Plant Cell, Tissue and Organ Culture (PCTOC) (2025) 163:63
https://doi.org/10.1007/s11240-025-03267-0

ORIGINAL ARTICLE

Micropropagation of blackberry and blueberry: assessing the effects of subculture duration and explant density through the integration of traditional measurements and smartphone 3D imaging

Luca Regni[1] · Silvia Calisti[1] · Arianna Cesarini[1] · Laura Marconi[2] · Primo Proietti[1] · Sara Zollini[3] · Raffaella Brigante[2]

Received: 9 September 2025 / Accepted: 18 October 2025

Abstract

The cultivation and consumption of blackberry and blueberry are expanding, with micropropagation emerging as the dominant propagation method. In the present study the influence of explant density and subculture duration on blackberry (*Rubus* spp. cv. 'Thornfree') and blueberry (*Vaccinium corymbosum* L. cv. 'Brigitta') was investigated. Four initial densities (6.64, 4.43, 3.32, and 2.21 cm² per explant, considering a vessel base area of 66.48 cm²) and two subculture durations (30 and 45 days for blackberry; 45 and 60 days for blueberry) were tested. Hereafter, the four densities will be referred to as 10, 15, 20, and 30 explants per vessel. Growth performance was assessed through shoot viability, number and length, callus formation, fresh and dry biomass, chlorophyll content, and canopy area, the latter quantified via high-resolution 3D modeling using an iPhone 15 Pro Max, viDoc RTK Rover, and Pix4Dcatch. In blackberry, subculture duration was the primary determinant of growth. Rooting occurred only at 45 days, while mean shoot length was lower at 45 days and this was due to increased shoot number and total shoot length. Explant density affected the dry weight that was higher with 10 explants due to a higher callus development. The chlorophyll content decreased at the higher explant density tested. The covered area per explant increased with explant density and subculture duration, reaching maximum values at 30 explants and at 45 days; shoot density followed the same trend. In blueberry, subculture duration significantly affected shoot length, callus fresh weight, and biomass accumulation. Longer subcultures (60 days) enhanced shoot length, callus weight, and dry weight, although shoot fresh weight remained unchanged. Chlorophyll content was not affected by either the number of explants or the duration of subculture. The highest values of the covered area per explant were observed with 10 explants and the lowest with 30 explants. Covered area also increased at 60 days of subculture. Shoot density decreased with increasing explant number but increased with subculture duration. These findings identify subculture duration as the key driver of micropropagation efficiency and demonstrate, for the first time, the potentiality of integrating digital 3D phenotyping to optimize protocols for blackberry and blueberry micropropagation. These findings identify subculture duration as the key driver of micropropagation efficiency and demonstrate, for the first time, the potentiality of integrating digital 3D phenotyping to optimize protocols for blackberry and blueberry micropropagation. Digital imaging further revealed species-specific responses: in blackberry, shoot-covered area and shoot density increased with both explant number and subculture duration; in blueberry, instead, shoot-covered area and density decreased with increasing explant number but increased with subculture duration.

Communicated by Melekşen Akın

✉ Luca Regni
luca.regni@unipg.it

[1] Department of Agricultural, Food and Environmental Sciences, University of Perugia, 06121 Perugia, Italy

[2] Department of Engineering, University of Perugia, Via G. Duranti 93, 06125 Perugia, Italy

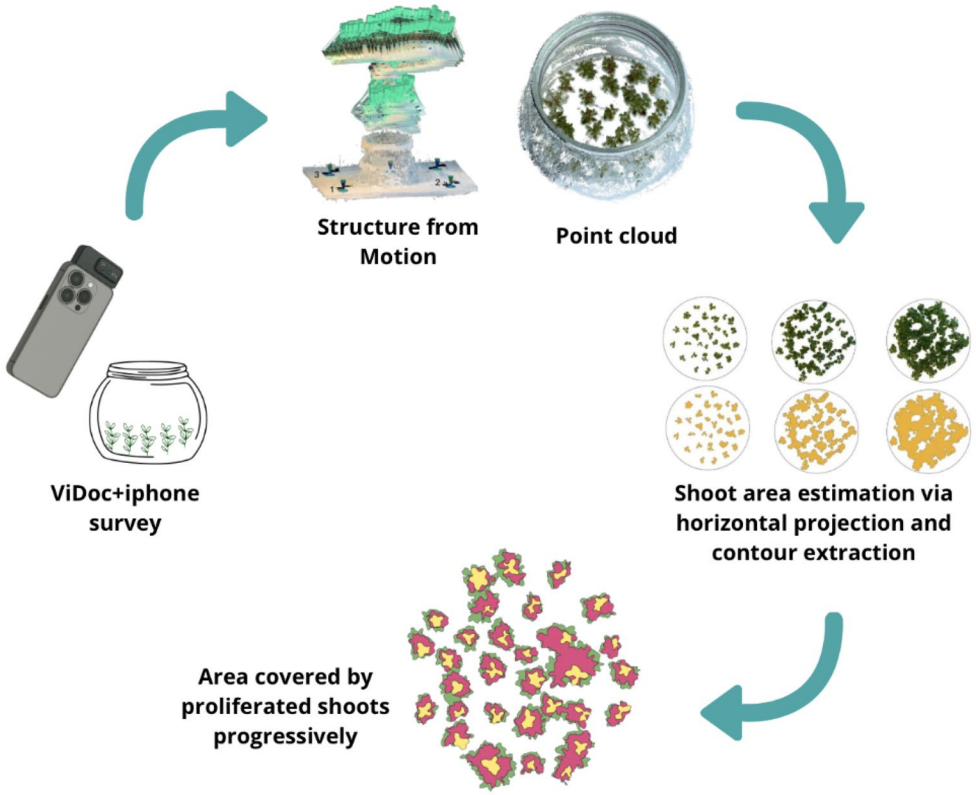
[3] Department. of Civil, Construction-Architectural & Environmental Engineering, University of L'Aquila, Piazzale Ernesto Pontieri 1, Monteluco di Roio, 67100 L'Aquila, Italy

Published online: 30 October 2025

Key message

The integration of digital 3D images and traditional measurements enables data-driven optimization of blackberry and blueberry micropropagation, identifying subculture duration as a key parameter and revealing distinct, species-specific growth responses.

Graphical abstract



Keywords *Rubus* spp. · *Vaccinium* spp. · *In vitro* culture · viDoc RTK rover · SfM · LiDAR

Introduction

Over the past two decades, blackberry and blueberry consumption has increased, resulting in a corresponding rise in production (Peano et al. 2017; Ștefănescu et al. 2020). This growth in demand can be attributed to the distinctive flavor of berries, while health-conscious consumers increasingly recognize the beneficial effects of phenolic compounds, which exhibit significant biological activity and contribute to human health through their antioxidant properties. (Cho et al. 2005; Wu et al. 2023). Phenolic compounds are naturally occurring secondary metabolites in plants and are known for antioxidant, anti-inflammatory, and anticancer properties, making them of considerable interest in food and pharmaceutical sectors (Kodikara et al. 2024).

For both blueberries and blackberries, the traditional agamic propagation methods by stem cuttings and layering have long been employed to maintain genetic fidelity and desirable agronomic traits (Reed et al. 2017; Vujović et al. 2017; Harutyunyan et al. 2022; Mazurek et al. 2024). However, these techniques present several limitations, including low rooting success, labor-intensive procedures, and reliance on seasonal and climatic factors, which hinder large-scale and year-round seedling production (Gomes et al. 2017; Mazurek et al. 2024). Highbush blueberries propagated through semi-woody cuttings often exhibit low rooting percentages, making this method inefficient for the rapid multiplication of new cultivars (Fan et al. 2017). Similarly, blackberry propagation through layering or cuttings requires extensive plantation areas and substantial labor input, posing challenges in weed management and overall scalability (Gomes et al. 2017).

To overcome these limitations, micropropagation—an in vitro culture technique—has emerged as an alternative, offering several advantages over traditional methods (Bobrowski et al. 1996; Gomes et al. 2017; Reed et al. 2017), including

the production of genetically homogeneous and pathogen-free plant material, as well as the ability to rapidly generate large numbers of plants in a small space and in a short time (Kavand et al. 2011). Additionally, this technique supports germplasm conservation, pathogen elimination, and genetic manipulations while optimizing resource utilization for cost-effective plant production (Dönmez et al. 2024).

In blackberry micropropagation, Murashige and Skoog (MS) medium (Murashige and Skoog 1962) at full and half strength was commonly used for stabilization, while multiplication favored MS at half, full, or double strength, with Woody Plant Medium (WPM) (Lloyd and McCown 1980) as an alternative (Regni et al. 2025; Regni and Cesarini 2025).Rooting was mainly achieved with MS (full or half strength) and WPM, both effective in promoting root development (Regni et al. 2025; Regni and Cesarini 2025).

Similarly, in blueberry micropropagation, WPM medium has proven to be the most effective for in vitro culture. Its composition offer better results than other media such as MS or Driver and Kuniyuki Woody Plant Medium (Driver and Kuniyuki 1984) (DKW) (Phillips and Garda 2019; Correia et al. 2024).

