

Article

Non-Destructive Evaluation of In Vitro Blackberry Shoot Architecture Under Different Sucrose Levels Through Smartphone-Derived 3D Reconstruction

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Abstract

Blackberry micropropagation enables the rapid production of pathogen-free and genetically uniform plant material, although the evaluation of in vitro shoot development still relies on destructive and time-consuming measurements. This study investigated a low-cost smartphone-based 3D imaging approach for the non-destructive characterization of in vitro blackberry shoots (cultivar ‘Thornfree’) grown under different sucrose concentrations in the media (0, 7.5, 15, and 30 g L⁻¹). Explants were cultured for 30 days under controlled environmental conditions in ventilated vessels containing 15 explants. Three-dimensional reconstructions generated using the viDoC RTK rover system coupled with an Apple iPhone 15 Pro Max were used to extract geometric traits, including shoot height, projected area, and shoot volume estimated through three complementary approaches, together with voxel-derived structural descriptors of shoot spatial organization and compactness. The proposed approach enabled the quantitative assessment of shoot architectural responses to sucrose availability, revealing differences in volumetric development and internal structural organization among treatments that would not be detectable by conventional measurements. The results highlight the potential of smartphone-based 3D phenotyping as a rapid, low-cost, and non-destructive tool for monitoring structural traits in micropropagated plant material and for supporting the optimization of in vitro culture conditions.

Keywords: micropropagation; non-destructive phenotyping; smartphone photogrammetry; voxel-based analysis; shoot architecture



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1. Introduction

Blackberry (*Rubus* spp.) is an economically important small fruit crop widely cultivated across temperate regions for fresh consumption, processing, and pharmaceutical applications due to its high content of bioactive compounds, including anthocyanins, polyphenols, and vitamins [1–3]. Traditional methods for blackberry propagation (layering and cuttings) are constrained by the need for large cultivation areas and high labor requirements, which also complicate weed control and limit scalability [4–6]. To address these drawbacks, micropropagation, an in vitro culture technique, has been adopted as an alternative approach, providing several advantages over conventional methods [7]. These include the rapid production of large quantities of genetically uniform and pathogen-free

plant material within a limited space and time [7–10]. Moreover, micropropagation facilitates germplasm conservation, pathogen eradication, and genetic manipulation, while improving resource efficiency and enabling cost-effective plant production [6,7,11–13].

In *in vitro* culture systems, sucrose is commonly added to the culture medium as the primary carbon and energy source, as shoot explants cannot yet sustain fully autotrophic growth under the low light intensities typically used in growth chambers [14,15]. The concentration of sucrose in the medium has been shown to significantly influence shoot proliferation, elongation, leaf development, and overall biomass accumulation in several woody and herbaceous species [15–21].

The assessment of shoot growth and architectural development during the micropropagation process has traditionally relied on destructive or manual measurements, including shoot height, number of nodes, fresh and dry weight, and leaf area [11]. These approaches are time-consuming, operator-dependent, and incompatible with repeated monitoring of the same plant material over time. The development of non-destructive phenotyping methods capable of capturing three-dimensional structural information represents a key step toward more efficient and objective monitoring of *in vitro* plant development.

Three-dimensional reconstruction techniques have been widely adopted in plant phenotyping applications at the field and greenhouse scale, enabling the extraction of morphological and architectural traits with high spatial resolution and temporal repeatability. Terrestrial Laser Scanning (TLS) provides submillimetre accuracy and high point cloud density, enabling precise characterization of canopy structure, volume, and growth habit in woody species [22] including hazelnut [23], olive [24,25], cherry [26], and peach [27]. However, TLS systems are expensive, require specialized operators, and are logistically impractical for routine use in controlled laboratory environments such as growth chambers [28].

Unmanned aerial vehicle (UAV)-based photogrammetry has similarly been employed to derive geometric descriptors of tree architecture in intensive orchard systems [25,29–31], offering wide-area coverage at spatial scales incompatible with the characterization of individual *in vitro* shoots. More recently, close-range SfM photogrammetry [32,33] using consumer-grade cameras has demonstrated point cloud accuracy and completeness comparable to laser scanning for individual plant characterization [34], at substantially lower hardware and operational costs. Smartphone-based platforms integrating RGB cameras with RTK (real-time kinematic) GNSS (Global Navigation Satellite System) positioning further reduce operational complexity while maintaining geometric accuracy, making them accessible for routine use in laboratory and field settings [11,35,36].

Voxel-based approaches have also been proposed as a complementary method for quantifying plant spatial occupancy and canopy structural complexity from point cloud data. By discretizing three-dimensional space into regular volume units, voxel-based models enable the estimation of leaf area density and the characterization of canopy internal architecture, including vertical distribution of plant material and spatial occupancy at different canopy layers. Originally developed for terrestrial laser scanning applications in forest and agricultural contexts [37,38], these methods have subsequently been adapted for use with photogrammetric point clouds derived from SfM workflows [39], extending their applicability to a wider range of platforms and acquisition systems.

In the context of *in vitro* plant material, however, the application of three-dimensional imaging techniques remains largely unexplored, and the potential of smartphone-based photogrammetry for monitoring shoot architectural development under controlled culture conditions has not yet been systematically investigated.

The first applications of smartphone-based SfM photogrammetry to *in vitro* plant material were reported by Regni et al. [11], who applied the iPhone–viDoc RTK system to the non-destructive monitoring of shoot development in blackberry and blueberry

micropropagation. In that study, however, the three-dimensional information of the point cloud was only partially exploited, as the analysis was limited to planar descriptors derived from the horizontal projection of the reconstruction, specifically canopy-covered area and shoot density.

Subsequently, Regni et al. [36] extended the approach to in vitro-grown kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F. Liang & A.R. Ferguson) at different explant densities and subculture durations, integrating height, projected area, and convex hull-based volume with shape indices such as the area-to-volume and height-to-area ratios. While this study represented a substantial advancement towards a comprehensive three-dimensional characterization of in vitro shoot architecture, voxel-based structural analysis, which enables the quantification of spatial occupancy, canopy density, and internal architectural complexity, was not included.

The present study builds on this methodological framework by introducing, for the first time, voxel-based structural descriptors for the non-destructive characterization of in vitro blackberry shoots (*Rubus* spp. cv. Thornfree), together with a systematic comparison of three volumetric estimators under four sucrose concentrations in ventilated vessels as the experimental variable.

Point cloud reconstructions generated using the viDoc RTK rover system coupled with an iPhone 15 Pro Max were used to extract geometric and voxel-based structural descriptors of shoot architecture at two time points: at the beginning of the subculture (t_0) and after 30 days of culture (t_{30}). The specific objectives of this study were: (i) to evaluate the effect of sucrose concentration (0, 7.5, 15, and 30 g L⁻¹) on the geometric and structural traits of in vitro blackberry shoots; (ii) to assess the suitability of smartphone-derived point clouds for the quantification of shoot height, projected area, and volume using both Digital Surface Model (DSM)-based and voxel-based approaches; and (iii) to evaluate derived structural indices, including the voxel occupancy density index and a shoot projected area index (SPAI), as non-destructive descriptors of shoot spatial organization and compactness.

2. Materials and Methods

2.1. Plant Material and In Vitro Culture Conditions

In vitro-propagated blackberry explants (cultivar ‘Thornfree’) were obtained from stock material cultured at the Micropropagation and In Vitro Biotechnology Laboratory of the Research Unit ‘Tree Science’ at the Department of Agricultural, Food and Environmental Sciences, University of Perugia (Italy). The culture medium consisted of half-strength Murashige and Skoog formulation supplemented with sucrose (0, 7.5, 15, and 30 g L⁻¹), IBA (0.1 mg L⁻¹), BAP (0.4 mg L⁻¹), and agar (8 g L⁻¹), adjusted to pH 5.7. Autoclavable plastic jars (height 8 cm, basal diameter 9.7 cm, volume 565 mL; Sac O2, Deinze, Belgium), equipped with HEPA filters, were filled with 100 mL of the described medium. Both the vessels and the culture medium were sterilized by autoclaving at 115 °C for 20 min prior to use. The initial explants consisted of nodal segments 1.2–1.5 cm in length, with 0.7–0.9 cm positioned above the substrate, each bearing 3–4 leaves. The jars, each containing fifteen explants, were placed in a growth chamber at a constant temperature of 22 ± 2 °C and a 16-h photoperiod of light with an intensity of 40 µE m⁻² s⁻¹ for 30 days.

