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Image analysis of sweet potato calluses in vitro

Abstract – The objective of this work was to evaluate the use of the GroundEye equipment to characterize calluses from sweet potato explants. Sweet potato calluses from different explants (leaf, stem, and root) were grown in a culture medium with four growth regulators (naphtaleneacetic acid, 6-benzylaminopurine, 2,4-dichlorophenoxyacetic acid, and kinetin) and under two cultivation conditions (light and dark). The GroundEye system was used to analyze callus geometry and color, capturing color variation efficiently across different samples. In addition, the measurement of the area by the software directly reflects callus mass. Therefore, GroundEye is efficient in evaluating the geometry and color of calluses from explants of the Brazlândia Branca sweet potato cultivar.

Index terms: *Ipomoea batatas*, GroundEye, micropropagation, tissue culture.

Análise de imagem de calos de batata doce in vitro

Resumo – O objetivo deste trabalho foi avaliar o uso do equipamento GroundEye na caracterização do calo de explantes de batata-doce. O calo de batata-doce de diferentes explantes (folha, caule e raiz) foi cultivado em meio de cultura com quatro reguladores de crescimento (ácido naftalenoacético, 6-benzilaminopurina, 2,4-diclorofenoxiacético e cinetina) e sob duas condições de cultivo (luz e escuro). O sistema GroundEye foi utilizado para analisar a geometria e a cor do calo, tendo capturado eficientemente a variação de coloração entre as diferentes amostras. Além disso, a medição da área pelo programa reflete diretamente a massa do calo. Portanto, o GroundEye é eficiente para avaliar a geometria e a cor do calo de explantes da cultivar Brazlândia Branca de batata-doce.

Termos para indexação: *Ipomoea batatas*, GroundEye, micropropagação, cultura de tecidos.

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The development of calluses is influenced by factors such as explant type, growth regulators, and environmental conditions as light intensity, which directly affect their physical and biochemical properties (Din et al., 2019). These properties are crucial for selecting robust calluses, with a high regenerative potential and bioactive-compound production (Wu et al., 2024).

In the literature, calluses are commonly categorized based on color variations, which reflect physiological and biochemical changes during their development. Therefore, callus coloration serves as an important indicator in plant tissue culture, reflecting physiological, biochemical, and morphological changes in cells (Kirakosyan et al., 2022). Widely accepted classifications include: green callus, often associated with the

presence of chlorophyll and a higher metabolic activity; cream-colored callus, indicating an undifferentiated cell proliferation; and brown callus, frequently linked to oxidative stress and a reduced viability (Reis et al., 2018; Kirakosyan et al., 2022).

Although some studies have characterized calluses based on color and geometry, they often rely on subjective photographic documentation (Ali et al., 2018; Chen et al., 2021), evaluating callus coloration either visually or using an image analysis software, often without specifying the adopted methodology. Reis et al. (2018) and Ashokhan et al. (2020), for example, performed visual assessments, assigning scores based on the intensity of pigmentation, whereas Al-Saeedi & Al-Rekaby (2022) and Kirakosyan et al. (2022) did not clarify the applied methodologies. This lack of transparency complicates the comparability and reproducibility of the obtained results in the field of plant tissue culture, reinforcing the need for standardized and automated evaluation methods. To address this issue, the use of image analysis software is recommended for more objective evaluations (Mamdouh & Smetanska, 2022; Zhang et al., 2022), but manual image capture still poses challenges related to lighting and background consistency.

In this context, an alternative is the GroundEye equipment, which is already widely used in the assessment of seed and seedling quality (Batista et al., 2022), but whose potential for plant tissue culture applications remains underexplored. GroundEye reduces the need for manual intervention, making the process more efficient and rapid as it is equipped with a camera for image capture and performs analyses autonomously. By using an automated system for image capture, the equipment ensures consistency in lighting conditions and capture angles, which is often a challenge in manual methods, enhancing the reproducibility of the obtained results.

The objective of this work was to evaluate the use of the GroundEye equipment to characterize calluses from sweet potato [*Ipomoea batatas* (L.) Lam] explants.

