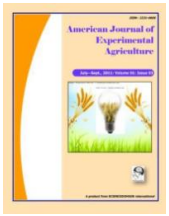

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Increase of Spinach Growth Through the use of Larger Plug Cell Volume and an Exogenous BAP Spray

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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: In spinach, transplanting has replaced direct seeding but small containers may cause root restriction effects that would be decreased post-transplant yield. Although a single hormonal regulator (BAP) spray is effective in overcoming root restriction, the mechanisms involved are unknown. The aim of this work was to analyze spinach growth changes by the use of different plug cell volumes and BAP as foliar spray.
Study Design: A randomized complete block factorial design with three blocks was used.
Place and Duration of Study: The experiments were conducted at the INTA Balcarce Experimental Station campus, Argentina (37º 45′ S, 58° 18′ W and altitude 130 m) from 5th March to 24th May 2008 and repeated once from 15th March to 19th May 2009.
Methodology: We analyzed growth changes by the use of different plug cell volumes and BAP as foliar spray on dry weight accumulation and partitioning in *Spinacea oleracea* plants in two field experiments.
Results: The use of large plug cell volumes and BAP sprays at the pre-transplant stage increased

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plant growth, through an increase in the relative growth rate (RGR) and the rate of leaf area expansion (RLAE). We found a close direct relationship between RGR and net assimilation rate (NAR) but an inverse relationship between RGR and leaf area ratio (LAR). In addition, we found that NAR significantly increased as leaf thickness, intercellular spaces and stomatal density increased. A change in dry weight partitioning toward shoots was found as well.
Conclusion: From a grower's point of view, the use of large plug cell volume would increase spinach growth. On the other hand, a promising approach to increasing crop productivity is the use of plant growth and development regulators such as BAP.

Keywords: Leaf traits; nursery; photoassimilate partitioning; productivity; root restriction.

1. INTRODUCTION

Technological advances in transplanting have contributed to the growth of the industry by reducing costs and increasing the reliability of production. However, small containers may cause root restriction effects, i.e. a physical stress imposed on a root system which leads to a significant decrease in post-transplant growth [1-3]. Surprisingly, the root restriction associated with container volume seems to have received little consideration in the scientific literature [4].

We have previously shown that a single exogenous application of cytokinin 6-benzylaminopurine (BAP) to plants grown in small pots at the pre-transplant stage may override the shoot growth limitation due to root restriction [2,5]. We have also shown that the pre-transplant application of BAP increases both lettuce and celery yield [1,3] through higher shoot fresh weight accumulation and the rate of leaf area expansion (RLAE) as a result of higher relative growth rate (RGR) and the rate of leaf appearance (RLA).

In addition, it has been shown that an endogenous decrease in cytokinins levels decreased the development of the aerial part of plants [6-9]). Root and shoot growth are closely coordinated, presumably by a cytokinin signaling pathway which is largely hormonal in nature with a major site of control located in the root system [10,11]. Thus, increasing root growth may lead to a corresponding increase in the synthesis of cytokinins [12].

Spinach (*Spinacea oleracea* L.), a leaf vegetable with a high nutritive value, is of considerable economic importance. Since transplanting has almost replaced direct seeding, the objective of this work was to analyze spinach growth changes by the use of different plug cell volumes and the hormonal regulator BAP through the physiological mechanisms involved.

2. MATERIALS AND METHODS

2.1 Plant Material, Treatments and Experiments

The experiments were conducted at the INTA Balcarce Experimental Station campus, Argentina (37º 45′ S, 58º 18′ W and altitude 130 m) from 5th March to 24th May 2008 and repeated once from 15th March to 19th May 2009.

Spinach (cv. 'Ballet') seeds (a flowering-resistant genotype) were sown in 288- (6.18 cm^3 $cell^{-1}$) or 200- (13.90 cm^3 $cell^{-1}$) cell trays and were transplanted to a Typic Argiudol soil. Soil containing clay-loam-sand at 17.5, 47.5 and 35% (v/v) respectively with pH=6.09; EC=0.16 dS/m; N=0.26% and P=0.91 mmol L^{-1}. Seedlings were sprayed with BAP (6-benzylaminopurine) (SIGMA EC 214-927-5) (Sigma-Aldrich Co., St. Louis, MO, USA) solutions (0, 5, 50, 100 and 200 mg L^{-1}) either when first true leaf pairs appeared (pre-transplant application) or one week after transplant (post-transplant application) [1]. BAP was previously diluted in alcohol 80%.