The traditional methods for growth monitoring are often destructive and time consuming. Recent advances in image-based analysis have demonstrated strong potential for non destructive, non-invasive, objective, and automated monitoring of plant cultures in vitro. Traditional approaches have utilized both microscopic and macroscopic imaging to evaluate cell and tissue parameters such as color, shape, growth rate, and aggregate size distribution (Ibaraki and Kenji 2001). Macroscopic imaging, in particular, offers advantages in acquisition simplicity, as it allows data collection from outside the culture vessel without specialized equipment (Ibaraki and Kenji 2001). Image analysis techniques have also been effectively integrated with neural network models to classify developmental stages in somatic embryogenesis, enabling accurate predictions of regeneration potential based on morphological traits such as area, circularity, and shape ratios (Ibaraki and Kenji 2001; Niazian et al. 2018). The development of 3D models further enhances digital plant phenotyping by enabling volumetric and spatial analyses of plant structures (Ivaschuk et al. 2023). Moreover, low-cost, automated systems have been proposed for in situ monitoring, combining image acquisition with sensor data to track culture progression under controlled conditions (Bethge et al. 2023). In parallel, Red Green Blue (RGB) based image analysis methods have proven effective in estimating physiological parameters such as chlorophyll content, offering a fast and non-destructive alternative to traditional chemical or optical measurements, although genotype-specific calibration may still be required (Treder et al. 2021). Since the 1990s, the availability of affordable CCD/CMOS sensors enabled the first applications of imaging in crop breeding, also related to GMO, providing non-invasive, objective trait measurements that complemented molecular selection, though large-scale industrial adoption was still limited by cost and logistics (Li et al. 2014; Walter et al. 2015; Feng et al. 2017; Kamle et al. 2017). Previous studies have also applied digital image analysis in vitro to derive physiological indices, such as MNDVI and G/R, correlating them with culture status (Aynalem et al. 2006). The methods did not allow to evaluate the three-dimensionality which can instead be assessed using other methods such as Light Detection and Ranging (LiDAR) and digital photogrammetric techniques. In this context, to the best of our knowledge, no studies to date have applied the smartphone LiDAR in combination with viDoc RTK Rover to monitor plant development in tissue culture. This methodology, originally developed for terrestrial and field surveying, provides centimeter-level positional accuracy and high-resolution imaging capabilities, making it suitable for precise measurements at the scale of individual explants. Some early applications of viDoc with Pix4D technologies include construction surveying and outdoor vegetation scanning (Zollini and Marconi 2025), but none within the context of in vitro plant phenotyping. To date, most implementations of this system have been concentrated in the field of cultural heritage documentation and preservation (Rapuca and Matoušková 2023; Aksoy 2025). Beyond that, only limited research has explored its potential in other domains, including roadway mapping (Suleymanoglu et al. 2023; Tamimi and Toth 2023), environmental monitoring (Chauvin 2023) and performance validation studies focused on assessing the system's measurement accuracy (Tamimi 2022). Our work addresses this gap by exploring the potential of this integrated system for non-destructive, high-resolution monitoring of plant development in tissue culture.

In this context, the aim of the present study was to analyze the effect of explant density and subculture duration on micropropagation for blackberry and blueberry also employing an innovative technique that integrates smartphone-based LiDAR scanning with photogrammetry through an iPhone equipped with the viDoc RTK Rover. Unlike traditional 2D imaging, this non-destructive and contactless method enables the reconstruction of accurate 3D models, allowing repeated, objective, and replicable measurements through automated processing. The proposed workflow offers a compact, low-cost solution for high-resolution digital phenotyping that, to our knowledge, has not yet been applied to in vitro plant culture, focusing on geometric traits, using both 2D and 3D analyses.

Materials and methods

Plant material

In vitro-cultured blackberry proliferated explants (′Thornfree′) and blueberry (′Brigitta′) from the 'Micropropagation and In Vitro Biotechnology Laboratory' of the Research Unit 'Tree Science' of Department of Agricultural, Food and Environmental Sciences -University of Perugia (Italy) were used.

′Thornfree′ is a vigorous cultivar with a late ripening period and an extended harvest season. Its fruits are large, oblong, glossy, and black in color. They have low firmness due to high juice content. This cultivar exhibits moderate resistance to pests and diseases while showing good tolerance to low temperatures (Stanisavljevic 1998). ′Brigitta′ is a very vigorous cultivar (Beccaro et al. 2011). It is a mid-season variety, ripens at middle July—first week of August (Spinardi et al. 2019) Berries are of good size and have high sugar content, they are suitable for cold storage. It is not very productive cultivar and is sensitive to winter colds (Beccaro et al. 2011).

Sub-culture set-up and growth conditions

Blackberry explants were grown on a medium consisting in an half-strength Murashige and Skoog macro and micro-nutrients and vitamins (Murashige and Skoog 1962), inositol (5 g L^{-1}), Indole-3-butyric acid (IBA) (0.1 mg L^{-1}), 6-Benzylaminopurine (BAP) (0.4 mg L^{-1}), sucrose (15 g L^{-1}), agar (8 g L^{-1}), pH of 5.7.

Blueberry explants were grown on a medium consisting in Woody Plant Medium (WPM) (Lloyd and McCown 1980) macro and micro-nutrients, Murashige and Skoog (Murashige and Skoog 1962) vitamins, inositol (10 g L^{-1}), IBA (0. 01 mg L^{-1}), zeatin (0.5 mg L^{-1}), sucrose (30 g L^{-1}), agar (7 g L^{-1}), pH 5.6.

Glass jars (8.5 cm high × 9.2 cm in diameter, 500 ml capacity), each containing 100 ml of the growth medium described above, were used. Jars and substrate were autoclaved for 20 min at 115 °C before utilization. The initial explants were represented by 1.5 cm long single shoots for blackberry and for blueberry. Explants were uniformly distributed within each vessel that were closed with a glass lid and covered with plastic film and four initial explant densities (10, 15, 20, and 30) were tested. Considering a vessel base area of 66.48 cm^2, the densities correspond to 6.64, 4.43, 3.32, and 2.21 cm^2 available per explant, respectively. Two subculture durations (30 and 45 days for blackberry and 45 and 60 days for blueberry) were considered. All the plant material manipulations were carried out in sterile conditions using a horizontal laminar flow cabinet. The jars containing the 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 each treatment, six jars (replicates) were established at the beginning of the experiment. Three vessels were used for destructive measurements, while the proliferated esplants of the other three vessels were used as starting material for the subsequent subcultures. The plant material was subcultured three consecutive times. At the end of each subculture, destructive measurements on the proliferated shoots of three jars were carried out and the plant material of the remaining vessels was used to start the subsequent subcultures.

Growth parameters

At the end of each subculture, on the proliferated explants the following parameters were measured:

- Viability (%): count of green and viable explants, referred to as total explants for each jar;
- Shoots (n): count of the number of shoots developed;
- Shoot length (mm): length of developed shoots;
- Callus (%): count of explants that produced basal callus, referred to as total explants for each jar;
- Rooting (%): count of explants that produced roots, referred to as total explants for each jar;
- Roots (n): count of the number of roots developed;
- Root length (mm): length of developed roots;
- Shoot fresh weight (FW) (mg): FW per explant of leaves and shoots;
- Callus FW (mg): FW per explant of callus masses;
- Root FW (mg): FW per explant of roots;
- Total dry weight (mg): dry weight per explant of leaves, shoots, callus masses, and roots measured with a precision balance after drying the plant material in an oven for three days at 105 °C.

Cholorophyll content

For each treatment three samples of 100 mg of proliferated shoots were extracted with 10 mL acetone/water (4:1, v: v) in a mortar using a pestle and liquid nitrogen and centrifuged at 5000 rpm for 10 min. The supernatant was collected and Chlorophyll a (Chl a), Chlorophyll b (Chl b) and Total Chlorophyll (Total Chl) contents were determined (Aly et al. 2022) spectrometrically (Aly et al. 2022).

IPhone + viDoc survey

All blackberry and blueberry samples, consisting of three replicates (A, B, and C) for each explant density (10, 15,

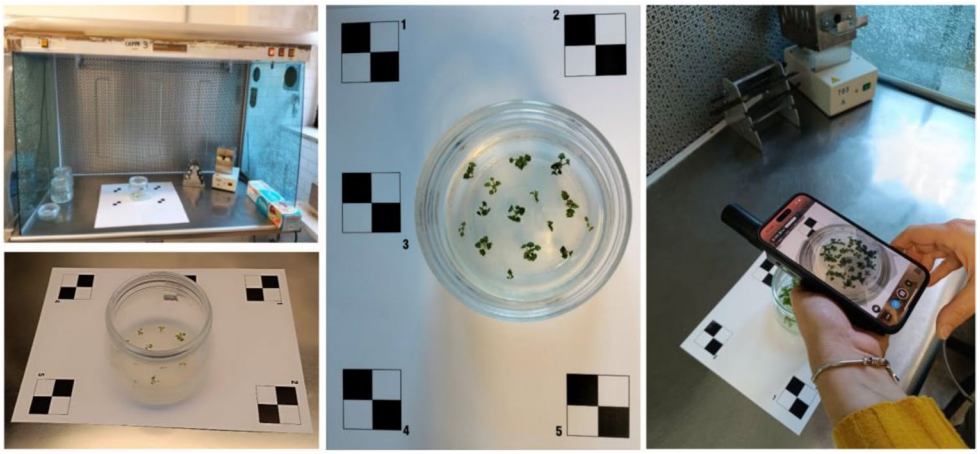


Fig. 1 Setup for 3D data acquisition: controlled capture environment, sample container on checkerboard target base, and viDoc RTK Rover with iPhone survey

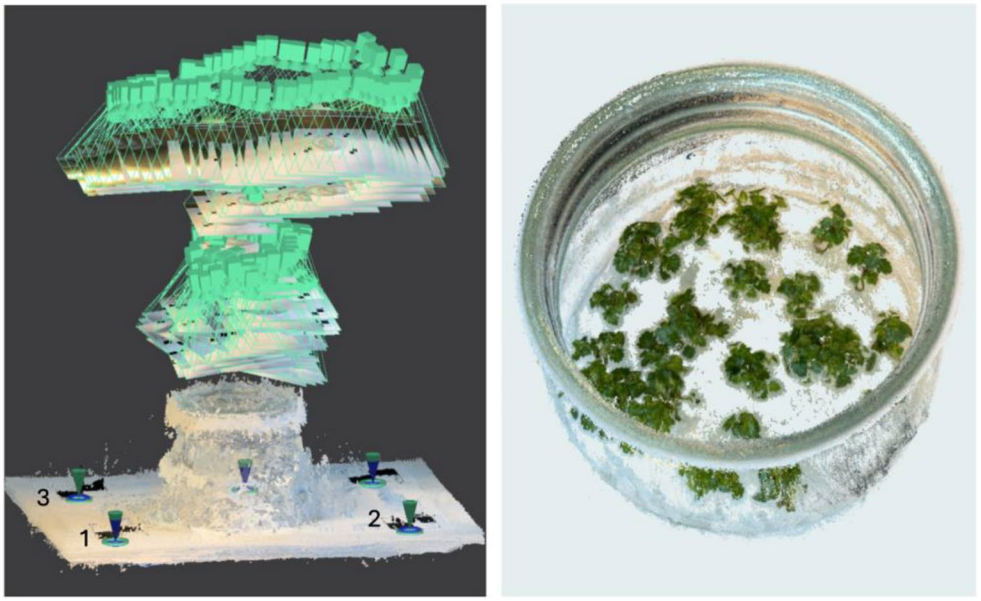


Fig. 2 Pix4Dmatic interface showing the image alignment and camera positions during the reconstruction process (left). Resulting dense 3D point cloud (right)

20, and 30 shoots), were surveyed at specific stages using the viDoc RTK Rover system connected to an Apple iPhone 15 Pro Max (Fig. 1). The viDoc RTK Rover (viDoc 2025), a GNSS device developed in Germany, is equipped with an RTK antenna and specifically designed to interface with Apple devices featuring LIDAR sensors (iPhone 2025). When used in combination with the Pix4Dcatch mobile application (Pix4D 2025), this setup enables the real-time acquisition of high-precision, geo-referenced images, suitable for generating accurate 3D models.

