

Enhancing germination and seedling development in lettuce and tomato using sustainable organic substrates

 Ricardo S. de Sousa^{1*},  Larissa M. de P. Barbosa²,  Luís A. P. L. Nunes¹,  Valber M. Ferreira¹,  Carlos J. G. de S. Lima¹,  Herbert M. M. Ramos³ and  Carlos H. A. Matos-Filho⁴

¹Department of Agricultural Engineering and Soil Science, Federal University of Piauí, Teresina, PI, 64049–550, Brazil.

²Postgraduate Program in Agronomy, Federal University of Piauí, Teresina, PI, 64049–550, Brazil.

³Center for Agricultural Sciences, State University of Piauí, Teresina, PI, 64002–150, Brazil.

⁴Department of Plant Science, Federal University of Piauí, Teresina, PI, 64049–550, Brazil.

*Correspondence should be addressed to Ricardo Silva de Sousa: ricardoss@ufpi.edu.br

Abstract

Aim of study: The aim of this study was to evaluate the effects of organic substrates formulated with different proportions of acerola (*Malpighia glabra*) bagasse and carnauba palm (*Copernicia prunifera*) bagana on the initial development of tomato and lettuce seedlings, with a focus on enhancing the sustainability of horticultural practices.

Area of study: This research was conducted in a controlled greenhouse environment at the Center for Agricultural Sciences, Federal University of Piauí, in Teresina, Piauí, Brazil.

Material and methods: The experiment involved 12 different substrates, each formulated from an organic compost mixed with acerola bagasse and carnauba palm bagana. Basaplant®, a commercially available substrate, was used as the control. This study evaluated the following variables: seed emergence (SE), emergence speed index (ESI), total dry mass (TDM), shoot dry mass (SDM), root volume (RV), dry root mass (DRM), seedling height (SH), leaf area (LA), stem diameter (SD), fresh root mass (FRM), fresh shoot mass (FSM), and Dickson quality index (DQI). The statistical analysis included principal component analysis (PCA) and cluster analysis to determine the effectiveness of the substrates.

Main results: For tomato, substrates with 50% carnauba bagana displayed higher volumetric density, which positively correlated with RV and DRM. This benefited seedling stability and water retention. Fine particles (<0.25 mm) enhanced TDM, while particles between 0.5–0.25 mm negatively affected SE. In lettuce, substrates with 50% acerola bagasse promoted higher aeration and drainage, which was beneficial for this crop. Larger particles (2.0–1.0 mm) correlated positively with LA, while finer particles reduced root dry mass. PCA and clustering grouped substrates by growth performance, with the G3 cluster favoring tomato and the G5 cluster favoring lettuce.

Research highlights: This study demonstrates that organic substrates with 50% carnauba bagana improve root growth and water retention in tomato seedlings, while substrates with 50% acerola bagasse enhance aeration and drainage, thereby benefiting lettuce growth. Fine particles positively influence biomass accumulation in tomato but limit root development in lettuce. Conversely, larger particles support greater leaf area in lettuce while reducing root depth. PCA revealed distinct substrate preferences for tomato and lettuce, underscoring the importance of tailoring substrate composition to crop-specific needs.

Keywords: *Copernicia prunifera*; *Malpighia glabra*; organic substrates; seedling development; sustainable.

Fomento de la germinación y el desarrollo de plántulas de lechuga y tomate mediante sustratos orgánicos sostenibles

Resumen

Objetivo del estudio: El objetivo de este estudio fue evaluar los efectos de sustratos orgánicos formulados con diferentes proporciones de bagazo de acerola (*Malpighia glabra*) y bagana de palma carnauba (*Copernicia prunifera*) en el desarrollo inicial de plántulas de tomate y lechuga, con un enfoque en mejorar la sostenibilidad de las prácticas hortícolas.

Área de estudio: La investigación se llevó a cabo en un ambiente controlado de invernadero en el Centro de Ciencias Agrícolas de la Universidad Federal de Piauí, ubicado en Teresina, Piauí, Brasil.

Material y métodos: El experimento incluyó 12 sustratos diferentes, cada uno formulado a partir de un compost orgánico mezclado con bagazo de acerola y bagana de palma de carnauba. Basaplant®, un sustrato comercial disponible, se utilizó como

control. En este estudio se evaluaron las siguientes variables: porcentaje de emergencia de semillas (ES), índice de velocidad de emergencia (IVE), masa seca total (MST), masa seca de la parte aérea (MSPA), volumen radicular (VR), masa seca de la raíz (MSR), altura de las plántulas (AP), área foliar (AF), diámetro del tallo (DT), masa fresca de la raíz (MFR), masa fresca de la parte aérea (MPPA) e índice de calidad de Dickson (ICD). El análisis estadístico incluyó un análisis de componentes principales (ACP) y un análisis de agrupamiento para determinar la efectividad de los sustratos.

Principales resultados: En el caso del tomate, los sustratos con un 50% de bagana de carnauba mostraron una mayor densidad volumétrica, lo cual se correlacionó positivamente con el VR y la MSR, beneficiando la estabilidad de las plántulas y la retención de agua. Las partículas finas (<0,25 mm) aumentaron la MST, mientras que las partículas entre 0,5 y 0,25 mm afectaron negativamente la EP. En el caso de la lechuga, los sustratos con un 50% de bagazo de acerola promovieron una mayor aireación y drenaje, lo cual resultó beneficioso para este cultivo. Las partículas más grandes (2,0–1,0 mm) se correlacionaron positivamente con el área foliar (AF), mientras que las partículas finas redujeron la masa seca de la raíz. El ACP y el análisis de agrupamiento clasificaron los sustratos según el rendimiento de crecimiento, con el grupo G3 favoreciendo al tomate y el grupo G5 favoreciendo a la lechuga.