2.2. Experimental Design

The experiment included the above mentioned four sucrose treatments corresponding to 0, 7.5, 15, and 30 g L⁻¹, each consisting of three biological replicates represented by one vessel containing 15 explants. Within each vessel, explants were arranged according to a standardized spatial design to ensure uniform inter-shoot and shoot-to-wall distances (Figure 1).

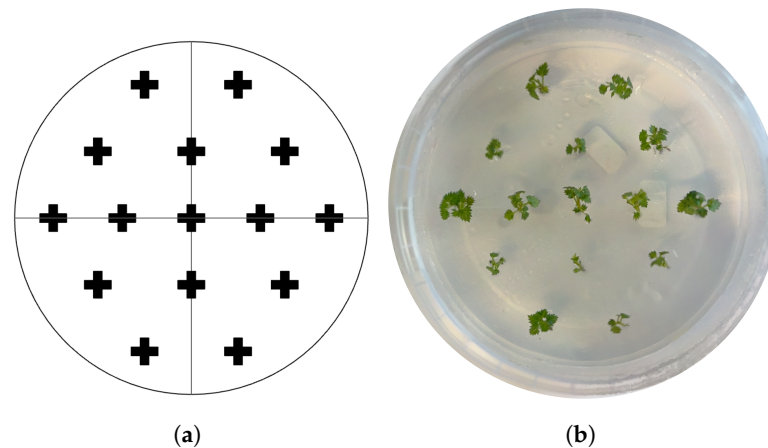


Figure 1. Explant distribution within the culture vessel: (a) Schematic layout. (b) Representative sample at t_0 .

Non-destructive 3D surveys were performed on the same vessels at two experimental time points: immediately after explant establishment (t_0) and after 30 days of in vitro growth (t_{30}). This approach enabled the temporal monitoring of shoot architectural development under different sucrose concentrations while preserving the plant material.

2.3. Image Acquisition and Photogrammetric Reconstruction

Image acquisition was performed inside a horizontal laminar flow cabinet using an Apple iPhone 15 Pro Max coupled with a viDoc RTK rover (vigram GmbH, Krauchenwies, Germany), a GNSS device equipped with an RTK antenna specifically designed to interface with Apple devices featuring LiDAR (Light Detection and Ranging) sensors (Figure 2a). The survey was carried out under natural illumination conditions, with all surveys performed in the same indoor environment to ensure consistent illumination across survey sessions. For each vessel, approximately 115 images were acquired following a circular trajectory around the sample with predominantly nadir camera orientation combined with additional oblique acquisitions to improve the reconstruction of lower and partially occluded plant structures (Figure 2b), requiring only a few minutes per vessel. The average acquisition distance between the camera and the sample was approximately 15 cm and the overlap between images was set to 90%. Each vessel was positioned on a calibrated reference base containing five ground control targets with known coordinates (Figure 2b).

Photogrammetric reconstruction and scaling were performed using Agisoft Metashape Professional (version 1.7.4, Agisoft LLC, St. Petersburg, Russia). Reconstruction was based on the Structure-from-Motion (SfM) approach, a photogrammetric technique that simultaneously estimates three-dimensional scene geometry and camera parameters from a set of overlapping images acquired from multiple viewpoints [32]. The algorithm identifies and matches common feature points across images, enabling the reconstruction of both camera trajectories and a dense point cloud representing the surveyed object (Figure 3). The targets were manually identified and used for spatial scaling and model optimization, resulting in a reconstruction error of approximately 1 mm.

Dense point clouds resulting from the SfM reconstruction were used for subsequent geometric and voxel-based analyses.

Image acquisition and photogrammetric reconstruction followed the general approach described by Regni et al. [11], using the same hardware and a similar acquisition protocol, but differing in experimental setup (type of vessel and variable studied), the analytical framework, which fully exploits the three-dimensional information of the SfM reconstruc-

tion to extract volumetric estimators, shape indices, and voxel-based structural descriptors, and SfM reconstruction software.

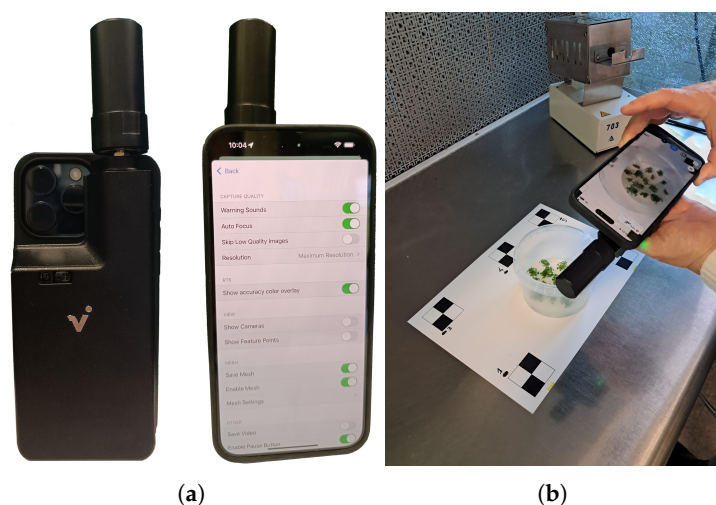


Figure 2. Experimental setup and image acquisition: (a) Apple iPhone 15 Pro Max coupled with the viDoc RTK rover. (b) Image acquisition of the culture vessel positioned on the calibrated target base.

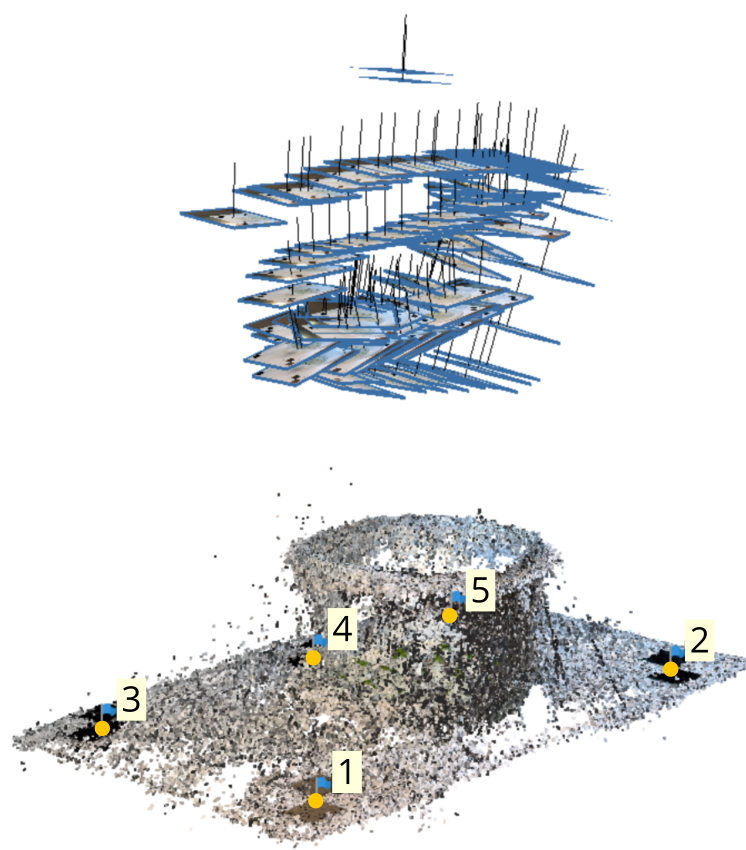


Figure 3. Point cloud reconstruction in Agisoft Metashape with camera positions and the ground control targets (yellow points).

