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The Effect of the Pre-transplant Pot Media Quality on Pansy Garden Performance

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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

Although there is a great amount of information about the production of bedding plants, little is documented on their post-production performance. In order to determine how the crop management affects the post-production performance, two growing media with significant differences in both physical and chemical properties during the pot culture were tested. The aim of this work was to understand how the substrate quality change the physiological mechanism related to biomass accumulation both during the pot culture and during the post-production handling. The hypothesis tested was that the growing medium at the pre-transplant stage, as an abiotic stress source, affect the performance during the post-production cropping as well. These novelty data show that plant quality and garden performance are dependent on the growing media quality during the pot culture as well as the plant genotype. The physiological changes involved included leaf area accumulation and biomass accumulation as a result of both carbohydrate production and partition. The positive relationships between the rate of leaf area expansion (RLAE), the rate of leaves appearance (RLA), the relative growth rate (RGR), the net assimilation rate (NAR) and root dry weight, which would involve the synthesis of cytokinins are discussed.

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Keywords: Bedding plants; biomass accumulation; growth parameters; shelf performance.

1. INTRODUCTION

Annual sales of flowering potted plants keep setting new records as the popularity of these crops continues to soar among most of the developed and non-developed countries consumers. Bedding plants are the most rapidly growing segment of the Green Industries. The garden pansy (*Viola* × *wittrockiana* Gams.) is a plant of moderate climate, which belongs to the most popular early spring ornamental plants for garden beds and borders.

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 [1]. Recently, it has been suggested that growing medium gives not only a matrix for water and nutrient absorption but also a source of external signaling for bedding plants [2], including pansy [3]. Under a poor quality-growing medium, ornamental bedding plants decreased shoot fresh weight in response to the root system restrictions [4-7].

Plant roots can sense adverse soil conditions and, via some internal signal, transmit the condition of the soil to extending leaves, with the typically net result of a decrease in leaf elongation rates [8]. It has been claimed that the close coordination between root and shoot growth is controlled by a signaling pathway which is largely hormonal and located in the root system [9,10,7]. These physiological and metabolic processes involve gene activation [11].

The quality of flowering bedding plants at the time of sale (as evaluated by plant size, flower color and size, plant form, leaf color, and the absence of blemishes due to mechanical damage and insect or disease infestation) is determined by the grower and is directly affected by environmental conditions and cultural practices used in production. However, post-production performance is dependent on the production practices but on the handling procedures used by the shipper, retailer and consumer. The relative impact that each segment of the industry has on plant quality during the post-transplant to a garden bed is not well known. However, a poorly produced plant cannot be improved by the most carefully designed handling procedure. Any practice that decreases plant quality during production also decreases shelf life.

The effects of environmental conditions [12,13] and culture management [14,15,3] on pansy plant growth and development during pot culture is limited. The specific requirements for the post-production management of the most bedding pot plants is scarce as well [16-20]. As example, the pre-transplant growing media effects on post-production performance of bedding pot plants is fully lacking.

The aim of this work was to assess the performance of three pansy genotypes grown in two growing media with significant differences in both physical and chemical properties during the pot production culture, aiming to understand how the substrate quality change the physiological mechanism related to biomass accumulation both during the pot culture and during the post-production handling. The hypothesis tested was that the growing medium at the pre-transplant stage, as an abiotic stress source, affect the performance during the post-production cropping as well.

2. MATERIALS AND METHODS

2.1 Plant Material and Experimental Design

The experiments were carried out at the Faculty of Agronomy campus, University of Buenos Aires, Argentina (34°28' S) from March 25th to July 29th 2015 and repeated once from March 23th to July 25th 2016. The pot experiments were carried out inside a greenhouse with automatic acclimatization but the post-transplant experiments were carried out in a field surrounding it.