Surveys were conducted at the beginning of the experiment and at 30, and 45 days for blackberry and at 45, and 60 days for blueberry.

Each sample was placed on a base containing five checkerboard targets with known coordinates, which were used for the data post-processing and point clouds extraction. Data acquisition was carried out under a horizontal laminar flow cabinet. On average, 150 images per sample were captured with the device manually moved over the culture vessel, at various distances and inclined views to also reconstruct areas hidden by upper leaves. The containers were never touched directly, and the entire data collection process required only a few minutes per sample.

3D point cloud reconstruction and shoot extraction

The data collected with the iPhone and viDoc system were processed using Pix4Dmatic software and the Structure-from-Motion (SfM) algorithms (Eltner and Sofia 2020) which allow accurate reconstruction of both camera positions and orientations during acquisition, as well as the 3D model. RGB images were used to generate a detailed dense point cloud, while LiDAR data provided the corresponding depth maps. These datasets were subsequently merged to produce a single, fused point cloud, averaging approximately 2 million points per sample (Fig. 2).

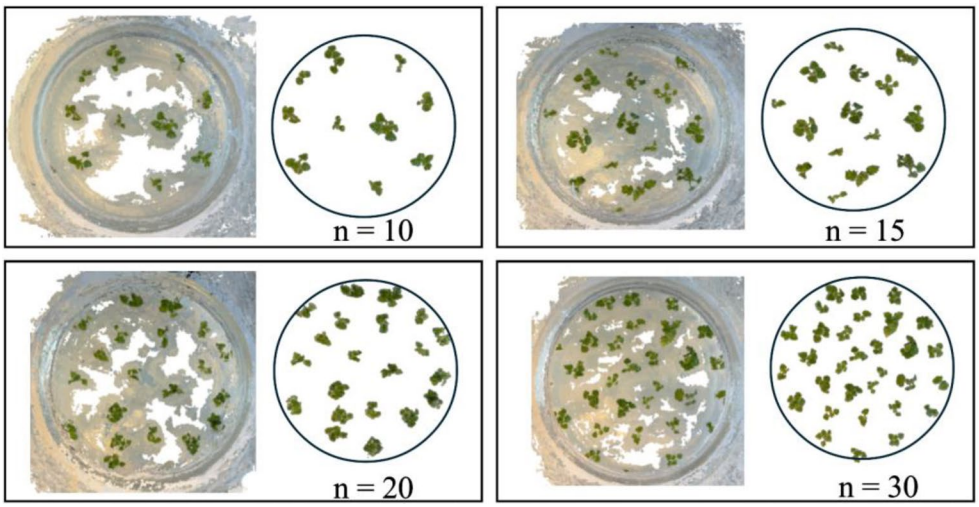


Fig. 3 Shoot isolation from original point clouds for blackberry samples containing 10, 15, 20, and 30 shoots, respectively

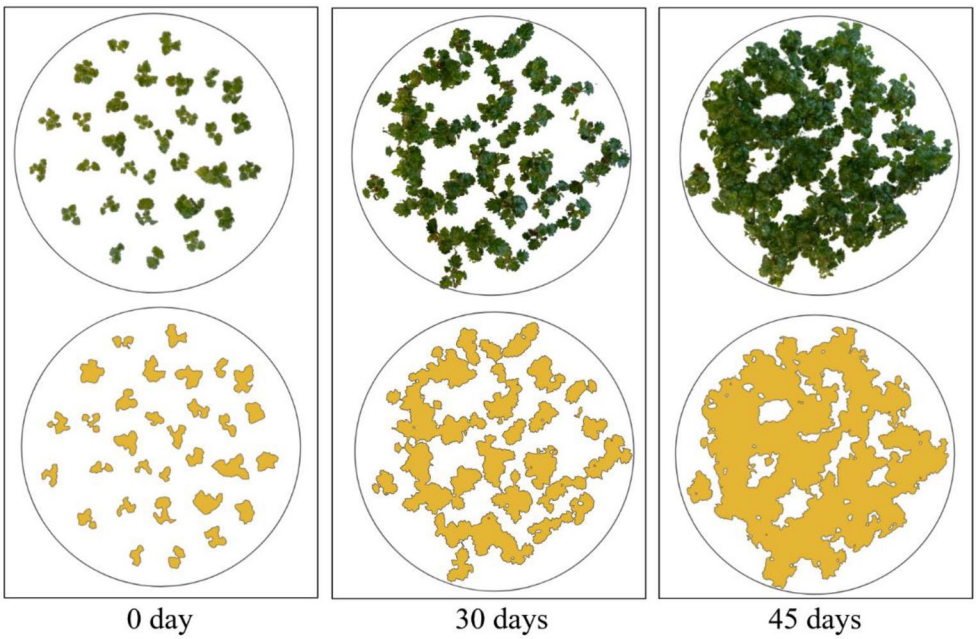


Fig. 4 Horizontal projection of isolated shoots (green points) with extracted contours (orange polygons) for shoot area estimation (blackberry samples with 30 shoots at the beginning of the experiment, and at 30 and 45 days)

To improve data quality, all point clouds were subjected to noise filtering to remove outliers and non-representative data points. Shoot isolation and canopy surface estimation were performed using Cyclone 3DR software, v. 2024.0. The original point cloud of each sample was initially filtered by color using the "Real Colors" tool which splits clouds according to the real color on each point. A few manually selected light-colored points, corresponding to the container, base, and surrounding elements, were used as reference for filtering. Points with similar color values were automatically grouped into a separate point cloud, allowing the removal of all non-green elements and the retention of only the green points corresponding to the shoots. The resulting shoot-only point cloud was then noise-filtered to eliminate isolated or erroneous points (Fig. 3).

The cleaned point cloud, consisting exclusively of shoot points, was projected onto a horizontal plane. From this projection, the planar contour was extracted to compute the covered surface area (Fig. 4).

To account for initial size differences among explants, the covered canopy surface of each sample was first divided by the number of explants to estimate the average surface area per shoot, and then further normalized to the container surface to calculate shoot density; this procedure allowed direct comparison of growth and morphological traits across the different density treatments.

Impact of GCPs on point cloud quality

In order to evaluate the impact of Ground Control Points (GCPs) on point cloud extraction, two independent

processing workflows were carried out: one using the known coordinates of GCPs (Fig. 2) during the image alignment and point cloud generation phases, and the other without incorporating GCPs. This approach aimed to verify whether the point clouds generated without the use of GCPs could still achieve a high level of accuracy relative to the size of the surveyed object.

First of all, the GCPs positions extracted from each point cloud derived from the GCP-based workflow were compared with a priori known positions, resulting in a zero mean error in both planimetry and elevation, with standard deviations of 0.3 mm and 1.2 mm, respectively.To assess the feasibility of a faster processing workflow, the analysis aimed to determine whether the point cloud generated without GCPs was sufficiently accurate in metric terms for the type of application considered in this study—namely, the survey of extremely small-scale objects. Therefore, the distances between GCPs (1–2 and 1–3 in Fig. 2) were measured in both resulting point clouds (with and without GCPs), and for each distance, the ratio between the measured value and the known reference distance was calculated. For the model processed without GCPs, the distances were slightly underestimated, with an average ratio of 0.97, whereas for the model processed with GCPs, the distances closely matched the actual dimensions, showing a ratio of 1.00. These results confirm that both approaches provide sufficient metric accuracy for small-scale objects, supporting the use of the viDoc RTK Rover and iPhone LiDAR system in in vitro shoot analyses. In the present study, the GCP-based model wasused for subsequent analyses of shoot-covered area measurements, in order to base the data processing on the most accurate model possible.

Data analysis

The trial was organized according to a completely randomized design. The experiment was conducted three times with three replicates ($n=9$). The data collected were subjected to various tests to verify the variance hypotheses and in particular homogeneity of variance was assessed by Levene's test and normal distribution by D'Agostino-Pearson omnibus normality test. The significance of differences was analysed using the Duncan's test ($p<0.05$) after analysis of the variance according to a two-way ANOVA analysis (split-plot design). Data on percentages were arcsine-transformed before performing statistical analysis.

Results

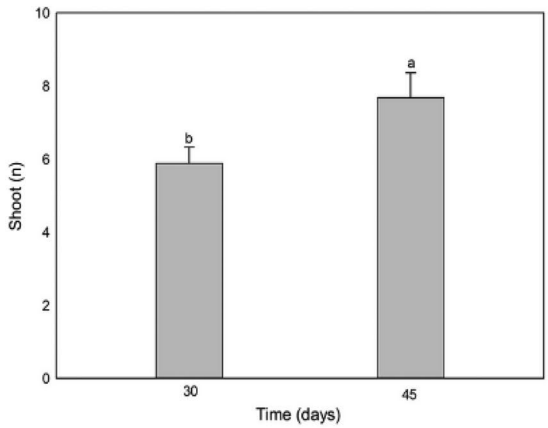
Blackberry

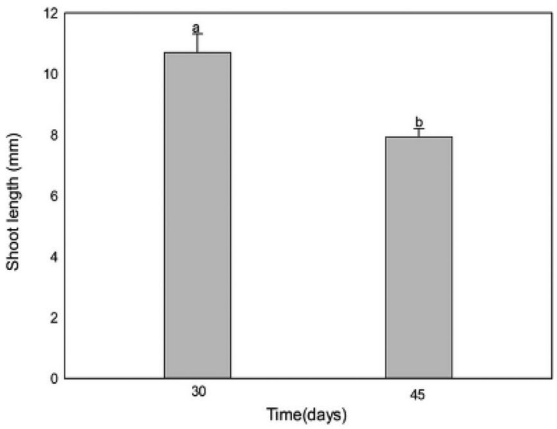
The duration of subculture and to a lesser extent the number of explants significantly influenced key parameters of blackberry micropropagation (Table S1). Subculture duration had a significant effect on many of the measured parameters. Specifically, it influenced the number of shoots, shoot length, total shoot length, callus percentage, rooting percentage, number of roots, root length, root weight, callus fresh weight, shoot fresh weight, total dry weight and Chl b, highlighting its central role in both shoot and root development as well as biomass accumulation (Table S1). In contrast, explant density only had a significant effect on callus fresh weight and as a consequence the total dry weight was higher at a density of 10 explants per vessel and on Chl a, and Total Chl content (Table S1). The interaction between explant density and subculture duration was significant only for the total shoot length (Table S1). Overall, subculture duration appeared to be the dominant factor in optimizing in vitro propagation efficiency in blackberry. In particular, callus formation percentage increased at 45 days of subculture but no significant differences were observed in callus weight (Fig. 5). Root development was only recorded at 45 days, suggesting a minimum subculture duration requirement for rooting induction (Fig. 5). The increase in total shoot length at 45 days was mainly due to the higher number of shoots per explant, as the individual shoots were shorter but more numerous, resulting in enhanced overall proliferation (Fig. 5).