Plants were grown under greenhouse facilities and were irrigated as needed (to compensate 80% relative evapo-transpiration) with high quality tap water; a weekly fertilization (Stage 2: 50 mg L^{-1} N; Stage 3-4: 100 mg L^{-1} N; post-transplant: 150 mg L^{-1} N) [13] was used.

Half hourly averages air temperature was measured using a HOBO H08-001-02 data logger (Onset Computer Corporation, MA, U.S.A) protected from direct radiation by aluminum foil shades. Mean temperatures during the experiments ranged from 8.9 to 18.2ºC.

2.2 Growth Evaluations

For destructive measurements, plants (10 per treatment and block) were harvested at the transplant stage and both in the middle and at

the end of the experiments. The numbers of new leaves that had appeared were also recorded on the same dates and the rate of leaf appearance (RLA) was calculated as the slope of the number of new (visible) leaves *vs.* time (in weeks). The fresh weight (FW) of roots and shoots was determined separately for each plant. Separate plant parts were dried at 80ºC for 48 hours and weighed to obtain the dry weight of shoots (including leaf blades and stems) and roots. Leaves were oven-dried at 80ºC for 48 hours. Leaf area was determined with a LI-COR 3000A automatic leaf area meter (LI-COR BioSciences, Lincoln, NE, USA).

The relative growth rate (RGR) was calculated as the slope of the regression of the natural logarithm (ln) of the whole plant on a dry weight basis *vs.* time (in days), while the rate of leaf area expansion (RLAE) was calculated as the slope of the regression of the ln of total leaf area *vs.* time (in days). Mean net assimilation rate (NAR) and leaf area ratio (LAR) were calculated according with Potter and Jones [14] as follows:

$$NAR = (k_w W_0 e^{k_w t}) / (A_0 e^{k_a t})$$
$$LAP = (k_a A_0 e^{k_a t}) / (k_w W_0 e^{k_w t})$$

Where: k_w: RGR (days^{-1}); W_0: extrapolated value of total dry weight at time zero (g); A_0: extrapolated value of leaf area at time zero (cm^2); k_a: RLAER (days^{-1}); t: time (in days) at the midpoint of the experimental period and e: base of natural logarithms.

The specific leaf area on a FW basis (SLA_{FW}) was calculated as the ratio between area of the new individual leaf and leaf FW. The leaf weight rate (LWR) values measured biomass allocated to leaves *vs.* biomass allocated to other plant parts. The allometric coefficients between roots and shoots were calculated as the slope of the straight-line regression of the ln of root dry weight *vs.* the ln of shoot dry weight.

Tissue from the middle region of the lamina was fixed in formalin-acetic-alcohol. The size and densities of epidermal stomata size were recorded on this tissues discolored with ethyl alcohol (96º) at boiling temperature. Leaf thickness was determined from leaf lamina tissues embedded in paraffin, sectioned at 20 µm on a rotary microtome. Leaf samples were stained with safranin-crystal violet-fast green. Data are the mean of three leaves per treatment and block from ten leaf cross-sections per leaf. An image analysis system (Image Pro Express v 6.0, Media Cybernetics, USA) facilitated quantitative anatomical measurements.

2.3 Statistical Analysis

We used a randomized complete factorial design with three blocks of each treatment: Cell size, BAP concentration (BAP_c) and BAP application time (BAP_{at}). Since we found no significant differences between the two experiments, we considered them together. Data were subjected to two way analysis of variance and means were separated by Tukey's test ($P<0.05$); STATISTICA 8 (StatSoft) software was used. Straight-line regression slopes were tested for parallelism (test for equal slope) [15].

3. RESULTS

3.1 Leaf area and biomass accumulation

The shoot fresh weight at the end of the experiments was higher for control plants grown in 200-cell trays than for those grown in 288-cell ones and for those sprayed at the pre-transplant stage with 5 to 100 mg L^{-1} BAP. The higher the BAP dose, the higher the shoot fresh weight. On the other hand, a high (200 mg L^{-1}) pre-transplant or post-transplant dose decreased aerial fresh weight (plants grown in 200-cell trays) or showed little changes (Fig. 1).

Spinach plants from 200-cell trays showed higher total leaf area than those from 288-cell trays at the end of the experiment. In general, total leaf area in control plants was increased by a single BAP spray, but was highest when BAP was sprayed at the pre-transplant stage. The higher leaf area was the result of an increase in both the individual leaf area and RLA (Table 1).