2.4. Point Cloud Processing and Geometric Trait Extraction

Point cloud processing was performed using Cyclone 3DR (version 2025.2.1, Leica Geosystems, Heerbrugg, Switzerland), CloudCompare (version 2.13.1), and QGIS (version 3.34). To isolate plant material from the background, dense point clouds (Figure 4a)

were filtered by color using the “Real Colors” tool in Cyclone 3DR with an intermediate tolerance. A set of manually selected points corresponding to the vessel, base, and surrounding non-plant elements, characterized by a very light grey coloration, were used as color reference. Points matching the reference color within the defined tolerance were automatically grouped and removed, retaining only the green points corresponding to shoot tissue. The resulting shoot-only point cloud (Figure 4b) was subsequently subjected to noise filtering to eliminate isolated or erroneous points. The color-based filtering procedure adopted here follows the same approach described by Regni et al. [11] for the isolation of shoot material from the vessel background in point clouds acquired with the same hardware system.

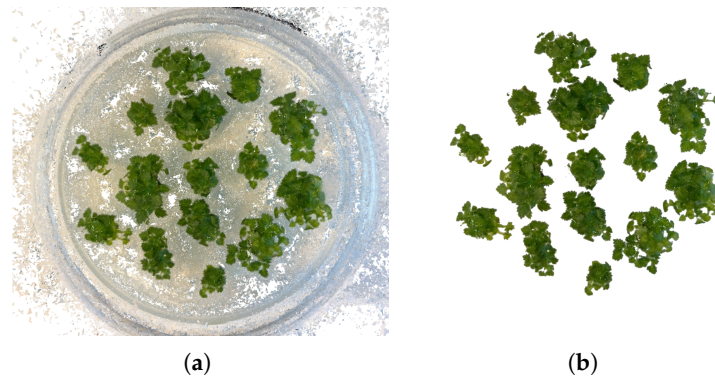


Figure 4. Example of dense point cloud at the end of the experiment: (a) Complete model. (b) Filtered point cloud after removal of non-plant elements.

Individual shoot segmentation was performed using the “Separate by Distance” function in Cyclone 3DR, which automatically partitioned spatially distinct shoot clusters based on inter-point distance thresholds. At t_0 , full automatic separation was achieved for all treatments. At t_{30} , where partial overlap occurred due to shoot expansion, automatic separation was complemented by manual editing to ensure accurate delineation of individual shoots.

Shoot height (h in Figure 5a) was calculated as the difference between the maximum Z-coordinate of the reconstructed point cloud and the reference substrate level, corresponding to 1.5 cm above the vessel base. Projected shoot area (A in Figure 5a) was obtained by orthogonally projecting the point cloud onto a horizontal plane followed by automatic vectorization of the external boundary. Shoot volume was estimated using a digital surface model (DSM)-based approach. The filtered point cloud was imported into CloudCompare and horizontally segmented into consecutive 2 mm slices. For each slice, a raster layer with a grid spacing of 0.5×0.5 mm was generated. The raster layers were then imported into QGIS and merged into a single DSM by retaining, for each pixel, the maximum height value across all slices, representing the upper surface of the shoot (Figure 5b). The DSM was subsequently masked using the projected shoot area in order to restrict the analysis to the actual shoot extent, and the height value (h_i) of each pixel centroid was extracted. Shoot volume was finally calculated as Equation (1):

$$V_{DSM} = \sum_{i=1}^n (h_i \times A_{pix}) \quad (1)$$

where h_i represents the height value of each pixel centroid, corrected with respect to the reference substrate level (1.5 cm above the vessel base), and A_{pix} corresponds to the pixel area ($0.5 \text{ mm} \times 0.5 \text{ mm}$).

A slice thickness of 2 mm was selected as a compromise among vertical resolution, structural completeness, and point cloud noise. Considering that SfM reconstruction

generally provides lower vertical accuracy than planimetric accuracy [40,41], selecting a slice thickness below 2 mm would risk confounding structural voids with reconstruction inaccuracies, generating artifactual empty layers for thin obliquely oriented structures. Thicker slices would reduce this sensitivity but result in reduced structural detail and an overestimation of occupied volume. Moreover, 2 mm slices are consistent with the typical width of blackberry petioles and leaf veins, which range between approximately 1 and 3 mm. The raster resolution of 0.5×0.5 mm was selected considering the sub-millimetric average inter-point distance of the reconstructed point cloud, ensuring minimal spatial undersampling while maintaining computational feasibility.

As a complementary approach, shoot volume was also estimated using the convex hull method. For each segmented shoot point cloud at t_{30} , a convex hull surface was generated directly in Cyclone 3DR using the “Create Convex Surface” tool, and the enclosed volume was extracted from the resulting mesh (V_{hull} in Figure 5a). This method approximates shoot volume as the minimum convex solid enclosing all reconstructed points, and therefore tends to overestimate the actual occupied volume by including void spaces between leaf and stem structures. For a limited number of shoots, the convex hull could not be computed due to geometric constraints of the point cloud, and the corresponding V_{hull} values were excluded from the comparative analysis. The convex hull approach was applied exclusively at t_{30} , as shoot geometry at t_0 was insufficient to generate reliable convex surfaces for most shoots.

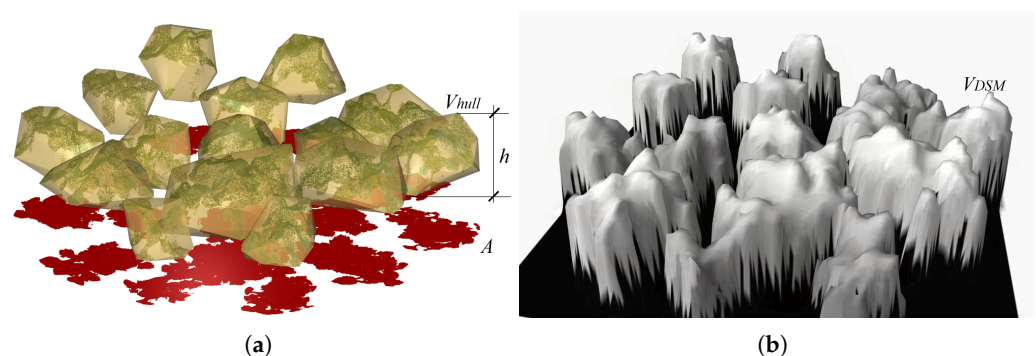


Figure 5. Example of 3D reconstruction models at the end of the experiment: (a) shoot point cloud with geometric parameters (height, projected area, and convex hull volume). (b) Digital surface model (DSM) used for shoot volume estimation.

The three volumetric estimators (V_{DSM} , V_{vox} , and V_{hull}), showed in Figure 6, were subsequently compared to assess the consistency among methods and to characterize their respective tendencies toward over or underestimation of shoot volume.

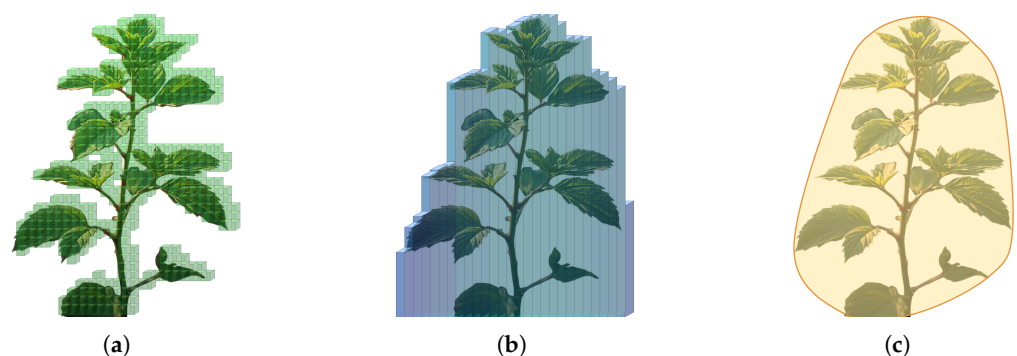


Figure 6. Three different volumetric estimators: (a) Voxel-derived volume (V_{vox}). (b) Digital Surface Model (V_{DSM}). (c) Convex hull volume (V_{hull}).

