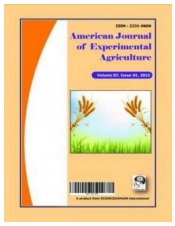

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Responses of Pansy (*Viola wittrockiana* Gams.) to the Quality of the Growing Media

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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

The quality of the growing medium stands out as one of the most important factors affecting the success of annual plants, especially when grown in potted culture. Recently, we have suggested that growing medium gives not only a matrix for water and nutrient absorption but also a source of external signalling. The aim of this work was to assess the performance of six pansy (*Viola wittrockiana* Gams.) genotypes grown in two growing media with significant differences in both physical and chemical properties, aiming to understand how substrate quality change the physiological mechanism related to biomass accumulation. The responses of the six pansy genotypes tested to the two growing media were significantly different. The mechanisms involved included fresh weight accumulation, leaf area expansion; RGR, RLA, glucose content and photo assimilate partitioning. On the other hand, RGR, RLA and glucose content were associated with root dry weight at the end of the experiments. Since the responses to the different growing media were the same as those found in plants grown with root restriction related to container volume, we speculated that cytokinins might act as endogenous signals.

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Keywords: Bedding plants; biomass accumulation; growth parameters; photo assimilate partitioning.

1. INTRODUCTION

Aerial environmental factors such as temperature, photoperiod and irradiance are of major concern in determining the success of annual bedding plants. However, factors associated with the root environment are critical, as they would determine both water and oxygen availability for plants. Under a set of environmental conditions, the availability of water and oxygen is mainly governed by the physical properties of the growing medium [1-4].

The most common organic substrate used for plant growth is peat moss and most of the crop technology available has been calibrated according with it. However, peat moss stocks in most countries are nearly depleted, because of continuous degradation of the peat-lands. The need to develop new substrates for the horticulture industry to replace peat moss is an issue that is being addressed by researchers around the world [5-7,3,4]. Thus, although some of these new types of growing media are limited in quality in terms of physical and chemical properties, a fact that negatively affects the development of plant roots, researchers have developed several commercialized products currently available to growers [8].

The growing medium gives not only a matrix for water and nutrient absorption but also a source of external signalling [9]. Different plants respond to the growing medium through different physiological mechanisms [4]. The traditional approach to select new growing media has been focused on selecting different organic and inorganic materials [1]. The lack of a clear understanding of how plants adapt to different growing media and of the physiological mechanisms involved in this plant growth regulation limits our efforts to find an alternative to peat moss for bedding pot plants [10,9].

We have previously shown that the shoot fresh weight of the bedding plant *Impatiens wallerana* is mainly determined by the root system size [11]. This is in agreement with the fact that there is a close coordination between roots and shoot growth, controlled by a signalling pathway, which is largely hormonal in nature with a major site of control located in the root system [12].

Several types of criteria can appraise the quality of ornamental plants: tolerance to biotic and abiotic stresses, development of potentialities and aesthetics. This last criterion is specific to ornamental plants and objective measurements are required [13].

Pansy (*Viola wittrockiana* Gams.) is a commercially important cool season garden crop for landscape, and one of the five best-selling bedding plants in both developed and undeveloped countries. However, information about the effects of environmental [14,15] and management [16-19] conditions on plant growth and development is limited. Thus, the aim of this work was to assess the performance of six pansy genotypes grown in two growing media with significant differences in both physical and chemical properties, aiming to understand how the substrate quality change the physiological mechanism related to biomass accumulation. The hypothesis tested was that the growing medium, as an abiotic stress source, would allow changing the medium-based paradigm to optimize both the growth and productivity of bedding pot plants.

2. MATERIALS AND METHODS

2.1 Plant Material and Experimental Design

The experiments were carried out in a greenhouse at the Faculty of Agronomy, University of Buenos Aires, Argentina (34°28'S), from March 28th to July 4th 2014 and from March 15th to June 28th 2015.