The experiment was conducted at the Plant Tissue Culture Laboratory and the Central Seed Research Laboratory of the Department of Agriculture of Universidade Federal de Lavras, located in the municipality of Lavras, in the state of Minas Gerais, Brazil. The Brazlândia Branca sweet potato cultivar was used due to its ease of handling and good in vitro development (Masekesa et al., 2021). Leaf, stem, root explants (5.0 mm^2 segments without the central vein, 5.00 mm internodes, and 5.00 mm segments, respectively) were collected after 45 days of in vitro growth. All explants were randomly selected and subcultured to maximize contact with the Murashige & Skoog (MS) medium.

The experimental design was planned to generate calluses with extreme and contrasting characteristics across the treatments. A combined data approach was used to highlight the ability of the GroundEye equipment to analyze diverse samples. Eighteen treatments were evaluated in a 3×3×2 factorial arrangement, with three explant types (leaf, stem, and root), three growth regulator recommendations [1.0 mg L^{-1} naphtaleneacetic acid + 10 mg L^{-1} 6-benzylaminopurine (Carswell & Locy, 1984), 3.0 mg L^{-1} 2,4-dichlorophenoxyacetic acid (Henderson et al., 1984), and 2.0 mg L^{-1} naphtaleneacetic acid + 2.0 mg L^{-1} kinetin (Schwenk, 1981) in MS medium with 30 g L^{-1} sucrose and 6.0 g L^{-1} agar, at pH 5.8], and two culture conditions (light and dark). A total of 90 test tubes were incubated, as follows: 45 at 25°C under a 16 hour photoperiod, and another 45 in a BOD chamber in the dark, with five replicates per treatment.

After 90 days, the callus samples were carefully removed from the culture tubes, washed under running water to eliminate excess culture medium, and gently dried with paper towels to remove surface moisture. Each sample was weighed on an analytical balance to determine fresh mass. Subsequently, the calluses were placed individually on the transparent acrylic tray in the GroundEye equipment (Figure 1), being positioned flat to avoid shadow formation and maintain an uniform image capture. Background calibration was performed based on preliminary tests using the YCbCr model, where: luma, 0–1; blue, 0.09–0.20; and red, -0.50–0.50.

The evaluated callus characteristics included color dominance (black, blue, cyan, green, magenta, orange, red, and yellow, for example) and geometric parameters (area, circularity, sphericity, maximum and minimum diameters, and perimeter). The data were analyzed using the analysis of variance with the SISVAR software (Ferreira, 2011), and means were compared via the Scott-Knott test, at 5% probability. The principal component analysis was carried out using the XLSTAT software (Addinsoft, 2017).

The obtained data suggests a direct correlation between the mass of the calluses and the area

measured by GroundEye (Table 1), in which, typically, the larger the callus area, the greater the mass. The treatments stem callus with naphtaleneacetic acid + 6-benzylaminopurine, stem callus with 2,4-dichlorophenoxyacetic acid, and leaf callus with naphtaleneacetic acid + 6-benzylaminopurine, all under light conditions, resulted in higher average masses, as well as in larger average areas, perimeters,