Additional geometric descriptors, including area-to-volume ratio (A/V) and height-to-area ratio (h/A), were subsequently derived from the measured parameters. These indices were computed using V_{DSM} as the reference volumetric estimator.

2.5. Voxel-Based Structural Analysis

Voxel-based structural analysis was conducted to quantify shoot spatial occupancy and structural organization. The following processing steps were applied to the raster layers generated as described in Section 2.4:

- Reclassification of each raster layer in QGIS, assigning a value of 1 to occupied pixels and 0 to empty pixels;
- Stacking of the reclassified layers into a single multi-band raster, with each band corresponding to one horizontal slice, resulting in voxel dimensions of $0.5 \text{ mm} \times 0.5 \text{ mm} \times 2 \text{ mm}$;
- Masking of the stacked raster using the same shoot projection polygons adopted in Section 2.4;
- Extraction of pixel centroids from the masked stacked raster and sampling of binary values across all bands through the “Sample Raster Values” function in QGIS, yielding for each centroid a series of 0/1 values corresponding to the occupancy state of each slice along the vertical column;
- Calculation of the total number of occupied voxels by summing all values equal to 1 across all bands and all centroids using a spreadsheet-based data processing workflow.

Voxel-derived shoot volume was calculated from the total number of occupied voxels (n) multiplied by the voxel volume (Equation (2)).

$$V_{vox} = [n \times (0.5 \times 0.5 \times 2)] \text{ mm}^3 \quad (2)$$

A voxel occupancy density index (D) was subsequently computed as the ratio between voxel-derived shoot volume and DSM-derived shoot volume (Equation (3)):

$$D = \frac{V_{vox}}{V_{DSM}} \quad (3)$$

where V_{vox} is the volume of occupied voxels and V_{DSM} is the shoot volume estimated from the DSM.

A Shoot Projected Area Index ($SPAI$) describing shoot layering and spatial occupation was also calculated as Equation (4):

$$SPAI = \frac{A_{vox}}{A_{proj}} \quad (4)$$

where A_{vox} corresponds to the cumulative occupied voxel area and A_{proj} represents the projected shoot area. A_{vox} was calculated as the total number of occupied voxels multiplied by the voxel base area (Equation (5)), summed across all slices.

$$A_{vox} = [n \times (0.5 \times 0.5)] \text{ mm}^2 \quad (5)$$

All calculations and index derivations were performed using spreadsheet-based data processing. Figure 7 summarizes the overall methodological workflow, including image acquisition, point cloud reconstruction, data processing, geometric traits and index extraction.

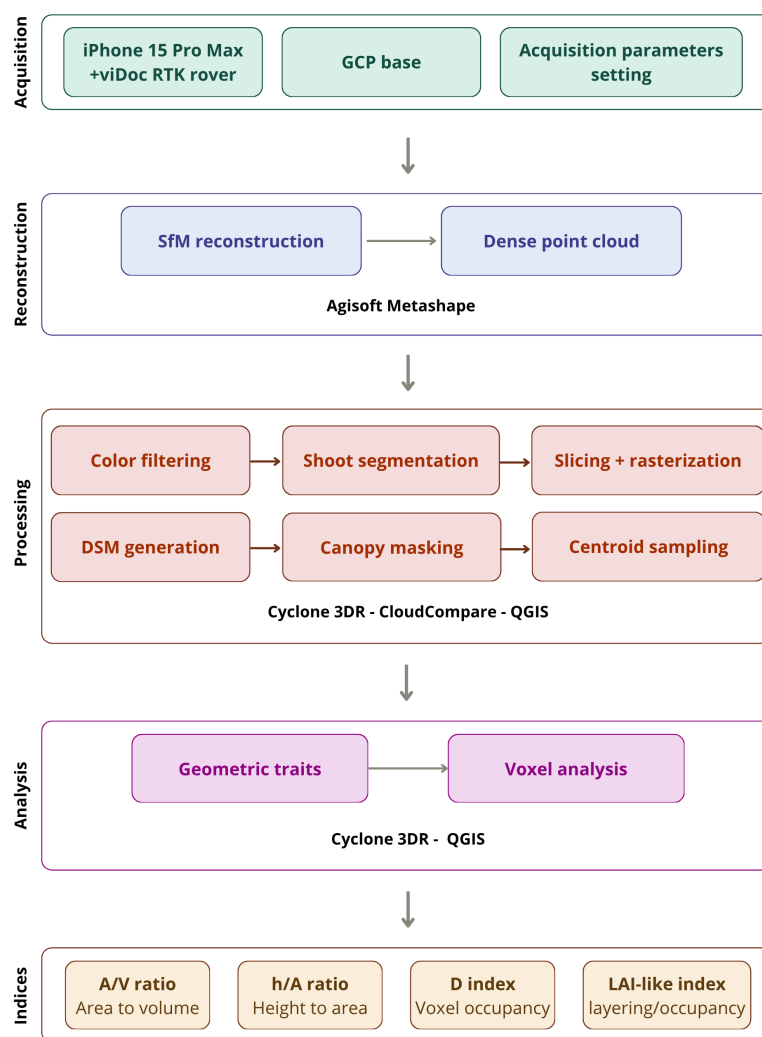


Figure 7. Methodological workflow of the proposed approach.

2.6. Statistical Analysis

Statistical analyses were performed to evaluate the effects of sucrose concentration on the geometric and voxel-derived structural parameters of blackberry shoots. Prior to analysis, data distribution was assessed using the D'Agostino–Pearson omnibus normality test and homogeneity of variances was verified using Levene's test. Differences among treatments were assessed by one-way analysis of variance (ANOVA). Where significant effects were detected, post hoc multiple comparisons were performed using Tukey HSD test ($p < 0.05$).

3. Results

3.1. Geometric Traits

At t_0 , all geometric parameters were comparable across sucrose treatments, confirming the absence of any pre-existing treatment effect at the time of explant transfer. Mean shoot height ranged from 0.49 ± 0.07 cm (30 g L⁻¹) to 0.78 ± 0.04 cm (0 g L⁻¹), projected shoot area from 0.27 ± 0.04 cm² (0 g L⁻¹) to 0.32 ± 0.03 cm² (30 g L⁻¹), and V_{DSM} from 0.09 ± 0.01 cm³ (7.5 g L⁻¹) to 0.16 ± 0.03 cm³ (0 g L⁻¹).

After 30 days of culture (t_{30}), clear differences in shoot architecture emerged among treatments (Figure 8). Shoot height increased in sucrose-supplemented treatments relative to the sucrose-free treatment (Figure 8a), with the highest values recorded at 15 g L⁻¹

(1.07 ± 0.03 cm) and 30 g L^{-1} (1.00 ± 0.06 cm), followed by 7.5 g L^{-1} (0.94 ± 0.10 cm), while shoots grown without sucrose showed virtually no height increase compared to t_0 (0.78 ± 0.00 cm). Projected shoot area increased markedly in all sucrose-supplemented treatments (Figure 8b), with values approximately 2.3–2.7 times greater than the sucrose-free treatment ($0.57 \pm 0.05 \text{ cm}^2$); the highest values were observed at 7.5 g L^{-1} ($1.55 \pm 0.25 \text{ cm}^2$) and 15 g L^{-1} ($1.54 \pm 0.06 \text{ cm}^2$), with a slight reduction at 30 g L^{-1} ($1.32 \pm 0.11 \text{ cm}^2$). V_{DSM} followed the same trend (Figure 8c), with the highest values at 15 g L^{-1} ($1.14 \pm 0.04 \text{ cm}^3$) and 7.5 g L^{-1} ($0.96 \pm 0.23 \text{ cm}^3$), an intermediate value at 30 g L^{-1} ($0.84 \pm 0.07 \text{ cm}^3$), and a markedly lower value in the sucrose-free treatment ($0.31 \pm 0.02 \text{ cm}^3$), suggesting a possible inhibitory effect of high sucrose concentration on volumetric shoot development. One-way ANOVA revealed significant differences among treatments at t_{30} for all three geometric parameters (Figure 8). Shoot height was significantly lower in the sucrose-free treatment (group a) compared to the 15 and 30 g L^{-1} treatments (group b), with the 7.5 g L^{-1} treatment showing an intermediate position (group ab). Projected shoot area was significantly lower in the 0 g L^{-1} treatment (group a) compared to all sucrose-supplemented treatments, which did not differ from each other (group b). V_{DSM} showed a similar pattern, with the sucrose-free treatment significantly lower than the 7.5 and 15 g L^{-1} treatments (group b), while the 30 g L^{-1} treatment occupied an intermediate position (group ab).