Aspectos destacados de la investigación: Este estudio demuestra que los sustratos orgánicos con un 50% de bagana de carnauba mejoran el crecimiento radicular y la retención de agua en plántulas de tomate, mientras que los sustratos con un 50% de bagazo de acerola favorecen la aireación y el drenaje, beneficiando el crecimiento de la lechuga. Las partículas finas influyen positivamente en la acumulación de biomasa en el tomate, pero limitan el desarrollo radicular en la lechuga. Por el contrario, las partículas más grandes aumentan el área foliar en la lechuga, aunque reducen la profundidad de las raíces. El ACP reveló preferencias de sustrato distintas para tomate y lechuga, destacando la importancia de adaptar la composición del sustrato a las necesidades específicas de cada cultivo.

Palabras clave: *Copernicia prunifera*; desarrollo de plántulas; *Malpighia glabra*; orgánicos sostenibles; sustratos.

Citation: Sousa, RS; Barbosa, LMP; Nunes, LAPL; Ferreira, VM; Lima, CJGS; Ramos, HMM; Matos-Filho, CHA (2025). Enhancing germination and seedling development in lettuce and tomato using sustainable organic substrates. Spanish Journal of Agricultural Research, Volume 23, Issue 2, 21256. <https://doi.org/10.5424/sjar/2025232-21256>

Received: 21/06/2024. **Accepted:** 27/11/2024. **Published:** 05/11/2025.

Copyright © 2025 CSIC. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License.

Introduction

Vegetable production is critical for meeting the nutritional needs of the growing world population (Godfray et al., 2010). In recent years, there has been a notable increase in interest surrounding the use of organic substrates as viable alternatives to conventional substrates. This shift is largely driven by the perceived environmental and economic benefits of these organic materials (Mo et al., 2018; Parada et al., 2021). Composting of organic residues, including agricultural byproducts and municipal solid waste, provides a valuable source of organic matter for substrate formulation (Bernal et al., 2009). This type of composting has the potential to significantly reduce environmental impact by allowing for the beneficial reuse of waste that would otherwise be disposed of inappropriately. Furthermore, it can contribute to the sustainability of cultivation systems by repurposing waste materials (Mahmoud et al., 2024).

Seedling development directly influences subsequent growth and crop productivity (Silva et al., 2021). The selection of an appropriate substrate is crucial, as it significantly impacts root development, nutrient uptake, and overall plant health. Organic substrates have been shown to promote seedling growth due to their high organic matter content, which provides a favorable environment for root growth and nutrient availability (Olaría et al., 2016; Mahmoud et al., 2024). Furthermore, the incorporation of organic waste can contribute to the preservation of ecological balance and long-term environmental health (Karsinah et al. 2023).

Vegetables, such as lettuce (*Lactuca sativa* L.) and tomato (*Solanum lycopersicum* L.), have significant economic and nutritional value. Seedling quality is critical for the successful cultivation of these crops. Therefore, it is essential to optimize growth conditions and substrate composition to ensure the production of healthy and vigorous seedlings (Funes-Pinter et al., 2022). Substrate particle size distribution is a critical element, as it influences water retention, porosity, and nutrient availability. A harmonious blend of fine and coarse particles can enhance water retention and porosity, facilitating root growth and nutrient uptake (Gutiérrez-Castorena et al., 2011; Giuffrida & Consoli, 2016).

The utilization of agro-industrial waste, such as carnauba palm bagana (*Copernicia prunifera* (Mill.) H.E. Moore) and acerola (*Malpighia glabra* L.) bagasse, represents a pioneering approach for substrate formulation. Carnauba bagana, a by-product of the carnauba wax industry, has been demonstrated to enhance the physical and chemical characteristics of substrates, including increased porosity, water retention, and essential nutrient content (Sousa et al., 2020; Leite et al., 2023). Similarly, acerola pomace, a waste product from juice production, has the potential to enrich substrates with nutrients and offers a sustainable solution to improper waste disposal (Silva et al., 2023). Evidence suggests that composts derived from agricultural and agro-industrial waste can impart ideal physical and chemical properties to substrates, facilitating seedling growth and serving as viable alternatives to peat and other commercial substrates (MirseyedHosseini et al., 2023; Mahmoud et al., 2024).

The aim of this study was to evaluate the effect of organic substrates formulated with different proportions of acerola bagasse and carnauba bagana on the initial development of tomato and lettuce seedlings. The hypothesis tested was that properties such

as bulk density and particle size distribution (granulometry) of the substrates would have a differential impact on the growth and vigor of the seedlings. The use of these alternative organic substrates represents a sustainable and economical option for the production of vegetable seedlings. This study seeks to contribute to more efficient and ecologically responsible practices in the production of tomato and lettuce seedlings.

Material and methods

Location and duration of the experiment

The experiments were conducted between November 2019 and March 2020, spanning a total of 150 days. The location of the experiments was the Agricultural Sciences Center of the Federal University of Piauí (UFPI), situated in Teresina, Piauí (05°05' S; 42°48' W, 75 m). The climate of the region is classified as Aw (tropical with dry season) according to the Köppen system, with two well-defined seasons: a rainy summer and a dry winter. The average annual temperature is 30 °C, and the average annual rainfall is between 1300 and 1600 mm (Alvares et al., 2013).

Study stages

The study was conducted in three main stages to facilitate monitoring and evaluation. The first stage involved the setup and monitoring of composting of rumen digesta (RD), chicken litter (CL), tree pruning residue (TPR), and fresh cattle manure (CM). The second stage entailed the formulation of substrates with varying compositions using the produced composts. The third stage involved the simultaneous evaluation of lettuce and tomato seedling development in a greenhouse, with the aim of assessing the potential of the organic substrates for seedling production.