RLAE was higher in control plants from 200-cell than from 288-cell trays and was significantly increased with a BAP spray at almost all doses tested at both pre- and post-transplant stages. RGR showed similar results except when BAP was applied at the post-transplant stage in plants grown in 200-cell trays. NAR was higher in plants grown in 200-cell trays than in those grown in 288-cell ones. A single BAP spray (at all doses) increased NAR plants grown in 288-cell trays both at the pre- and at the post-transplant stage; BAP failed to increase NAR at the post-transplant stage in 200-cell grown plants. Results for LAR were opposite. Specific leaf area (SLA_{FW}) was higher in control plants grown in 288-cell trays than in those grown in 200-cell

ones, but in both cases was significantly decreased by a BAP spray. Leaf weight ratio (LWR) increased when plants were sprayed with BAP but even was higher in plants grown in 200-cell plug trays than in those grown in 288-cell ones (Table 2).

3.2 Dry Weight Partitioning

The allometries from Table 3 show that controls plants grown in 288- and 200-cell trays partitioned a similar photo-assimilate proportion to roots and that a single BAP application significantly changed photo-assimilate partitioning towards shoots.

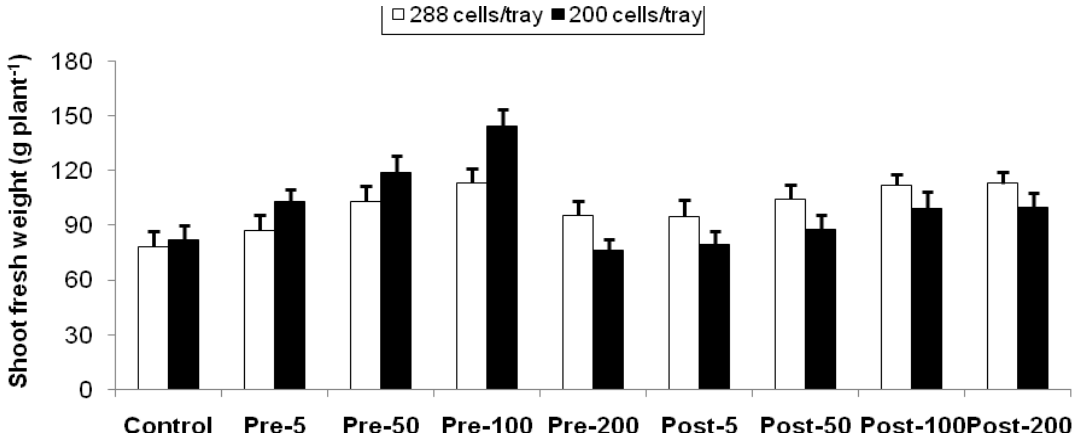
3.3 Anatomical Traits

Leaf thickness was higher in plants grown in 200-cells trays than in those grown in 288-cell ones. Leaf thickness was significantly increased by a BAP spray, but the response was different according to the BAP dose applied and application time; the same response was found when size and density of stomata were recorded (Table 4).

3.4 Growth Rates and Anatomical Changes Ratios

When plotting the data from all treatments, we found a close direct relationship (r^2=0.822) between RGR and NAR (Fig. 2a) and an inverse relationship between RGR and LAR (r^2=0.761) (Fig. 2b). Nevertheless, control plants grown in 200-cell trays showed higher NAR and lower LAR values than those grown in 288-cell trays.

On the other hand, NAR significantly increased according to an increase in leaf thickness (r^2=0.694) (Fig. 3a), intercellular space proportion (r^2=0.727) (Fig. 3b) and stomata density (r^2=0.495) (Fig. 3c). NAR relationships were higher in control plants grown in 200-cell trays than in those grown in 288-cells ones.



ANOVA	
Significantly	
PCV	***
BAP_c	***
BAP_{at}	***
PCV x BAP_c	***
PCV x BAP_{at}	***
PCV x BAP_c x BAP_{at}	***

*Significance *** 0.001*

Fig. 1. The effects of two plug cell volume (288- and 200-cells tray^{-1}) (PCV) and a pre-transplant or post-transplant BAP sprays (5, 50,100 or 200 mg L^{-1}) on spinach shoot fresh weight at the end of the experiment (n=6). Both, standard errors and the analysis of variance (ANOVA) are indicated

Table 1. Changes in the total leaf area, the individual leaf area and the rate of leaf appearance (RLA) at the end of the experiment in spinach plants grown at two plug cell volume (PCV) and sprayed with different concentrations of BAP solutions at the pre- or post-transplant stage (data are the mean of two experiment) (n=6)±SE. Different lower-case letters indicate statistically significant differences (*P*=.05) between BAP treatments for each cell volume while different capital letters indicate statistically significant differences (*P*=.05) for each BAP treatment between different cell volumes. The probability of the RLA slope being zero was *P* = .001. The significance of interactions (ANOVA) has been indicated as well