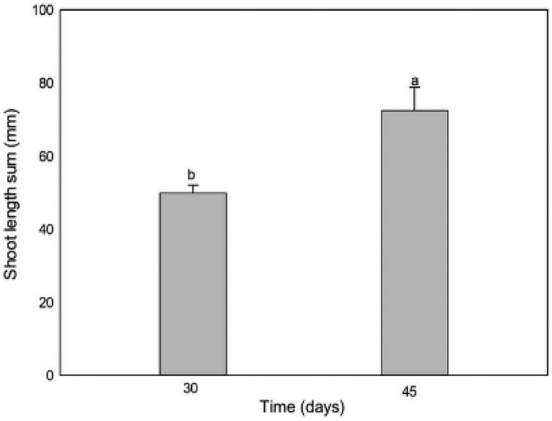
In contrast, shoot fresh weight per explant was significantly higher at 30 days, likely due to the production of fewer but more robust shoots (Fig. 6). Total dry weight did not differ significantly between the two subculture durations (Fig. 6). The Chl a and as a consequence Total Chl content decreased at the highest explant density tested (30 explants) (Fig. 7). Figure 8 reports photos illustrating the development of blackberry explants under different subculture durations and explant densities.

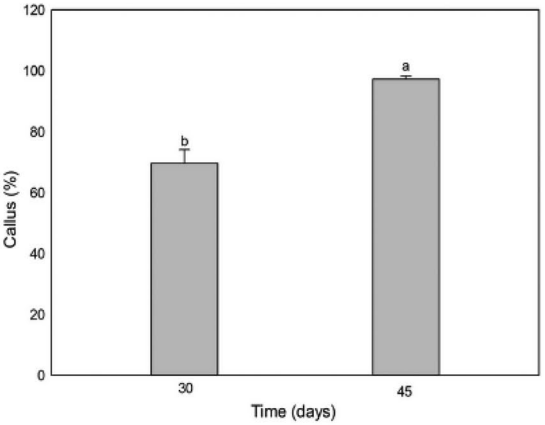
The shoot coverage areas were compared across the four initial densities and survey stages. The covered area per explant was influenced by both density and subculture duration, showing the lowest values at 10 explants and the highest at 30 (Table 1). It also increased at 45 days of subculture (Table 2). The highest values for shoot density were found with 30 explants (Table 1) and at 45 days of subculture (Table 2).

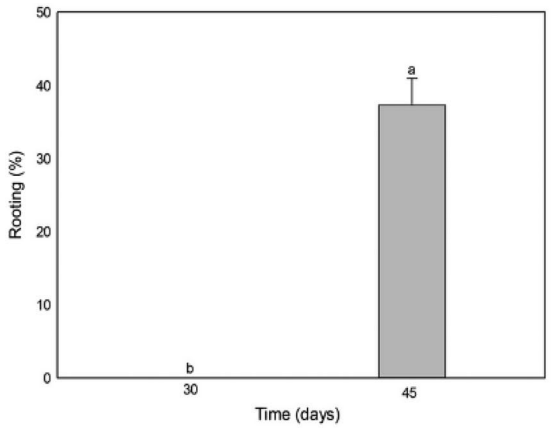
To visually assess shoot development dynamics across different explant densities, the shoot-covered areas extracted from the point clouds were superimposed for all four densities (10, 15, 20, and 30 shoots) (Fig. 9).

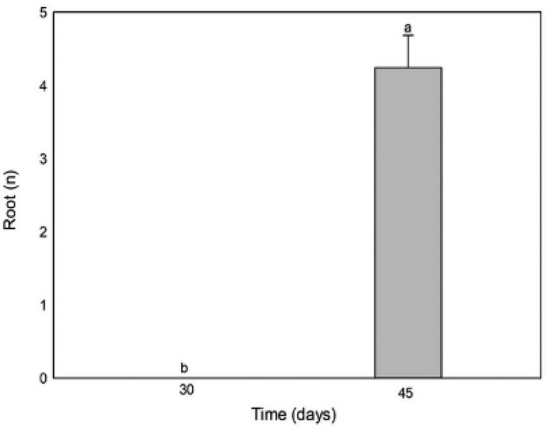

Shoot (n)
10
8
6
4
2
0
b
a
30
45
Time (days)


Shoot length (mm)
12
10
8
6
4
2
0
a
b
30
45
Time(days)


Shoot length sum (mm)
100
80
60
40
20
0
b
a
30
45
Time (days)


Callus (%)
120
100
80
60
40
20
0
b
a
30
45
Time (days)


Rooting (%)
50
40
30
20
10
0
b
a
30
45
Time (days)


Root (n)
5
4
3
2
1
0
b
a
30
45
Time (days)

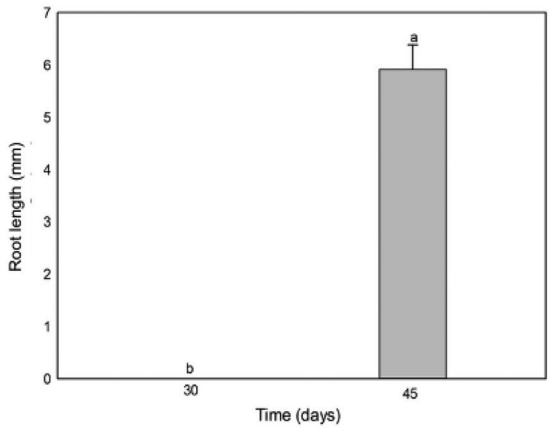

Root length (mm)
7
6
5
4
3
2
1
0
b
a
30
45
Time (days)

Fig. 5 Shoot (n), shoot length (mm), shoot length sum (mm), callus (%), rooting (%), root (n), and root length (mm) values ± SE of the blackberry proliferated explants. Mean values followed by different letters were significantly different ($p < 0.05$)

The overlays show a marked increase in shoot-covered area over time, with progressively larger expansions at higher densities, indicating an intensification of shoot proliferation during the observed period (Fig. 9).

Blueberry

Subculture duration had a significant influence on shoot length, callus fresh weight, shoot fresh weight, and total dry weight, indicating a strong role of subculture duration in shoot development and biomass accumulation (Table S2). In contrast, explant density did not significantly affect any of the measured variables, and no interaction effects between explant density and subculture duration were observed (Table S2). No rooting was observed at any explant density or under either subculture duration tested. Chl a and Total Chl content were affected by subculture duration (Table S2).

Shoot length significantly increased between day 45 and day 60, indicating enhanced shoot elongation over this period (Fig. 10). Similarly, callus fresh weight was higher at 60 days, as well as Total DW (Fig. 10). The increase in total DW was attributed to the greater accumulation of callus biomass, as shoot fresh weight at 60 days was not higher than that observed at 45 days (Fig. 10). These findings highlight that while shoot elongation continues with extended culture, the gain in total biomass is primarily driven by callus development rather than shoot tissue growth. A decrease in total Chl content was observed at 45 days of subculture (Fig. 11). In Fig. 12 the photos showing the development of explants of the blueberry related to subculture duration and explant density are presented.

The covered area per explant varied with density and subculture duration, with the highest values observed at 10 explants and the lowest at 30 (Table 3). It also increased by 60 days of subculture (Table 4). The shoot density decreased with the increase of explant number (Table 3) and increased with the subculture duration (Table 4).

The overlays of shoot-covered areas extracted from the point clouds across all four densities and survey times illustrated a consistent and gradual expansion of shoot-covered areas over time, with relatively uniform growth patterns observed among the different densities (Fig. 13).

Discussion

The findings regarding the duration of subculture and the density of explants in blackberry and blueberry micropropagation reveal significant insights into optimizing plant propagation in vitro. It has been observed that longer subculture duration positively influences various growth parameters including shoot number, shoot length, callus percentage, and root development emphasizing its critical role in successful micropropagation outcomes. In particular, it is important to highlight that at 45 days of subculture in the blackberry proliferated shoots rooted spontaneously, thus making it possible to obtain plants ready for the acclimatisation phase. This finding is supported by literature which indicates that prolonged subculturing can enhance both shoot and root development as well as overall biomass accumulation, thereby improving propagation efficiency (Vujović et al. 2012; Kefayati and Kafkas 2018; Aly et al. 2022). However, attention must be paid since too long subculture duration can led hyperhydricity, which is frequently caused by the accumulation of ethylene in in vitro cultures (Park et al. 2004).

The density of explants during in vitro culture also plays a crucial role in determining the efficiency and outcome of micropropagation protocols. In the present study, explant density influenced total dry weight and chlorophyll content in blackberry, with higher values observed at lower densities, and affected covered area per explant and shoot density, which were higher at greater densities. Overall, the findings in the present study highlight that low explant densities in blackberry favor shoot multiplication per explant and this is particularly relevant for micropropagation efficency. Comparable multiplication rates have been reported for blackberry cv 'Thornfree' by (Lepse and Laugale 2009) who observed a multiplication rate of 4.0 for the 'Thornfree' cultivar on MS medium supplemented with 1.0 mg L^{-1} BAP, 3.6 with MS containing 0.5 mg L^{-1} BAP and 0.25 mg L^{-1} IAA, and 4.0 when MS was enriched with Fe-EDTA, 1.0 mg L^{-1} BAP, and 0.05 mg L^{-1} IBA.