Viola wittrockiana Colossus Series ('Purple with blotch', 'White with blotch', 'Rose with blotch' and 'White purple') and Mammoth Series ('Viva La Violet' and 'Sangria punch') seeds (Goldsmith Inc., NY, USA) were germinated and grown in 288-plastic plug trays (6.18 cm^3 $cell^{-1}$) in Klasmann 411® medium (Klasmann-Deilmann, GmbH, Germany). When seedlings reached the transplant stage, 20 plants per block and treatment (growing medium and genotype) were transplanted into 1,200 cm^3 pots filled with two different growing media as follows:

1) *Sphagnum maguellanicum*-organic soil-perlite (40-40-20, v/v/v) medium (**S_1**) [20]. At the beginning of the experiments total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm^{-3}) were 63.50, 17.06, 10.06 and 0.35

respectively. Organic matter (%), pH, electrical conductivity (dS m^{-1}) and cation exchange capacity were 45.3, 5.2, 0.71 and 58.9 respectively.

2) *Sphagnum maguellanicum*-river waste-perlite (40-40-20, v/v/v) medium (**S_2**) [16]. At the beginning of the experiments total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm^{-3}) were 20.17, 4.33, 15.83 and 0.84 respectively. Organic matter (%), pH, electrical conductivity (dS m^{-1}) and cation exchange capacity were 11.8, 6.5, 0.11 and 35.2 respectively.

The two growing media tested were chosen with the aim to compare growing media with significant differences in their physical and chemical properties.

S_1 (field soil) came from the campus of the Faculty of Agronomy of the University of Buenos Aires. **S_2** (river waste or 'temperate peat') was collected from the Paraná River bank (Argentina). This sedimentary organic matter is derived from the delta plain vegetation and is highly dominated by phytoplasts (plant debris). The result is a fine-grained, black, oozy sediment deposited in the bottom of the coasts [21].

Plants were irrigated as needed, using intermittent overhead mist, and weekly soil fertilization (Stage 2: 50 ppm N; Stage 3-4: 100 ppm N; pot: 150 ppm N) (nitric acid, phosphorus acid, potassium nitrate, and calcium nitrate; Agroquímica Larocca S.R.L., Buenos Aires, Argentina) was included according to Styer and Koranski [22].

Daily mean temperatures (13.25 to 15.57℃) and daily photosynthetic active radiation (6.51 to 7.37 mol photons m^{-2} day^{-1}) for the two experiments were recorded with a HOBO sensor (H08-004-02) (Onset Computer Corporation, MA, USA) connected to a HOBO H8 data logger. The plants were arranged at a density of 25 plants m^{-2}, which avoided mutual shading.

Plants were harvested at the transplant stage and at 15, 30, 45, and 60 days after transplanting. Roots were washed and roots, stems, leaves and flower fresh weights (FW) were recorded. Dry weights (DW) were obtained after drying roots, stems, leaves and flowers to constant weight at 80℃ for 96 h. The number of leaves was recorded, and each leaf area was determined using a LI-COR 3000A automatic leaf area meter (LI-COR, Inc., Lincoln, NE, USA).

2.2 Data Analysis

The rate of leaf appearance (RLA) was calculated as the slope of the number of fully expanded leaves versus time (in weeks). The relative growth rate (RGR) was calculated as the slope of the regression of the natural logarithm (ln) of the whole plant DW versus time (in days). The allometric coefficients between roots and shoots were calculated as the slope (**β**) of the straight-line regression of ln root DW versus ln shoot DW (ln root DW = a **+** b x ln shoot DW).

Glucose content was analysed at the final sampling of the pot experiments using the Nelson-Somogyi method.

2.3 Statistical Analysis

The experimental design was a randomized factorial with three blocks of twenty single-pot replications of each treatment combination (growing medium × genotype). Since there were no significant differences between the two experiments, they were considered together (n = 6). Data were subjected to two-way analysis of variance (ANOVA). STATISTICA 8 (Stat Soft) software was used and the assumptions of ANOVA were checked. Least significant differences (LSD) values were calculated. Means were separated by Tukey's tests ($P \leq 0.05$). Slopes from straight-line regressions of RLA, RGR and allometric values were tested using the SMATR package [23].