PCV	Total leaf area (cm^2 $plant^{-1}$)	Individual leaf area (cm^2 $leaf^{-1}$)	RLA (leaves $week^{-1}$)
288 cells $tray^{-1}$			
Control	499.37^{cB}	34.07^{cB}	1.122^{cB}
Pre-5	598.74^{bB}	42.71^{bB}	1.262^{aB}
Pre-50	681.25^{aB}	45.18^{bA}	1.233^{aB}
Pre-100	670.18^{aB}	53.72^{aA}	1.292^{aB}
Pre-200	656.61^{aA}	41.50^{bB}	1.142^{bB}
Post-5	595.80^{bA}	41.48^{bA}	1.108^{bB}
Post-50	598.93^{bA}	42.43^{bA}	1.157^{bB}
Post-100	610.34^{bA}	38.90^{bA}	1.131^{bB}
Post-200	638.42^{bA}	43.02^{bA}	1.147^{bB}
200 cells $tray^{-1}$			
Control	562.04^{cA}	39.21^{cA}	1.219^{cA}
Pre-5	743.23^{aA}	50.94^{aA}	1.358^{aA}
Pre-50	768.69^{aA}	46.04^{aA}	1.384^{aA}
Pre-100	773.18^{aA}	51.30^{aA}	1.350^{aA}
Pre-200	649.18^{bA}	49.12^{aA}	1.202^{bA}
Post-5	604.75^{bA}	45.76^{bA}	1.272^{bA}
Post-50	642.65^{bA}	48.80^{bA}	1.224^{cA}
Post-100	639.43^{bA}	45.58^{bA}	1.212^{cA}
Post-200	654.23^{bA}	42.58^{bA}	1.225^{cA}

ANOVA			
Significantly	**Total leaf area**	**Individual leaf area**	**RLA**
PCV	***	***	***
BAP_c	***	***	***
BAP_{at}	*	*	***
PCV x BAP_c	***	***	***
PCV x BAP_{at}	**	**	***
PCV x BAP_c x BAP_{at}	**	**	***

*Significance *** 0.001 ** 0.01 * 0.05*

4. DISCUSSION

Leaf expansion and synthesis of photo-assimilates are essential for plant growth. Our results showed that a higher total leaf area in spinach (Table 1) as a result of higher RLAE (Table 2) was closely related to plug cell volume and both BAP spray concentration and BAP application time (pre- or post-transplant stage). Previous reports have shown similar increases in total leaf area in other leafy vegetables such as lettuce and celery [1,3], but the fact that this result would be associated with a higher individual leaf area and RLA (Table 1) has not been previously published in vegetables. We have recently suggested that an increase in plug cell size or exogenous BAP would increase leaf size or leaf appearance as an expression of meristem growth and phyllochron decrease respectively in ornamental foliage plants [16,5,17]. Cytokinins control growth mainly through the regulation of cell division and differentiation in the stem apical meristem [18]. Similarly, the size of the apical meristem is in turn related to the rate of leaf appearance, as shown in experiments with cytokinins deficient *Arabidopsis* mutants [19].

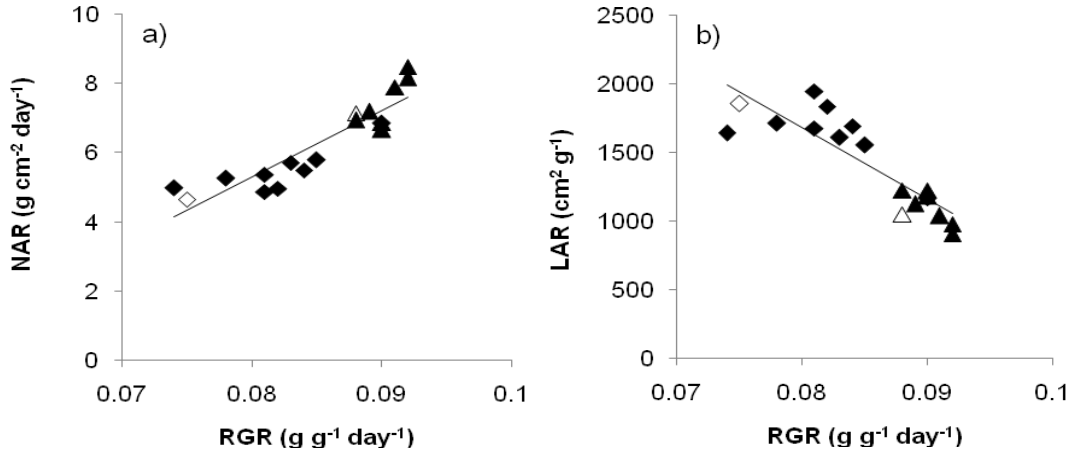


Fig. 2. The net assimilation rate (NAR) (a) and the leaf area ratio (LAR) (b) related to the relative growth rate (RGR). The straight-line regressions were: NAR = 192.11 RGR - 10.07 (r^2= 0.822) and LAR = - 52,175.00 RGR + 5,856.20 (r^2= 0.761). ◊: 288-grown controls; ∆: 200-grown controls