In blueberry, explant density affected covered area per explant and shoot density, with lower values observed at higher densities. For blueberry 'Brigitta' fresh biomass reached approximately 45 mg at 45 days and about 55 mg at 60 days of subculture. These values are consistent with those reported by (Regni et al. 2024), who observed a fresh biomass of around 25 mg at 30 days of culture. Similarly, dry biomass increased from about 15 mg at 45 days to approximately 30 mg at 60 days, whereas at 30 days it was around 8 mg. Several studies have demonstrated that optimal explant density can enhance shoot proliferation, improve plantlet quality, and reduce the duration and cost

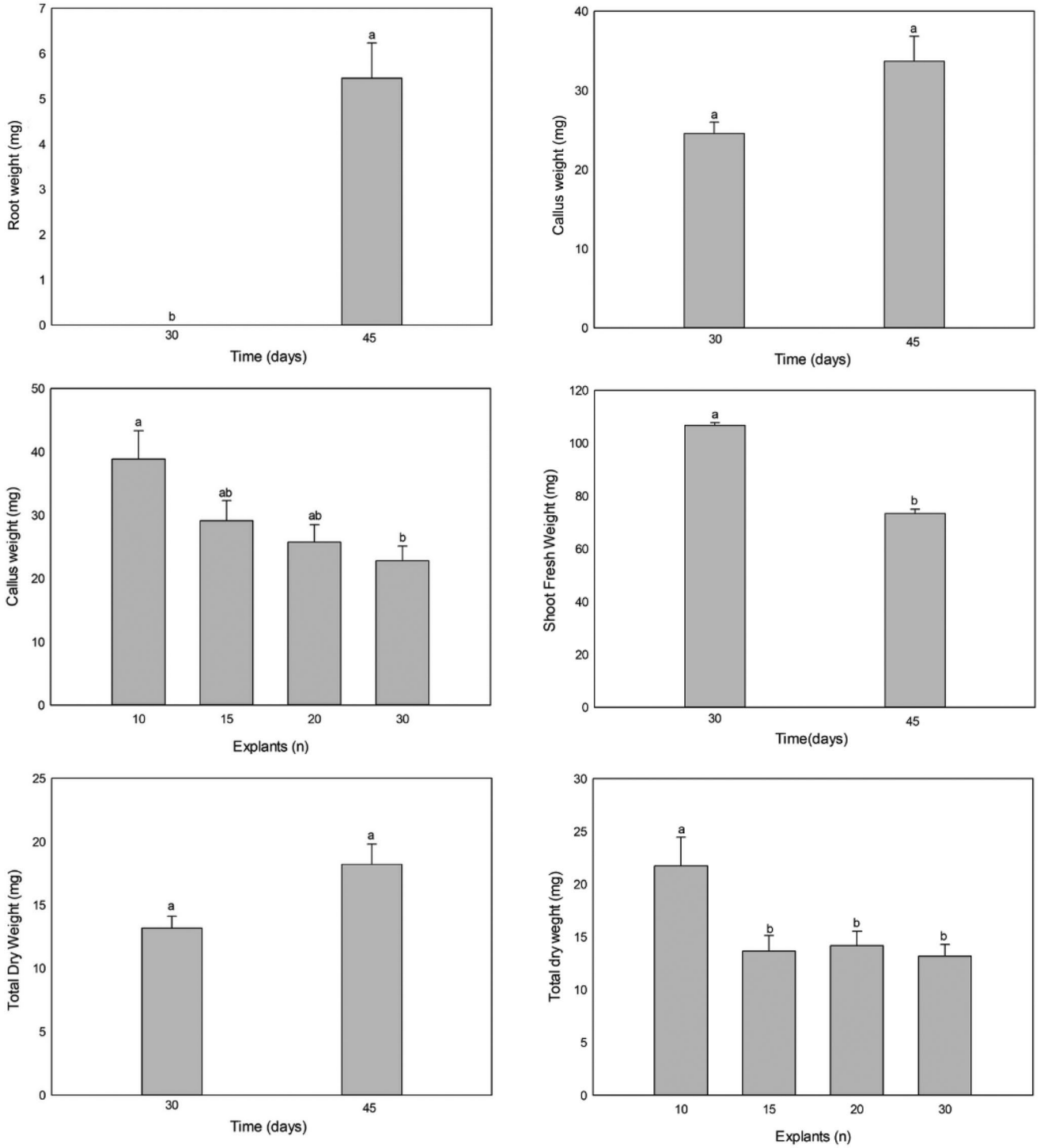


Fig. 6 Root weight (mg), callus weight (mg), shoot fresh and dry weight (mg) values ± SE of the of the blackberry proliferated explants. Mean values followed by different letters were significantly different ($p < 0.05$)

of micropropagation cycles, although the effects are often species- and system-specific. In *Gynura procumbens* low explant densities promoted higher shoot proliferation and growth index, whereas higher densities favored total biomass accumulation but reduced growth efficiency (Saadah et al. 2019). In *Solanum tuberosum*, the increase in explant density enhanced shoot length and rooting during the early stages, although differences diminished over time, likely due to competition effects (Sarkar et al. 1997). In *Curcuma longa* the explant density influenced

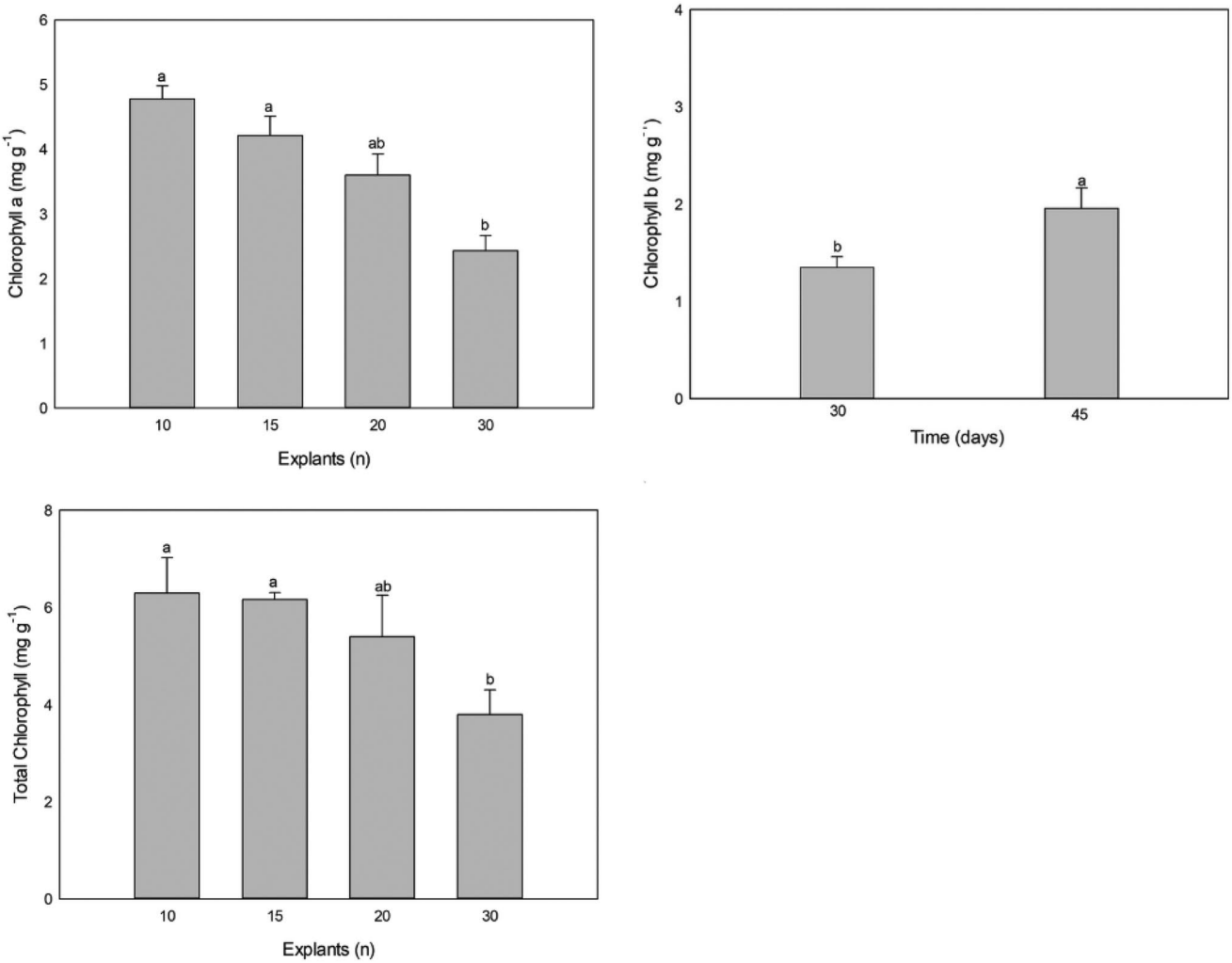


Fig. 7 Chlorophyll a, chlorophyll b, and total chlorophyll (mg g^{-1}) of the blackberry proliferated explants. Mean values followed by different letters were significantly different ($p < 0.05$)

multiplication and biomass production, with low densities favoring shoot proliferation under specific nutrient conditions, while high densities optimized total biomass under nutrient-rich regimes (El-Hawaz et al. 2016). In woody species such as *Populus alba* × *P. grandidentata*, (Chun et al. 1986) showed that increased shoot density reduced proliferation per explant, with optimal results achieved

Table 2 Covered area (cm^2) and shoot density related to subculture duration (30 and 45 days) in blackberry

Days	Covered area/ *n* of explants (cm^2)	Shoot density
30	0.73 ± 0.04 b	1.10 ± 0.06 b
45	1.16 ± 0.08 a	1.75 ± 0.12 a

Mean values followed by different letters were significantly different ($p < 0.05$)