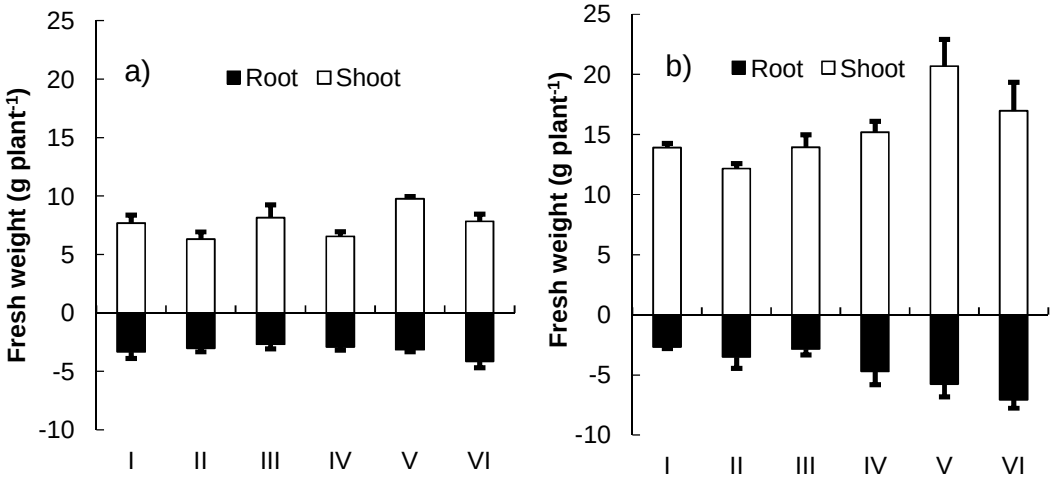
3. RESULTS

3.1 Biomass Accumulation and Leaf Area

Pansy plants grown in S_2 (Fig. 1b) showed significantly higher FW accumulation than those grown in S_1 (Fig. 1a). Differences between genotypes were higher when plants were grown in S_2.

Total leaf area was highest in pansy plants grown in S_2 for all the genotypes tested, although there were significant differences between genotypes in both growing media (Table 1).

RLA and RGR showed significant differences, associated with the different growing media. Table 2 shows that the highest RLA and RGR were achieved in S_2. RLA and RGR also showed significant differences between pansy genotypes, regardless of the growing medium used.



ANOVA	
Significantly	
Growing media	***
Genotype	***

*Significance *** 0.001*

Fig. 1. Mean fresh weight at the end of the experiments in six pansy (*V. wittrockiana* Gams.) plants (I: Colossus 'Purple with blotch'; II: Colossus 'White with blotch'; III: Colossus 'Rose with blotch'; IV: Colossus 'White purple'; V: Mammoth 'Viva La Violet' and VI: Mammoth 'Sangria punch') grown in two post-transplant growing media: S_1 (a) and S_2 (b) (n = 6). The standard errors over each bar and the significance of interactions (ANOVA) are indicated

Table 1. Total leaf area at the end of the experiments for pansy plants grown in two post-transplant growing media (S_1 and S_2) (n = 6) at the end of the experiments. Different lower-case letters indicate significant differences ($P \leq 0.05$) between growing media, while different capital letters indicate significant differences ($P \leq 0.05$) between different pansy genotypes

Pansy genotypes	Total leaf area (cm^2 $plant^{-1}$)	
	S_1	S_2
Colossus 'Purple with blotch'	179.65 ^{bC}	302.62 ^{aD}
Colossus 'White with blotch'	132.02 ^{bD}	257.65 ^{aD}
Colossus 'Rose with blotch'	195.90 ^{bB}	329.57 ^{aC}
Colossus 'White purple'	143.78 ^{bB}	341.31 ^{aC}
Mammoth 'Viva La Violet'	271.08 ^{bA}	541.86 ^{aA}
Mammoth 'Sangria punch'	176.77 ^{bB}	362.66 ^{aB}

Total glucose content shows significant differences in favour of plants grown in S_2 and significant differences between genotypes as well (Fig. 2).

3.2 Dry Weight Partitioning

The allometries between roots vs. shoots (Table 3) showed a partition to shoots in plants grown in S_2. Once again, significant differences between genotypes were also found.

3.3 Growth Rates and Root Dry Weight Relationships

When data from all the genotypes tested were plotted together, positive relationships between RLA (Fig. 3a), RGR (Fig. 3b), glucose content (Fig. 3c), and root DW were found at the end of the experiment. The highest control values were those of plants grown in S_2.

4. DISCUSSION

The commercial offer of bedding plants is very varied, but mainly related to aesthetic traits such

as colour and flower size (see Goldsmith Inc., NY, USA catalogue). However, growers need to offer the greater plants in the lower cropping times as well as plants resistant to different biotic and abiotic stresses [13]. Our results showed significant differences between the six pansy genotypes tested in agreement with that found by Johnson and Lenhard [24] who indicated that the growth of plant organs is under genetic control. Although organ size and shape can be modified by environmental factors, the genotype determines the limits within which such modification of growth and development can occur. Thus, different genotypes may achieve different final sizes and shapes when grown together across a range of environments.

