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Adaptive Response of *Petunia* × *hybrida* Plants to Water-Scarce Urban Substrates

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Abstract

The use of hydrogel and biostimulants holds great potential for plants' adaptation to stressful urban conditions, increasing their tolerance to drought stress. In this study, we investigated the plant performance and anatomical response of *Petunia* × *hybrida* hort. ex E. Wilm., cultivated under different substrate volumes and compositions, hydrogel amendments, and biostimulant treatments, as well as their interactions under drought stress. Namely, the plants were planted in pots with a substrate depth of 7 cm and 10 cm and cultivated under different combinations of organic (peat) and inorganic (perlite) substrates. Moreover, half of the plants were subjected to hydrogel and biostimulant treatments. Different watering intervals (24–96 h) were applied in combination with exposing the plants to direct sunlight for 8–10 h. The results showed that a larger substrate depth, along with hydrogel and biostimulant amendments in a mixture of perlite and peat, helps plants adapt to dry conditions when grown in shallow substrates, providing optimal water availability and thus contributing to the physiological adaptation of plants to water deficit. The study clearly demonstrates that substrate selection and irrigation frequency must be jointly optimized to ensure resilient urban greening. Hydrogels stand out as essential amendments, enabling significant water savings by extending irrigation intervals without compromising vascular growth or drought resilience. These water-efficient substrate strategies are vital for sustainable urban vegetation management, especially as cities face increasing environmental pressures and the imperative of climate adaptation, thereby supporting multiple Sustainable Development Goals.

Keywords: stem anatomy; substrate depth; hydrogel; drought tolerance; urban composition

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1. Introduction

Drought stress is recognized as one of the most significant abiotic factors limiting plant growth and development. Climate change, characterized by rising air temperatures and altered precipitation patterns, is expected to exacerbate drought conditions in many regions of Europe, negatively impacting plant survival and productivity [1–3]. The impacts of drought are especially severe in urban environments, where the maintenance of green spaces is challenged by the limited availability of high-quality water. According to Poje et al. [4], the combined effects of drought and urban expansion place growing pressure on urban green areas, which offer numerous essential benefits. Therefore, it is imperative to ensure their sustainability.

In this context, the activation of informal green spaces, such as rooftops, balconies, terraces, and vertical surfaces, can positively influence urban microclimates and enhance the

well-being of residents. Transforming rooftops into green spaces is not merely an innovative strategy; it is a vital step toward a sustainable future. This initiative reduces surface water runoff, mitigates the urban heat island effect, and improves air quality. It also supports biodiversity and contributes to a more pleasant and restorative urban environment [5–8]. In addition to green roofs, the integration of vertical greenery and vegetation on terraces, balconies, and other elevated green surfaces provides further value for sustainable urban planning. However, the effectiveness of these solutions is often limited by the plant material selected, the substrate that supports its growth, and the continuous exposure to sunlight. Moreover, plants frequently struggle to thrive under dry conditions, making it essential to address these challenges through appropriate cultivation strategies.

Petunia × *hybrida* hort. ex E. Wilm. (Solanaceae) is considered one of the most popular and widely used flower species in the world [9]. Besides its large, multicolored flowers and diverse morphology, petunia also shows notable drought resistance, as indicated by more intense flowering during higher temperatures [10]. *Petunia* × *hybrida* performs best in full sun when grown in an appropriate substrate, and is considered suitable for use in urban areas.

Plant growth and development begin with the proper selection of a compatible substrate. Among these substrates, perlite stands out for its valuable characteristics. The use of perlite enables satisfactory plant growth and development, primarily due to its ability to regulate water, oxygen [11] and nutrient availability. Perlite also promotes adequate root aeration by improving substrate structure [12]. Since soil reflectance depends on the substrate, perlite largely reflects UV radiation due to its white color, lowering substrate temperature and reducing heat stress on plant roots.

Numerous studies have shown the great potential of plants' adaptation to stressful conditions fortified by a synthetic superabsorbent polymer—hydrogel, which, when added to the substrate, promotes plant growth, postpones the negative effects of drought, and thus reduces the need for irrigation, making substrate compaction more difficult [13–18]. This photo-stable material improves the physical condition of the soil and has a high water absorption capacity, making it an ideal solution for use in lighter substrates constantly exposed to stressful drought conditions. Substrates with hydrogel supplementation mitigate water stress by retaining water longer in the plant [19], inducing anatomical adaptive changes [20,21] that increase cell membrane stability and improve safe absorption of water and nutrients across vascular bundles [22]. Additionally, one solution to reduce water stress is the use of biostimulants—products formulated with active ingredients free of pesticides, designed to enhance plant growth and development while providing protection against drought, salinity, freezing and the negative impacts of bacteria and viruses [23–25]. Plants treated with biostimulants, along with the correct use of hydrogel, are more resistant to water deficit and diseases. These treatments reduce the need for fertilizers and make plants better adapted to water deficit by assimilating nutrients and increasing growth rate [26–28].

The aim of this study was to evaluate the effects of pot size, substrate composition, hydrogel amendment, and biostimulant application on the growth performance and stem anatomical traits of *P.* × *hybrida* cultivated in container-based systems under semi-controlled urban conditions.

To frame the investigation, the study addressed the following research questions (RQs):

(RQ1) How do pot size (substrate depth and volume) and substrate composition influence the vitality, vegetative growth, and stem anatomical traits of *P.* × *hybrida* under limited irrigation conditions?

(RQ2) What is the effect of hydrogel amendment on plant development and internal stem tissue organization in shallow substrate systems?

(RQ3) To what extent do biostimulant applications affect anatomical adaptations and growth performance of *P.* × *hybrida* under water-limited container cultivation conditions?

(RQ4) How do different treatment combinations interact to influence the vitality and drought-adaptive anatomy of *P.* × *hybrida*?

The ultimate objective was to identify treatment combinations that maximize the visual and functional performance of *P.* × *hybrida*, ensuring sustainable cultivation through optimized resource management. The findings are intended to inform substrate and amendment strategies for container-based cultivation of *P.* × *hybrida* in urban conditions characterized by shallow substrates and variable microclimates, such as green roofs, balconies and terraces.

2. Materials and Methods

2.1. Experimental Setup and Materials

Pot experiment was conducted from May to September 2021 in the Botanical Garden of the Faculty of Agriculture, University of Novi Sad, in Novi Sad, Serbia (N 45.2472; E 19.8506; alt. 81 m a.s.l.). The experiment took place in an open field fully exposed to direct sunlight. The climate of the area is moderate-continental to continental, characterized by very hot summers and cold winters. The lowest average maximum air temperature recorded during the experimental period was 21.9 °C, while the minimum of 10.6 °C was recorded in May (Figure 1). The warmest month was July, with an average maximum daily temperature of 32.0 °C. June and August showed similar daily temperature variations, with maximum temperatures of 29.8 °C and 28.9 °C, and minimum temperatures of 23.3 °C and 22.2 °C, respectively. The summer of 2021 was marked by tropical days in June, July, and August, with a peak air temperature of 39.0 °C. In September, a significant drop in average air temperature was recorded, falling to 17.9 °C, signaling the transition from summer to autumn.

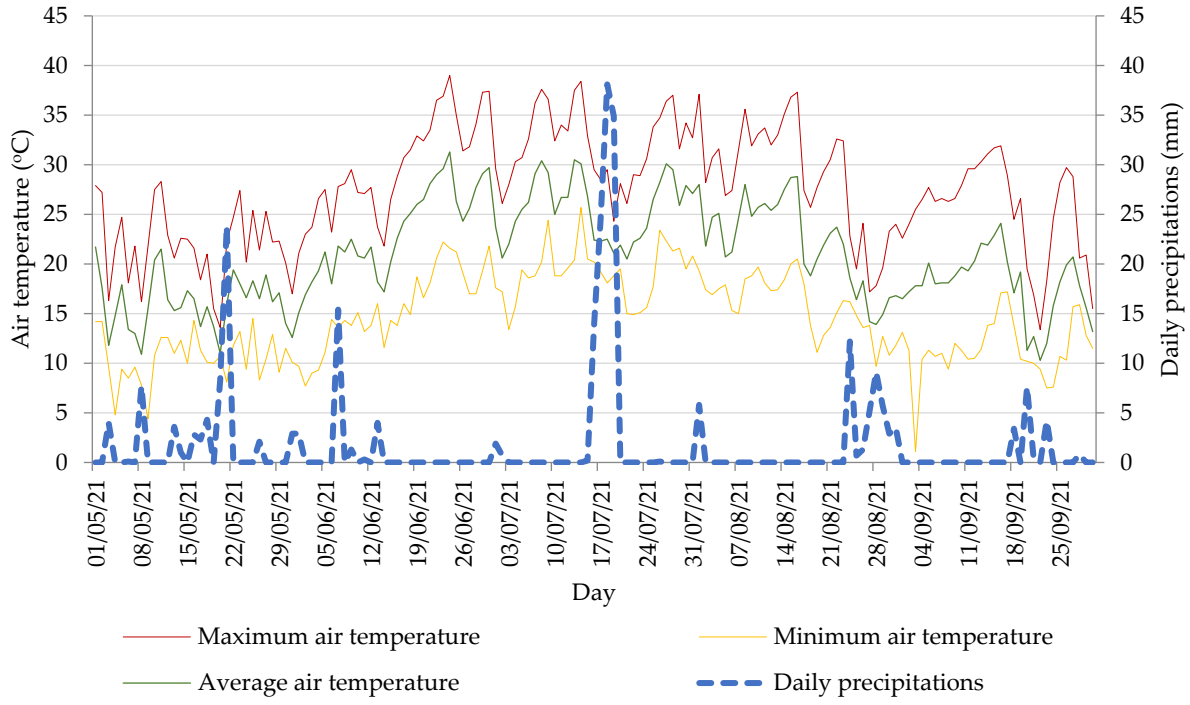


Figure 1. The daily temperature and precipitation data over a 5-month period (May–September 2021). Source: RHSS (Republic Hydrometeorological Service of Serbia) [29].

Precipitation from May to September showed seasonal patterns consistent with the climate of the studied area, characterized by monthly precipitation fluctuations. Figure 1 illustrates that dry days predominated throughout the study period, with limited precipitation, except in July. May experienced moderate rainfall, with recorded amounts of 7.7 mm, 8.3 mm, and 23.8 mm. June was notably dry, with precipitation occurring on only three days. On 7 June, 15.4 mm of rainfall was recorded, likely indicating a storm event, while the remaining days had minimal precipitation. The most intense rainy period occurs in mid-July when large amounts of rain fell (38.1 mm and 34.9 mm, which may be linked to extreme temperature variations as shown in Figure 1. In August, precipitation levels stabilized, with a maximum daily value of 12.2 mm. September was predominantly dry, with lower precipitation values of 7.6 mm and 4.2 mm, suggesting a gradual transition to autumn [29].

The seeds of *Petunia* × *hybrida* hort. ex E. Vilm. 'Grandiflora mix' were sown in nursery seedbeds in the second half of February 2021. When the true leaves (five to seven) appeared after the cotyledons, usually within 6–8 weeks, and the plants reached a height of 8–10 cm, a total of 108 plants were transplanted into pots and moved to the experimental site.

A split-plot design was employed, considering four factors: pot size, substrate type, hydrogel addition, and biostimulant application. The experimental setup included two parts (A and B). In both parts, pots were used (A—9 × 9 × 9 cm and B—12 × 12 × 12 cm), arranged into 6 columns and three substrate groups (group 1—perlite treatment; group 2—peat treatment; group 3—mixture of 30% peat and 70% perlite treatment). The substrate depths were 7 cm and 10 cm, respectively, for the smaller and larger pots (Figure 2). The plants were foliar-treated with different biostimulants every 7–10 days, due to their ability to increase plant growth and vitality, and increase plant tolerance to drought through improved water use efficiency and reduced cellular damage. Specifically, columns a and d were treated with the biostimulant Stim pure AA Liquid (SP) (Ascophyllum nodosum, Van Iperen International, Westmaas, The Netherlands) at a concentration of 0.3 mL/L, while columns b and e were treated with the biostimulant WAKE-up (WU) Liquid (potassium carboxylate, a salt of organic acids with potassium, Van Iperen International, Westmaas, The Netherlands) at a concentration of 0.5 mL/L.