Table 2. Changes in the rate of leaf area expansion (RLAE), the relative growth rate (RGR), the net assimilation rate (NAR), the leaf area ratio (LAR), the specific leaf area (SLA_{FW}) and the leaf weight rate (LWR) in spinach plants grown at two plug cell volume (PCV) and sprayed with different concentrations of BAP solutions at the pre- or post-transplant stage (n = 6) ± SE. Different lower-case letters indicate statistically significant differences between BAP treatments for each cell volume while different capital letters indicate statistically significant differences for each BAP treatment between different cell volumes at *P* = .05 by Tukey's test (SLA_{FW} and LWR) or according the test for equal slope (testing for parallelism) (RLAE, RGR, NAR and LAR); the probability of these slopes being zero were *P* = .001

PCV	RLAE ($cm^2\ cm^{-2}\ day^{-1}$)	RGR ($g\ g^{-1}\ day^{-1}$)	NAR ($g\ cm^{-2}\ day^{-1}$) x 10^5	LAR ($cm^2\ g^{-1}$)	SLA_{FW} ($cm^2\ g^{-1}$)	LWR ($g\ g^{-1}$)
288 cells tray^{-1}						
Control	0.028^{bB}	0.076^{bB}	4.65^{bB}	1856.20^{aA}	3.53^{aA}	1.389^{dB}
Pre-5	0.036^{aA}	0.084^{aB}	5.97^{aB}	1540.71^{bA}	3.11^{bA}	1.532^{cB}
Pre-50	0.036^{aA}	0.082^{aB}	5.96^{aB}	1535.37^{bA}	2.61^{bA}	2.822^{bA}
Pre-100	0.037^{aA}	0.083^{aB}	5.71^{aB}	1511.63^{bA}	2.86^{bA}	2.531^{bB}
Pre-200	0.038^{aA}	0.075^{bB}	5.78^{aB}	1556.20^{bA}	2.76^{bA}	2.838^{aB}
Post-5	0.037^{aA}	0.074^{bB}	5.49^{aB}	1594.26^{bA}	2.73^{bA}	1.450^{cA}
Post-50	0.037^{aA}	0.071^{bB}	5.37^{aB}	1577.40^{bA}	2.61^{bA}	1.542^{cA}
Post-100	0.036^{aA}	0.071^{bB}	5.27^{aB}	1548.86^{bA}	2.77^{bA}	1.790^{cA}
Post-200	0.035^{aA}	0.070^{bB}	5.26^{aB}	1511.29^{aB}	2.87^{bA}	1.587^{cA}
200 cells tray^{-1}						
Control	0.032^{bA}	0.082^{bA}	7.15^{bA}	1248.38^{aB}	3.13^{aB}	1.590^{dA}
Pre-5	0.038^{aA}	0.092^{aA}	8.16^{aA}	906.87^{bB}	2.66^{bB}	2.005^{cA}
Pre-50	0.040^{aA}	0.091^{aA}	7.90^{aA}	1007.95^{bB}	2.64^{bA}	2.507^{bA}
Pre-100	0.041^{aA}	0.092^{aA}	8.49^{aA}	977.50^{bB}	2.84^{bA}	3.021^{aA}
Pre-200	0.035^{aA}	0.084^{bA}	6.94^{bA}	1224.15^{aB}	2.80^{bA}	3.008^{aA}
Post-5	0.036^{aA}	0.080^{bA}	6.86^{bA}	1224.15^{aB}	2.88^{bA}	1.754^{cA}
Post-50	0.036^{aA}	0.080^{bA}	6.85^{bA}	1182.04^{aB}	2.71^{bA}	1.776^{cA}
Post-100	0.034^{bA}	0.079^{bA}	7.20^{bA}	1124.39^{aB}	2.42^{bB}	1.729^{cA}
Post-200	0.033^{bA}	0.080^{bA}	6.67^{bA}	1170.28^{aB}	2.57^{bB}	1.753^{cA}