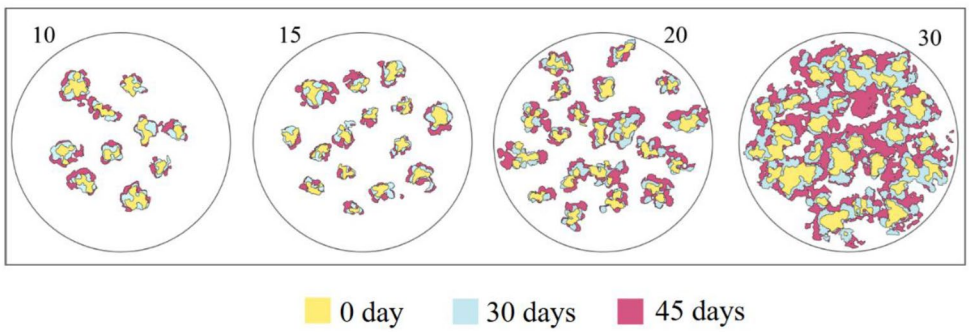


Fig. 9 Overlays of shoot-covered areas extracted from 3D models for blackberry at different densities (10, 15, 20, and 30 explants) over time

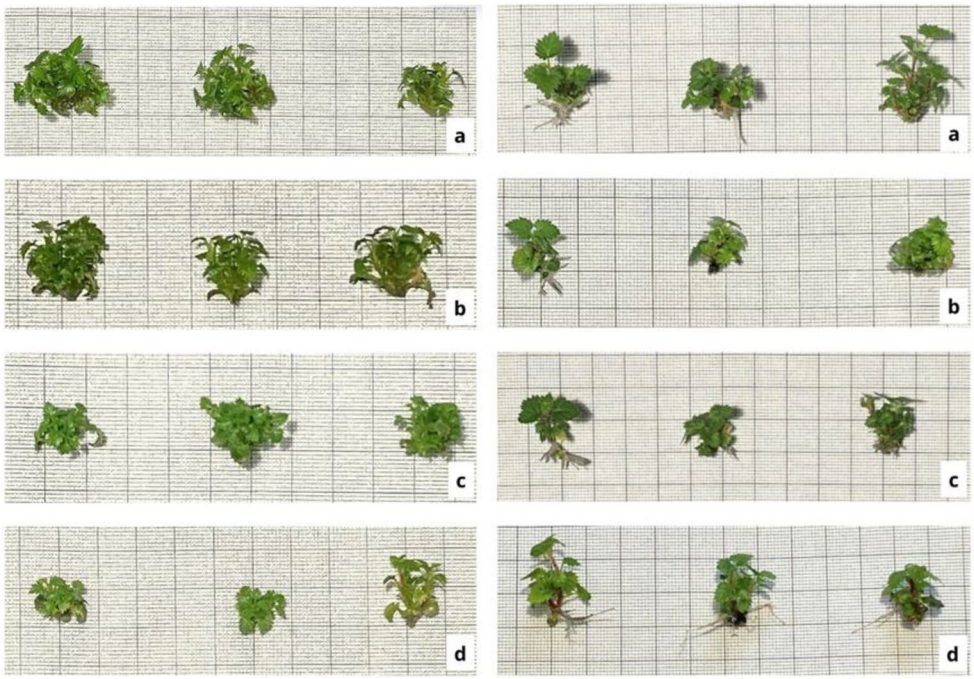


Fig. 8 Explants of the blackberry cultivar 'Thornfree' at 30 days (left): 10 explants (**a**); 15 explants (**b**); 20 explants (**c**), and 30 explants (**d**). Explants of blackberry at 45 days (right): 10 explants (**a**); 15 explants (**b**); 20 explants (**c**), and 30 explants (**d**)

Table 1 Covered area (cm^2) and shoot density related to the number of explants in blackberry

N of explants	Covered area/ *n* of explants (cm^2)	Shoot density
10	0.81 ± 0.06 b	1.21 ± 0.09 bc
15	0.75 ± 0.09 b	1.13 ± 0.13 c
20	1.00 ± 0.13 ab	1.51 ± 0.20 b
30	1.23 ± 0.14 a	1.85 ± 0.21 a

Mean values followed by different letters were significantly different ($p < 0.05$)

at intermediate densities in liquid media. As reported, total chlorophyll content in blackberry decreased as the number of explants increased, while in blueberry it decreased with longer subculture duration, although the values remained within satisfactory ranges. However, the decrease in total chlorophyll content was not related to the growth parameters monitored as already observed in willow (Regueira et al. 2018). Several factors, including the shading between explants can influence and modulate chlorophyll a and b contents, underscoring a strong correlation between accumulation and specific abiotic conditions (Sonobe et al. 2020).

The observed changes in the top-view covered area per explant and shoot density (defined as canopy surface divided by the number of explants and normalized to the container surface area) were not always attributable to the number of shoots, but rather may reflect differences in cluster compactness.

Other studies on blueberry and blackberry have applied advanced techniques such as UAV and LiDAR to generate 3D point clouds for phenotyping (Patrick and Li 2017; Jiang et al. 2019; Tagoe et al. 2024). However, to date, no imaging-based studies have been reported for in vitro cultures of these two species. Relevant works on other plants in vitro have been conducted using multi-sensor digital imaging systems or 2D image analysis approaches (Mestre et al. 2017; Bethge et al. 2023), though these studies did not utilize 3D models.

The proposed methodology offers several key advantages, including objectivity, high accuracy, and non-destructiveness, based on 3D data rather than simple 2D images. The non-destructive nature of the approach allows repeated measurements during subculture including at intermediate time points, providing accurate data and additional temporal information. Moreover, the integration of imaging data with traditional measurements enhances understanding of growth dynamics and phenotyping accuracy. The workflow, encompassing shoot isolation and canopy surface calculation, relies on semi-automated or fully automated processes, significantly reducing the influence of operator subjectivity. In particular, the estimation of the shoot-covered surface area, extracted through projection and contour analysis, is based on consistent geometric criteria and does not rely on manual tracing or visual scoring. Compared to conventional photographic top views, the use of a 3D point cloud enables metrically correct area estimation, free from perspective distortions and independent of lighting conditions, with the potential to capture elevation information. To our knowledge, this study represents the first application of the viDoc RTK Rover and iPhone LiDAR system in in vitro plant culture analysis, allowing fully

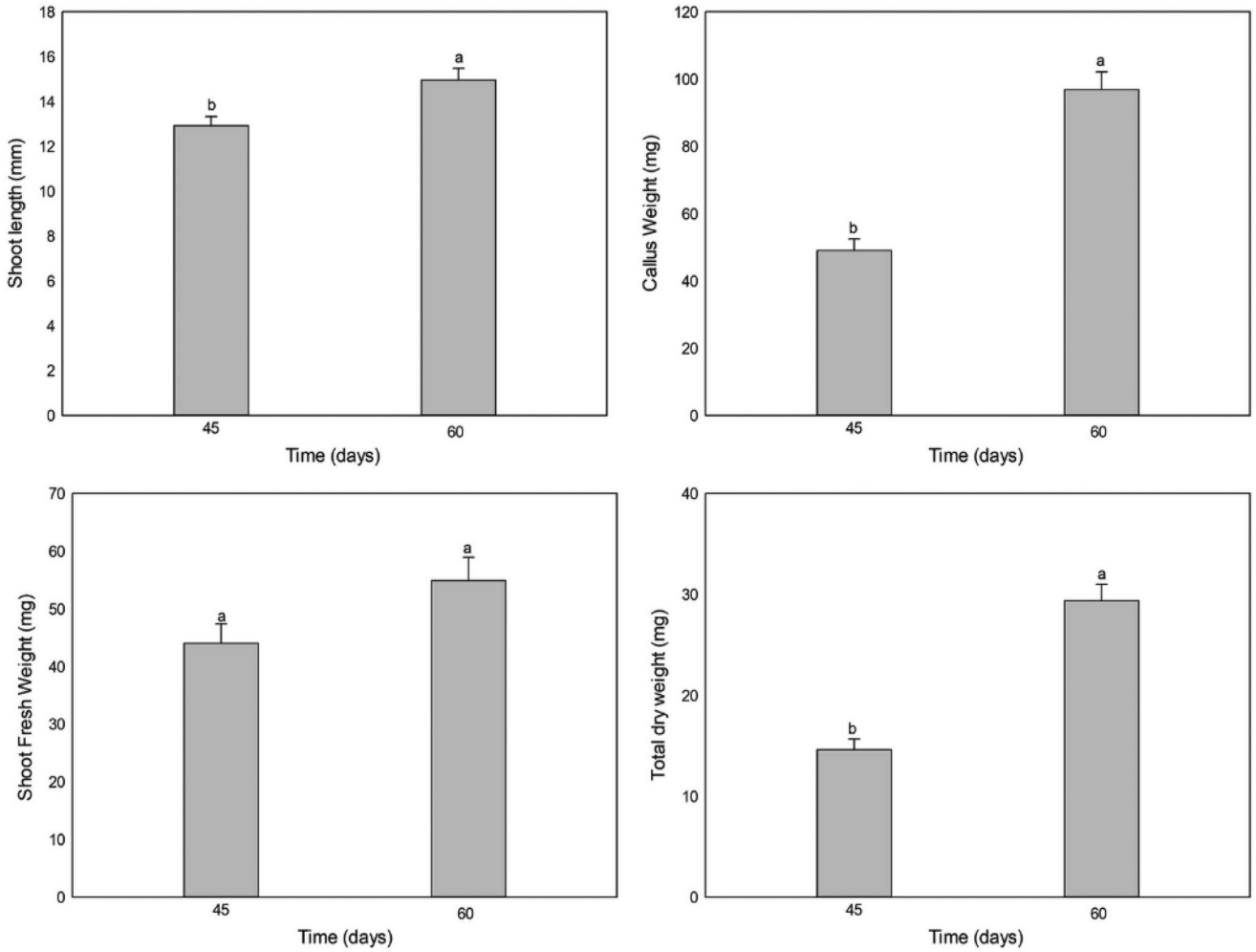


Fig. 10 Shoot length (mm), callus weight (mg), shoot fresh and dry weight (mg) values ± SE of the blueberry proliferated explants. Mean values followed by different letters were significantly different ($p < 0.05$)