Progress in improving abiotic stress tolerance of crop plants using classic breeding and selection approaches has been slow in most higher plants and almost absent in ornamental bedding plant. This has been generally blamed on the lack of reliable traits and phenotyping methods to quantify stress tolerance. Adaptation of plants to extreme environments requires complex morphological, developmental and metabolic adaptations [25], most of which have been documented in bedding pot plants only recently [3,4,11,10,9].

Although numerous factors affect the growth and productivity of bedding plants, the quality of the growing medium stands out as one of the most important ones, especially when plants grow in potted culture. When the production of a bedding plant is started, a critical decision that must be made is the choice of the growing medium [11]. The traditional approach to select a new growing medium has been focused on selecting organic and inorganic materials [1]. However, the aim of this work was to identify the physiological mechanism involved when a pansy plant is grown in two growing media that thoroughly differed in both physical and chemical properties. The hypothesis tested was that, the growing medium as an abiotic stress source would allow changing the media-based paradigm to optimize both the growth and productivity of bedding pot plants. Our results showed that the RGR (Table 2) and final size (Fig. 1) of pansy plants are determined by both genetic constraints and environmental factors, in agreement with that previously reported by Bögre et al. [26] and Powell and Lenhard [27].

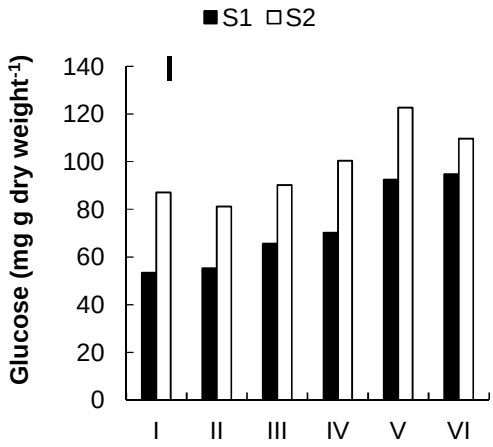


Fig. 2. Total glucose content at the end of the experiments in six pansy plants (I: Colossus 'Purple with blotch'; II: Colossus 'White with blotch'; III: Colossus 'Rose with blotch'; IV: Colossus 'White purple'; V: Mammoth 'Viva La Violet' and VI: Mammoth 'Sangria punch') grown in two post-transplant growing media (S_1 and S_2) (n = 6). The vertical line indicates least significant differences (LSD)

Table 2. Changes in the rate of leaf appearance (RLA) and the relative growth rate (RGR) for pansy plants grown in two post-transplant growing media (S_1 and S_2) (n = 120). Different lower-case letters indicate significant differences ($P \leq 0.05$) between growing media while different capital letters indicate significant differences ($P \leq 0.05$) between different pansy genotypes. The probability of the slope being zero was $P \leq 0.001$ for all growth parameters

Pansy genotypes	RLA (leaves week^{-1})		RGR (g g^{-1} day^{-1})	
	S_1	S_2	S_1	S_2
Colossus 'Purple with blotch'	0.322 ^{bD}	0.361 ^{aE}	0.025 ^{bB}	0.032 ^{aC}
Colossus 'White with blotch'	0.314 ^{bD}	0.502 ^{bD}	0.031 ^{bA}	0.040 ^{aB}
Colossus 'Rose with blotch'	0.367 ^{aC}	0.597 ^{bC}	0.027 ^{bB}	0.032 ^{aC}
Colossus 'White purple'	0.371 ^{aC}	0.686 ^{bB}	0.030 ^{bA}	0.040 ^{aB}
Mammoth 'Viva La Violet'	0.539 ^{aA}	0.942 ^{bA}	0.020 ^{bB}	0.039 ^{aB}
Mammoth 'Sangria punch'	0.469 ^{aB}	0.653 ^{bB}	0.031 ^{bA}	0.046 ^{aA}

Table 3. Changes in allometric relationships between roots and shoots of pansy plants, using a straight-line regression analysis between the natural logarithm of root and shoot dry weight. Treatments included two post-transplant growing media (S_1 and S_2) (n = 120). The straight-line regression slopes (β) and the coefficients of determination r^2 are indicated. Different lower-case letters indicate significant differences ($P \leq 0.05$) between growing media, while different capital letters indicate significant differences ($P \leq 0.05$) between different pansy genotypes. The probability of the slope being zero was $P \leq 0.001$ for all allometric relationships.