Viola wittrockiana Colossus Series ('Ocean 2' and 'Yelow Blocht') and *Viola cornuta* Patiola Series ('Pure Yellow') seeds (Goldsmith Inc., NY, USA) were germinated and grown in 288-plastic plug trays (6.18 cm3 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**) [21]. 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**) (Di Benedetto et al., 2006). 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 (mile equivalents 100 g^{-1}) were 11.8, 6.5, 0.11 and 35.2 respectively.

During pot cropping, the two growing media tested were chosen with the aim to compare the effect of growing media with significant differences in their physical and chemical properties during the pot cropping on the post-transplant pansy field performance. For the **S_1** growing media, field soil came from the campus of the Faculty of Agronomy of the University of Buenos Aires while for the **S_2** growing media, river waste or 'temperate peat' was collected from the Paraná River bank (Argentina). River waste was a sedimentary organic matter, which 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 [22]. Plants were transplanted to a field location 60 days after the beginning of the experiment. Original field soil properties were changed with the *Sphagnum maguellanicum* peat amendment. At the beginning of the experiments field total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm^{-3}) were 25.02, 6.60, 31.56 and 0.90 respectively, while pH and electrical conductivity (dS m^{-1}) were 6.1 and 0.16 respectively.

Plants were irrigated as needed, using intermittent overhead mist, and weekly soil fertilization (Stage 2: 50 mg L^{-1} N; Stage 3-4: 100 mg L^{-1} N; pot: 150 mg L^{-1} 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 [23]. Plants grown in **S_2** growing medium received 30% water than plants in **S_1** growing medium.

Daily mean temperatures (14.28 to 16.50°C) and daily photosynthetic active radiation (6.41 to 7.17 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 plug tray transplant stage and at 20-days intervals. 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°C for 96 hours. The number of leaves was recorded and each leaf area was determined using the ImageJ® (Image Processing and Analysis in Java) software.

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 on a DW basis *vs.* time (in days) [24]. The rate of leaf area expansion (RLAE) was calculated as the slope of the regression of the ln of total leaf area versus time (in days) [25]. The mean net assimilation rate (NAR), and the leaf area ratio (LAR) [26] were calculated as follows:

$$NAR = \frac{k_w W_0 e^{k_w t}}{A_0 e^{k_a t}}$$

$$LAR = k_a \,/\, \frac{A_a e^{k_a t}}{k_w W_0 e^{k_w t}}$$

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: RLAE ($days^{-1}$); t: time (in days) at the midpoint of the experimental period and e: base of natural logarithms.

The allometric coefficients between root and shoot were calculated as the slope (**β**) of the straight-line regression of the ln of the root DW *vs*. the ln of the shoot DW (ln root DW = a **+** b x ln shoot DW) [27].

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 through the Pearson correlations [28]. 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 [29].

3. RESULTS

3.1 Biomass Accumulation and Leaf Area

At transplant stage, total fresh weight was higher in plants grown at the **S_2** growing media because of higher shoot fresh weight and no significant differences in root fresh weight (Fig. 1a, b and c). Fresh weight differences increased at the end of the experiments and included both shoot and root values for the three pansy genotypes tested (Fig. 1d, e and f).

Total leaf area increased between the transplant stage and the end of the experiments but the relative effect of the growing media be dependent of the pansy genotype. The significant total leaf area differences were associated to the rate of leaf appearance (RLA) but not in the individual leaf area (Table 1).

RLAE showed no significant differences between growing media but a decrease when plants were cropping in a field bed. On the other hand, RGR was higher in plants potted with the **S_2** growing media from *V. cornuta* and *V. wittrokiana* 'Ocean 2' at the pre-transplant stage with no significant differences at the post-transplant cropping. RGR values were the result of a higher NAR and a lower LAR when plants were cropping in pots (Table 2).

3.2 Dry Weight Partitioning

The plant allometries from Table 3 showed a partition to shoots in plants grown in **S_2** so during both the pot culture and the field cropping. Once again, significant differences between both genotypes and pre- or post-transplant stage were also found.