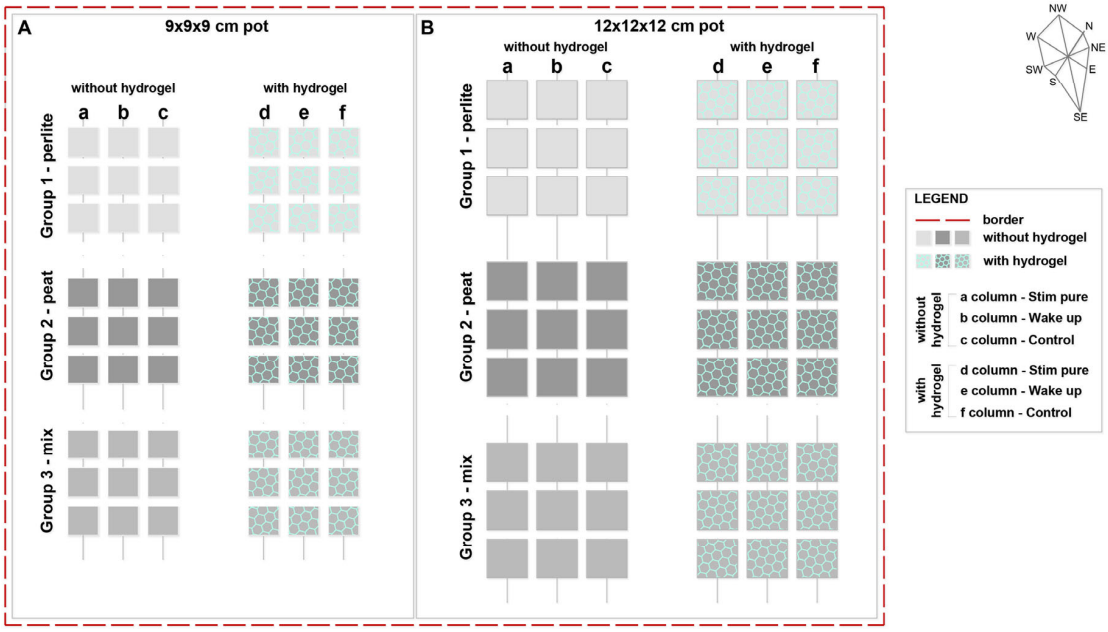


Figure 2. Experimental design scheme. (**A**): Part with 7 cm substrate depth; (**B**): Part with 10 cm substrate depth.

Columns c and f served as control treatments (without biostimulant application). In addition, one of the treatments involved the application of hydrogel, due to numerous benefits: water absorption and retention, nutrient recovery, microbial control, improved root development, and plant resistance to drought. Following the methodology of Ljubojević et al. [17], 2 g/dm^3 of hydrogel was added to the substrate in the d, e, f columns, while the a, b, c columns of the experiment did not contain hydrogel. In the second week of May, slow-release Ocmocote fertilizer (5 g per pot with a diameter of 9–12 cm) was added to the sterile substrate as a source of nutrients (nitrogen (N)—16%, phosphorus—7%, potassium—18% and macroelements). The granules dissolve under optimal growth conditions, and a single application was sufficient for the entire growing season. A special membrane containing nutrients prevents direct contact between the roots and the fertilizer components, thus preventing possible damage caused by direct exposure [30].

The plants were watered at 7 o'clock in the morning, according to the established schedule, shown in Table 1 (24–96 h intervals). In case of rainfall, the irrigation cycle was reset and then resumed following the same schedule. The amount of water applied per irrigation event was sufficient to fully saturate the substrate. The exact volume varied depending on the treatment, with irrigation stopped once the substrate was completely moistened. Although exact water consumption per event was not measured, evapotranspiration was monitored to estimate water loss during the experiment. In dry conditions, peat retains significantly more water than perlite, with over 100% greater mass, particularly in smaller pots. However, it has a slower rate of evapotranspiration (20 g over 24 h in both pot sizes—9 cm and 12 cm substrate depth). The mixture of peat and perlite in a deeper substrate depth (12 cm) retains 51.6% higher water compared with peat. Also, has a higher evapotranspiration (50 g—smaller substrate depth and 90 g—deeper substrate depth, over 24 h), the reason might be the combination of peat's water retention capacity with perlite's drainage, making it a more efficient substrate for plant growth.

Table 1. Frequency of irrigation during the experimental period.

	Substrate Treatment	Frequency of Irrigation
T1	Perlite without hydrogel	24 h
T2	Perlite with hydrogel	48 h
T3	Peat and a mixture of perlite and peat without hydrogel	72 h
T4	Peat and a mixture of perlite and peat with hydrogel	96 h

2.2. Visual Assessment and Mortality Rate Calculation

Plant adaptability was assessed during and at the end of the drought period based on five parameters. Survival rate refers to the percentage of surviving plants out of the total number per treatment. With nine plants per treatment, a survival rate above 50% was defined as at least 5 surviving plants. The assessment of visual moderate and good vitality and decorativeness value of plants followed Anastasijević et al. [31]. Each plant was graded on a scale from 1 to 5: 5—excellent, no damage or stress, 4—very good, minor or non-significant damage, 3—good, grows under conditions but not optimally, 2—bad, clear signs of poor adaptation, and 1—very bad, unsustainable, withering. Average values were analyzed. Mortality (complete withering) was recorded as the absence of survival or regrowth. Regenerative potential means 100% recovery after stress. Data were used to evaluate the combined effects of substrate type, hydrogel supplementation, biostimulants' application and container depth on drought resilience in *P.* × *hybrida*. Figure 3 shows the experimental plot and variable decorativeness of plants under different treatments.

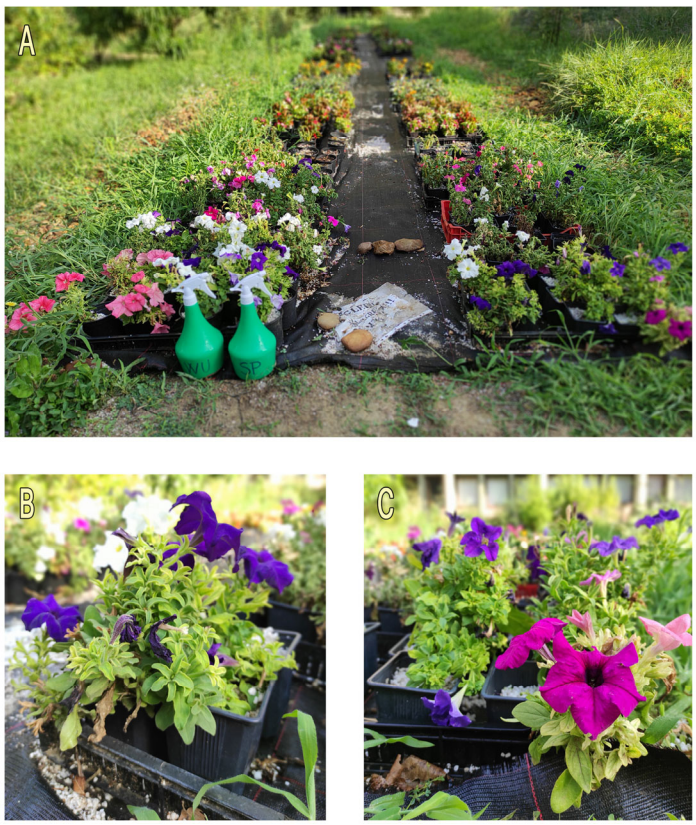


Figure 3. (**A**): experimental plot; (**B**,**C**): decorativeness of plants under different treatments.

2.3. Morphological Parameters

Measurement of the morphological parameters was conducted on the surviving plants in all treatments, after which the mean value of the field was calculated. Plant height was measured with a ruler from the base of the stem (at ground level) to the highest point of the plant. Plant width was measured in two perpendicular directions (width and depth), and then their mean values were calculated. The first dimension (width) was measured as the maximum horizontal spread from the widest tips of the leaves, and then the same method was applied for the other side of the plant (depth). Prior to root volume measurement, mature plants were removed from containers and root balls were rinsed under running water to eliminate all growth medium. The cleaned root systems were blotted dry. The balance was tared, and the plant tissue designated for measurement was placed in water in a laboratory plastic cup. For root system volume determination, the plant was submerged until the water surface reached 2 mm above the uppermost lateral root, which served as a reference point for repeated measurements and minimized experimental error. After immersion, the new balance reading was recorded as the estimated volume of the plant part [32].

2.4. Anatomical Traits

At the end of the experimental period (15 September 2021), all the plants were harvested and separated into plant organs—roots and stems. Prior to anatomical analyses, the stems (plant organ of interest in this study) were stored in glass tubes containing: 36 mL ethanol, 10 mL glycerin and 54 mL distilled water for tissue preservation. The laboratory analyses were conducted at the University of Novi Sad, Faculty of Agriculture, the Department for Fruit Science, Viticulture, Horticulture and Landscape Architecture.

Subsequently, measurements of stem anatomical characteristics on the cross-sections were made using a Motic Digital BA310 microscope with a built-in digital camera, and

software Motic Images Plus 2.0 (Motic China Group Co., Ltd., Xiamen, China). Images of stem macro and micro cross-sections were taken at 40× and 400× magnifications.

Cross-section and secondary wood measurements were performed on each sample, whereas studied traits were determined on four radial segments, 90° apart, following the methodology explained by Narandžić et al. [33]. Following anatomical characteristics were measured on the cross-section: stem diameter (mm), cross-section area (mm^2), percentage of pith area per stem cross-section (%), percentage of xylem per stem cross-section (%), xylem area (mm^2), percentage of phloem per stem cross-section (%), xylem-phloem ratio, inner vessel lumen area (mm^2), outer vessel lumen area (mm^2), inner vessel frequency (mm^2), outer vessel frequency (mm^2), average of inner and outer vessel frequency (mm^2), percentage of total vessel area per stem cross-section (%), percentage of total vessel area relative to secondary wood area (%), percentage of total ray area relative to secondary wood area (%), percentage of total ray area per stem cross-section (%), percentage of xylem area relative to secondary wood area (%), and percentage of xylem area per stem cross-section (%). Since the presence of calcium oxalate crystals was noticeable, their determination included the measurement of the crystal area (CaOx, expressed in μm over the entire cross-sectional area by multiplying the number and area of each specific crystal).

2.5. Statistical Analysis

The results were processed with the program STATISTICA 13 (TIBCO Software Inc., 2017, Palo Alto, CA, USA). The factorial analysis of variance (ANOVA) was used to test the significant differences among different treatments (pot size, hydrogel, substrate, and biostimulant), followed by Tukey's honest significant difference (HSD) test for the comparison of means. Correlation analysis was performed using Pearson's correlation coefficients, with statistically significant values determined at $p < 0.05$.

3. Results

3.1. Visual Assessment and Mortality Rate

The adaptability of *P. × hybrida* under varying water stress conditions was evaluated through a comprehensive assessment of survival rates, vitality indices, decorative quality, and regenerative potential during and after a no-irrigation period over a four-month period post-planting. Treatments varied according to substrate depth (D1, D2), hydrogel presence (H0—absent, H1—present), substrate composition (S1, S2, S3), and additional treatments with biostimulators (SP, WU, C). The data indicate high survival percentages (>50%) across most treatments, reflecting substantial resilience of *P. × hybrida* to water-limited conditions. High survival was generally maintained regardless of hydrogel supplementation, though differences in vitality and decorative attributes were evident (Table 2). Importantly, the absence of marks in the table—referring to mortality or complete plant withering—was predominantly observed in treatments with shallower substrate depths (D1) combined with peat-based substrate (S2) and in some cases without hydrogel amendment (H0). The highest mortality was observed in plants cultivated in peat-based substrate—7 out of 12 combinations, while in perlite, no mortality was observed, and in the perlite-peat mixture, it occurred in 2 out of 12 combinations. This pattern suggests that limited root zone volume and inadequate moisture buffering critically compromise plant survival under drought stress.