Pansy genotypes	Roots vs. shoots			
	S_1		S_2	
	β	r^2	β	r^2
Colossus 'Purple with blotch'	0.904 ^{aD}	0.942	0.800 ^{bD}	0.853
Colossus 'White with blotch'	1.132 ^{aA}	0.928	1.022 ^{bA}	0.928
Colossus 'Rose with blotch'	1.031 ^{aB}	0.915	0.959 ^{bB}	0.916
Colossus 'White purple'	0.965 ^{aC}	0.954	0.905 ^{bC}	0.964
Mammoth 'Viva La Violet'	0.983 ^{aC}	0.944	0.926 ^{bC}	0.962
Mammoth 'Sangria punch'	1.124 ^{aA}	0.955	0.975 ^{bB}	0.916

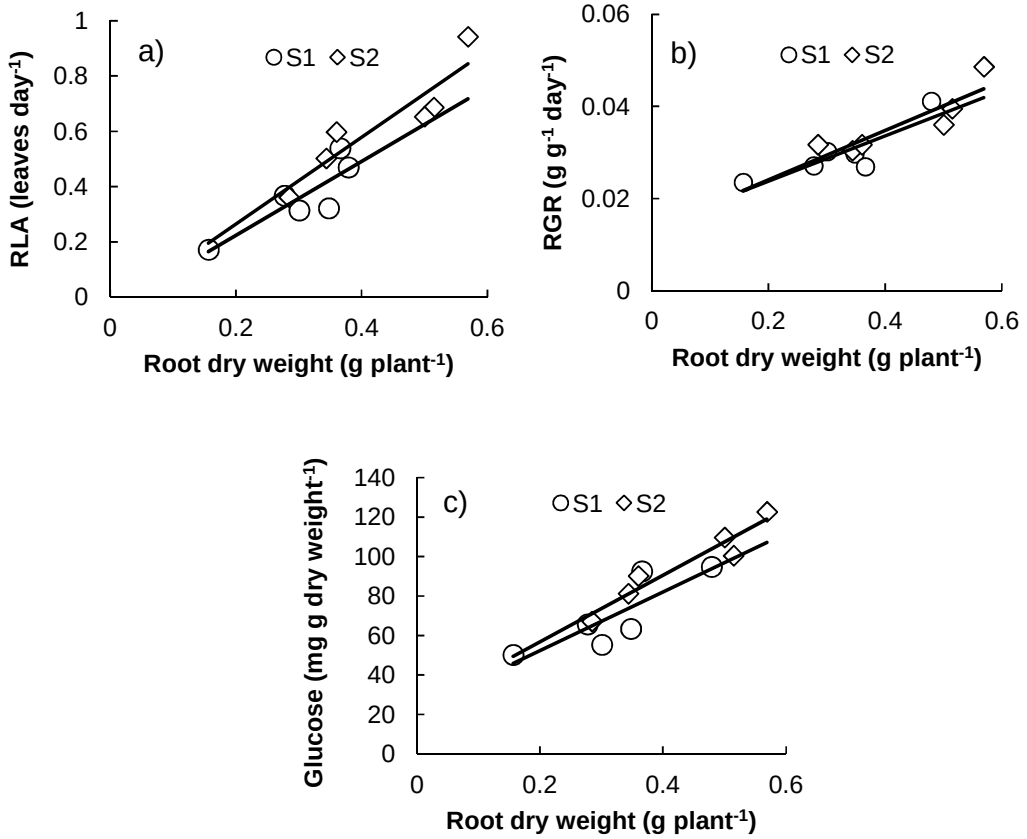


Fig. 3. Relationships between the rate of leaf appearance (RLA) (a), the relative growth rate (RGR) (b), the glucose content (c) and the root dry weight (RDW) in six pansy plants grown in two post-transplant growing media (S_1 and S_2). Linear regression equations are: RLA-S_1 = 1.34 RDW - 0.04 (r^2 = 0.731; $P \leq 0.001$); RLA-S_2 = 1.57 RDW - 0.05 (r^2 = 0.843; $P \leq 0.001$); RGR-S_1 = 0.049 RDW + 0.014 (r^2 = 0.749; $P \leq 0.001$); RGR-S_2 = 0.53 RDW + 0.013 (r^2 = 0.773; $P \leq 0.001$); Glucose content-S_1 = 148.81 RDW + 22.50 (r^2 = 0.705; $P \leq 0.001$); Glucose content-S_2 = 168.09 RDW + 23.16 (r^2 = 0.918; $P \leq 0.001$)