Table 1. Changes in both total and individual leaf area and RLA at the transplant stage to field (pre-transplant stage) and at the end of the experiments (post-transplant stage) for three pansy plants grown in two pre-transplant growing media (S_1 and S_2) (n = 6). Different lower-case letters indicate significant differences (P < .05) between growing media, while different capital letters indicate significant differences (P < .05) between the pre- and the post-transplant stage

Pansy genotypes	Total Leaf area (cm^2 $plant^{-1}$)		Individual leaf area (cm^2 $leaf^{-1}$)		RLA (leaves $week^{-1}$)	
	Pre-transplant	Post-transplant	Pre-transplant	Post-transplant	Pre-transplant	Post-transplant
V. cornuta 'Pure Yellow'						
S_1	88.21^{bB}	154.37^{bA}	2.36^{aA}	2.76^{aA}	0.428^{bB}	1.098^{bA}
S_2	133.79^{aB}	215.84^{aA}	2.46^{aA}	2.13^{aA}	0.499^{aB}	1.266^{aA}
V. wittrokiana 'Ocean 2 '						
S_1	209.52^{aB}	328.51^{bA}	7.62^{aA}	5.66^{aB}	0.456^{aB}	0.858^{bA}
S_2	238.67^{aB}	589.57^{aA}	7.87^{aA}	5.26^{aB}	0.512^{aB}	0.910^{aA}
V. wittrokiana 'Yellow Blotch'						
S_1	208.80^{aB}	365.53^{aA}	8.95^{aA}	4.59^{aB}	0.415^{bB}	1.108^{bA}
S_2	189.55^{aB}	354.28^{aA}	4.72^{bA}	4.50^{aA}	0.917^{aB}	1.171^{aA}