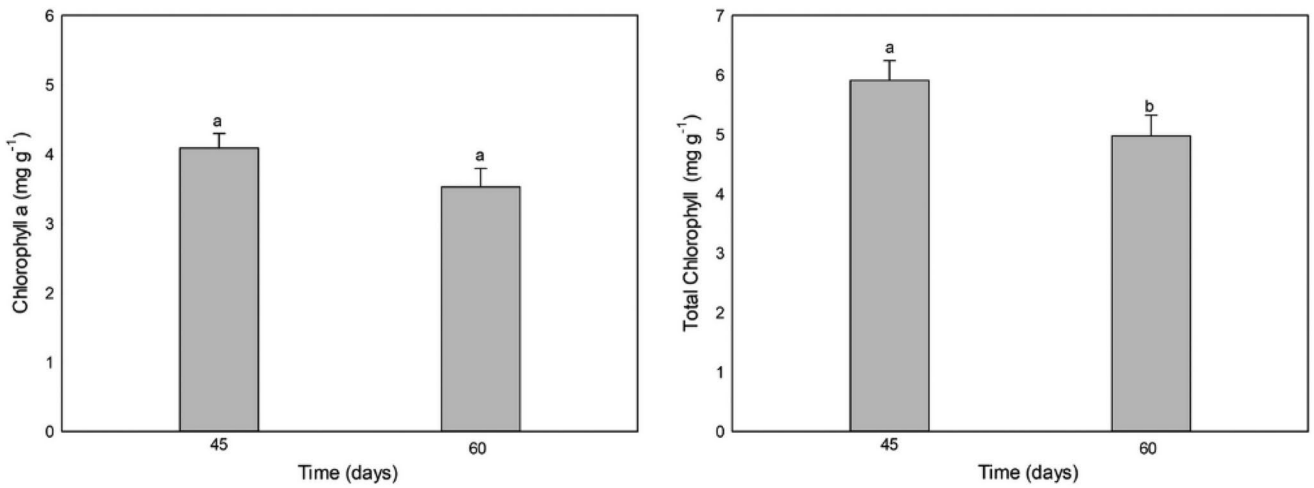


Fig. 11 Chlorophyll a and total chlorophyll (mg g^{-1}) values ± SE of the blueberry proliferated explants. Mean values followed by different letters were significantly different ($p < 0.05$)

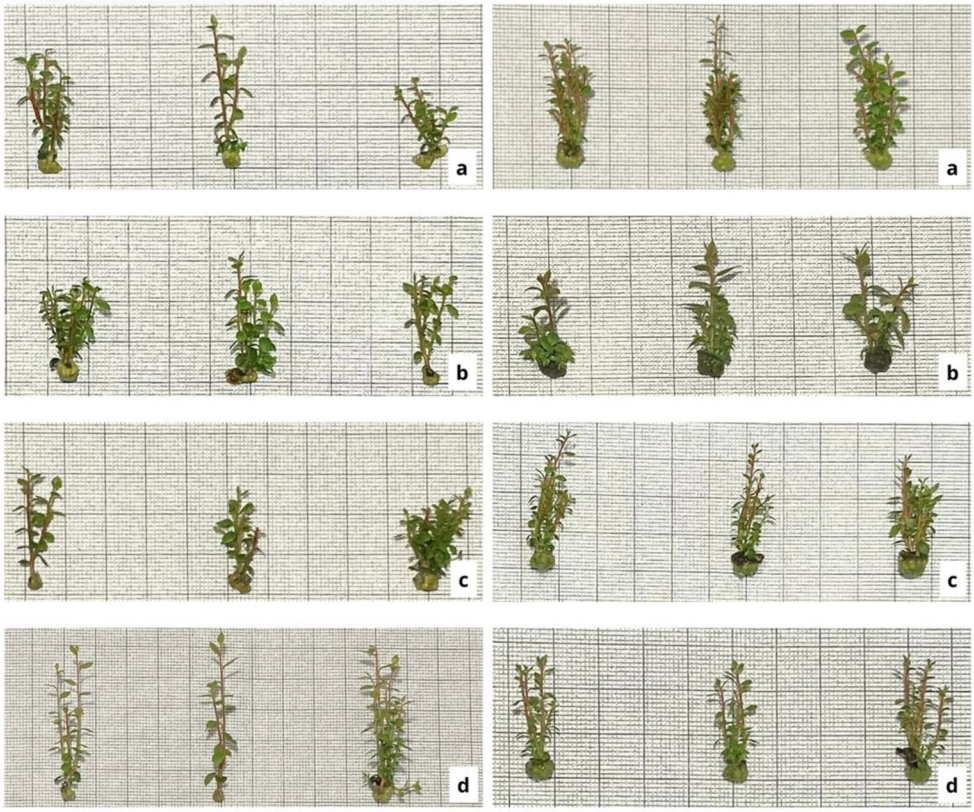


Fig. 12 Explants of blueberry cultivar 'Brigitta' at 45 days (left): 10 explants (**a**); 15 explants (**b**); 20 explants (**c**), and 30 explants (**d**). Explants of blueberry at 60 days (right): 10 explants (**a**); 15 explants (**b**); 20 explants (**c**), and 30 explants (**d**)

Table 3 Covered area (cm²) and shoot density related to the number of explants in blueberry

N of explants	Covered area/ *n* of explants (cm^2)	Shoot density
10	1.46 ± 0.20 a	2.19 ± 0.30 a
15	1.21 ± 0.14 ab	1.81 ± 0.21 ab
20	1.04 ± 0.10 b	1.57 ± 0.15 b
30	0.72 ± 0.07 c	1.08 ± 0.10 c

Mean values followed by different letters were significantly different ($p < 0.05$)

Table 4 Covered area (cm²) and shoot density related to subculture duration (45 and 60 days) in blueberry

Days	Covered area/ *n* of explants (cm^2)	Shoot density
45	0.84 ± 0.05 b	1.27 ± 0.08 b
60	1.37 ± 0.12 a	2.05 ± 0.18 a

Mean values followed by different letters were significantly different ($p < 0.05$)

non-invasive data acquisition, with no physical contact with the samples, and clear quantification of growth differences between stages. Even without GCPs, millimetric accuracy was achieved, demonstrating that the viDoc RTK Rover combined with the iPhone LiDAR system provides sufficient spatial resolution for surveying small-sized objects, as in the present case study. This high level of accuracy, together with the largely automated workflow, considerably reduces processing time and enables fast, efficient data acquisition.

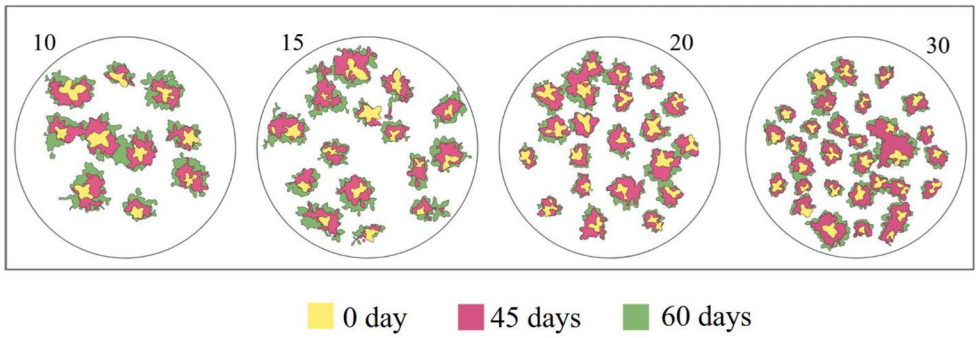


Fig. 13 Overlays of shoot-covered areas extracted from 3D models for blueberry at different densities (10, 15, 20, and 30 explants) over time

Conclusion

Integrating traditional measurements with smartphone 3D imaging, this study evaluated how subculture duration and explant density affect blackberry and blueberry micropropagation. Subculture duration was the primary factor influencing micropropagation performance, whereas explant density had a more limited effect. In blackberry, longer subculture duration promoted an increase in shoot number and rooting, while higher explant density reduced total dry weight. In blueberry, extended subculture duration enhanced most monitored growth parameters. The non-destructive, rapid, and repeatable measurements of the covered area and shoot density obtained through the viDoc RTK Rover and a LiDAR-equipped iPhone allow near real-time monitoring of in vitro plant growth. The system captures high-resolution RGB images to generate dense point clouds improving spatial accuracy and repeatability. The LiDAR sensor provides additional depth information, enhancing point cloud quality in areas with low texture or variable illumination. Integrating visual and depth data enables both 2D surface measurements and potential volumetric or morphological analyses, such as shoot height, curvature, and compactness, thereby complementing and reducing the need for time-consuming traditional measurements. The system proved to be user-friendly, portable, and cost-effective, while its automated data processing pipeline ensures objective and replicable monitoring of micropropagation efficiency. Overall, this study demonstrates that combining traditional and 3D imaging methods provides a powerful framework for optimizing in vitro plant propagation and monitoring growth dynamics.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11240-025-03267-0.

Funding Open access funding provided by Università degli Studi di Perugia within the CRUI-CARE Agreement. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval Not applicable.

Competing interests The authors have no relevant financial or non-financial interests to disclose.

References

Aksoy E (2025) Determining the usability of the vidoc device, which integrates with smart phones, in documenting historical structures. Measurement 242:116156

Aly AA, El-Desouky W, El-Leel OFA (2022) Micropropagation, phytochemical content and antioxidant activity of gamma-irradiated blackberry (*Rubus fruticosus* L.) plantlets. Vitro CellDevBiol-Plant 58:457–469. https://doi.org/10.1007/s11627-021-10244-7

Aynalem HM, Righetti TL, Reed BM (2006) Non-destructive evaluation of in vitro-stored plants: A comparison of visual and image analysis. Vitro Cell Dev Biol - Plant 42:562–567. https://doi.org/10.1079/IVP2006816

Beccaro GL, Giongo L, De Salvador R et al (2011) Scegliere La cultivar Di Lampone, Mirtillo e Rovo per Il 2011. L'Informatore Agrario 67:58–61

Bethge H, Winkelmann T, Lüdeke P, Rath T (2023) Low-cost and automated phenotyping system phenomenon for multi-sensor in situ monitoring in plant in vitro culture. Plant Methods 19:42. https://doi.org/10.1186/s13007-023-01018-w

Bobrowski VL, Mello-Farias P, Petters J (1996) Micropropagation of blackberries (*Rubus* sp.) cultivars. Curr Agricultural Sci Technol 2

Chauvin S (2023) Water table height and microtopography in swamps of southeastern Michigan as influences of black Ash tree establishment and survival in the Presence of Emerald Ash Borer

Cho M, Howard L, Prior R, Clark J (2005) Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry. J Sci Food Agric 85:2149–2158. https://doi.org/10.1002/jsfa.2209