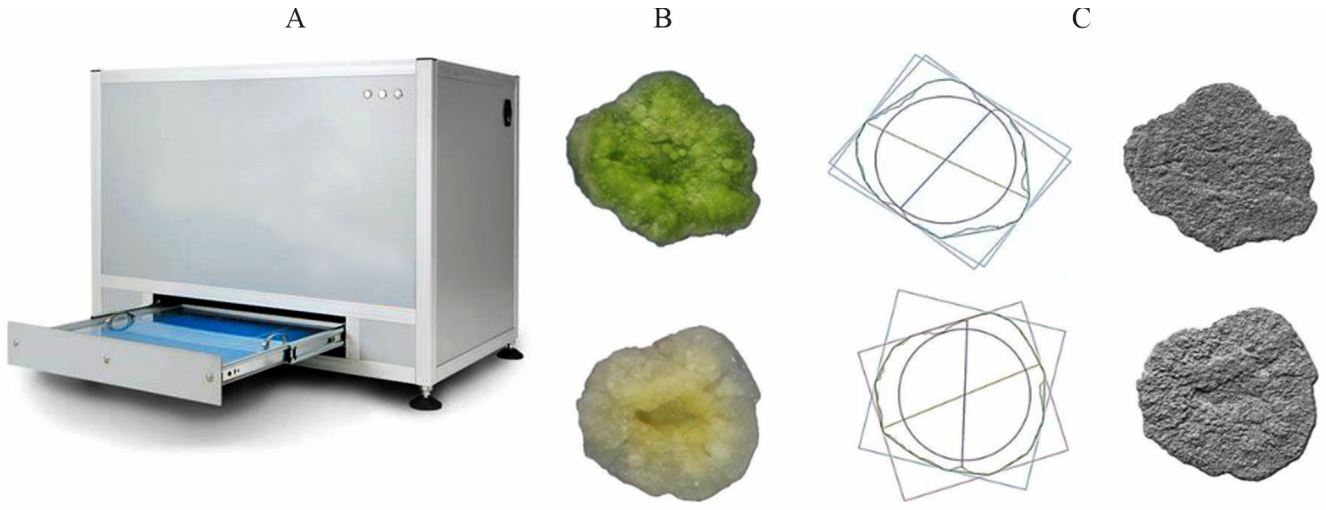

Figure 1. GroundEye equipment (Tbit, 2025) with open sampling tray (A), as well as representative images obtained with its integrated camera, showing calluses from leaf explants of the Brazlândia Branca sweet potato (*Ipomoea batatas*) cultivar under light (green) and dark (light beige) conditions (B), with geometry and texture analysis performed by the software (C).

Table 1. Average values of the area, circularity, sphericity, maximum diameter, minimum diameter, perimeter, and mass of callus samples from explants obtained from the leaves, stems, and roots of the Brazlândia Branca sweet potato (*Ipomoea batatas*) cultivar, developed in an environment under a 16-hour photoperiod and in the dark for 90 days[(1)].

Treatmen[t(2)]	Area (cm^{-2})	Maximum diameter (cm)	Minimum diameter (cm)	Perimeter (cm)	Mass (g)
C1L	4.19a	4.06a	1.78a	25.72a	7.224a
C2L	5.41a	2.97b	1.71a	20.53b	10.301a
C3L	3.16b	2.22c	1.75a	13.00c	5.185b
C1D	2.75b	2.15c	1.57a	9.31d	3.504c
C2D	2.92b	1.60c	0.62d	11.94c	2.346c
C3D	2.46b	1.97c	1.54a	8.89d	5.219b
F1L	4.41a	3.01b	1.39b	16.00c	7.139a
F2L	0.84c	1.21d	0.70d	5.81d	1.995d
F3L	2.45b	2.10c	1.40b	12.67c	4.051b
F1D	3.27b	2.26c	1.72a	10.03d	5.553b
F2D	0.21c	0.65d	0.40e	2.47e	0.212e
F3D	2.65b	1.91c	1.47b	11.07d	3.973b
R1L	2.70b	2.07c	1.63a	9.12d	4.690b
R3L	2.06b	1.92c	1.21c	9.84d	2.450c
R1D	2.40b	1.99c	1.44b	9.96d	4.084b
R2D	0.11c	0.48d	0.23e	1.91e	0.141e
R3D	2.19b	1.91c	1.35b	9.41d	3.336c
CV (%)	36.62	19.26	11.42	29.96	20.97
General average	1.92	1.68	1.10	8.11	4.524

[(1)]Means followed by equal letters do not differ significantly by the Scott-Knott test, at 5% probability. [(2)]C, stem; F, leaf; R, root; 1, naphtaleneacetic acid + 6-benzylaminopurine; 2, 2,4-dichlorophenoxyacetic acid; 3, naphtaleneacetic acid + kinetin; L, light; and D, dark for 90 days.

and maximum diameters according to the GroundEye system. In contrast, the treatments under dark conditions, especially those using root explants, presented a lower mass and reduced geometric development. Taratima et al. (2022) also observed an association between a greater callus mass and size in rice (*Orzya sativa* L.) calluses. This suggests that the area measurement provided by GroundEye is a reliable indicator of callus growth, showing the technology's capability to deliver consistent and reproducible data.