Preparation and characterization of the composts

The organic waste was collected from local sources: the RD was obtained from a slaughterhouse in the municipality of Teresina, the CL from local producers, and the CM and TPR from the Agricultural Sciences Center of the Federal University of Piauí. Four composting windrows were set up in different combinations to create the organic compost. The four composting windrows were designated as OC 1 [RD+CM (3:1; v:v)]; OC 2 [TPR+CM (2:1; v:v)]; OC 3 [RD+CL (2:1; v:v)]; and OC 4 [TPR+CL (2:1; v:v)]. The compost windrows were situated within a covered area to circumvent direct contact with solar radiation, thereby preventing excessive drying and ensuring optimal conditions for humification (Kiehl, 2004).

The windrows were monitored on a triweekly basis to ascertain the temperature and humidity levels, with the contents being rotated every 20 days. This was performed to ensure optimal conditions for the organisms involved in the composting process. The temperature was gauged with the aid of a digital thermometer, with a range of -50 °C to +300 °C and an accuracy of ± 1 °C, while the humidity was determined using the gravimetric method. The compost windrows were irrigated regularly to maintain the moisture content within the range of 40% to 60%. The maturation of the composts was completed within a period of 90–120 days, during which the C/N ratio reached a value below 18. Following the composting period, compost samples were dried in a forced convection oven at 65 °C for 72 h. Thereafter, dry matter and nutrient concentrations were determined. Chemical attributes were analyzed in accordance with Normative Instruction No. 17 of the Ministry of Agriculture, Livestock, and Food Supply (Brasil, 2017). The analyses were conducted at the Soil Quality Laboratory of the Federal University of Piauí and encompassed macronutrients (N, P, K, Ca, and Na), pH, carbon content, and C/N ratio (Table 1).

Table 1. Chemical properties of sustainable organic composts used in substrate formulations for lettuce and tomato seedling production.

Attributes		Organic compost ^a			
		OC 1	OC 2	OC 3	OC 4
pH	(H ₂ O)	6.6	6.8	5.8	6.7
N	(g kg ⁻¹)	19.7	10.8	15.2	10.1
P	(g kg ⁻¹)	5.2	7.7	5.0	6.3
K	(g kg ⁻¹)	18.0	16.0	18.0	36.0
Ca	(g kg ⁻¹)	9.75	10.0	8.4	12.0
Mg	(g kg ⁻¹)	3.2	3.3	2.8	4.0
Na	(g kg ⁻¹)	16.0	5.0	20.0	8.0
Organic matter	(%)	41.09	26.44	29.87	25.94
C/N	-	12.1	14.2	11.4	14.9

^aOC 1 [RD+CM (3:1, v:v)], OC 2 [TPR+CM (2:1, v:v)], OC 3 [RD+CL (2:1, v:v)], and OC 4 [TPR+CL (2:1, v:v)]. RD: ruminal digesta, CL: chicken litter, TPR: tree pruning residue, and CM: fresh cattle manure.

Formulation and evaluation of substrates for seedling production

Once the organic composts reached the requisite maturity, 12 sustainable organic substrates were formulated in accordance with the specifications set forth in Table 2. A commercial substrate for seedlings (Basaplant®) was used as a control (CK) for comparison. The granulometry of the substrates was evaluated using dry sieving. At the outset of the process, each material was passed through a 5.0 mm sieve to achieve homogenization. Subsequently, 100 g of each air-dried material was sieved through a series of sieves with mesh sizes of 2.0 mm, 1.0 mm, 0.5 mm, and 0.25 mm, and agitated for a period of five minutes. The fractions retained on each sieve were subsequently weighed and the percentage of the total weight of each sample was calculated for each fraction (Fig. 1). The density was determined by weighing samples of soaked, drained, and dried substrates in a cylinder of known volume. The densities of the substrates, expressed in g cm^{-3} , are presented in the following: S1=0.13, S2=0.11, S3=0.13, S4=0.07, S5=0.08, S6=0.11, S7=0.13, S8=0.12, S9=0.13, S10=0.11, S11=0.09, S12=0.09, and CK=0.19.

Table 2. Composition and volumetric ratios of sustainable organic substrates for lettuce and tomato seedling production.

Substrate	Composition ^a	Ratio (v:v:v)
S1	OC 1 + acerola bagasse + carnauba palm bagana	1:1:1
S2	OC 1 + acerola bagasse	1:2
S3	OC 1 + carnauba palm bagana	1:2
S4	OC 2 + acerola bagasse + carnauba palm bagana	1:1:1
S5	OC 2 + acerola bagasse	1:2
S6	OC 2 + carnauba palm bagana	1:2
S7	OC 3 + acerola bagasse + carnauba palm bagana	1:1:1
S8	OC 3 + acerola bagasse	1:2
S9	OC 3 + carnauba palm bagana	1:2
S10	OC 4 + acerola bagasse + carnauba palm bagana	1:1:1
S11	OC 4 + acerola bagasse	1:2
S12	OC 4 + carnauba palm bagana	1:2
CK	Commercial substrate (Basaplant®)	-

^a OC 1 [RD+CM (3:1, v:v)], OC 2 [TPR+CM (2:1, v:v)], OC 3 [RD+CL (2:1, v:v)], and OC 4 [TPR+CL (2:1, v:v)]. RD: ruminal digesta, CL: chicken litter, TPR: tree pruning residue, and CM: fresh cattle manure.