Table 2. Adaptability assessment of *Petunia* × *hybrida* to water stress based on the qualitative and quantitative characteristics for a period of four months after planting.

Treatments	Survival Rate > 50%	Moderate Vitality	Good Vitality	High Decorativeness	100% Regenerative Potential
D1_H0_S1_SP	☑	☑	☑	☑	☑
D1_H0_S1_WU	☑	☑	☑	☑	☑
D1_H0_S1_C	☑	☑	☑	☑	☑
D1_H0_S2_SP		☑			
D1_H0_S2_WU	☑		☑		☑
D1_H0_S2_C		☑			
D1_H0_S3_SP	☑		☑	☑	☑
D1_H0_S3_WU	☑	☑		☑	☑
D1_H0_S3_C	☑		☑	☑	☑
D1_H1_S1_SP	☑		☑	☑	☑
D1_H1_S1_WU	☑		☑	☑	☑
D1_H1_S1_C	☑	☑	☑	☑	☑
D1_H1_S2_SP		☑			
D1_H1_S2_WU	☑		☑		☑
D1_H1_S2_C		☑			
D1_H1_S3_SP		☑			
D1_H1_S3_WU		☑			☑
D1_H1_S3_C	☑	☑			☑
D2_H0_S1_SP	☑	☑	☑	☑	☑
D2_ H0_S1_WU	☑	☑		☑	☑
D2_ H0_S1_C	☑	☑	☑	☑	☑
D2_ H0_S2_SP	☑		☑		☑
D2_ H0_S2_WU	☑		☑	☑	☑
D2_ H0_S2_C	☑	☑		☑	☑
D2_ H0_S3_SP	☑		☑	☑	☑
D2_ H0_S3_WU	☑		☑		☑
D2_ H0_S3_C	☑	☑	☑	☑	☑
D2_H1_S1_SP	☑	☑		☑	☑
D2_ H1_S1_WU	☑	☑		☑	☑
D2_ H1_S1_C	☑		☑	☑	☑
D2_ H1_S2_SP		☑			☑
D2_ H1_S2_WU		☑			
D2_ H1_S2_C		☑			
D2_ H1_S3_SP	☑	☑	☑	☑	☑
D2_ H1_S3_WU	☑	☑		☑	☑
D2_ H1_S3_C	☑	☑		☑	☑

Treatments abbreviations: less and greater depth (D1, D2), without hydrogel and with hydrogel amendment (H0, H1), substrates: perlite (S1), peat (S2), perlite and peat (S3), biostimulants: Stim pure (SP), Wake UP (WU) and without application of biostimulants (C).

Conversely, treatments featuring deeper substrate (D2) and hydrogel incorporation (H1), excluding the peat substrate, demonstrated markedly reduced mortality, higher vitality scores, and improved regenerative potential. These results emphasize the protective role of increased substrate depth and water-retentive amendments in sustaining plant health during prolonged dry periods. Good vitality and high decorative quality were most often associated with perlite or perlite–peat substrate mixtures (S1 and S3), further supporting their suitability for urban planting schemes requiring drought resilience. A combination of peat and hydrogel amendment (H1_S2) caused the highest mortality, noted a few weeks after the trial setup, indicating overheating of a saturated substrate, presumably leading to root zone anoxia and potential thermal damage to the root system ('cooking').

3.2. Vegetative Growth

Figure 4 shows the effects of the four treatment groups on plant morphological parameters, specifically plant height, plant width and root volume. Plants grown in deeper pots (D2; 10 cm) showed greater height (17.89 cm) and root volume (38.51 cm^3) than those grown in shallower substrates (D1; 7 cm). The application of hydrogel significantly increased root volume (49.39 cm^3), although plant width was smaller compared to the treatment without

hydrogel, which showed the opposite trend. The perlite substrate resulted in the highest root volume (34.22 cm^3), while the peat substrate produced the highest plant height and width. The mixture of perlite and peat supported optimal overall plant development. The application of Stim pure biostimulator resulted in the greatest root system volume and plant height compared to Wake UP and the control group (no biostimulator was applied). However, plant width did not differ significantly between these treatments.

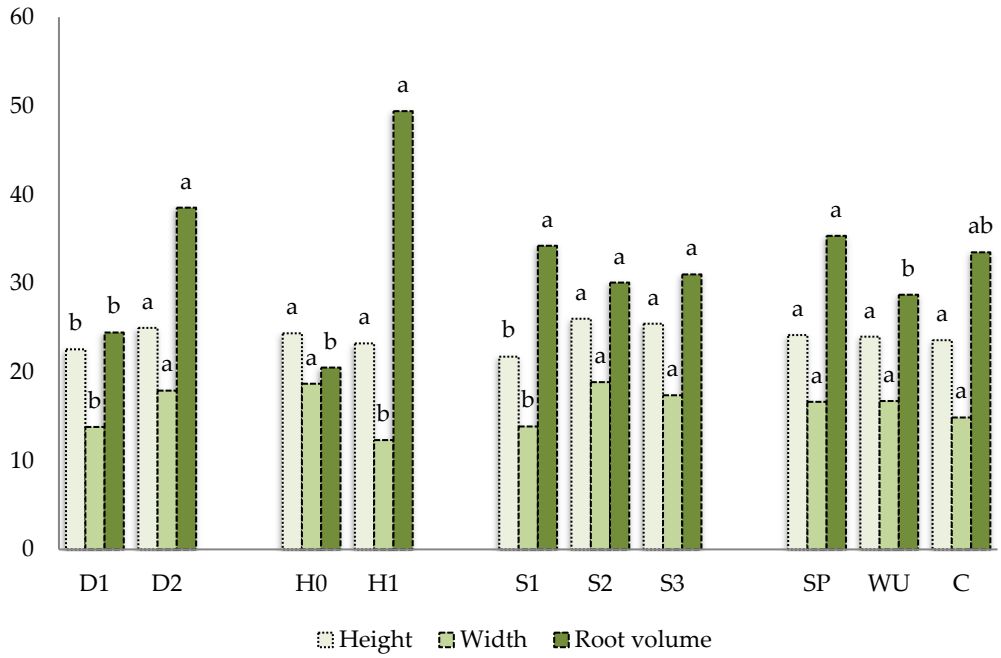


Figure 4. The effects of the four treatment groups on plant morphological parameters (plant height, plant width, and root volume). Grouped by treatments: less and greater depth (D1, D2), without hydrogel and with hydrogel amendment (H0, H1), substrates: perlite (S1), peat (S2), perlite and peat (S3), biostimulants: Stim pure (SP), Wake UP (WU) and without application of biostimulants (C). Differences between means marked with the same letter within the treatment group are not statistically significant, according to the Tukey HSD homogeneity test ($p \leq 0.05$).

3.3. Macro Anatomical Characterization

The anatomical assessment of stem traits revealed that increased substrate depth (D2, 10 cm) significantly enhanced most of the measured parameters (Figure 5). Specifically, plants grown in D2 exhibited a mean stem diameter of 3.85 mm, statistically significantly greater than D1 (3.54 mm), and achieved the highest mean cross-sectional stem area (11.89 mm^2) compared to 10.04 mm^2 in D1. Similarly, xylem area was elevated in D2 (3.93 mm^2) compared to D1 (3.51 mm^2), indicating a positive correlation between rooting depth and overall stem tissue development. Hydrogel supplementation (H1) promoted an increase in key stem traits. Plants in H1 treatments showed a mean stem diameter of 3.81 mm, higher than in H0 (3.64 mm), with a corresponding increase in cross-sectional area (11.71 mm^2 vs. 10.62 mm^2) and phloem area (4.05 mm^2 vs. 3.64 mm^2).

These increases were statistically significant. Interestingly, although the xylem-to-phloem ratio was significantly higher in H0 (1.08) than in H1 (0.96), the xylem area itself remained similar between the two treatments (~3.7 mm^2), suggesting that hydrogel primarily enhances phloem expansion rather than reducing xylem development. Substrate composition markedly affected stem anatomy, as shown in Figure 6.

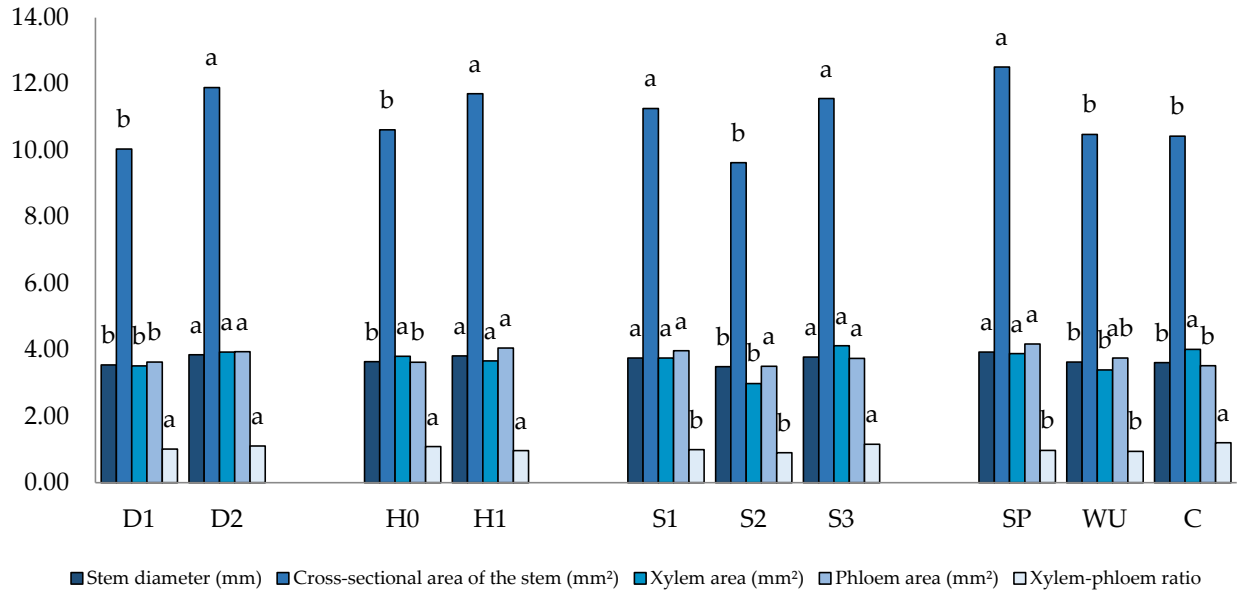


Figure 5. Anatomical characteristics of the cross-section of the stem, grouped by treatments: less and greater depth (D1, D2), without hydrogel and with hydrogel amendment (H0, H1), substrates: perlite (S1), peat (S2), perlite and peat (S3), biostimulants: Stim pure (SP), Wake UP (WU) and without application of biostimulants (C). Differences between means marked with the same letter within the treatment group are not statistically significant, according to the Tukey HSD homogeneity test ($p \leq 0.05$).