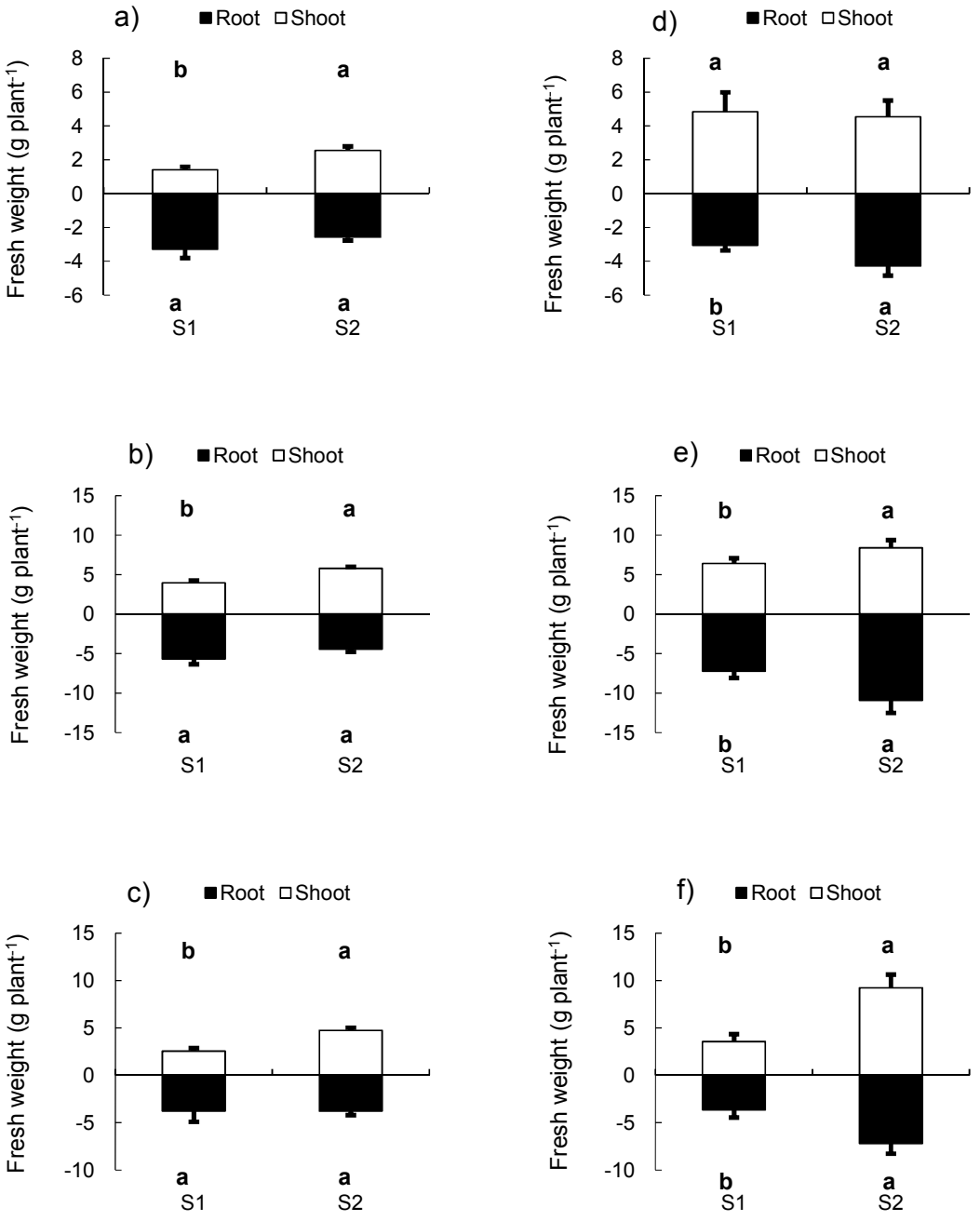

Fig. 1. Mean fresh weight at the transplant stage to field (A, B and C) and at the end of the experiments (D, E and F) in three pansy plants. *Viola cornuta* Patiola 'Pure Yellow' (A and D) and Viola *wittrockiana* (Gams.) Colossus 'Ocean 2' (B and E) and 'Yellow Blotch' (C and F) were grown in two growing media (S_1 and S_2) (n = 6) at the pre-transplant stage. The standard errors over each bar are indicated. Different lower-case letters indicate statistical significant differences al P < .05

Table 2. Changes in RLAE, RGR, NAR and LAR at the transplant stage to field (pre-transplant stage) and at the end of the experiments (post-transplant stage) for three pansy plants grown in two pre-transplant growing media (S_1 and S_2) (n = 6). Different lower-case letters indicate significant differences (P < .05) between growing media, while different capital letters indicate significant differences (P < .05) between the pre- and the post-transplant stage

Pansy genotypes	RLAE ($cm^2\ cm^{-2}\ day^{-1}$)		RGR ($g\ g^{-1}\ day^{-1}$)		NAR ($g\ cm^{-2}\ day^{-1}$) (x 10^{-4})		LAR ($cm^2\ g^{-1}$)	
	Pre-transplant	Post-transplant	Pre-transplant	Post-transplant	Pre-transplant	Post-transplant	Pre-transplant	Post-transplant
V. cornuta 'Pure Yellow'								
S_1	0.0406^{aA}	0.0222^{aB}	0.0538^{bA}	0.0366^{aB}	3.68^{bA}	3.59^{aA}	146.34^{aA}	115.58^{aB}
S_2	0.0457^{aA}	0.0256^{aB}	0.0599^{aA}	0.0385^{aB}	7.20^{aA}	3.33^{aB}	83.20^{bB}	101.87^{aA}
V. wittrokiana 'Ocean 2'								
S_1	0.0441^{aA}	0.0259^{aB}	0.0545^{bA}	0.0378^{aB}	3.28^{bA}	3.52^{aA}	166.09^{aA}	125.57^{aB}
S_2	0.0485^{aa}	0.0283^{aB}	0.0609^{aA}	0.0393^{aB}	6.75^{aA}	3.01^{aB}	90.18^{bB}	111.62^{aA}
V. wittrokiana 'Yellow Blotch'	0.0400^{aA}	0.0244^{aB}	0.0517^{aA}	0.0365^{aB}	3.94^{aA}	2.51^{aB}	131.05^{aA}	138.12^{aA}
S_1	0.0400^{aA}	0.0242^{aB}	0.0517^{aA}	0.0346^{aB}	4.19^{aA}	3.14^{aB}	123.35^{aA}	116.08^{bA}
S_2								