The statistical analysis of the presented data reveals significant differences between treatments (Table 1). Although the relationship between callus mass and size is complex and varies across species and growth conditions (Nguyen et al., 2020), the measurements obtained via imaging and direct mass showed consistency, indicating that GroundEye is sufficiently sensitive to detect variations in callus growth under different experimental conditions, which validates the use of this equipment as a complementary tool to traditional weighing methods.

Cavallaro et al. (2022) highlighted that variations in light exposure and other environmental factors can significantly influence callus growth within the same species, reinforcing the importance of adopting standardized and automated image analysis to minimize potential measurement errors. In the present study, the use of GroundEye ensures such standardization due to a uniform image capture and processing criteria across all samples.

The groupings by the principal component analysis showed variations in callus coloration (Figure 2), which is in alignment with the findings of previous studies, indicating that the type of growth regulator and light exposure significantly affect callus coloration (Reis et al., 2018; Al-Saeedi & Al-Rekaby, 2022). In the present work, GroundEye proved effective in capturing these variations, with a consistent and accurate color analysis.

The GroundEye system standardizes key parameters, such as lighting conditions, camera angle, image analysis software, and color scale, ensuring that the results from different studies are comparable

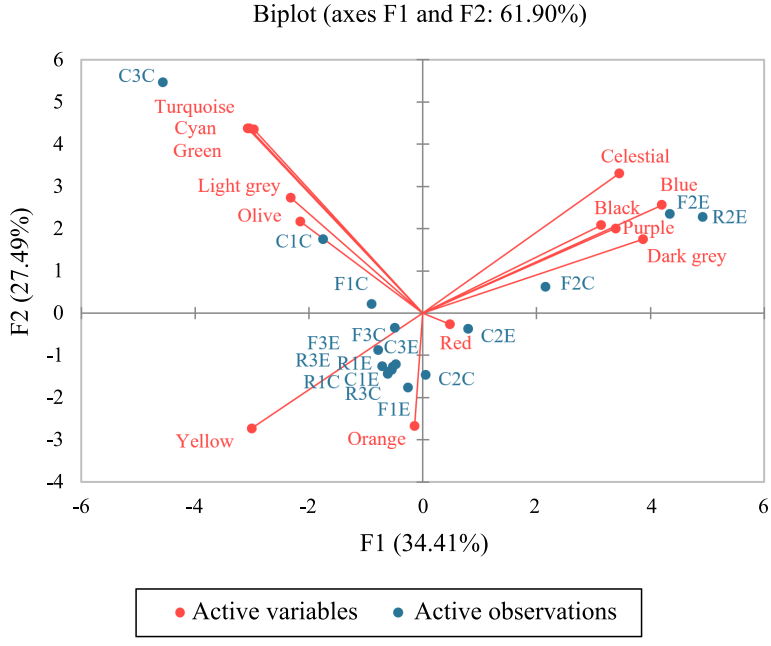

Figure 2. Two-dimensional projection and scoring of the color characteristics of callus samples from explants obtained from the leaf, stem, and root of the Brazlândia Branca sweet potato (*Ipomoea batatas*) cultivar, developed in culture medium supplemented with different growth regulators under a photoperiod of 16 hours or in the dark for 90 days. C, stem; F, leaf; R, root; 1, naphtaleneacetic acid + 6-benzylaminopurine; 2, 2,4-dichlorophenoxyacetic acid; 3, naphtaleneacetic acid + kinetin; L, light; and D, dark for 90 days.

and reliable, much like the established practices in seed and seedling analyses (Batista et al., 2022). However, although GroundEye can inform subsequent experiments due to the valuable data provided, its assessment is destructive, limiting the continuity of callus development.

The findings of the present work indicate that GroundEye is highly effective for characterizing calluses of the Brazlândia Branca sweet potato cultivar, providing standardized data that aligns with traditional methods such as weighing, making it a reliable and automated alternative to complement conventional measurements. This innovative tool, therefore, holds great potential for advancing research in plant tissue culture. However, future studies involving other cultivars are essential to further validate the general applicability of the equipment, and exploring non-destructive applications could broaden its utility in plant biotechnology.

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