, the physiological mechanisms involved would be different. For example, while a BAP spray decreased the root/shoot ratio, a PBZ application showed either no changes or lesser response (Figs. 1 and 3).

5. CONCLUSIONS

In summary, the present results suggest that larger cell size may change biomass accumulation through a decrease in T_b and that a single BAP or PBZ application at the pre-transplant stage and would be a tool to improve the yield of leafy vegetables without negative changes in their quality (data not shown). The higher fresh weight accumulation was the result of a higher RGR, an increase in the rate of leaf appearance and a change in photo-assimilate partitioning towards the shoots. However, this investigation line needs additional experiments, some of which are already in progress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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