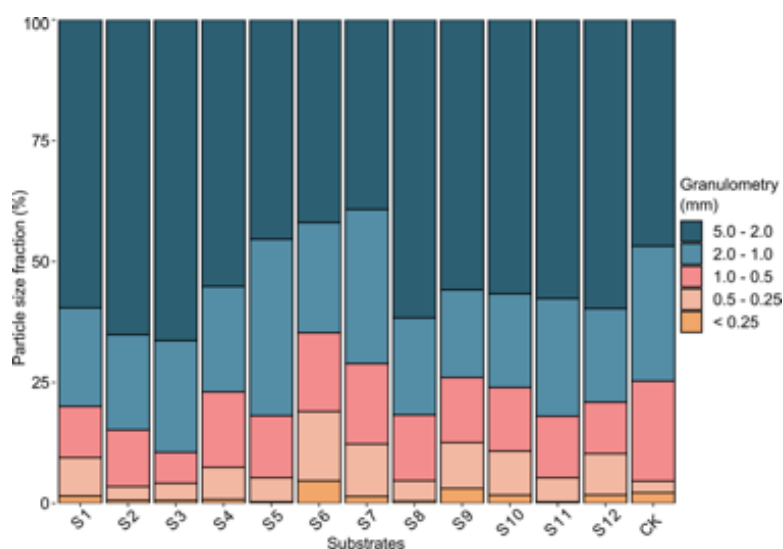


Figure 1. The proportion of particles in different particle size intervals on different substrates for lettuce and tomato seedling production. CK: Commercial substrate (Basaplant®).

Seeding and monitoring the development of tomato and lettuce plants

Lettuce (cv. Verdinha), and tomato (cv. Caline IPA) seedlings were cultivated concurrently in a greenhouse setting. The experimental design was entirely randomized, with 13 substrates and four replicates. Each experimental plot consisted of eight seedlings. The sowing process was initiated on March 20, 2020, utilizing expanded polystyrene trays (128 cells) arranged in a linear configuration on two perpendicular benches, measuring 4.9 m in length, 1.0 m in width, and 1.0 m in height. Each cell type was populated with a single seed. One week after sowing, seedling emergence was assessed for both crops. The emergence speed index (ESI) was calculated as the ratio of the number of emerging plants to the number of days since sowing, as proposed by Maguire (1962).

Once the seedlings reached the optimal size for commercial transplantation (approximately 10–15 cm for lettuce and 15–20 cm for tomato), which occurred approximately 25 days after sowing, they were harvested and separated into aerial parts and roots. Root volume (RV) was evaluated in both crops by displacing the water column in a graduated cylinder, in accordance with the methodology proposed by Basso (1999). Subsequently, the shoot dry mass (SDM) and dry root mass (DRM) were determined following a 72-hour drying period at 60 °C in a forced ventilation oven. Total dry mass (TDM) was calculated as the sum of DSM and DRM. Additionally, the fresh mass of shoots (FSM) and roots (FRM) were determined using a 0.001 g precision scale. In the lettuce seedlings, leaf area (LA) was quantified using the ImageJ® software (Rasband, 2004). In tomato plants, the shoot height (SH), expressed in cm, was measured from the neck to the apical bud, and the diameter of the stem (SD), in mm, was measured at the base of the neck using a digital caliper with a precision of 0.01 mm.

Statistical analysis

The experimental design was completely randomized, with 13 substrates and four replications. Each plot consisted of eight seedlings. All statistical analyses and graphical representations were conducted using the R software, v. 4.1.3 (R Core Team, 2022). Prior to conducting the primary analyses, we verified that the requisite assumptions for the analysis of variance were satisfied. The Shapiro-Wilk test indicated that the residuals followed a normal distribution, and Bartlett's test confirmed the homogeneity of the variances between the groups, eliminating the need for data transformation. Substrate comparisons were conducted using the Scott-Knott test ($p \leq 0.05$), with the “easynova” package (Arnhold, 2013). Additionally, box plots were generated for each measured characteristic using the “ggpubr” package (Kassambara, 2020), facilitating visual representation of the data distribution and intra- and inter-group variances.

To further examine the relationships and groupings between the variables and observations of lettuce and tomato seedlings, multivariate analyses were conducted. Principal component analysis (PCA) was employed to ascertain the principal axes of variation and illustrate the grouping of observations. Subsequently, k-means cluster analysis was conducted to categorize the observations into distinct groups. The results of the cluster analysis were illustrated using a PCA biplot for comprehensive visual interpretation. These analyses were conducted with the “stats” and “factoextra” packages (Kassambara & Mundt, 2020), as well as the “cluster” package (Maechler et al., 2021), which provide advanced clustering algorithms and visualization options. An additional figure to help interpret the results is presented in the Supplementary Material (Supplementary Fig. S1).

Results

Evaluation of substrate suitability for tomato cultivation

The evaluation of different substrates on tomato seedling morphology and quality parameters indicated statistically significant effects ($P < 0.001$). Notably, substrates S4, S8, S11, and the control (CK) had the highest seed emergence rates, indicating their efficacy in supporting germination (Fig. 2A). Conversely, S9 showed diminished performance in the emergence speed index, suggesting reduced effectiveness in the initial seedling growth stages (Fig. 2B).

Stem diameter analyses revealed that substrates S3 and S7 were the most beneficial, suggesting they provide optimal nutrition and structural conditions for seedling development (Fig. 2C). In contrast, substrates S5, S11, and CK were less conducive to seedling height, possibly limiting elongation during the early growth phase (Fig. 2D).

Significant differences were observed in root development. The CK substrate significantly enhanced root volume (Fig. 2E), while S3 led to root dry mass accumulation, indicating its superior capability to support root biomass development (Fig. 2F). Conversely, substrates S5, S10, and S11 showed the lowest root dry mass, underscoring the need for detailed substrate composition analysis to optimize seedling outputs.

Shoot dry mass varied significantly across substrates, with S3 displaying the highest values, suggesting it provides an optimal nutrient balance and vegetative growth conditions. Both the total dry mass and Dickson quality index were the highest in substrates S3 and S12 (Fig. 2G–H), indicating these substrates offer an ideal mix of conditions for root and shoot growth essential for high-quality seedling production (Suppl. Fig. S1).