ANOVA						
Significantly	**RLAE**	**RGR**	**NAR**	**LAR**	**SLA_{FW}**	**LWR**
PCV	***	***	***	***	***	***
BAP_c	***	***	***	***	***	**
BAP_{at}	ns	**	**	**	***	**
PCV x BAP_c	***	***	***	***	***	**
PCV x BAP_{at}	**	**	**	**	***	**
PCV x BAP_c x BAP_{at}	**	**	**	**	***	**

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

Table 3. Changes in the allometric relationships between shoots and roots for spinach plants grown at two plug cell volume (PCV) and sprayed with different BAP solutions at the pre- or post-transplant stage using a straight-line regression analysis between the natural logarithm of the roots dry weight and the natural logarithm to the shoot dry weight (ln Root dry weight = $\alpha + \beta$ x ln Shoot dry weight). Mean values (n=6) ± SE. The coefficients of determination (r^2) are indicated. Different lower-case letters indicate statistically significant differences between BAP treatments for each cell volume while different capital letters indicate statistically significant differences for each BAP treatment between different cell volumes according the test for equal slope (testing for parallelism). The probability of the slope being zero was *P* = .001

PCV	α	β	r^2
288 cells $tray^{-1}$			
Control	-1.77	0.69^{aA}	0.675
Pre-5	-1.97	0.58^{bA}	0.788
Pre-50	-2.25	0.55^{bA}	0.807
Pre-100	-2.17	0.60^{bA}	0.748
Pre-200	-2.33	0.59^{bA}	0.661
Post-5	-1.77	0.60^{bA}	0.782
Post-50	-2.04	0.53^{bA}	0.722
Post-100	-1.92	0.60^{bA}	0.836
Post-200	-1.76	0.61^{bA}	0.822
200 cells $tray^{-1}$			
Control	-1.65	0.65^{aA}	0.771
Pre-5	-1.76	0.50^{bB}	0.730
Pre-50	-1.93	0.60^{bA}	0.663
Pre-100	-2.01	0.55^{bB}	0.750
Pre-200	-1.91	0.55^{bA}	0.821
Post-5	-1.50	0.60^{bA}	0.854
Post-50	-1.70	0.56^{bA}	0.841
Post-100	-1.78	0.58^{bA}	0.841
Post-200	-1.81	0.52^{bB}	0.836

ANOVA	
Significantly	
PCV	***
BAP_c	***
BAP_{at}	**
PCV x BAP_c	**
PCV x BAP_{at}	**
PCV x BAP_c x BAP_{at}	**

*Significance *** 0.001 ** 0.01*

The productivity of most typical field grown leafy vegetables is closely associated with a high both fresh and dry weight per plant. The possibility to reach a higher plant yield was strongly restricted by the plug cell volume used, perhaps related to insufficient cytokinins supply [1,3]. Fig. 1 shows that the higher spinach fresh weight was found in control plants transplanted from 200-cell trays and those which were sprayed with a single 100 mg L^{-1} BAP at the pre-transplant stage. These

results are in agreement with the fact that availability of hormones synthesized in the root apex and reallocated to shoots would be reduced when the vertical cell root growth is impeded by the container base [20].

Since there were no significant differences in dry weight content for control or treated plants (data not shown), it is possible to describe the photo-assimilate acquisition and partition rates on a dry weight base. Thus, RGR increased in plants grown in 200-cell trays and when a 5 to 100 mg L^{-1} BAP spray was applied at the pre-transplant stage (Table 2). RGR can be described as the product between NAR or 'physiological component', and LAR or 'morphological component'. In our experiment, the higher spinach RGR would be explained by an increase in NAR (Table 2) and by the close positive relationship between NAR and RGR (r^2=0.822) (Fig. 2a). An increased efficiency of carbon fixation would be associated with higher NAR values, since this variable is largely the net result of carbon gain and carbon loss [21].

Both direct additions of cytokinins [22] and over expression in transgenic plants [23] have been reported to increase leaf thickness, which may further enhance carbon assimilation per unit leaf area. On the other hand, fresh biomass per unit leaf area is well correlated to leaf thickness [24].