Chun YW, Hall RB, Stephens LC (1986) Influences of medium consistency and shoot density on in vitro shoot proliferation of Populus Alba P. grandidentata. Plant Cell Tiss Organ Cult 5:179–185. https://doi.org/10.1007/BF00040128

Correia S, Matos M, Leal F (2024) Advances in blueberry (*Vaccinium* spp.) in vitro culture: a review. Horticulturae 10:533

Dönmez BA, Polat Ş, Hamakhan AM, Kafkas NE (2024) Methods of blackberry propagation in vitro condition. In: BIO Web of Conferences. EDP Sciences, p 01009

Driver JA, Kuniyuki AH (1984) In vitro propagation of paradox walnut rootstock. HortScience 19:507–509. https://doi.org/10.21273/HORTSCI.19.4.507

El-Hawaz R, Park D, Bridges WC, Adelberg J (2016) Optimizing in vitro mineral nutrition and plant density increases greenhouse growth of curcuma longa L. during acclimatization. Plant Cell Tiss Organ Cult 126:33–42. https://doi.org/10.1007/s11240-016-0974-9

Eltner A, Sofia G (2020) Structure from motion photogrammetric technique. Developments in Earth surface processes. Elsevier, pp 1–24

Fan S, Jian D, Wei X et al (2017) Micropropagation of blueberry 'Bluejay'and 'Pink lemonade'through in vitro shoot culture. Sci Hort 226:277–284

Feng X, Zhao Y, Zhang C et al (2017) Discrimination of Transgenic maize kernel using NIR hyperspectral imaging and multivariate data analysis. Sensors 17:1894

Gomes HT, Bartos PMC, de Andrade MT et al (2017) In vitro conservation of blackberry genotypes under minimal growth conditions and subsequent large-scale micropropagation. Pesquisa Agropecuária Brasileira 52:1286–1290

Harutyunyan ZE, Vardanian IV, Hoveyan ZH et al (2022) Biotechnology methods in study of vaccinium uliginosum L. and vaccinium myrtillus L. in Armenia. IOP Conf Ser: Earth Environ Sci 1045:012149. https://doi.org/10.1088/1755-1315/1045/1/012149

Ibaraki Y, Kenji K (2001) Application of image analysis to plant cell suspension cultures. Comput Electron Agric 30:193–203

iPhone (2025) https://support.apple.com/en-us/111828. Accessed 1 October 2025

Ivaschuk OA, Berezhnoy VA, Maslakov YN, Fedorov VI (2023) Creation and study of 3D models for digital plant phenotyping. Sci Tech Inf Proc 50:422–429. https://doi.org/10.3103/S0147688223050088

Jiang Y, Li C, Takeda F et al (2019) 3D point cloud data to quantitatively characterize size and shape of shrub crops. Hortic Res 6

Kamle M, Kumar P, Patra JK, Bajpai VK (2017) Current perspectives on genetically modified crops and detection methods. 3 Biotech 7:219. https://doi.org/10.1007/s13205-017-0809-3

Kavand S, Kermani MJ, Haghnazari A et al (2011) Micropropagation and medium-term conservation of Rosa pulverulenta. Acta Scientiarum Agron 33:297–301

Kefayati S, Kafkas E (2018) Micropropagation of 'Chester thornless' blackberry cultivar using axillary bud explants. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 47:162. https://doi.org/10.15835/nbha47111280

Kodikara C, Netticadan T, Bandara N et al (2024) A new UHPLC-HRMS metabolomics approach for the rapid and comprehensive analysis of phenolic compounds in blueberry, raspberry, blackberry, cranberry and Cherry fruits. Food Chem 445:138778. https://doi.org/10.1016/j.foodchem.2024.138778

Lepse L, Laugale V (2009) Micropropagation, rooting and acclimatization of blackberry AGAVAM. Acta Hort 43–49. https://doi.org/10.17660/ActaHortic.2009.839.2

Li L, Zhang Q, Huang D (2014) A review of imaging techniques for plant phenotyping. Sensors 14:20078–20111

Lloyd G, McCown B (1980) Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture

Mazurek M, Siekierzyńska A, Piechowiak T et al (2024) Comprehensive analysis of highbush blueberry plants propagated in vitro and conventionally. Int J Mol Sci 25:544. https://doi.org/10.3390/ijms25010544

Mestre D, Fonseca JM, Mora A (2017) Monitoring of in-vitro plant cultures using digital image processing and random forests. In: 8th International Conference of Pattern Recognition Systems (ICPRS 2017). Institution of Engineering and Technology, Madrid, Spain, p 8 (6)

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497

Niazian M, Sadat-Noori SA, Abdipour M et al (2018) Image processing and artificial neural Network-Based models to measure and predict physical properties of embryogenic callus and number of somatic embryos in Ajowan (Trachyspermum Ammi (L.) Sprague). Vitro CellDevBiol-Plant 54:54–68. https://doi.org/10.1007/s11627-017-9877-7

Park SW, Jeon JH, Kim HS et al (2004) Effect of sealed and vented gaseous microenvironments on the hyperhydricity of potato shoots in vitro. Sci Hort 99:199–205. https://doi.org/10.1016/S0304-4238(03)00097-9

Patrick A, Li C (2017) High throughput phenotyping of blueberry Bush morphological traits using unmanned aerial systems. Remote Sens 9:1250

Peano C, Girgenti V, Baudino C, Giuggioli NR (2017) Blueberry supply chain in italy: Management, innovation and sustainability. Sustainability 9:261. https://doi.org/10.3390/su9020261

Phillips GC, Garda M (2019) Plant tissue culture media and practices: an overview. Vitro CellDevBiol-Plant 55:242–257. https://doi.org/10.1007/s11627-019-09983-5

Pix4D (2025) https://www.pix4d.com/product/pix4dcatch/. Accessed 1 October 2025

Rapuca A, Matoušková E (2023) Testing of close-range photogrammetry and laser scanning for easy Documentation of historical objects and buildings parts. Stavební obzor-Civil Eng J 32:504–518

Reed B, Poothong S, Hall HK (2017) Propagation of blackberries and related Rubus species. Blackberries and their hybrids 101–112

Regni L, Cesarini A (2025) Over half a century of research on blackberry micropropagation: A comprehensive review. Horticulturae 11:556. https://doi.org/10.3390/horticulturae11050556

Regni L, Del Buono D, Micheli M et al (2024) Biogenic zinc oxide nanoparticles improve in vitro growth of blueberries. Horticulturae 10:1234

Regni L, Cesarini A, Micheli M, Proietti P (2025) A bibliometric analysis of research on blackberry micropropagation. Plant Cell Tiss Organ Cult 160:33. https://doi.org/10.1007/s11240-025-02978-8

Regueira M, Rial E, Blanco B et al (2018) Micropropagation of axillary shoots of Salix viminalis using a temporary immersion system. Trees 32:61–71. https://doi.org/10.1007/s00468-017-1611-x

Saadah IN, Kristanti AN, Hardjo PH, Manuhara YSW (2019) Shoots culture of Gynura procumbens (Lour.) Merr. In balloon-type bubble-bioreactor Influenced by sucrose concentration and Inoculums density. Asian J Plant Sci 18:85–90

Sarkar D, Chandra R, Naik PS (1997) Effect of inoculation density on potato micropropagation. Planr Cell Tissue Organ Cult 48:63–66. https://doi.org/10.1023/A:1005759211058

Sonobe R, Yamashita H, Mihara H et al (2020) Estimation of leaf chlorophyll a, b and carotenoid contents and their ratios using hyperspectral reflectance. Remote Sens 12:3265

Spinardi A, Cola G, Gardana CS, Mignani I (2019) Variation of anthocyanin content and profile throughout fruit development and ripening of highbush blueberry cultivars grown at two different altitudes. Front Plant Sci 10:1045

Stanisavljevic M (1998) New small fruit cultivars from Cacak: 1. The new blackberry (Rubus sp.) cultivar 'Cacanska Bestrna.' In: VII International Symposium on Rubus and Ribes 505. pp 291–296

Ștefănescu B-E, Călinoiu LF, Ranga F et al (2020) The chemical and biological profiles of leaves from commercial blueberry varieties. Plants 9:1193. https://doi.org/10.3390/plants9091193

Suleymanoglu B, Tamimi R, Yilmaz Y et al (2023) Road infrastructure mapping by using iPhone 14 pro: an accuracy assessment. Int Archives Photogrammetry Remote Sens Spat Inform Sci 48:347–353

Tagoe A, Silva A, Koparan C et al (2024) Blackberry growth monitoring and feature quantification with unmanned aerial vehicle (UAV) remote sensing. AgriEngineering 6:4549

Tamimi R (2022) Relative accuracy found within iPhone data collection. Int Archives Photogrammetry Remote Sens Spat Inform Sci 43:303–308

Tamimi R, Toth C (2023) Performance assessment of a mini mobile mapping system: Iphone 14 pro installed on a e-Scooter. Int Archives Photogrammetry Remote Sens Spat Inform Sci 48:307–315

Treder W, Klamkowski K, Sowik I, Maciorowski R (2021) Possibilities of using RGB-based image analysis to estimate the chlorophyll content of micropropagated strawberry plants. Acta Scientiarum Polonorum Hortorum Cultus 20:105–115

viDoc (2025) https://data.pix4d.com/misc/viDoc_Pix4D/viDoc_Technical_Data_vigram_Pix4D.pdf. Accessed 1 October 2025

Vujović T, Ružić D, Cerović R (2012) In vitro shoot multiplication as influenced by repeated subculturing of shoots of contemporary fruit rootstocks. Hortic Sci 39:101–107. https://doi.org/10.17221/208/2011-HORTSCI

Vujović T, Ružić \DJur\djina, Cerović R et al (2017) An assessment of the genetic integrity of micropropagated raspberry and blackberry plants. Sci Hort 225:454–461

Walter A, Liebisch F, Hund A (2015) Plant phenotyping: from bean weighing to image analysis. Plant Methods 11:14. https://doi.org/10.1186/s13007-015-0056-8

Wu Y, Han T, Yang H et al (2023) Known and potential health benefits and mechanisms of blueberry anthocyanins: A review. Food Bioscience 55:103050. https://doi.org/10.1016/j.fbio.2023.103050

Zollini S, Marconi L (2025) Evaluation of positioning accuracy using smartphone RGB and lidar sensors with the vidoc RTK Rover. Sensors 25:3867