The natural environment of plants is composed of a complex set of abiotic and biotic stresses. Plant responses to these stresses are equally complex. Cramer et al. [28] defined abiotic stress as an environmental condition that reduces growth and yield below optimal levels. Our results showed that the quality of the growing medium should be considered as an abiotic stress source.

Fig. 1 shows significant differences in both shoot and root FW between the six genotypes tested but larger differences between the two growing media tested for each pansy genotypes. Ghosh and Xu [29] indicated that abiotic stress responses in plants occur at various organ levels among which root specific processes are of particular importance. On the other hand, Feller et al. [30] showed that for optimal development of the plant as a whole, both roots and shoot biomass must be balanced. In a given environment, the root fraction (the ratio of root mass relative to the mass of the entire plant) lies within certain species-specific limits, suggesting that the relative growth of the root and the shoot has a genetic basis. Under varying environmental conditions, however, the relative growth of the shoot and the root can change. For example, when light is limiting, the root fraction can change in favour of the shoot; a similar pattern may be observed in different limiting growing media such as those tested in the present and previous experiments [11,9].

When total leaf area was analysed (Table 1), we found a pattern similar to that shown in Fig. 1, i.e., greater photosynthetic leaf area in S_2 than in S_1 and significant differences between pansy genotypes. Since results for RLA were similar (Table 2), we could suppose that the higher total leaf area and RLA, the higher the shoot apical meristem. A decrease in the plastochron (i.e. the time between successive leaf initiation events) needs an increase in apex size [31], the presence of non-limiting sugar availability [32,33] and the regulation of relative assimilate allocation [30]. The plant allometries from Table 3 show that most of the significant differences found between growing media and pansy genotypes would be related to a change in photo assimilate partitioning. On the other hand, sink organs can potentially stimulate sugar supply by activating their consumption rate, thereby increasing their sink strength; the relative carbon allocation to a particular organ must be regarded as a function of source and sink activities of all parts of the plant [34]. The differences in glucose content shown in Fig. 2 are in agreement with these assumptions.

A higher leaf area and a change in photo assimilate to favour shoots rather than roots necessarily determine higher RGR (Table 2) and allow achieving higher bedding plant productivities, in agreement with that reported by Osone et al. [35] who indicated the importance of shoot-root interactions in understanding genotype differences in RGR. Pansy productivity would be mainly determined by the root system size (Fig. 3), in agreement with previous reports in other bedding pot plants [11].

Puig et al. [36] and Chen et al. [37] have concluded that plants can sense the volume of the rooting space available, and a limited number of studies on individual roots have shown that plant roots may sense the identity of neighbouring roots and respond accordingly [38,24]. Cytokinins are root-synthesised molecules, which are transported via the xylem to the shoot [39,40]. Some authors have claimed that the plastochron may be altered in transgenic plants with reduced cytokinin levels [41,42]. Similar results have been found in vegetables [43-45] and ornamental plants [11,46-50].

Although the higher the root system the higher the zeatin ribosides [51], it is not easy to show quantitative changes in endogenous cytokinin concentration [52] because plants synthesise different cytokinin-ribosides and not all have biological activity. Nevertheless, in the present study, when the root system increased, positive relationships with RLA (Fig. 3a), RGR (Fig. 3b), and glucose content (Fig. 3c) were found.

5. CONCLUSIONS

The physiological mechanisms (photo assimilate partitioning, higher RLA, higher leaf area as a feasible apical meristem growth increase and higher RGR) found to be involved in the response to the different growing media were the same as those found in plants grown with a root restriction related to container volume. To validate these conclusions and offer both adequate suggestions to growers and key traits for breeding programs, we should test the effect of exogenously applied cytokinins in plants grown in different growing media in the same way as when we tried to assess the volume root restriction on biomass accumulation. However, this investigation line needs additional experiments, which are already in progress.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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