3.3 Growth Rates and Root Dry Weight Relationships

When data from all the genotypes tested and cropping environment were plotted together, positive relationships between RLAE (Fig. 2a), RLA (Fig. 2b), RGR (Fig. 2c), NAR (Fig. 2d) and root DW were found. The highest control values were those of plants grown in **S_2**.

4. DISCUSSION

The quality of flowering ornamental plants is essentially defined by visual appearance. One of the main criteria for the visual quality of an ornamental potted plant is its shape, which should be compact and well branched [30]. However, Santagostini et al. [31] indicated that the quality of ornamental plants must be appraised with other criteria, which include tolerance to biotic and abiotic stresses, development potentialities and aesthetics. In this way, horticulturists are faced with the problem of defining the quality of plants in terms of aesthetic criteria, stress resistance, longevity and after-sales development [32]. Abiotic stress is defined as environmental conditions that reduce growth and yield below optimum levels [33]. Abiotic stress responses in plants occur at various organ levels among which the root specific processes are of particular importance [34]. Adaptation of plants to extreme environments requires complex morphological, developmental and metabolic adaptations [35]. Recent reports [3,5,7] indicate that the quality of growing media should be considered as an abiotic stress source.

A pansy plant increase in size, which depend on the environmental and cultural pot production facilities and the genotype. Data from Fig. 1 showed that the fresh weight of *V. cornuta* at the pot transplant stage was the half of the *V. wittrockiana* genotypes. Johnson & Lenhard [36] indicate that the growth of plant organs is under genetic control, although organ size and shape can be modified by environmental factors. At the same time, genotype sets the limits within which such modification of growth and development can occur.

A correct growing media to optimize plant growth is demanding, and represents a production cost about 4-6% for bedding plants [37]. A large growing media offer is available [38] and it has been indicated that potting media plays a key role in quality and production of bedding plants [1,39,2] including pansy [3] as well. Fig. 1 results are in agreement with these previous reports during the pot production culture. Data from Fig. 1 indicate that the variation of the quality parameters such as plant size during post-production depends on the genotype as well as on the potting media during the pot-production stage, even though plant size significantly increased under a decreased in soil root restriction.

Table 3. Changes in allometric relationships between roots and shoots of three 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) at the transplant stage to field (pre-transplant stage) and at the end of the experiments (post-transplant stage) (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 < .05) between growing media, while different capital letters indicate significant differences (P < .05) between the pre- and the post-transplant stage

Pansy genotypes	Pre-transplant		Post-transplant	
	β	r^2	β	r^2
V. cornuta 'Pure Yellow'				
S_1	0.980^{aA}	0.855	0.963^{aA}	0.858
S_2	0.157^{bB}	0.527	0.904^{aA}	0.869
V. wittrokiana 'Ocean 2'				
S_1	1.286^{aA}	0.953	1.112^{aB}	0.931
S_2	0.458^{bB}	0.507	0.757^{bA}	0.610
V. wittrokiana 'Yellow Blotch'				
S_1	1.150^{aA}	0.824	1.103^{aB}	0.873
S_2	0.668^{bA}	0.695	0.512^{bB}	0.690