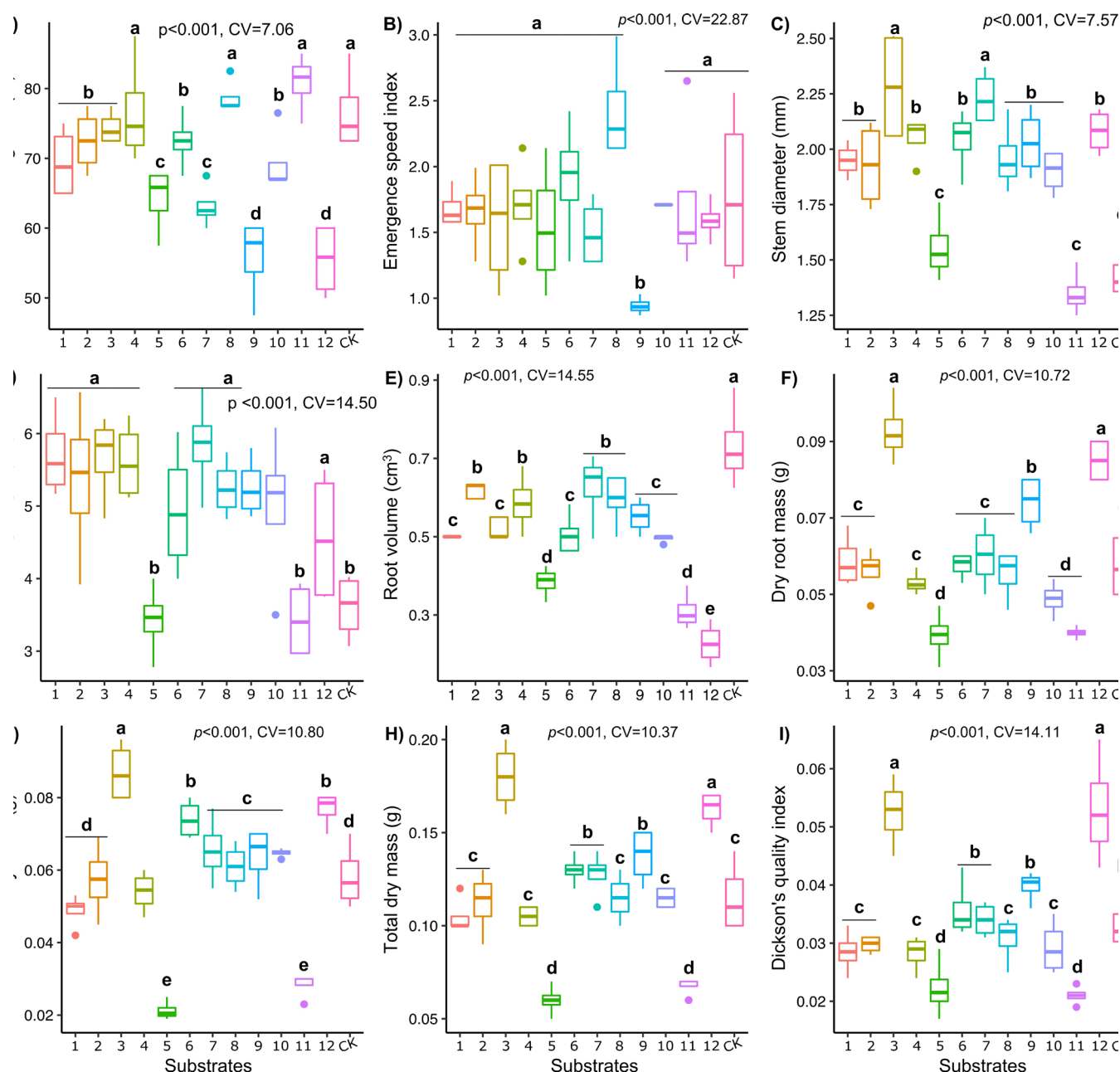


Figure 2. Boxplots for A) seed emergence, B) emergence speed index, C) stem diameter, D) seedling height, E) root volume, F) dry root mass, G) shoot dry mass, H) total dry mass, and I) Dickson's quality index of tomato seedlings produced on different substrates 25 days after sowing. Different letters above the box indicate significant differences according to the Scott Knott test ($p < 0.05$). CK: Commercial substrate (Basaplant®).

Principal component analysis (PCA) explained 75.8% of the total variation in growth performance across substrates through the first two principal components, PC1 (53.7%) and PC2 (22.1%), effectively grouping the substrates into three distinct clusters (Fig. 3A-C). Substrates in G1 (S5 and S11) were identified as underperformers in growth metrics, suggesting their composition may not be optimal for plant development. Conversely, G2 (S1, S2, S4, S6, S7, S8, S10, and CK) exhibited a more balanced performance with satisfactory emergence rates. G3 (S3, S9, and S12) exhibited superior performance in growth and seedling quality variables, including TDM, SDM, DRM, DQI, and SD. This enhanced performance indicates a more favorable physicochemical substrate composition. Analysis of the contributions of the variables to the first two principal components (Fig. 3B) indicated that the TDM, SDM, DRM, DQI, and SD were the most influential. These variables are crucial for evaluating the quality of tomato seedlings and their substrate performance, suggesting that a multifaceted approach is essential for selecting effective substrates.