Table 4. Changes in leaf thickness, stomata density and stomata size in the last expanded leaf at the end of the experiments in spinach plants grown at two plug cell volume (PCV) and sprayed with different concentrations of BAP solutions at the pre- or post-transplant stage. Mean values (n=6) ± SE. Different lower-case letters indicate statistically significant differences (*P* = .05) between BAP treatments for each cell volume while different capital letters indicate statistically significant differences (*P* = .05) by Tukey's test for each BAP treatment between different cell volumes

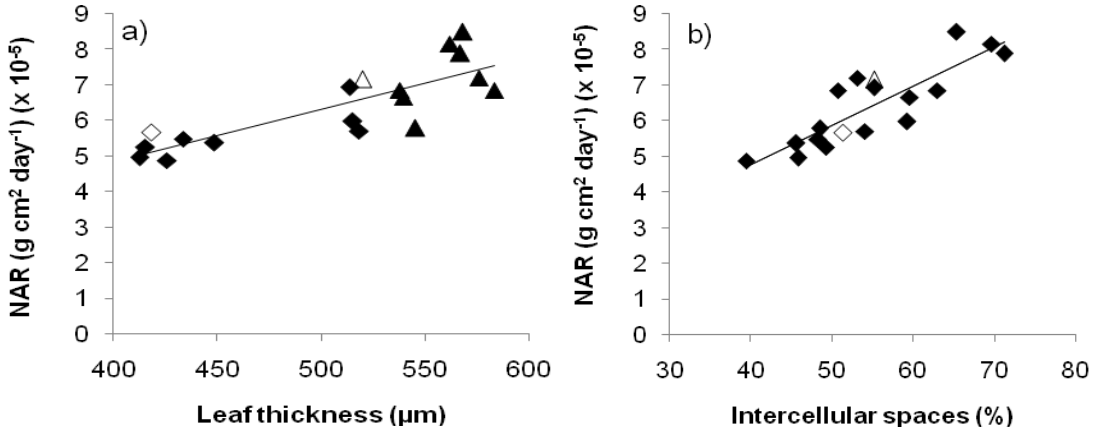
	Leaf thickness (µm leaf^{-1})	Stomata density (stomata mm^{-2})	Stomata size (µm stomata^{-1})
288 cells tray^{-1}			
Control	418.18^{cB}	55.73^{cB}	904.80^{aB}
Pre-5	535.15^{aB}	87.58^{aB}	723.76^{bB}
Pre-50	542.42^{aB}	79.62^{aB}	837.33^{bA}
Pre-100	538.18^{aB}	79.85^{aB}	842.00^{bA}
Pre-200	445.45^{bB}	79.78^{aB}	659.40^{cB}
Post-5	433.33^{bB}	85.80^{aB}	586.13^{cA}
Post-50	448.48^{bB}	79.83^{aB}	628.00^{cA}
Post-100	425.76^{cB}	85.54^{aB}	732.67^{bA}
Post-200	415.15^{cB}	71.66^{bB}	779.20^{bA}
200 cells tray^{-1}			
Control	469.70^{dA}	63.69^{cA}	1014.10^{aA}
Pre-5	562.12^{aA}	87.58^{aA}	921.07^{bA}
Pre-50	566.97^{aA}	111.46^{aA}	805.93^{cA}
Pre-100	568.18^{aA}	108.50^{aA}	853.67^{cA}
Pre-200	513.64^{bA}	87.80^{bA}	879.20^{bA}
Post-5	483.33^{cA}	99.62^{aA}	565.20^{eA}
Post-50	457.88^{cA}	87.58^{bA}	733.70^{cA}
Post-100	475.76^{cA}	95.54^{aA}	659.40^{eA}
Post-200	439.39^{dA}	88.51^{bA}	742.30^{cA}

ANOVA			
Significantly	**Leaf thickness**	**Stomata density**	**Stomata size**
PCV	***	***	***
BAP_c	***	***	***
BAP_{at}	***	***	***
PCV x BAP_c	***	***	***
PCV x BAP_{at}	***	***	***
PCV x BAP_c x BAP_{at}	***	***	***