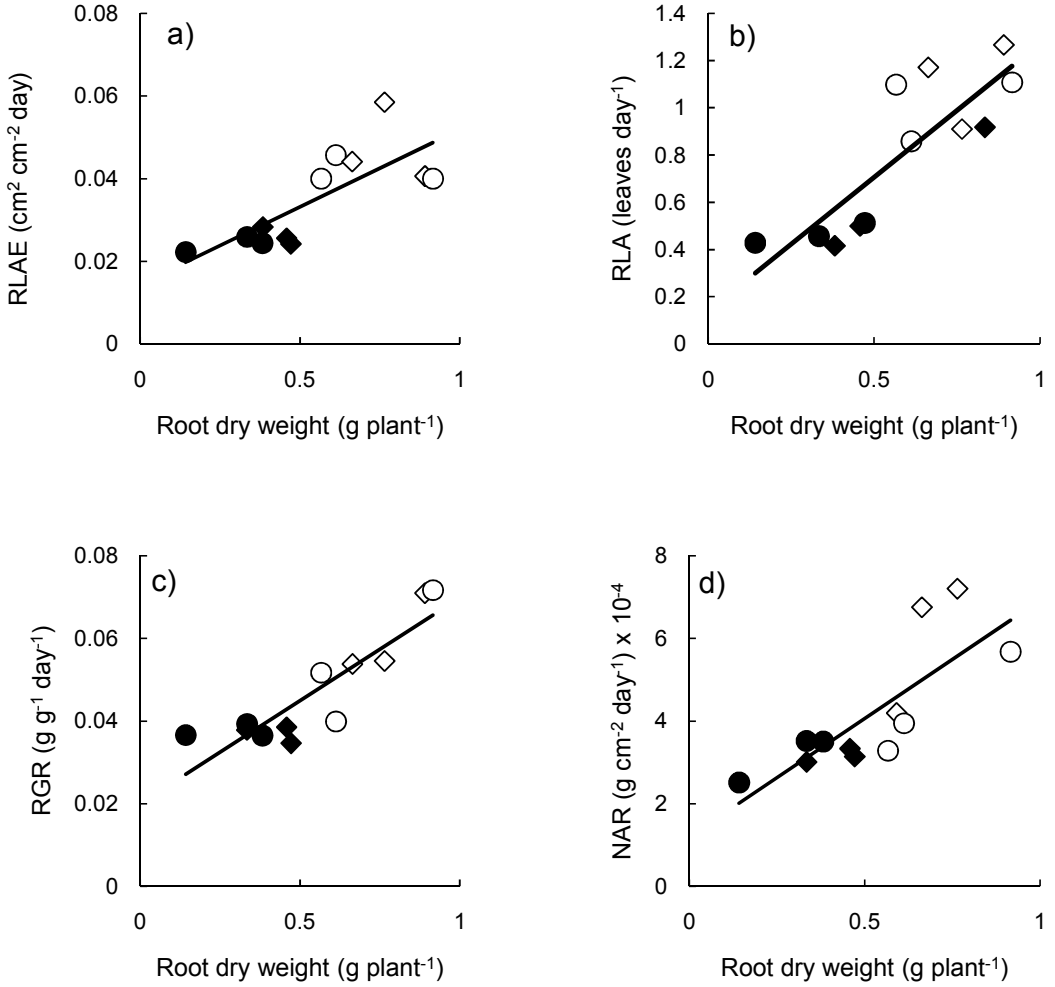

Fig. 2. Relationship between RLAE (A), RLA (B), RGR (C), NAR (D) and the root dry weight (RDW) in three pansy plants grown in two post-transplant growing media (S_1: ○ and S_2: ◊) at the transplant stage to field (pre-transplant stage) (full symbols) and at the end of the experiments (post-transplant stage) (empty symbols). Linear regression equations are RLAE = 0.037 RDW + 0.014 (r^2 = 0.681; P < .001); RLA = 1.133 RDW + 0.139 (r^2 = 0.811; P < .001); RGR = 0.050 RDW + 0.020 (r^2 = 0.783; P < .001); NAR = 5.72 RDW + 1.120 (r^2 = 0.625; P < .001).