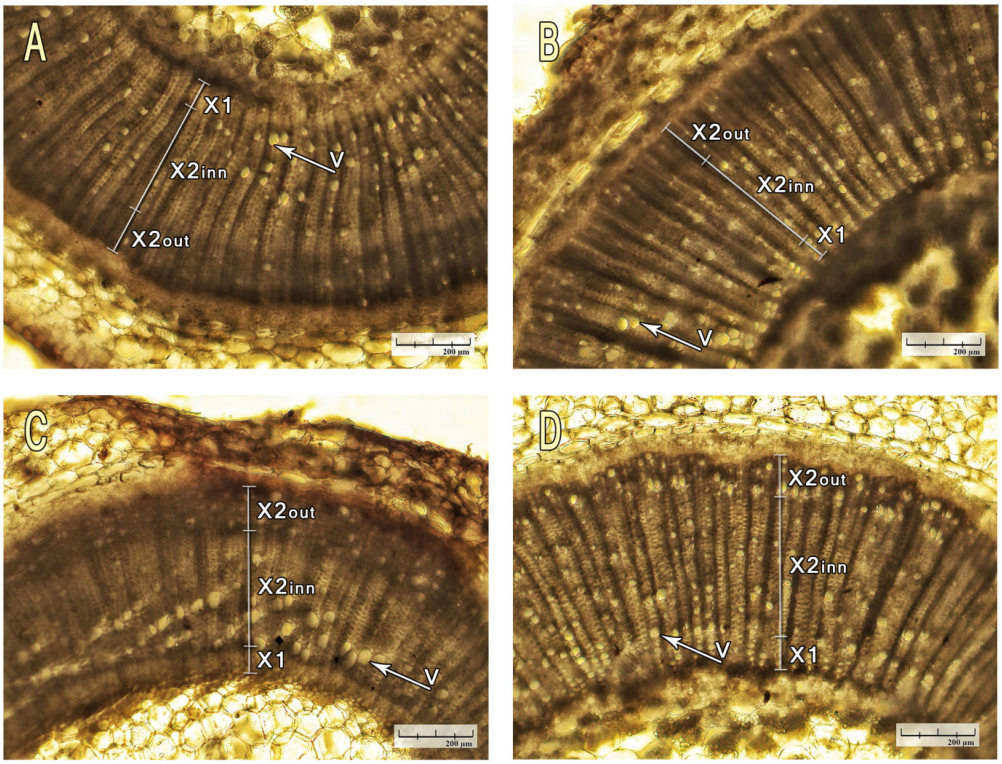


Figure 6. Stem cross-section, appearance of xylem (primary xylem (X1), secondary xylem, inner zone (X2inn), secondary xylem, outer zone (X2out), vessels (V)) in plants grown in: (**A**): mixture of peat and perlite, with hydrogel amendment and application of Stim pure biostimulant, 10 cm substrate depth; (**B**): mixture of peat and perlite, without hydrogel, subjected to the Stim pure biostimulant, 7 cm substrate depth; (**C**): control plants, cultivated in perlite substrate, with hydrogel, 7 cm substrate depth; (**D**): control plants, cultivated in perlite substrate, with hydrogel, 10 cm substrate depth.

Plants cultivated in perlite (S1) and perlite–peat mixture (S3) showed consistently higher values for four out of five parameters. For example, S3 yielded the highest mean stem cross-sectional area (11.56 mm^2), stem diameter (3.78 mm), and xylem-to-phloem ratio (1.15). In contrast, peat-only substrate (S2) resulted in reduced values of stem diameter (3.49 mm), cross-sectional area (9.63 mm^2), xylem area (2.98 mm^2), and a lower xylem-to-phloem ratio (0.9), although the differences in xylem area were not statistically significant. These findings suggest that substrates with better aeration and drainage promote vascular tissue development more effectively than peat alone.

Among biostimulant treatments, Stim Pure (SP) induced the greatest anatomical expansion, producing the highest stem cross-sectional area (12.51 mm^2) and elevated values for stem diameter (3.93 mm) and phloem area (4.17 mm^2). However, xylem area (3.88 mm^2) and the xylem-to-phloem ratio (0.97) were lower than those observed in the control treatment (C), where xylem area reached 4.01 mm^2 and the ratio peaked at 1.2. This suggests a potential 'trade-off'—while SP enhances overall stem growth and phloem development, control conditions may favor structural allocation toward xylem tissue, which could be critical for water transport and mechanical strength.

Cultivation in deeper substrates consisting of a perlite–peat mixture, amended with hydrogel and the biostimulant Stim Pure, exhibited the highest pith percentage (44%), as shown in Figure 7. In contrast, the lowest pith values were observed in shallow substrate treatments such as D1_H0_S3_SP (17.9%), D1_H0_S1_C (18.9%), and D1_H1_S1_WU (18.7%). These results indicate a strong influence of substrate depth and biostimulant type on central parenchyma development. Xylem percentage followed a positive trend with increasing substrate depth, particularly in control treatments using the perlite–peat mixture, reaching up to 46%. The lowest xylem development (22%) occurred in D2_H0_S2_WU. Other treatments generally ranged between 26% and 42%, suggesting that deeper substrates support more extensive secondary tissue formation.

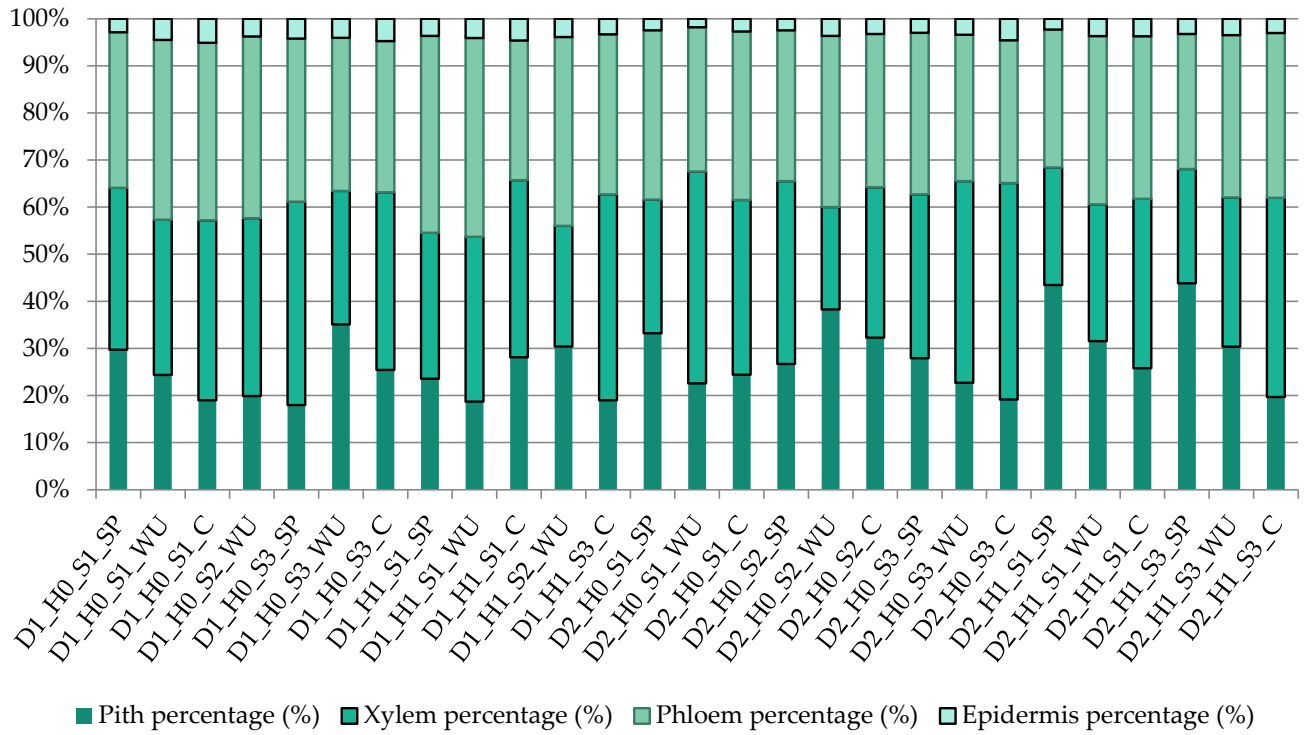


Figure 7. Percentage representation of pith, xylem, phloem, and epidermis on a cross-section of the stem. Treatments abbreviations: less and greater depth (D1, D2), without hydrogel and with hydrogel amendment (H0, H1), substrates: perlite (S1), peat (S2), perlite and peat (S3), biostimulants: Stim pure (SP), Wake UP (WU) and without application of biostimulants (C).

Phloem tissue appeared to be most responsive to hydrogel supplementation in shallow perlite-based substrates, especially when combined with Wake UP (WU) or Stim Pure (SP), reaching values of 42%. In contrast, control treatments in shallow perlite-hydrogel substrates and Stim Pure applications in deeper substrates resulted in reduced phloem percentages (approximately 30%), indicating potential interactions between substrate composition, moisture retention capacity, and biostimulant activity. Epidermal tissue percentage remained relatively stable across treatments, with values ranging from 2.0% to 5.1%. The highest epidermis percentage was observed in D1_H0_S1_C (5.1%), and the lowest in D2_H0_S1_WU (2.0%). These minimal fluctuations suggest that epidermal (protective tissue) development is less sensitive to substrate configuration and biostimulant treatment than internal tissues.

3.4. Micro Anatomical Characterization

The anatomical evaluation of vessel traits revealed a distinct trade-off between vessel size and vessel density across treatments (Figure 8). In particular, increasing substrate depth from D1 to D2 led to a significant shift in vessel architecture. Plants grown in D2 (10 cm) exhibited a higher number of vessels per mm^2 cross-section (229 vessels/mm^2), while simultaneously forming significantly smaller average vessel areas (337 μm^2) compared to D1, where vessel area reached 372 μm^2 and vessel density was lower (197 vessels/mm^2). This pattern suggests an adaptive adjustment in vessel formation, favoring density over size to potentially enhance hydraulic redundancy and reduce cavitation risk. Hydrogel supplementation (H1) promoted the formation of larger xylem vessels than the untreated group (H0), with significantly higher average vessel area (H1: 381 μm^2 vs. H0: 332 μm^2), with no differences in vessel density (H1: 211 vs. H0: 217).

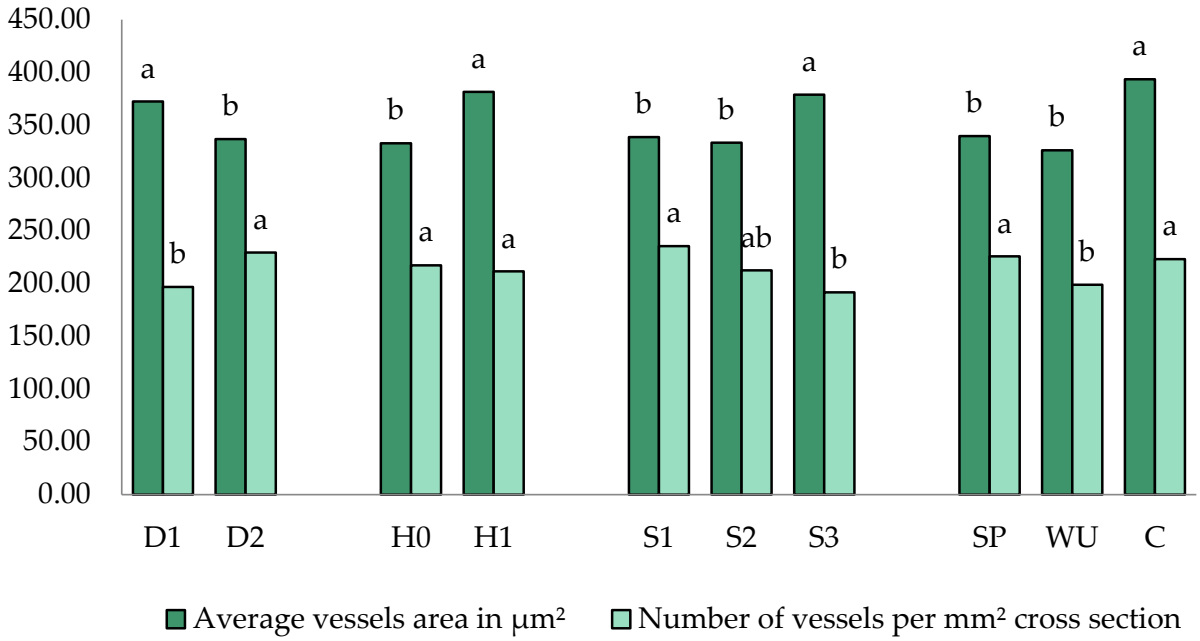


Figure 8. Average vessel area in μm^2 and number of vessels per mm^2 cross section. Grouped by treatments: less and greater depth (D1, D2), without hydrogel and with hydrogel amendment (H0, H1), substrates: perlite (S1), peat (S2), perlite and peat (S3), biostimulants: Stim pure (SP), Wake UP (WU) and without application of biostimulants (C). Differences between means marked with the same letter within the treatment group are not statistically significant, according to the Tukey HSD homogeneity test ($p \leq 0.05$).