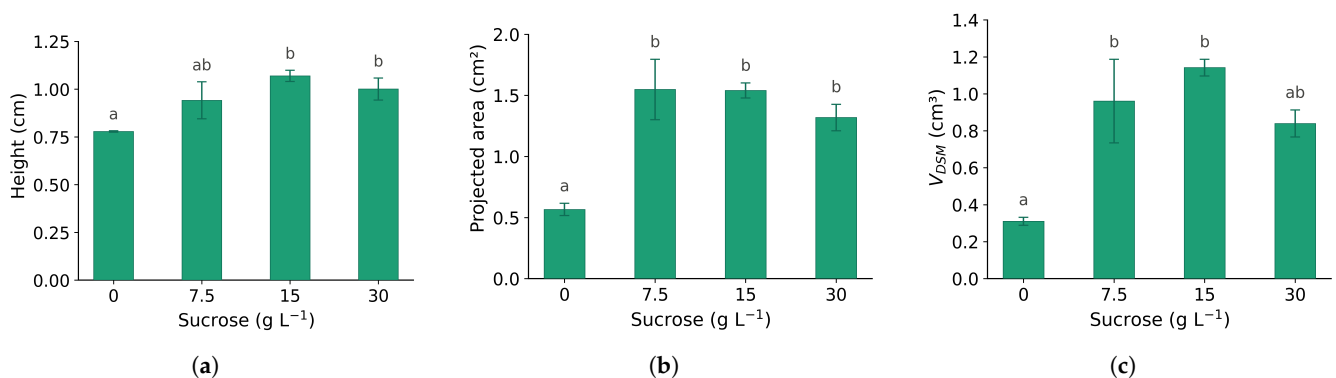


Figure 8. Mean values ($\pm$ standard error) of the geometrical traits measured at t_{30} for blackberry shoots (*Rubus* spp. cv. Thornfree) grown under four sucrose concentrations (0 , 7.5 , 15 , and 30 g L^{-1}): (a) Shoot height. (b) Projected shoot area. (c) DSM-derived shoot volume (V_{DSM}). Different lowercase letters indicate statistically significant differences among treatments (one-way ANOVA followed by Tukey HSD test, $p < 0.05$).

3.2. Derived Geometric Indices

At t_0 , shoot coverage density ranged from $0.33 \pm 0.05\%$ (0 g L^{-1}) to $0.39 \pm 0.04\%$ (30 g L^{-1}). The A/V ratio showed wider numerical variation, ranging from $1.92 \pm 0.15 \text{ cm}^{-1}$ (0 g L^{-1}) to $4.82 \pm 0.78 \text{ cm}^{-1}$ (7.5 g L^{-1}), and the h/A ratio from $1.93 \pm 0.13 \text{ cm}^{-1}$ (30 g L^{-1}) to $3.44 \pm 0.74 \text{ cm}^{-1}$ (0 g L^{-1}), reflecting the irregular geometry of freshly inoculated explants.

At t_{30} , shoot coverage density increased substantially in sucrose-supplemented treatments relative to the sucrose-free treatment (Figure 9a), with the highest values recorded at 7.5 g L^{-1} ($1.89 \pm 0.30\%$) and 15 g L^{-1} ($1.88 \pm 0.07\%$), followed by 30 g L^{-1} ($1.61 \pm 0.13\%$), while the sucrose-free treatment remained markedly lower ($0.69 \pm 0.06\%$). The area-to-volume ratio (A/V) decreased with increasing sucrose concentration, indicating a proportionally greater volumetric development relative to projected area in supplemented treatments (Figure 9b); the lowest value was observed at 15 g L^{-1} ($1.51 \pm 0.07 \text{ cm}^{-1}$), while the sugar free treatment showed the highest value ($2.15 \pm 0.10 \text{ cm}^{-1}$), with 7.5 and 30 g L^{-1} showing intermediate values (1.97 ± 0.17 and $1.76 \pm 0.12 \text{ cm}^{-1}$, respectively). The height-to-area ratio (h/A) was markedly higher in the sucrose-free treatment ($1.47 \pm 0.11 \text{ cm}^{-1}$)

compared to all supplemented treatments (Figure 9c), which showed similar and considerably lower values (7.5 g L^{-1} : $0.69 \pm 0.04 \text{ cm}^{-1}$; 15 g L^{-1} : $0.85 \pm 0.03 \text{ cm}^{-1}$; 30 g L^{-1} : $0.91 \pm 0.12 \text{ cm}^{-1}$), reflecting the limited lateral expansion of shoots grown without sucrose relative to their height.

Statistical analysis confirmed significant differences among treatments at t_{30} for all three parameters (Figure 9). Shoot density was significantly lower in the sucrose-free treatment (group a) compared to all sucrose-supplemented treatments, which did not differ from each other (group b). The A/V ratio was significantly lower at 15 g L^{-1} (group a) compared to the sucrose-free treatment (group b), with the 7.5 and 30 g L^{-1} treatments showing intermediate values (group ab). The h/A ratio was significantly higher in the 0 g L^{-1} treatment (group b) compared to all sucrose-supplemented treatments, which did not differ from each other (group a).

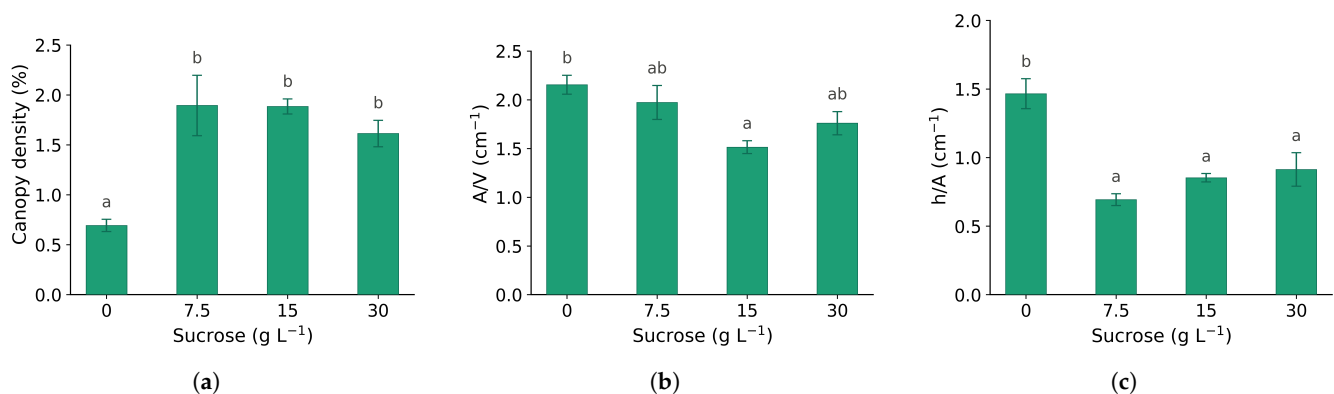


Figure 9. Mean values ($\pm$ standard error) of the derived geometric indices measured at t_{30} for blackberry shoots (*Rubus* spp. cv. Thornfree) grown under four sucrose concentrations (0 , 7.5 , 15 , and 30 g L^{-1}): (a) Density. (b) Area to Volume. (c) Height to Area. Different lowercase letters indicate statistically significant differences among treatments (one-way ANOVA followed by Tukey HSD test, $p < 0.05$).