The primary meristem in a plant are the shoot apical meristem (SAM), responsible for generating all aboveground organs [40]. It define growth rate and final size of plant organs [41]. The size of plant organs, such as leaves, is determined by an interaction of genotype and environmental influences as well [42]. It can explain that the higher fresh biomass accumulation (Fig. 1) the higher pansy total leaf area (Table 1). Results from Table 1 showed that changes in total leaf area during the experiments were related mainly to significant differences in RLA. Both total leaf area and RLA increased during the post-production time and are in agreement with the fact that leaf growth and development responds to hormone, nutritional and environmental conditions such as soil water content, incident light and leaf temperature. However, combined with this regulation there are genotype-specific differences between species and ecotypes of the same species [43].

When RGR were estimated (Table 2), we found significant differences related to growing media

quality and plant genotype, which were associated to higher NAR and lower LAR values. RGR is the product of LAR, the so-called 'morphological component' and NAR, the 'physiological component'. A change in dry weight partitioning towards the development of leaf area would be reflected as an increased LAR, while an increased efficiency of dry weight fixation would be associated with higher NAR values [44,45]. In plants grown under an abiotic stress such as the pot size, a direct relationship between RGR and NAR but an inverse relationship between RGR and LAR were found [46-48]. These results are in agreement with Poorter et al. [49], who found that, net photosynthesis is the process more strongly affected by pot size. De Lojo et al. [7] showed that growing media quality can be seen as an abiotic stress of the same magnitude than pot size.

In a previous report on the ornamental foliage plant *Epipremnum aureum*, RGR increase in response to an exogenous hormonal spray was explained by a large increase in NAR, which in turn was associated with a higher net photosynthetic rate. Increased accumulation of biomass by cytokinin or auxin exogenous sprays was observed earlier than the effects on leaf development [50]. Cytokinins is known to stimulate the expression of photosynthetic enzymes like Rubisco [51].

We found a significant partition increase to shoots in plants grown in $\mathbf{S_2}$ growing media (Table 3). Changes in the allometric slopes (β) reflect variations in carbon partitioning in response to biotic and abiotic environment interactions [52]. Benincasa et al. [53] indicated that crop management, such us the nitrogen supply (intimately related to growing media quality) modify the source-sink relationships. Carbohydrate partitioning between competing sites is explained by the fact that plants are capable of modifying their resource allocation to favor the growth of their growing parts [54, 55]. Plant tissues and organs rich in cytokinins are known to attract assimilate translocation [56]. Ron'zhina [57] 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.

Plants are able to detect the presence of their neighbours below-ground through the root apical meristem (RAM), responsible for producing all underground organs [40]. The associated root responses may affect plant performance, plant–plant interactions and community dynamics, but the extent and direction of these responses is heavily debated [58]. Root growth and differentiation in plants has been intimately linked with plant hormones [59]. O'Hare and Turnbull [60] showed that, an increase in root growth might lead to a corresponding increase in the synthesis of cytokinins. On the other hand, 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 [61]. The hypothesis that endogenous cytokinins are involved in these responses is supported by the fact that the mentioned physiological changes are similar to those found when ornamentals [4, 25,62,47,50,46] and vegetables [63,64,48] were sprayed with exogenous cytokinins. Our results showed positive relationships between RLAE, RLA, RGR, and NAR related to root DW (Fig. 3A, B, C and D respectively) when data from different growing media quality and pansy genotypes responses were plotted together and partially support this previous hypothesis, although validation of it exceed the objective of our work and it is matter for future research.

5. CONCLUSIONS

The novelty of our work is to show that plant quality and garden performance are dependent on genotype and the growing media quality during the pot culture, which must be considering as a pansy abiotic pre-transplant stress to a garden bed. The physiological mechanisms involved included differences in leaf area expanded (estimated mainly through RLA), differences in CO_2 fixation capacity (estimated through NAR) and differences in carbohydrate partitioning (estimate through root: shoot allometries). The sum of these responses determine significant differences in total fresh weight related to two growing media during the pot culture which were amplified when plants were transplanted to a field bed. The positive relationships between RLAE, RLA, RGR and NAR related to DW let us to speculate with the involvement of endogenous cytokinins, which are mainly synthesized in root apical meristems.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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