Substrate composition also exerted a substantial influence on vessel traits. The perlite–peat mixture (S3) induced the highest average vessel area among the S-treatments (379 μm^2),

while also showing the lowest vessel density (192 vessels/mm^2). This supports the notion that well-aerated substrates promote the development of wider xylem conduits, possibly due to reduced hypoxic stress. Conversely, peat-only substrate (S2) resulted in a moderate vessel area (333 μm^2) and intermediate vessel density (213 vessels/mm^2), indicating a balanced but less optimized vessel anatomy. Interestingly, vessel density in S2 did not differ significantly from other treatments, suggesting less anatomical specialization. Biostimulant application had divergent effects depending on formulation. Stim Pure (SP) enhanced vessel density (225 vessels/mm^2) while producing moderate vessel sizes (339 μm^2), indicating a possible anatomical strategy to maximize water transport capacity through redundancy rather than conduit width. In contrast, WU slightly favored vessel enlargement (326 μm^2) at the expense of density (199 vessels/mm^2), resembling the pattern observed in H1. Notably, the control treatment (C) exhibited significantly large vessel areas (392 μm^2) with relatively low vessel density (222 vessels/mm^2), aligning with a strategy of maximizing flow efficiency—conduction of all available water at its disposal. These findings collectively suggest that anatomical plasticity in vessel formation responds sensitively to substrate depth, composition, and biostimulant application.

The analysis of vessel surface area across the entire xylem revealed pronounced variation among treatments (Table 3), with mean values ranging from 0.09 mm^2 (D2_H0_S2_WU) to 0.49 mm^2 (D2_H0_S3_SP). The highest vessel area was observed in D2_H0_S3_SP (0.49 ± 0.11 mm^2), followed closely by D2_H1_S3_SP (0.41 ± 0.09 mm^2) and D1_H1_S3_C (0.40 ± 0.07 mm^2). These results indicate that substrates with perlite or perlite–peat mixtures (especially S3) in combination with D2 depth and hydrogel treatments (H1) are associated with enhanced vessel development. Conversely, the lowest vessel area values were consistently found in treatments combining S2 substrate with WU biostimulant, such as D2_H0_S2_WU (0.09 ± 0.03 mm^2) and D2_H1_S1_C (0.14 ± 0.05 mm^2), suggesting a restrictive effect of peat-dominant substrates on vessel expansion. Ray area followed a similar pattern, with the highest values recorded in perlite-based substrates (S1, S3) combined with hydrogel and biostimulant treatments. The maximum ray area was observed in D2_H1_S3_WU (0.99 ± 0.08 mm^2), D1_H0_S3_C (0.95 ± 0.13 mm^2), and D2_H0_S1_C (0.75 ± 0.41 mm^2). These findings underscore the positive influence of aerated substrates on parenchymatous tissue development within the xylem. Notably, treatments with WU frequently promoted root development, especially when combined with deeper rooting conditions and perlite-containing media. Xylem porosity values varied from 4.50% (D2_H1_S3_WU) to 13.23% (D2_H0_S1_C), reflecting differences in both vessel frequency and size. Increased porosity was generally associated with control (C) treatments, implying a more conductive vascular architecture, as stated above. The highest porosity (13.23%) observed in D2_H0_S1_C suggests the intrinsic petunia ability to cope with altered water availability.

In our research, the surface area of calcium oxalate (CaOx) crystals showed substantial variation across treatments, ranging from only 19.35 μm^2 (D2_H0_S1_WU) to 125.69 μm^2 (D2_H0_S1_C). Crystals were more abundant in control (C) treatments and in combinations with S1 or S3 substrates, possibly reflecting a role in regulating ion balance or mechanical protection under stressful growth conditions (Figure 9). Treatments with biostimulants, particularly SP and WU, also showed elevated CaOx levels, though with higher variability (CV up to 70.9%).

Table 3. Vessel area on the whole xylem (mm^2), ray area on the whole xylem (mm^2), xylem porosity on the stem cross-section (%) and surface area of calcium oxalate (CaOx) crystals in the field of vision (μm^2).

Treatments	Vessel Area on the Whole Xylem (mm^2)	Ray Area on the Whole Xylem (mm^2)	Xylem Porosity on the Stem Cross-Section (%)	Surface Area of Calcium Oxalate (CaOx) Crystals in the Field of Vision (μm^2)
D1_H0_S1_SP	$0.26 \pm 0.09^{b–h}$	$0.56 \pm 0.20^{a–f}$	$7.10 \pm 0.70^{b–f}$	$65.12 \pm 55.73^{a–c}$
D1_H0_S1_WU	$0.19 \pm 0.02^{e–h}$	$0.45 \pm 0.03^{c–f}$	$6.04 \pm 0.35^{c–f}$	$52.26 \pm 2.22^{a–c}$
D1_H0_S1_C	$0.24 \pm 0.05^{b–h}$	$0.53 \pm 0.02^{a–f}$	$6.58 \pm 1.39^{c–f}$	$41.07 \pm 9.28^{a–c}$
D1_H0_S2_SP	/	/	/	/
D1_H0_S2_WU	$0.30 \pm 0.13^{a–g}$	$0.47 \pm 0.08^{b–f}$	$8.17 \pm 2.29^{b–f}$	$45.60 \pm 27.29^{a–c}$
D1_H0_S2_C	/	/	/	/
D1_H0_S3_SP	$0.19 \pm 0.02^{d–h}$	$0.38 \pm 0.06^{c–f}$	$6.69 \pm 0.28^{c–f}$	117.49 ± 33.41^{ab}
D1_H0_S3_WU	$0.16 \pm 0.14^{f–h}$	$0.37 \pm 0.10^{c–f}$	4.98 ± 2.66^{f}	31.24 ± 13.55^{bc}
D1_H0_S3_C	$0.37 \pm 0.03^{a–e}$	0.95 ± 0.13^{ab}	$5.85 \pm 0.12^{d–f}$	$47.22 \pm 3.86^{a–c}$
$\overline{x}$	0.24	0.53	6.49	57.14
D1_H1_S1_SP	$0.30 \pm 0.11^{a–g}$	$0.70 \pm 0.34^{a–e}$	$7.35 \pm 0.60^{b–f}$	$45.35 \pm 5.21^{a–c}$
D1_H1_S1_WU	$0.19 \pm 0.04^{e–h}$	$0.34 \pm 0.07^{c–f}$	$7.13 \pm 1.36^{b–f}$	$70.68 \pm 25.75^{a–c}$
D1_H1_S1_C	$0.32 \pm 0.03^{a–g}$	$0.29 \pm 0.01^{c–f}$	11.54 ± 1.94^{ab}	31.03 ± 0.96^{bc}
D1_H1_S2_SP	/	/	/	/
D1_H1_S2_WU	$0.22 \pm 0.03^{c–h}$	$0.29 \pm 0.08^{c–f}$	$8.25 \pm 0.64^{b–f}$	$40.54 \pm 3.69^{a–c}$
D1_H1_S2_C	/	/	/	/
D1_H1_S3_SP	/	/	/	/
D1_H1_S3_WU	/	/	/	/
D1_H1_S3_C	$0.40 \pm 0.07^{a–d}$	$0.51 \pm 0.09^{a–f}$	$10.36 \pm 1.86^{a–d}$	30.19 ± 13.42^{bc}
$\overline{x}$	0.29	0.43	8.93	43.56
D2_H0_S1_SP	0.13 ± 0.05^{gh}	0.22 ± 0.09^{ef}	$7.84 \pm 2.32^{b–f}$	$44.41 \pm 4.13^{a–c}$
D2_ H0_S1_WU	$0.26 \pm 0.03^{b–h}$	$0.58 \pm 0.07^{a–f}$	$6.14 \pm 0.76^{c–f}$	19.35 ± 1.17^{c}
D2_ H0_S1_C	$0.36 \pm 0.09^{a–f}$	$0.75 \pm 0.41^{a–c}$	13.23 ± 1.62^{a}	125.69 ± 29.09^{a}
D2_ H0_S2_SP	$0.20 \pm 0.12^{d–h}$	$0.49 \pm 0.29^{b–f}$	5.60 ± 0.36^{ef}	31.87 ± 11.09^{bc}
D2_ H0_S2_WU	0.09 ± 0.03^{h}	0.13 ± 0.04^{f}	$7.09 \pm 1.59^{b–f}$	31.20 ± 7.34^{bc}
D2_ H0_S2_C	$0.18 \pm 0.03^{e–h}$	$0.27 \pm 0.03^{c–f}$	$6.40 \pm 0.75^{c–f}$	27.50 ± 6.34^{c}
D2_ H0_S3_SP	0.49 ± 0.11^{a}	$0.74 \pm 0.22^{a–d}$	$9.92 \pm 2.26^{a–e}$	$73.40 \pm 55.59^{a–c}$
D2_ H0_S3_WU	$0.20 \pm 0.02^{d–h}$	$0.47 \pm 0.18^{b–f}$	5.16 ± 0.48^{f}	$91.96 \pm 22.34^{a–c}$
D2_ H0_S3_C	0.43 ± 0.03^{ab}	$0.50 \pm 0.16^{a–f}$	$10.45 \pm 0.63^{a–c}$	$79.60 \pm 43.68^{a–c}$
$\overline{x}$	0.26	0.46	7.98	58.33
D2_H1_S1_SP	$0.17 \pm 0.02^{e–h}$	$0.27 \pm 0.03^{c–f}$	$8.77 \pm 1.23^{a–f}$	$81.45 \pm 39.04^{a–c}$
D2_ H1_S1_WU	$0.18 \pm 0.02^{e–h}$	$0.38 \pm 0.06^{c–f}$	$8.22 \pm 0.64^{b–f}$	$63.04 \pm 27.25^{a–c}$
D2_ H1_S1_C	0.14 ± 0.05^{gh}	$0.26 \pm 0.06^{d–f}$	$7.21 \pm 2.02^{b–f}$	$73.75 \pm 50.78^{a–c}$
D2_ H1_S2_SP	/	/	/	/
D2_ H1_S2_WU	/	/	/	/
D2_ H1_S2_C	/	/	/	/
D2_ H1_S3_SP	$0.41 \pm 0.09^{a–c}$	$0.65 \pm 0.15^{a–e}$	$8.98 \pm 1.82^{a–f}$	$55.23 \pm 16.50^{a–c}$
D2_ H1_S3_WU	$0.18 \pm 0.02^{e–h}$	0.99 ± 0.08^{a}	4.50 ± 0.00^{f}	$39.45 \pm 10.17^{a–c}$
D2_ H1_S3_C	$0.25 \pm 0.05^{b–h}$	$0.33 \pm 0.08^{c–f}$	$7.36 \pm 1.99^{b–f}$	$56.98 \pm 48.85^{a–c}$
$\overline{x}$	0.22	0.48	7.50	61.65

Legend: $\overline{x}$—mean value; /—plants were not subjected to anatomical analyses due to mortality; $\pm$ standard deviation. Treatments abbreviations: less and greater depth (D1, D2), without hydrogel and with hydrogel amendment (H0, H1), substrates: perlite (S1), peat (S2), perlite and peat (S3), biostimulants: Stim pure (SP), Wake UP (WU) and without application of biostimulants (C). Differences between means marked with the same letter in the same column are not statistically significant, according to the Tukey HSD homogeneity test ($p \leq 0.05$).