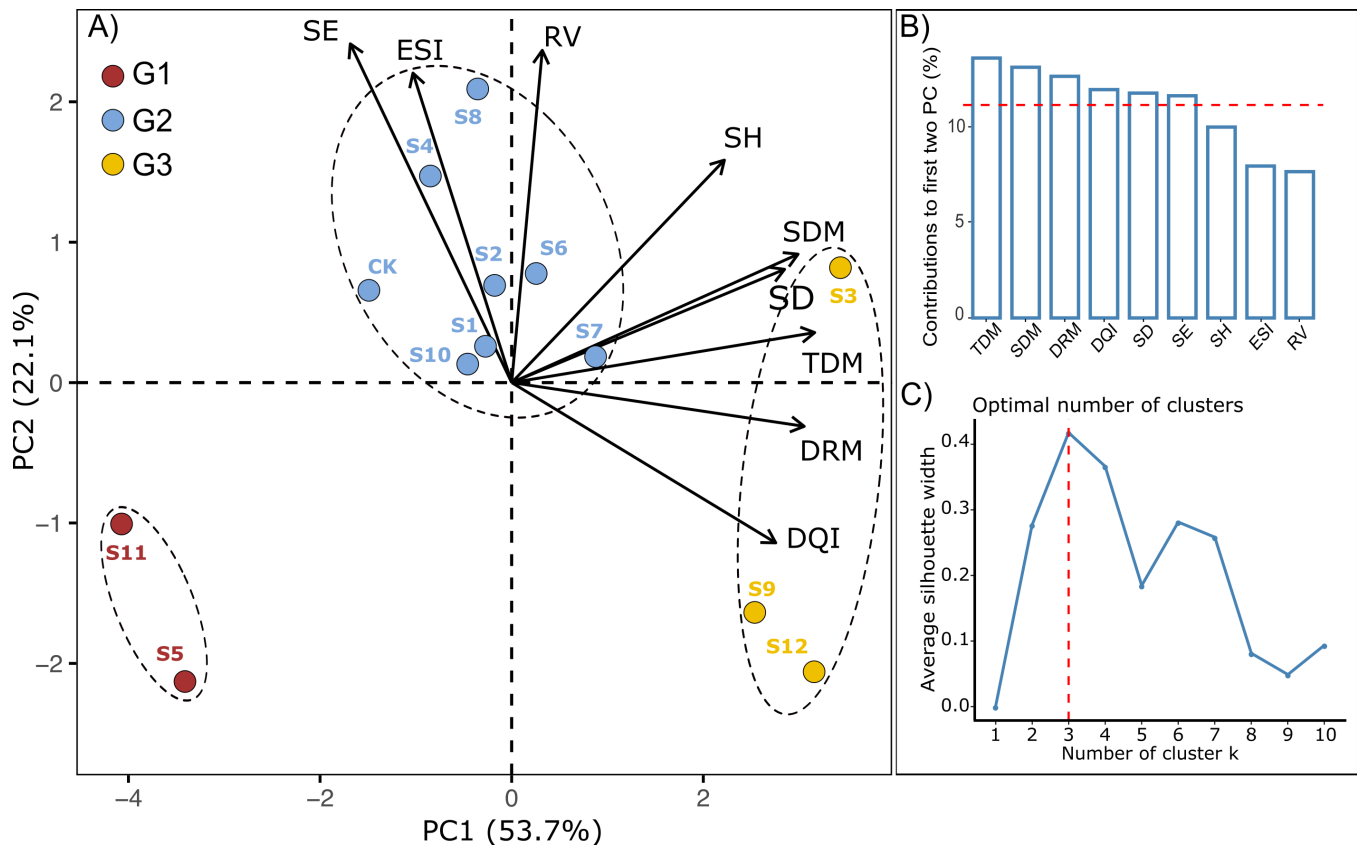


Figure 3. Principal component analysis of the tomato seedlings produced on different substrates at 25 d after sowing (A).

Contribution of each morphological variable of tomato seedlings to the first two principal components (B), and the optimal number of clusters to the substrates (C). The black dashed ellipses in panel “A” indicate nonhierarchical cluster formation by the k-means method. The red dashed line in panel “B” indicates the theoretical contribution of the morphological variables of the tomato plants. S, substrate; SE, seed emergence (%); ESI, emergence speed index; SD, stem diameter (mm); SH, seedling height (cm); RV, root volume (cm³); DRM, dry root mass (g); SDM, shoot dry mass (g); TDM, total dry mass (g); DQI, Dickson quality index.

Evaluation of substrate suitability for lettuce cultivation

Germination and subsequent development of lettuce seedlings exhibited significant variability across different substrates (Fig. 4A). The highest emergence percentage was observed for substrate S8, comparable to CK, indicating its efficacy in supporting germination. In contrast, substrates S4 and S7 exhibited the lowest emergence percentages, potentially due to suboptimal physical or chemical properties.

Notable variations were observed in the emergence speed index. Substrates S2, S3, and S9 facilitated rapid and uniform seedling emergence (Fig. 4B), while S1 and S6 exhibited lower indices, indicating deficiencies in their composition that impeded early seedling vigor.

Further analysis revealed significant differences in vegetative development. The CK substrate exhibited the largest leaf area, promoting robust initial growth, while substrate S12 demonstrated the least effectiveness, potentially impeding overall biomass accumulation.

Significant disparities were observed in root volume, with substrates S2 and S9 providing optimal conditions for root development, crucial for effective nutrient and water uptake (Fig. 4D). This robust root development is indicative of the suitability of the substrate to support overall plant health and productivity.

The fresh mass measurements of the roots and shoots exhibited considerable variation across substrates. The results indicated that S2, S5, S6, and S12 were superior for root mass, whereas S1, S10, S11, and CK were superior for shoot mass (Fig. 4E-F). These findings suggest that certain substrates effectively support both below- and aboveground development, which is crucial for balanced and vigorous plant growth.

Furthermore, notable discrepancies were observed in root dry mass, with substrates S1, S2, S3, S4, S5, S6, S7, S8, S9, and S11 facilitating root growth (Fig. 4G-H). Shoot dry mass and total dry mass also exhibited significant variation among the substrates, with S3 and S5 displaying the highest values, suggesting their effectiveness in promoting overall plant growth (Fig. 4I). Conversely, substrates S10 and S12 exhibited the lowest total dry mass, indicating the need for improvements in their formulation to optimize performance.