3.3. Voxel-Based Structural Parameters

At t_0 , V_{vox} was uniformly low across all treatments, ranging from $0.073 \pm 0.007 \text{ cm}^3$ (7.5 g L^{-1}) to $0.077 \pm 0.004 \text{ cm}^3$ (15 g L^{-1}). The D index ranged from 0.096 ± 0.013 (0 g L^{-1}) to 0.150 ± 0.007 (30 g L^{-1}), and the SPAI from 1.16 ± 0.04 (30 g L^{-1}) to 1.40 ± 0.10 (0 g L^{-1}), reflecting the limited shoot development at the time of explant establishment.

At t_{30} , V_{vox} increased substantially in sucrose-supplemented treatments (Figure 10a), following the same pattern observed for V_{DSM} : highest values at 15 g L^{-1} ($0.46 \pm 0.03 \text{ cm}^3$) and 7.5 g L^{-1} ($0.44 \pm 0.08 \text{ cm}^3$), an intermediate value at 30 g L^{-1} ($0.39 \pm 0.04 \text{ cm}^3$), and the lowest value in the sucrose-free treatment ($0.16 \pm 0.01 \text{ cm}^3$). The voxel occupancy density index (D) showed an inverse trend relative to the volumetric parameters (Figure 10b): the lowest value was recorded at 15 g L^{-1} (0.44 ± 0.01), while the sucrose-free treatment showed the highest value (0.57 ± 0.02), with 7.5 and 30 g L^{-1} showing intermediate values (0.54 ± 0.04 and 0.51 ± 0.02 , respectively). This pattern suggests that shoots grown at higher sucrose concentrations, despite their greater absolute volume, develop a more open and relative spatial occupation within the DSM-derived envelope. The SPAI showed modest and consistent variation across all treatments at t_{30} (Figure 10c), ranging from 1.36 ± 0.00 (0 g L^{-1}) to 1.50 ± 0.05 (15 g L^{-1}), with no clear trend in relation to sucrose concentration.

One-way ANOVA confirmed significant differences among treatments for V_{vox} and the D index at t_{30} , while no significant differences were detected for the SPAI (Figure 10). V_{vox} was significantly lower in the sucrose-free treatment (group a) compared to all sucrose-supplemented treatments, which did not differ from each other (group b). The D index

showed an inverse pattern: the 15 g L⁻¹ treatment showed the significantly lowest value (group a), the sucrose-free treatment the significantly highest (group b), with the 7.5 and 30 g L⁻¹ treatments in an intermediate position (group ab).

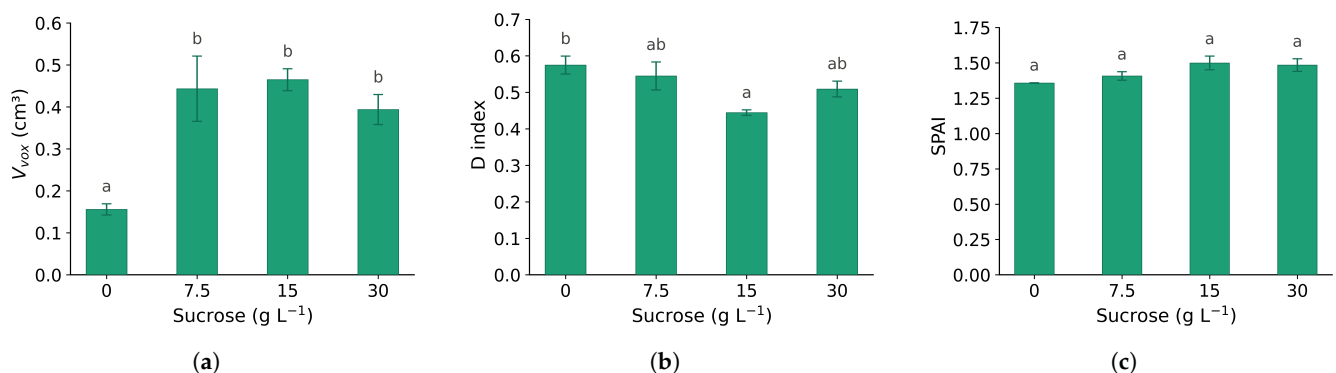


Figure 10. Mean values ($\pm$ standard error) of the voxel indices measured at t_{30} for blackberry shoots (*Rubus* spp. cv. Thornfree) grown under four sucrose concentrations (0, 7.5, 15, and 30 g L⁻¹): (a) Voxel-derived shoot volume V_{vox} . (b) Voxel occupancy density index D . (c) SPAI. Different lowercase letters indicate statistically significant differences among treatments (one-way ANOVA followed by Tukey HSD test, $p < 0.05$).

3.4. Comparison of Volumetric Estimation Methods

The three volumetric estimators (V_{DSM} , V_{vox} , and V_{hull}) were compared at t_{30} to assess the consistency among methods and characterize their respective tendencies toward over- or underestimation of shoot volume. As expected from their respective geometric assumptions, the three methods yielded systematically different absolute values across all treatments, with $V_{vox} < V_{DSM} < V_{hull}$ in all cases (Figure 11). At t_{30} , mean V_{hull} values ranged from 0.36 ± 0.02 cm³ (0 g L⁻¹) to 1.44 ± 0.15 cm³ (15 g L⁻¹), consistently exceeding both V_{DSM} and V_{vox} due to the inclusion of void spaces within the convex envelope. Mean V_{vox} values were consistently the lowest, ranging from 0.16 ± 0.01 cm³ (0 g L⁻¹) to 0.46 ± 0.03 cm³ (15 g L⁻¹), as the voxel approach accounts only for actually occupied spatial units.

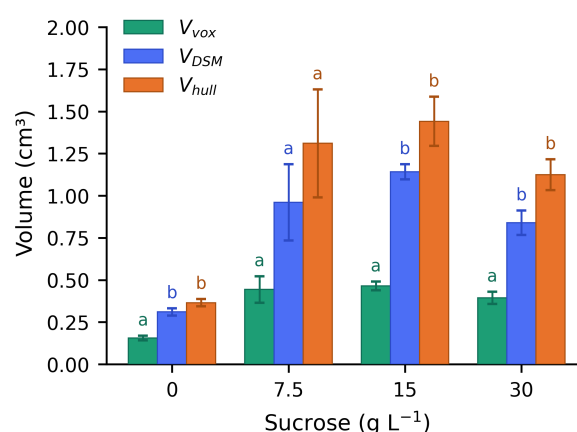


Figure 11. Comparison of three volumetric estimators—DSM-derived volume (V_{DSM} , green), voxel-derived volume (V_{vox} , blue), and convex hull volume (V_{hull} , orange)—measured at t_{30} for blackberry shoots (*Rubus* spp. cv. Thornfree) grown under four sucrose concentrations (0, 7.5, 15, and 30 g L⁻¹). Error bars represent standard error. The three methods differ in their geometric assumptions and systematically yield $V_{vox} < V_{DSM} < V_{hull}$ across all treatments. Different lowercase letters indicate statistically significant differences among treatments for each sugar concentration.

Despite these absolute differences, all three methods showed strong mutual correlations when assessed at the replicate level across treatments (Figure 12). V_{vox} and V_{DSM} were highly correlated ($R^2 = 0.967$, $p < 0.001$), with a regression slope of 0.385 and intercept of 0.010 cm^3 , indicating that V_{vox} estimates approximately 38.5% of V_{DSM} across the observed range. The slope below unity reflects the systematic underestimation of occupied volume by the voxel approach relative to the DSM-based method, consistent with the geometric assumptions of each estimator. V_{hull} was strongly correlated with V_{DSM} ($R^2 = 0.954$, $p < 0.001$, slope = 1.352, intercept = -0.018 cm^3), indicating that the convex hull systematically overestimates DSM-derived volume by approximately 35%, due to the inclusion of void spaces within the convex envelope. The correlation between V_{hull} and V_{vox} ($R^2 = 0.953$, $p < 0.001$, slope = 3.452, intercept = -0.030 cm^3) reflects the cumulative effect of both sources of systematic difference. The high R^2 values suggest that any of the three methods can reliably discriminate among treatments, while the systematic differences in absolute values reflect the distinct geometric assumptions underlying each approach. The near-zero intercepts in all three regressions confirm the proportional nature of the relationships across the full range of shoot sizes observed, with no evidence of systematic offset.