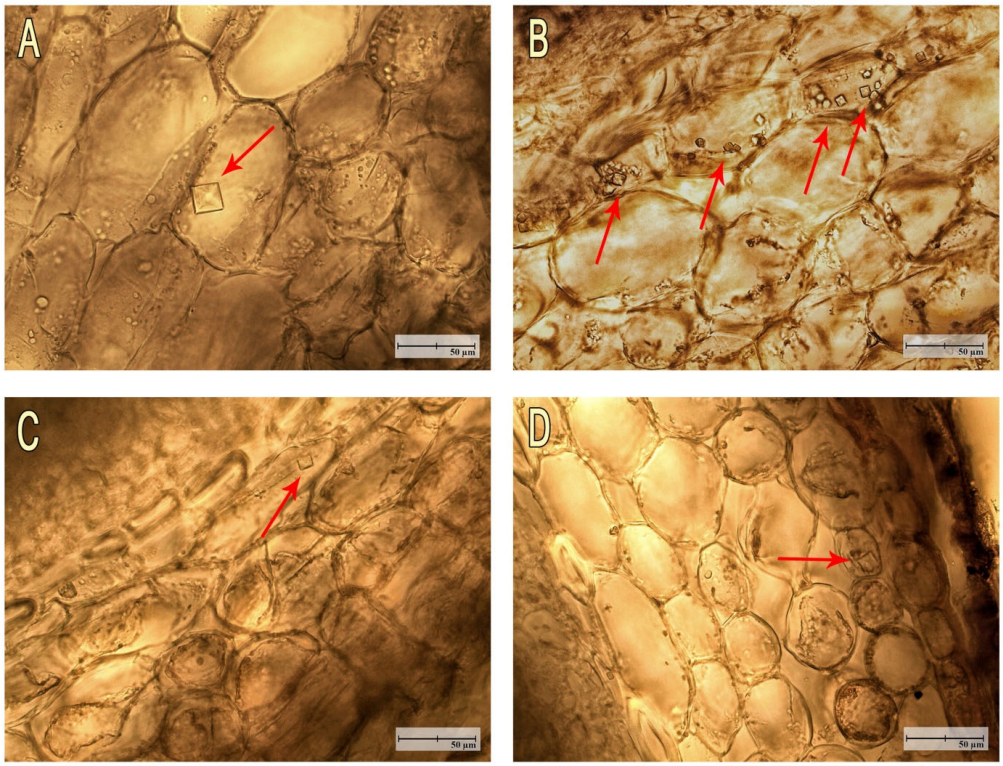


Figure 9. Calcium oxalate crystals in the phloem tissue of the stem of *Petunia* × *hybrida* ((**A**,**B**): plants cultivated in the mixture of peat and perlite, without hydrogel amendment, treated by Stim Pure biostimulant, 7 cm pots; (**C**,**D**): control plants cultivated in the mixture of peat and perlite, with hydrogel amendment, 7 and 10 cm pots).

The anatomical distribution of tissues within the total stem cross-section further reinforced the trends observed in the xylem-specific measurements (Table 4). The percentage of total vessel area across the stem cross-section varied considerably among treatments, ranging from 0.76% (D2_H0_S2_WU) to 4.80% (D2_H0_S3_C). The highest values were generally associated with control treatments (C) and with perlite-based substrates (S1, S3), particularly under shallow depth (D1) and with hydrogel addition (H1). For instance, D1_H1_S3_C achieved 4.52%, while D1_H1_S1_C reached 4.22%, mirroring their high xylem porosity values reported previously. These findings point to anatomical consistency across organizational levels, with well-aerated conditions and standard hydration regimes favoring greater investment in vessel tissue. Ray area, both within the xylem and across the whole cross-section, exhibited even greater plasticity. The percentage of total ray area on the stem cross-section peaked at 7.70% in D2_H1_S3_WU, a treatment that also demonstrated the highest absolute ray area in previous analyses. These results suggest that the combination of deep substrate, hydrogel supplementation, and a perlite–peat substrate mixture strongly promotes ray cell proliferation, potentially enhancing radial transport, carbohydrate storage, and mechanical flexibility. On average, the greatest proportional contribution of ray tissues was found in treatments with S3 substrates, regardless of biostimulant, indicating that substrate structure alone exerts a dominant influence on ray formation. Notably, the mean percentage of ray area was highest in plants grown at shallow substrate depth without hydrogel (D1_H0), where it averaged 5.07% on the whole cross-section and 14.07% within the xylem. This suggests that under more restricted water availability (i.e., without hydrogel), plants allocate a greater fraction of vascular tissue to rays, possibly as a compensatory strategy to maintain internal water redistribution and metabolic buffering.

Table 4. Percentage shares of vessel area, ray area, and xylem area in the total stem cross-sectional area and secondary wood area.

Treatments	Percentage of Total Vessel Area on the Stem Cross-Section (%)	Percentage of Total Ray Area on Xylem (%)	Percentage of the Total Ray Area on the Stem Cross-Section (%)	Percentage of Xylem Area on the Stem Cross-Section (%)
D1_H0_S1_SP	$2.43 \pm 0.07^{\text{d–i}}$	$15.07 \pm 0.89^{\text{bc}}$	$5.18 \pm 0.51^{\text{a–d}}$	$26.77 \pm 2.42^{\text{a–h}}$
D1_H0_S1_WU	$1.99 \pm 0.19^{\text{d–i}}$	$14.55 \pm 0.12^{\text{bc}}$	$4.79 \pm 0.15^{\text{a–d}}$	$26.15 \pm 0.97^{\text{a–h}}$
D1_H0_S1_C	$2.51 \pm 0.51^{\text{c–h}}$	$14.70 \pm 0.41^{\text{bc}}$	$5.62 \pm 0.07^{\text{ab}}$	$30.09 \pm 1.05^{\text{a–h}}$
D1_H0_S2_SP	/	/	/	/
D1_H0_S2_WU	$3.16 \pm 1.20^{\text{a–e}}$	$13.31 \pm 2.21^{\text{b–d}}$	$4.96 \pm 0.62^{\text{a–d}}$	$29.63 \pm 4.63^{\text{a–h}}$
D1_H0_S2_C	/	/	/	/
D1_H0_S3_SP	$2.89 \pm 0.17^{\text{b–g}}$	$13.43 \pm 2.33^{\text{b–d}}$	$5.79 \pm 0.96^{\text{ab}}$	$34.50 \pm 1.35^{\text{a–d}}$
D1_H0_S3_WU	$1.41 \pm 0.75^{\text{f–i}}$	$12.73 \pm 2.11^{\text{b–d}}$	$3.62 \pm 0.70^{\text{b–e}}$	$23.31 \pm 0.71^{\text{c–h}}$
D1_H0_S3_C	$2.20 \pm 0.16^{\text{d–i}}$	$14.70 \pm 0.72^{\text{bc}}$	$5.55 \pm 0.65^{\text{ab}}$	$29.96 \pm 2.64^{\text{a–h}}$
$\bar{x}$	2.37	14.07	5.07	28.63
D1_H1_S1_SP	$2.29 \pm 0.39^{\text{d–i}}$	$17.14 \pm 5.18^{\text{ab}}$	$5.32 \pm 1.79^{\text{a–c}}$	$23.37 \pm 3.17^{\text{b–h}}$
D1_H1_S1_WU	$2.49 \pm 0.45^{\text{c–i}}$	$12.66 \pm 2.09^{\text{b–d}}$	$4.42 \pm 0.69^{\text{a–e}}$	$28.06 \pm 1.79^{\text{a–h}}$
D1_H1_S1_C	$4.22 \pm 1.00^{\text{a–c}}$	$10.36 \pm 1.23^{\text{b–d}}$	$3.80 \pm 0.83^{\text{b–e}}$	$29.57 \pm 10.00^{\text{a–h}}$
D1_H1_S2_SP	/	/	/	/
D1_H1_S2_WU	$2.12 \pm 0.22^{\text{d–i}}$	$10.85 \pm 2.41^{\text{b–d}}$	$2.80 \pm 0.78^{\text{b–e}}$	$20.70 \pm 0.44^{\text{f–h}}$
D1_H1_S2_C	/	/	/	/
D1_H1_S3_SP	/	/	/	/
D1_H1_S3_WU	/	/	/	/
D1_H1_S3_C	$4.52 \pm 0.71^{\text{ab}}$	$13.16 \pm 2.24^{\text{b–d}}$	$5.75 \pm 0.96^{\text{ab}}$	$33.45 \pm 2.33^{\text{a–e}}$
$\bar{x}$	3.12	12.84	4.42	27.03
D2_H0_S1_SP	$1.12 \pm 0.37^{\text{hi}}$	$6.46 \pm 2.28^{\text{cd}}$	$1.84 \pm 0.72^{\text{c–e}}$	$25.41 \pm 1.43^{\text{a–h}}$
D2_H0_S1_WU	$2.76 \pm 0.32^{\text{c–h}}$	$13.48 \pm 1.93^{\text{b–d}}$	$6.04 \pm 0.64^{\text{ab}}$	$36.17 \pm 2.32^{\text{a}}$
D2_ H0_S1_C	$2.46 \pm 0.42^{\text{d–i}}$	$13.74 \pm 5.91^{\text{b–d}}$	$5.14 \pm 2.35^{\text{a–d}}$	$29.50 \pm 1.94^{\text{a–h}}$
D2_ H0_S2_SP	$2.19 \pm 0.70^{\text{d–i}}$	$14.67 \pm 3.86^{\text{bc}}$	$5.55 \pm 1.81^{\text{ab}}$	$31.09 \pm 9.52^{\text{a–g}}$
D2_H0_S2_WU	$0.76 \pm 0.12^{\text{i}}$	$5.27 \pm 1.20^{\text{d}}$	$1.13 \pm 0.18^{\text{e}}$	$19.87 \pm 2.03^{\text{gh}}$
D2_ H0_S2_C	$2.06 \pm 0.54^{\text{d–i}}$	$9.86 \pm 0.69^{\text{b–d}}$	$3.15 \pm 0.71^{\text{b–e}}$	$26.72 \pm 5.16^{\text{a–h}}$
D2_ H0_S3_SP	$3.43 \pm 0.99^{\text{a–d}}$	$15.16 \pm 5.42^{\text{bc}}$	$5.18 \pm 1.77^{\text{a–d}}$	$26.17 \pm 6.66^{\text{a–h}}$
D2_H0_S3_WU	$2.22 \pm 0.33^{\text{d–i}}$	$12.19 \pm 4.28^{\text{b–d}}$	$5.30 \pm 2.23^{\text{a–c}}$	$35.33 \pm 1.58^{\text{ab}}$
D2_ H0_S3_C	$4.80 \pm 0.29^{\text{a}}$	$12.14 \pm 4.08^{\text{b–d}}$	$5.58 \pm 1.86^{\text{ab}}$	$35.59 \pm 1.75^{\text{a}}$
$\bar{x}$	2.42	11.44	4.32	29.54
D2_H1_S1_SP	$1.09 \pm 0.16^{\text{hi}}$	$6.89 \pm 1.61^{\text{cd}}$	$1.70 \pm 0.28^{\text{de}}$	$22.16 \pm 1.82^{\text{e–h}}$
D2_H1_S1_WU	$1.19 \pm 0.10^{\text{g–i}}$	$8.59 \pm 0.95^{\text{b–d}}$	$2.49 \pm 0.33^{\text{b–e}}$	$25.38 \pm 3.22^{\text{a–h}}$
D2_ H1_S1_C	$1.33 \pm 0.57^{\text{g–i}}$	$7.07 \pm 0.71^{\text{cd}}$	$2.57 \pm 0.65^{\text{b–e}}$	$32.10 \pm 4.26^{\text{a–f}}$
D2_ H1_S2_SP	/	/	/	/
D2_H1_S2_WU	/	/	/	/
D2_ H1_S2_C	/	/	/	/
D2_ H1_S3_SP	$2.16 \pm 0.40^{\text{d–i}}$	$14.07 \pm 3.19^{\text{b–d}}$	$3.39 \pm 0.71^{\text{b–e}}$	$18.69 \pm 2.34^{\text{h}}$
D2_H1_S3_WU	$1.42 \pm 0.14^{\text{e–i}}$	$24.29 \pm 0.00^{\text{a}}$	$7.70 \pm 0.75^{\text{a}}$	$22.56 \pm 2.20^{\text{d–h}}$
D2_ H1_S3_C	$3.13 \pm 0.94^{\text{a–f}}$	$9.91 \pm 3.17^{\text{b–d}}$	$4.22 \pm 1.48^{\text{a–e}}$	$34.97 \pm 1.24^{\text{a–c}}$
$\bar{x}$	1.72	11.80	3.68	25.98

Legend: $\bar{x}$—mean value; /—plants were not subjected to anatomical analyses due to mortality; ± standard deviation. Treatments abbreviations: less and greater depth (D1, D2), without hydrogel and with hydrogel amendment (H0, H1), substrates: perlite (S1), peat (S2), perlite and peat (S3), biostimulants: Stim pure (SP), Wake UP (WU) and without application of biostimulants (C). Differences between means marked with the same letter in the same column are not statistically significant, according to the Tukey HSD homogeneity test ($p \leq 0.05$).