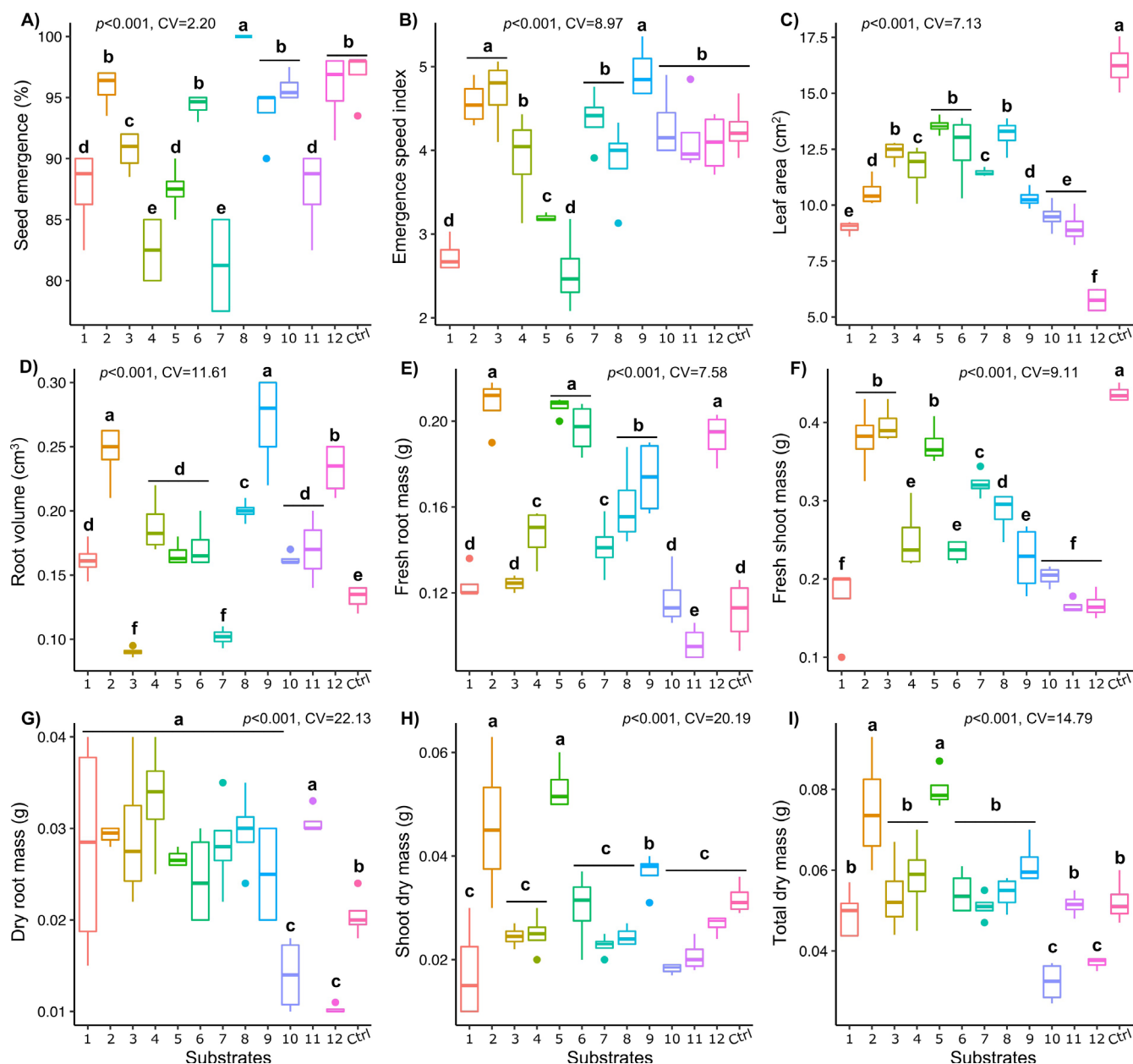


Figure 4. Boxplots for A) seed emergence, B) emergence speed index, C) leaf area, D) root volume, E) fresh root mass, F) fresh shoot mass, G) dry root mass, H) shoot dry mass, and I) total dry mass of lettuce seedlings produced on different substrates 25 days after sowing. The same letters above the box do not indicate significant differences according to the Scott–Knott test ($p < 0.05$). CK: Commercial substrate (Basaplant®).

Principal component analysis (PCA) and k-means cluster analysis delineated the substrates into five distinct groups (G1–G5), reflecting the significant impact of substrate composition on seedling development (Fig. 5A–C). The first two principal components (PC1 and PC2) explained 61.8% of the total variation in the data (35.5% and 26.3 %, respectively). The most significant contributions to the PCA were from SDM, TDM, RV, FRM, FSM, and LA, underscoring their importance in assessing seedling quality and growth potential.