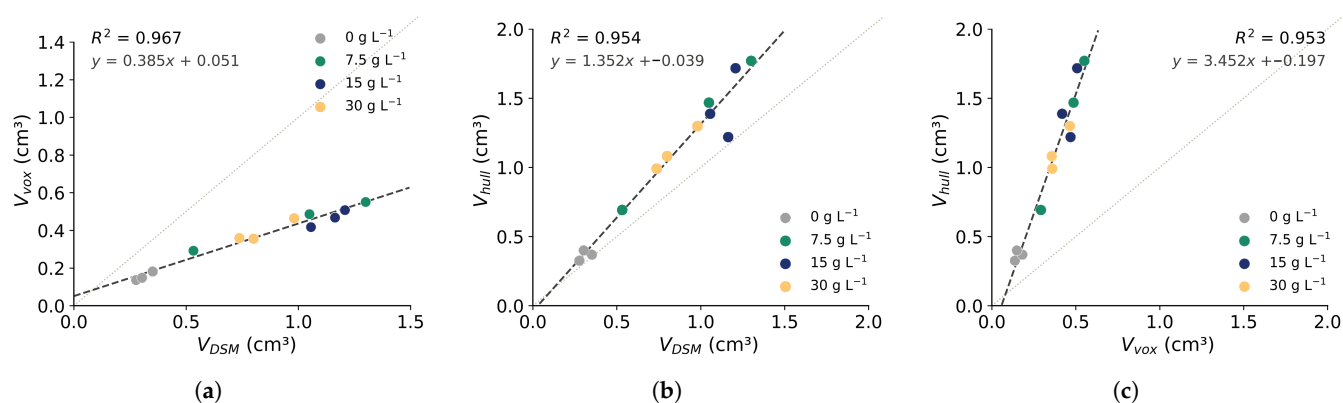


Figure 12. Scatter plots showing the relationships among the three volumetric estimators at t_{30} ($n = 12$ replicates): (a) Voxel-derived volume (V_{vox}) versus DSM-derived volume (V_{DSM}). (b) Convex hull volume (V_{hull}) versus V_{DSM} . (c) V_{hull} versus V_{vox} . Symbol colors indicate sucrose concentration (0 g L⁻¹: grey; 7.5 g L⁻¹: green; 15 g L⁻¹: blue; 30 g L⁻¹: orange). Dashed lines represent linear regression fits; dotted lines are 1:1 references. Regression statistics: (a) $R^2 = 0.967$, slope = 0.385; (b) $R^2 = 0.954$, slope = 1.352; (c) $R^2 = 0.953$, slope = 3.452; $p < 0.001$ in all cases.

4. Discussion

In plant tissue culture, carbohydrates, particularly sucrose, play a crucial role as an energy source and in regulating the osmotic pressure of the medium [42,43]. Therefore, optimizing sucrose concentration in conjunction with growth regulators is essential for achieving better outcomes in micropropagation [16].

At t_0 , the absence of significant differences among sucrose treatments for projected area and V_{DSM} confirms the structural homogeneity of the inoculated material, supporting the validity of the experimental design and ensuring that differences observed at t_{30} can be attributed to the effect of sucrose concentration rather than to pre-existing variability in explant size or architecture.

After 30 days of culture, shoot height, projected area, and volume (V_{DSM}) were significantly higher in sucrose-supplemented treatments compared to the sucrose-free treatment, consistent with the established role of sucrose as the primary carbon and energy source in heterotrophic and mixotrophic in vitro culture systems [15]. The sucrose-free treatment showed virtually no increase in height and markedly lower values of projected area and

volume at t_{30} , suggesting that shoot architectural development was strongly dependent on the availability of exogenous sucrose in the culture medium. Among sucrose-supplemented treatments, the 7.5 and 15 g L⁻¹ concentrations supported the greatest lateral expansion of the shoot cluster, while the 30 g L⁻¹ treatment showed intermediate volumetric values. Although this trend was not statistically significant, the numerical reduction at the highest concentration is consistent with the known inhibitory effect of elevated sucrose levels on tissue hydration and water uptake through osmotic stress [44,45].

The simultaneous quantification of height, projected area, and volume from a single 3D acquisition revealed that the response to sucrose availability manifests primarily as lateral cluster expansion rather than vertical elongation, a pattern that would not be detectable through height measurements alone and highlights the added informative value of three-dimensional phenotyping over conventional single-parameter measurements.

Shoot coverage density increased significantly in all sucrose-supplemented treatments relative to the sucrose-free treatment, reflecting a greater horizontal occupancy of the vessel surface and a more developed leaf area. This parameter, directly derived from the projected area of the shoot cluster relative to the vessel area, provides a rapid and non-destructive estimate of the degree of vessel colonization during the proliferation phase, with potential practical relevance for monitoring culture efficiency.

The area-to-volume ratio (A/V) was significantly lower at 15 g L⁻¹ compared to the sucrose-free treatment, indicating that at intermediate sucrose concentrations the volumetric development of the shoot cluster was proportionally greater than its projected footprint. This suggests a more three-dimensional, vertically developed cluster structure at 15 g L⁻¹, which would not be captured by two-dimensional projected area measurements alone [11,36].

The height-to-area ratio (h/A) was significantly higher in the sucrose-free treatment compared to all supplemented treatments, which did not differ from each other. This pattern reflects the limited lateral expansion of shoots grown without sucrose: these shoots maintained a narrow cluster geometry relative to their projected area, suggesting a growth form characterized by reduced leaf development and limited branching rather than by an increase in absolute shoot height [36].

V_{vox} followed the same pattern as V_{DSM} , with the sucrose-free treatment significantly lower than all supplemented treatments, confirming the consistency between the two volumetric estimators and the robustness of the voxel-based approach for discriminating among treatments.

The voxel occupancy density index (D) showed an inverse trend relative to the absolute volumetric parameters: the 15 g L⁻¹ treatment, which had the largest absolute volume, showed the lowest D value, while the sucrose-free treatment showed the highest. This pattern indicates that shoot clusters that developed at higher sucrose concentrations, despite their greater absolute volume, exhibit a more open and spatially complex three-dimensional structure, with a lower proportion of actually occupied voxels relative to the total DSM-derived envelope. In contrast, shoot clusters in the sucrose-free treatment, while structurally limited in size, maintained a relatively compact and dense spatial organization. These differences in internal structural complexity would be entirely undetectable by projected area or height measurements alone, and represent the specific contribution of voxel-based analysis to the characterization of in vitro shoot architecture. This pattern is consistent with a greater lateral and vertical canopy expansion in sucrose-supplemented treatments, where V_{DSM} increased proportionally more than V_{vox} , suggesting the development of larger and more open foliar structures rather than a denser accumulation of plant tissue within the canopy envelope. D, the ratio between V_{vox} and V_{DSM} , is affected by the estimation errors associated with both volumes' calculation. Therefore, its interpretation as an indicator

of relative spatial occupancy within the shoot cluster envelope should be considered as an approximation rather than an absolute measure of structural occupancy.

The SPAI did not show significant differences among treatments, suggesting that the degree of vertical layering and cumulative spatial occupancy per unit projected area was similar across sucrose concentrations regardless of absolute cluster size. This result may reflect the fact that, under the culture conditions used, leaf area development was proportional to cluster expansion across all supplemented treatments, maintaining a relatively constant ratio between cumulative voxel area and projected area.

The relatively large error bars observed for some parameters, particularly at 7.5 g L^{-1} (Figures 8–11), reflect biological variability among replicate vessels rather than technical measurement error. Each replicate consisted of an independent vessel containing 15 explants, and the observed variability is consistent with the inherent biological heterogeneity of in vitro plant material. The technical contribution to measurement variability is expected to be minimal, as the extraction of geometric parameters from the point cloud is fully objective and automated, eliminating the operator-dependent errors associated with manual measurements such as ruler-based shoot length or fresh weight determination.