Finally, the percentage of the total stem cross-section occupied by xylem ranged from 18.69% (D2_H1_S3_SP) to 36.17% (D2_H0_S1_WU). Higher values were frequently found in deeper rooting treatments (D2), especially when hydrogel was not applied (H0), suggesting that deep rooting under moderate water conditions promotes stronger structural investment in xylem tissues. This is consistent with earlier findings showing increased vessel number under D2 conditions (Figure 8), thus establishing a coherent picture of structural reinforcement driven by rooting environment.

3.5. Correlation Analysis

The obtained Pearson correlation coefficients revealed strong and statistically significant relationships ($p < 0.05$) between both root and stem development and anatomical traits, as shown in Table 5.

Table 5. Correlation analysis between vegetative growth and anatomical parameters.

Variable	Stem Diameter	Cross Section	Phloem Area	Xylem Area	Xylem/Phloem Ratio	Average Vessel Area	Number of Vessels	Pith Percentage (%)	Xylem Percentage (%)	Phloem Percentage (%)	Epidermis Percentage (%)
Height	0.90	0.12	−0.05	−0.11	0.66	−0.02	0.38	0.53	−0.10	−0.60	−0.66
Width	0.89	0.13	−0.05	−0.12	0.64	0.02	0.35	0.51	−0.08	−0.61	−0.64
Root volume	0.33	0.81	−0.12	−0.11	0.27	0.42	0.22	−0.24	0.70	−0.85	−0.06

Variable	Vessel area on the whole xylem (mm^2)	Ray area on the whole xylem (mm^2)	Xylem porosity on the stem cross-section (%)	Surface area of calcium oxalate (CaOx)	Percentage of total vessel area on the stem cross-section (%)	Percentage of total ray area on xylem (%)	Percentage of the total ray area on the stem cross-section (%)	Percentage of xylem area on the stem cross-section (%)
Height	0.34	0.43	0.31	0.79	−0.14	0.20	0.02	−0.12
Width	0.37	0.46	0.32	0.80	−0.11	0.23	0.06	−0.10
Root volume	0.83	0.83	0.56	0.90	0.56	0.41	0.62	0.68

Legend: Underlined values represent significant correlations at $p < 0.05$ significance. Correlation analysis was performed using Pearson's correlation coefficient.

Aboveground growth, reflected in plant height and width, was strongly associated with stem diameter ($r > 0.89$) and the xylem/phloem ratio ($r > 0.64$), suggesting that thicker stems with greater investment in xylem tissue support enhanced shoot development. Root volume was strongly correlated with xylem proportion ($r > 0.7$), as well as vessel and ray area ($r > 0.8$), indicating that changes in vascular anatomy may play a key role in supporting more extensive root system development. The strong positive correlation between CaOx area and plant growth traits ($r > 0.8$) suggests that CaOx deposition may be associated with enhanced tissue development.

4. Discussion

Climate change strongly indicates the inevitability of seeking green solutions in terms of sustainability and flexibility, especially within urban areas characterized by rainwater surface runoff, extremely high temperatures and reduced evaporation. According to research by Bombarely et al. [34], petunia is defined as a model plant and represents one of the world's most famous plants. Van Iersel, as well as Henson et al. [35,36], highlight the impressive adaptability of petunias to water scarcity. Therefore, this paper aimed to decipher anatomical adaptations in the conditions of reduced substrate volume, substrate lightness and fortification by hydrogel and biostimulants amendment. To our knowl-

edge, no previous study has investigated the combined influence of substrate depth and type, hydrogel, and biostimulants on both growth performance and anatomical traits of *P.* × *hybrida* exposed to drought in urban settings. These results offer important insights for drought-resilient urban planting, particularly in systems such as green roofs or containerized landscapes.

4.1. Factors Influencing Petunia × hybrida Survival and Ornamental Value in Container-Based Planting Systems

While *P.* × *hybrida* demonstrated notable adaptability to prolonged periods without irrigation, substrate composition and rooting volume proved to be critical factors influencing plant health and ornamental value. The highest mortality was recorded in peat-based substrates (7 out of 12 combinations), likely due to poor drainage, limited aeration, and inadequate moisture regulation, conditions that are especially problematic in shallow substrates. In contrast, perlite-based media showed no plant loss, and only minimal mortality (2 out of 12) was observed in perlite–peat mixtures, underscoring their superior performance under drought stress. The positive effects of increased substrate depth and hydrogel addition were particularly evident in perlite-containing treatments, which maintained high vitality, strong regenerative potential, and good decorative quality. However, the combination of peat and hydrogel proved detrimental, potentially due to overheating and root zone anoxia in saturated media. This is consistent with the research of Wang et al. [37], where the incorporation of hydrogel into the peat substrate improved the heat transfer through the medium. Also, according to Ghobashy [38], the polymers used in hydrogel formulations can swell to the point of clogging the pores in the soil responsible for aeration, which can reduce oxygen availability and, in severe cases, lead to plant and seedling mortality. These authors pointed out that water exhibits higher thermal conductivity than air; therefore, as the moisture content of the soil increases, the capacity of the substrate to conduct heat also increases. For urban applications where irrigation is limited or irregular, selecting well-aerated, water-retentive substrates, such as perlite or perlite–peat blends, and ensuring sufficient rooting depth are key strategies for enhancing plant performance and landscape resilience.

4.2. Deciphering Petunia × hybrida Adaptive Anatomical Responses in Water-Scarce Environment

Overall, the results demonstrate that substrate composition and hydrogel use are critical determinants of vascular and parenchymatous tissue development in the xylem. Treatments combining deeper rooting (D2), hydrogel (H1), and perlite-containing substrates (S1 or S3) consistently promoted larger total vessel and ray areas, while peat-dominant substrates (S2), particularly when combined with WU biostimulant, limited anatomical expansion. These patterns suggest that both physical (aeration, drainage) and chemical (water retention, biostimulant effect) properties interact to shape stem anatomical plasticity. Specifically, the volume of the pot significantly influenced all anatomical characteristics of the stem's cross-section, demonstrating that larger substrate depth (D2; 10 cm) is beneficial for plant growth. Research by Obede da Silva Aragão et al. [39] confirmed this relationship, showing that in an experiment with beans (*Phaseolus vulgaris* L.), plant growth increased as pot volume grew. The height of the pot is crucial for ensuring the availability of water and oxygen. As shown in Figure 2, all parameters were higher for plants grown in 12 cm pots (10 cm depth). A study by de Mello et al. [40] on cucumber plants indicated that a larger substrate volume is essential for better nutrient and water absorption during seedling growth, with stem diameter being a key characteristic for assessing seedling quality. This finding was also supported by earlier research from Haase [41], which recommended analyzing stem diameter as an indicator of plant growth under varying conditions. The results confirmed the expectation that larger substrate volumes promote

greater root development. However, no instances of physical instability, such as uprooting, were observed during the experiment, even in the smaller pots. This suggests that the root systems developed within the given volumes were sufficient to maintain mechanical stability under the tested conditions.

When assessing the effectiveness of various treatment combinations, it is possible to achieve successful growth in both pot depths (D1 and D2), but with the right substrate mix and the application of hydrogel. Adequate substrate selection plays an important role in plant adaptation to external influences [42]. In our paper, it was demonstrated that plants cultivated in the peat were characterized by the lowest values of all examined anatomical features, followed by the high plant mortality observed on this medium. This data can be justified by increasing the temperature of the substrate in darker containers/pots [43], which, in the presence of excessive water (like H1), leads to root overheating ('cooking'). On the contrary, perlite, with its ability to balance substrate aeration and substrate water retention, allowed plants to transport water more efficiently. In greater detail, the highest xylem porosity was recorded for control plants cultivated in a 10 cm depth of perlite substrate, without amendment of hydrogel and for plants cultivated in a 7 cm depth of perlite substrate, but with hydrogel application. Previously, Markoska et al. [44] indicated the positive effect of perlite, which improves drainage and provides the roots with a sufficient amount of oxygen, unlike the case of peat/hydrogel combination, where the root system might have experienced suffocation/root anoxia. Al-Shammari et al., as well as Al-Wazzan and Abdulrahman [45,46], both found that perlite substrate can mitigate the negative effects of water scarcity, enhancing growth and yield in crops like tomato and maize under deficit conditions. In our research, a mixture of perlite and peat also provided petunia with better adaptability to the lack of necessary water, which resulted in larger stem diameter, stem cross-sectional area, larger xylem area, and improved xylem to phloem ratio. Furthermore, perlite and a mixture of perlite and peat had a positive effect on radial transport and thus enabled adequate movement of water and nutrients, better mechanical stability of the tissue, and improved resistance to stress factors over time (Figure 3). Our findings corroborate the conclusions of earlier research on *Aloe vera* (L.) Burm.f. [47], *Rosmarinus officinalis* L. [48], and *Erythropalum scandens* [49], indicating that the optimal blend of perlite and peat maximized growth efficiency. Notably, the control treatment (C) exhibited significantly large vessel areas (392 μm^2) with relatively low vessel density (222 vessels/mm^2), aligning with a strategy of maximizing flow efficiency—conduction of all available water at its disposal, as previously demonstrated for other drought-tolerant semi-ring porous species [33].

By examining the individual effects of the treatment, the hydrogel had a positive effect on increasing stem diameter, stem cross-sectional area, and phloem surface area growth of *P.* × *hybrida* seedlings by retaining more water and enabling better nutrient absorption. Numerous studies have shown that the incorporation of hydrogel into the substrate had a positive impact through increased vegetative growth and flowering in the species: *Calendula officinalis* L. [50], *Verbena canadensis* (L.) Britton [51], *Dendranthema grandiflora* [52], *Trigonella foenum-graecum* L. and *Anethum graveolens* L. [53]. Further, the application of hydrogel in a mixture of perlite and peat helps plants adapt to dry conditions when grown in a shallow substrate, providing optimal conditions for water flow in the stem and thus contributing to the physiological adaptation of plants to water deficit. It is crucial to apply the correct amount of hydrogel, as applying too much can harm the plants. An amount of hydrogel unsuitable for the soil type can result in poor aeration, subsequently causing reduced plant growth and, in some cases, mortality [38]. Additionally, although the chemical toxicity associated with hydrogel is generally considered minimal, prolonged and excessive application may increase the likelihood of acrylamide release into

the environment [54]. Due to the aforementioned scientific findings, it is crucial to adjust the formulation and concentration of the hydrogel according to the application conditions as well as the plant species. Our findings are further supported by studies of Pontes Filho et al. [55] and Ljubojević et al. [17], who demonstrated that a similar dose of hydrogel (2 g L^{-1}) positively affected the growth of *Enterolobium contortisiliquum* (Vell.) Morong seedlings and three different Salvia species were grown in full sun, respectively.