*Significance *** 0.001*

Resistances encountered in the mesophyll from the intercellular spaces to the chloroplasts may relevantly decrease CO_2 concentration in the chloroplast and may contribute to limiting photosynthesis [25]. Aalto and Juurola [26] showed that large gradients in CO_2 concentrations can be observed in the stomatal capillary tube and in the mesophyll cells and that the concentration changes less than 1% across the airspaces of the mesophyll. This suggests that the gas phase diffusion is fast enough to provide a plentiful CO_2 supply, even in the narrowest passages between the cells [27]. Airspace effects may be larger in some species. It is profitable to have a large amount of absorbing surface because CO_2 transport is fast in air and slow in solution. The concentration decreased faster in the mesophyll cells than in the airspaces, because the diffusion coefficient is four orders of magnitude smaller in liquids than in gases. Due to the fast gas phase diffusion and equal distance from cell walls, chloroplasts in different parts of the leaf obtain almost equal quantities of CO_2 for photosynthesis. SLA and internal leaf anatomy often affect net gas exchange because of their effects on internal CO_2 conductance to the site of carboxylation, internal shading and competition for CO_2 among carboxylation sites. On the other hand, Magyar-Tábori et al. [28] have indicated that cytokinins can influence the leaf structure. Although a negative relationship between LAR and RGR (r^2= 0.761) (Fig. 2b) was found in the present study, there were statistically significant changes in the 'morphological component' of growth (LAR) through different leaf attributes such as leaf thickness, stomata density and stomata size related to cell size or BAP concentration and time of application (pre- or post-transplant) (Table 4). We also found a change in specific leaf area on a fresh weight basis (SLA_{FW}) (Table 2). A significant proportion of NAR values would be explained by the positive relationship between leaf thickness (r^2=0.694) (Fig. 3a), intercellular spaces (r^2=0.727) (Fig. 3b) and stomata density (r^2=0.495) (Fig. 3c). The BAP-mediated increase in NAR might be the consequence of different possible effects of cytokinin on the efficiency of carbon fixation. Another possibility would be that BAP could promote leaf blade thickening, thus leading to an increase in the levels of photosynthetic machinery per unit leaf area [29]. Mediavilla et al. [30] and Gandolfo et al. [31] showed that changes in leaf dry mass per unit area are caused by variations in internal anatomy and leaf tissue density, and are not simply a consequence of changes in leaf thickness. Our results are in agreement with these previous findings (Table 4, Fig. 3).

Photo-assimilates from leaves are used for root and stem growth. In addition, the size of the different plant sinks determines the photo-assimilate partitioning among the plant organs. Our results showed that a decrease in root restriction through the use of larger plug cell and an exogenous BAP application increased dry weight partitioning to shoots as indicated by both the allometric relationships between roots and shoots (Table 3) and the leaf weight rate (LWR), which measures the allocation of biomass to leaves *vs.* that to other plant parts [21] (Table 2). However, complementary information about sugar contents in different organs of the plant is needed.



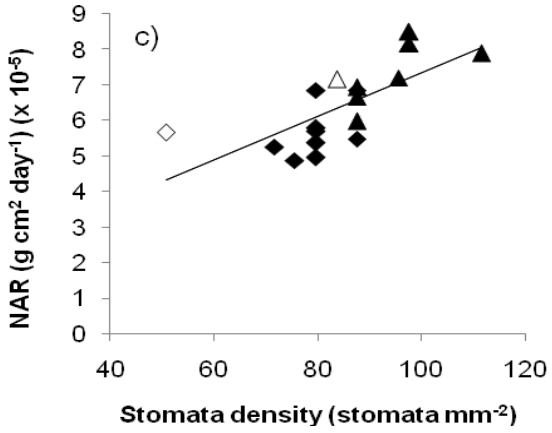


Fig. 3. The net assimilation rate (NAR) related to leaf thickness (a), intercellular spaces (b) and stomata density (c). The straight-line regressions were: NAR = 0.015 leaf thickness -0.95 (r^2= 0.694), NAR = 0.110 intercellular spaces + 0.40 (r^2= 0.727) and NAR = 0.061 stomata density + 1.22 (r^2= 0.495) respectively. ◊: 288-grown controls; ∆: 200-grown controls

Changes in the allometric slopes reflect variations in carbon partitioning [32], and carbohydrate partitioning between competing sites is fundamental to plant growth; especially because plants are capable of modifying their resource allocation to favor the growth of their growing parts [33]. Plant tissues and organs rich in cytokinins are known to attract assimilate translocation. Ron'zhina (2004) [34] showed that the sink-promoting effect of cytokinins in detached leaves was related to the source-to-sink transformation of the portion of the mature leaf and manifests itself by changing the direction of the phloem assimilate translocation. Incorporation of cytokinin-producing bacteria into the root zone of lettuce plants has been shown to double the speed of accumulation of shoot biomass at the normal level of water supply [35].

5. CONCLUSION

In summary, spinach growth is limited by root restriction during the plug tray propagation stages mainly associated with a decrease in dry weight accumulation. The use of larger plug cells and BAP sprays at the pre-transplant stage increased plant growth (expressed as RGR or RLAE) through an increase in NAR as a result of higher leaf thickness, stomata size, stomata density and intercellular spaces and a change in dry weight partitioning toward shoots.

From a grower's point of view, the use of large plug cell volume would increase spinach growth. On the other hand, a promising approach to increasing crop productivity is the use of plant growth and development regulators such as BAP. This compound, even at very low doses, is capable of changing the intensity and direction of plant physiological processes. Because BAP is not phytotoxic, it can be used in the nursery plant industry on a large scale.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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