The three volumetric estimators (V_{vox} , V_{DSM} , and V_{hull}) yielded systematically different absolute values, with $V_{vox} < V_{DSM} < V_{hull}$ across all treatments, consistent with their respective geometric assumptions. V_{vox} estimates only the volume of spatial units actually occupied by reconstructed points, and therefore provides the most conservative estimate of shoot volume, capturing the effective structural occupancy of the cluster. V_{DSM} integrates the upper surface of the cluster projected downward to the substrate level, including internal void spaces, and therefore represents an intermediate estimate between occupied volume and total envelope. V_{hull} , by enclosing the minimum convex solid containing all reconstructed points [36], systematically overestimates shoot volume by including void spaces both within and around the cluster periphery, and is therefore less suited for precise volumetric characterization of structurally complex shoot clusters.

The systematic hierarchy $V_{vox} < V_{DSM} < V_{hull}$ observed across all treatments is consistent with findings reported in comparable plant phenotyping studies. Convex hull and alpha-shape methods consistently overestimate actual plant volume due to their inability to represent concave surfaces and internal void spaces [46,47], while voxel-based approaches show greater stability when voxel size is appropriately matched to point cloud density [37,48]. Despite these systematic differences in absolute values, all three methods showed strong mutual correlations ($R^2 > 0.95$, $p < 0.001$ in all cases), with near-zero intercepts confirming proportional relationships across the full range of shoot sizes. The regression slopes quantify the systematic ratios among methods: V_{vox} corresponds to approximately 38.5% of V_{DSM} , while V_{hull} exceeds V_{DSM} by approximately 35%. These consistent scaling relationships suggest that all of the three methods can reliably discriminate among treatments, and that conversion factors derived from the regression equations could in principle be used to harmonize volumetric estimates across methods. This result demonstrates that smartphone-based SfM reconstruction produces point clouds of sufficient density and geometric accuracy to support multiple volumetric estimation approaches. The choice of method can therefore be adapted to the specific analytical objective: V_{DSM} as the primary reference estimator given its computational simplicity and robustness; V_{vox} when a more precise characterization of structural occupancy is required; and V_{hull} as a rapid first-order estimate when absolute precision is not critical.

The smartphone-based SfM approach presented in this study offers several advantages over both traditional manual measurements and established 3D sensing technologies. Compared to high-end systems such as terrestrial laser scanning and UAV-based photogrammetry, it offers substantially lower hardware costs, greater portability, and simpler

operational requirements, making it accessible for routine use in micropropagation laboratory settings [23,28,29], while still achieving comparable accuracy, as suggested by direct comparisons between the viDoc RTK system and TLS for plant geometric characterization [35]. The non-destructive nature of the method allows repeated measurements on the same vessels over time, enabling temporal monitoring of shoot architectural development while preserving the biological integrity of the material—an advantage that is particularly relevant in the context of *in vitro* culture, where plant material is often scarce and repeated destructive sampling is not feasible. In terms of operational efficiency, image acquisition required only a few minutes per vessel, while SfM reconstruction and geometric parameter extraction required approximately 20–30 min per vessel with minimal operator intervention during processing. Although the total time per vessel is comparable to that of conventional destructive measurements, which typically require around 30 min with two operators, the key advantage of the proposed approach lies not primarily in time savings but in its non-destructive nature. Unlike destructive methods, SfM data processing is not constrained to the subculture period: image acquisition and processing can be performed independently, and the resulting point clouds and extracted parameters can be revisited and re-analyzed at any time without the need for new sampling. This opens the possibility of monitoring shoot development at time intervals shorter than the subculture duration of retrospectively verifying measurements.

Beyond time efficiency, the three-dimensional nature of the point cloud enables the extraction of information that is fundamentally inaccessible through manual measurements. Shoot volume, spatial occupancy indices, shoot density, and shape descriptors such as the A/V and h/A ratios can only be derived from a full 3D reconstruction, providing an objective and operator-independent characterization of shoot architecture. Unlike manual measurements such as shoot length or fresh weight, which are subject to operator variability and require physical access to individual shoots, the geometric parameters extracted from the point cloud are computed automatically from the same dataset, ensuring full reproducibility and metrological consistency across repeated measurements and different operators. The relationships between smartphone-derived shape indices and manually measured growth traits were investigated by Regni et al. [36], highlighting consistent correlations between the two methods. Furthermore, the spatial distribution of shoots within the vessel, including inter-shoot distances and the degree of lateral overlap, can in principle be quantified from the point cloud, opening perspectives for the study of spatial competition and shoots cluster closure dynamics in *in vitro* culture systems.

In the previous study by Regni et al. [11], the analysis was limited to the planar projection of the 3D point cloud, which was used to estimate the shoot-covered area. In contrast, a key advancement of the present study is the full exploitation of the three-dimensional information provided by the SfM reconstruction, which enables the extraction of structural descriptors, including shape and density indices, thereby providing a more comprehensive characterization of shoot architecture.

Compared to high-end TLS systems and structured-light scanners, which achieve submillimetre accuracy [49] but at high costs and operational complexity not well suited to routine laboratory use, the smartphone-based SfM approach proposed here represents a cost-effective alternative, consistent with previous demonstrations of low-cost consumer-grade 3D acquisition systems for plant phenotyping [50,51], while retaining submillimetre reconstruction accuracy, as confirmed by the point cloud quality analysis reported in Regni et al. [11].

Some limitations must nonetheless be acknowledged. The method is sensitive to the degree of shoot overlap within the vessel: although this issue was observed in only a limited number of vessels, at t_{30} , manual correction of the automatic segmentation was required

for some cases due to lateral cluster expansion, introducing a subjective component in the delineation of individual shoots. Occlusions in the outer portions of the point clouds may occasionally occur for shoots positioned close to the vessel wall; however, these are effectively addressed through the color-based filtering procedure and do not introduce systematic errors in the extracted parameters.

Future developments should focus on the automation of the segmentation pipeline to improve throughput and reproducibility. The sensitivity of the voxel-based analysis to slice thickness and the comparative performance of the three volumetric estimators under different shoot architectures and culture conditions represent further methodological aspects that warrant systematic investigation in future studies. Furthermore, the acquired images could be used as a basis for deriving additional descriptors such as radiometric indices, further extending the analytical potential of a single acquisition session.

5. Conclusions

This study demonstrated the applicability of a low-cost, smartphone-based SfM approach for the non-destructive, three-dimensional characterization of *in vitro* blackberry shoot architecture. From a single photogrammetric survey, the workflow enabled extraction of geometric and voxel-based structural parameters including shoot height, projected area, volume, voxel occupancy density, and the SPAI without compromising the biological integrity of the cultured material.

The approach proved effective in detecting sucrose-dependent architectural responses that would not be captured by conventional single-parameter measurements, in particular distinguishing lateral cluster expansion from vertical elongation. The voxel occupancy density index and SPAI provided structural information complementary to volumetric parameters, capturing internal spatial organization beyond what projected area, height, or volume alone can describe. The three volumetric estimators tested (V_{vox} , V_{DSM} , V_{hull}) showed strong mutual agreement, confirming that smartphone-based SfM approach produces point clouds of proper density and accuracy to support multiple estimation strategies, with V_{DSM} recommended as a simple and robust default.

Overall, the proposed method offers a non-destructive, repeatable, and objective alternative to conventional measurements for monitoring shoot architectural traits in micro-propagation systems. Its integration into routine workflows could support culture medium optimization, early detection of treatment effects, and standardization of propagation protocols. Future work should focus on automating the segmentation pipeline and extending the method to a wider range of species, explant densities, and culture conditions.

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Abbreviations

The following abbreviations are used in this manuscript:

DSM	Digital Surface Model
GNSS	Global Navigation Satellite System
LiDAR	Light Detection and Ranging
RTK	Real-Time Kinematic
SfM	Structure from Motion
TLS	Terrestrial Laser Scanning
UAV	Unmanned Aerial Vehicle

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