Biostimulant SP demonstrated the highest average value for the cross-sectional area of the stem. Additionally, both biostimulants (SP and WU) positively affected the phloem in plants grown in shallower substrate depths when hydrogel was used. The brown seaweed *Ascophyllum nodosum* (active ingredient of SP) has shown promising effects in enhancing plant tolerance to urban drought stress due to its high content of bioactive compounds such as alginates, cytokinins, and betaines. Its application in urban horticulture, particularly in green roofs and containerized landscapes, can improve water-use efficiency, stimulate antioxidant activity, and support ornamental plant vitality under prolonged water limitation [56]. The environmental benefits of biostimulants are mainly reflected in their ability to improve plant resilience and enhance nutrient uptake from the soil, thereby reducing the need for chemical stimulants and pesticides, which ultimately contributes to environmental sustainability. Grammenou et al. [57] reported that biostimulants increase crop tolerance to various stressors and improve nutrient use efficiency, consequently lowering the dependence on synthetic agrochemicals. Moreover, they can mitigate soil degradation caused by potentially toxic elements through processes such as phytoextraction, translocation, or stabilization. Similarly, Mandal et al. [58] emphasized that biostimulants help reduce the agricultural chemical footprint and promote sustainable, resilient farming systems, thereby supporting environmental preservation. However, despite these benefits from the biostimulants, the control plants still exhibited the best results regarding the anatomical characteristics of plants subjected to drought conditions.

Taken all together, the integration of xylem-specific and whole-section anatomical data reveals coordinated developmental responses to substrate depth, hydrogel supplementation, and substrate type. Perlite and perlite–peat substrates, in combination with deep rooting and/or hydrogel use, enhance both vessel and ray development, while peat-only substrates and WU treatments tend to restrict both tissue types. These structural adjustments likely reflect adaptive strategies to balance hydraulic efficiency, mechanical support, and internal resource allocation under variable environmental conditions. Finally, calcium oxalate crystals were more numerous and larger in treatments without hydrogel, indicating that plants under greater drought stress activated defensive responses through increased crystal formation [59]. The major functions of CaOx crystal formation in plants include calcium regulation, plant protection, detoxification, ion balance, and providing tissue support and rigidity [60–62]. In species belonging to the Solanaceae family, particularly petunia, it has been reported that dissolved CaOx crystals may serve as a vital source of calcium ions for pollen grain development [63,64], and they have also been shown to modify anther structure in *Capsicum annuum* [65,66]. The results obtained in this study indicate that CaOx may also influence plant survival under drought-induced stress conditions in *P.* × *hybrida*. Specifically, we observed increased CaOx formation in plants grown without hydrogel, with the most pronounced effect in plants cultivated in perlite substrate. This response may represent a survival strategy, as the degradation of CaOx can release CO_2, which is subsequently utilized for low-intensity photosynthesis, known as “alarm photosynthesis” [67]. Such a mechanism, crucial under stomatal closure and reduced atmospheric CO_2 uptake [68], could help maintain minimal photosynthetic activity and protect the photosynthetic apparatus during drought stress.

4.3. Integration of Obtained Results into Practice

Given the intensifying challenges posed by urban sprawl, densification, and the associated environmental stressors, the findings of this study on *P.* × *hybrida* offer valuable insights into plant adaptability and substrate management for its sustainable cultivation in urban settings. In urban environments—often characterized by compact soil and water scarcity—plant resilience depends heavily on adaptive physiological and anatomical traits [69]. The anatomical assessment presented here demonstrates that even a 2 cm difference in substrate depth significantly enhances secondary vascular development, particularly xylem tissue, improving water transport capacity and mechanical strength in *P.* × *hybrida*—features essential for plant performance under fluctuating microclimates and prolonged drought.

This research supports a transition toward more resilient urban cultivation systems for *P.* × *hybrida* by informing substrate design for container planting, with potential application to green roofs and micro-greening interventions. Optimizing root zone conditions through thoughtful substrate and amendment choices becomes a practical tool for enhancing plant survival, ecosystem service provision, and climate adaptation [70]. The findings of this study underline the critical role of substrate composition and irrigation frequency in optimizing plant physiological and anatomical responses, particularly in the context of sustainable urban greening under water-limited conditions. Variations in irrigation frequency among treatments, aligned with substrate composition and hydrogel application, were directly linked to the anatomical and physiological performance of the studied species. More frequent irrigation (T1) compensates for substrates with lower water retention (perlite without hydrogel), but may increase water use and management costs in urban settings. Hydrogels effectively extend irrigation intervals without compromising plant vascular development, offering a practical means to reduce water consumption (T2). Peat-based substrates inherently reduce irrigation frequency needs but may have drawbacks related to sustainability and substrate aeration. The combination of aerated substrates with water-retentive amendments (peat-perlite + hydrogel T4) provides a dual benefit—ensuring oxygen availability to roots while sustaining moisture, thereby enhancing plant resilience to drought stress. These insights emphasize the importance of balancing substrate physical properties with irrigation scheduling to optimize plant health and water efficiency of *P.* × *hybrida* in urban plantings. Extending irrigation intervals through substrate amendments has also been recognized as an effective strategy to reduce water demand while supporting plant adaptability [71].

4.4. Aligning Research on Petunia × *hybrida with the Sustainable Development Goals (SDGs)*

This research on the anatomical and visual responses of *P.* × *hybrida* to varying substrate compositions, hydrogel supplementation, and irrigation regimes provides insights relevant to sustainable horticultural practices and aligns with the general objectives of the UN Sustainable Development Goals (SDGs). Although actual water consumption was not quantified, but expressed through evapotranspiration, this study is relevant to SDG6 (Clean Water and Sanitation) as proper substrate choice and amendment with hydrogel allowed for reduced irrigation frequency without compromising plant development. These findings may be relevant for informing irrigation strategies in water-limited environments, particularly in container-grown *P.* × *hybrida* under shallow substrate conditions. Furthermore, identifying drought-adaptive ornamental species such as *P.* × *hybrida* supports the development of resilient container-based plantings, which may also be applicable to green roofs and small-scale urban greening applications (SDG11). Such a planting strategy could support micro-scale greening efforts that contribute to improved urban microclimates and increased access to vegetation, even in highly built environments [72]. Identification of

plant traits (e.g., enhanced xylem development and regenerative capacity) that contribute to understanding species resilience under abiotic stress is essential. Cultivating ornamentals with improved drought tolerance supports climate adaptation strategies in urban horticulture (SDG13), while minimizing the environmental footprint of urban greening projects [73]. The improved performance of *P.* × *hybrida* under specific low-input substrate regimes also contributes to SDG12 (Responsible Consumption and Production). It encourages a shift toward resource-efficient horticultural practices by demonstrating that plant health and aesthetic value can be maintained with reduced irrigation and sustainable growing media, including peat alternatives and amendments like perlite and hydrogels. Furthermore, this research lays the groundwork for more inclusive green economy models (SDG8). The use of ornamental plants in urban greening not only creates aesthetic and ecological value but also provides opportunities for small-scale urban growers, plant nursery operators, and landscape professionals to adopt water-efficient, climate-smart cultivation practices [74]. Educationally, these findings can serve as demonstrative content in academic curricula and community training programs, helping to build capacity in sustainable horticulture and urban ecology, thereby supporting SDG4 (Quality Education). Integrating plant-based experimental models like *P.* × *hybrida* into school gardens, citizen science projects, or municipal green planning frameworks can raise awareness of plant-water relations and sustainable substrate management. While ornamental plants are often overlooked in the SDG framework in favor of food crops, their role in urban well-being (SDG3) is undeniable [75]. Resilient urban greenery enhances mental health, reduces stress, and provides opportunities for physical activity and connection with nature—particularly important in high-density urban areas. Finally, this research reflects the importance of interdisciplinary partnerships (SDG17), uniting horticultural science, urban ecology, landscape architecture, and water management. The integration of anatomical plant studies with practical substrate innovations exemplifies how targeted academic inquiry can inform policy, guide urban planning, and support grassroots greening initiatives across urban regions [76].

In summary, the study demonstrated that both pot size and substrate type significantly influenced plant vitality, above- and belowground growth, and stem anatomy of *P.* × *hybrida* (**RQ1**). Visual assessment highlighted the advantages of deeper containers and perlite-based substrates, while the perlite–peat mixture supported optimal overall development. From an anatomical perspective, the most favorable results were achieved with deeper rooting volume and substrates containing perlite. Hydrogel amendment significantly increased root volume, though its impact on aboveground growth was less consistent (**RQ2**). The best results occurred in perlite-based media, while peat-based combinations sometimes produced adverse effects. At the anatomical level, hydrogel supplementation promoted increases in stem diameter, cross-sectional area, phloem expansion, central parenchyma development, and the production of larger xylem vessels. Stim Pure was the most effective biostimulant in promoting vegetative growth and induced the greatest anatomical expansion, enhancing stem thickness and phloem development (**RQ3**). In contrast, control plants exhibited greater xylem investment, suggesting a shift toward structural reinforcement under drought stress. Significant effects of combined treatments on the vitality and drought-adaptive anatomical traits of *P.* × *hybrida* were observed (**RQ4**). The significance of this study lies in its integrated assessment of multiple key cultivation factors under semi-controlled, water-limited urban conditions, providing a comprehensive understanding of their interactive effects on plant development. Finally, optimal performance of *P.* × *hybrida* in container-based plantings can be achieved through a combination of deeper pots, perlite-containing substrates, hydrogel, and targeted biostimulant application.

5. Conclusions

This study is the first to examine the combined effects of pot size, substrate composition, hydrogel amendment, and biostimulant application on both growth performance and anatomical adaptations of *P.* × *hybrida* under water-limited urban conditions. Our findings showed that deeper substrates (10 cm) significantly enhance plant survival and vegetative growth, as well as xylem development and overall stem structure, contributing to improved water transport and mechanical stability. Plants grown in perlite had the highest root volume, while the peat substrate induced the highest plant height and width. Light substrates, particularly perlite and perlite–peat mixtures, were most beneficial for stem anatomy, supporting cambial activity and radial growth more effectively than peat alone, due to better aeration and drainage. Substrate amendment with hydrogel promoted phloem expansion and central parenchyma development, especially in shallower substrates, suggesting enhanced internal water distribution and buffering under drought stress. Application of hydrogel induced a twofold increase in root volume, without enhancing aboveground growth. Of the tested biostimulants, Stim Pure more effectively promoted stem thickening and phloem area, whereas control treatments favored greater xylem investment. The same biostimulant also triggered the greatest vegetative growth. Therefore, in water-limited urban settings, combining deeper containers, aerated substrates (such as perlite blends), and targeted biostimulant use (e.g., Stim Pure for biomass increase or no biostimulant for structural reinforcement) can substantially enhance anatomical resilience and adaptive performance in *P.* × *hybrida*. Results from correlation analysis confirmed that improvements in growth are strongly tied to changes in stem anatomy, reflecting the impact of the cultivation factors investigated. While water consumption for irrigation was not directly measured, the study highlights that substrate selection and irrigation frequency must be co-optimized for resilient cultivation of *P.* × *hybrida*. Hydrogels emerged as key amendments that may enable longer irrigation intervals without sacrificing vascular development or drought resistance. These findings support the potential of water-smart substrate strategies for improving drought adaptation in urban horticulture and advancing sustainable practices aligned with multiple SDGs.

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Abbreviations

SP	Stim pure AA Liquid (SP) (Van Iperen International, Westmaas, The Netherlands)
WU	WAKE-up (WU) Liquid (Van Iperen International, Westmaas, The Netherlands)

C	Control plants—without biostimulant application
D1	Substrate depth (7 cm)
D2	Substrate depth (10 cm)
H0	Without the hydrogel application
H1	With the hydrogel application
S1	Perlite substrate
S2	Peat substrate
S3	Mixture of peat and perlite substrate

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