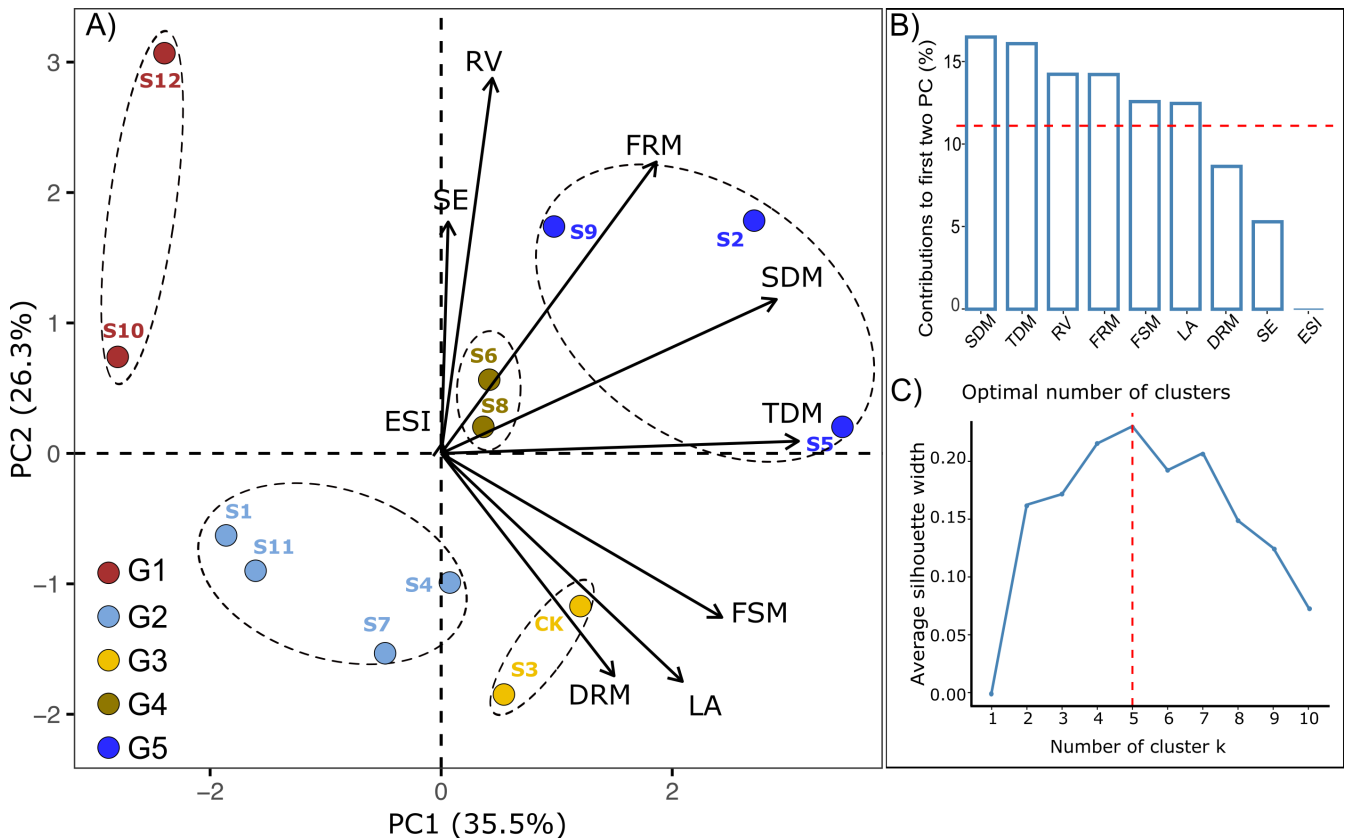


Figure 5. Principal component analysis of the lettuce seedlings produced on different substrates at 25 days after sowing (A). Contribution of each morphological variable of lettuce seedlings to the first two principal components (B), and the optimal number of clusters to the substrates (C). The black dashed ellipses in panel “A” indicate nonhierarchical cluster formation by the k-means method. The red dashed line in panel “B” indicates the theoretical contribution of the morphological variables of the lettuce seedlings. S, substrates; SE, seed emergence (%); ESI, emergence speed index; LA, leaf area (cm²); RV, root volume (cm³); FRM, fresh root mass (g); FSM, fresh shoot mass (g); DRM, dry root mass (g); SDM, shoot dry mass (g); TDM, total dry mass (g).

Discussion

Evaluation of substrate suitability for tomato cultivation

The findings indicate that substrates comprising 50% carnauba bagana tend to exhibit a higher volumetric density compared to substrates containing 50% acerola bagasse. The higher volumetric density demonstrated a positive correlation with root volume and root dry mass in tomato seedlings (Suppl. Fig. S1), indicating that the denser structure of the substrates with carnauba bagana provides favorable physical support and stability for root development. This characteristic also promotes optimized water retention, which benefits the availability of water in the regions of active root growth. This is essential for crops such as tomato, which have a moderate to high demand for water in the initial phase (Sousa et al., 2020; Mendonça et al., 2021).

The presence of fine particles (less than 0.25 mm) in the substrates was found to be beneficial for tomato, exhibiting a positive correlation with the total dry mass. This relationship indicates that a greater proportion of these particles may facilitate the retention of moisture and the availability of nutrients in the vicinity of the roots, which could be beneficial for the accumulation of total biomass. Conversely, particles between 0.5 mm and 0.25 mm exhibited a negative correlation with seedling emergence, suggesting that a high concentration of these particles may impede the initial germination process. This could be attributed to increased substrate compaction, which restricts oxygenation and hinders early root growth (Castro et al., 2016; Monteiro et al., 2020).

Evaluation of substrate suitability for lettuce cultivation

The results demonstrated that substrates with a lower volumetric density, such as those containing 50% acerola bagasse, were more conducive to optimal lettuce growth. The low density of these substrates facilitates greater aeration and drainage, which are beneficial

characteristics for the initial growth of lettuce seedlings. This crop is generally sensitive to excess moisture and compaction, and thus the aforementioned characteristics are advantageous. However, a notable negative correlation was identified between particles with a diameter of less than 0.25 mm and the root dry mass of lettuce seedlings, indicating that an excess of these fine particles may impede oxygenation and drainage, thereby limiting the root development of this crop (Albano et al., 2017; Mendonça et al., 2021).

Larger particles (between 2.0 mm and 1.0 mm) showed a negative correlation with root volume and a positive correlation with leaf area in lettuce seedlings. This positive correlation with LA indicates that larger particles enhance aeration and drainage, conditions that are conducive to leaf development in lettuce. Conversely, the negative correlation with RV indicates that these particles may restrict root growth in depth, which may be beneficial for lettuce, which typically develops a more superficial root system. These findings align with previous research, which suggests that substrates with larger particles provide an optimal balance between aeration and water retention, which are essential for robust leaf development in lettuce (Zuffo et al., 2020; Ripp et al., 2020).

Influence of different substrate components

The analysis of the substrates composed of acerola bagasse and carnauba bagasse revealed that these raw materials exerted disparate influences on the development of the seedlings. The results demonstrated that carnauba bagasse, which has a higher volumetric density, yielded superior outcomes for tomato, whereas acerola bagasse, which has a lower density, facilitated the growth of lettuce seedlings. These effects may be attributed to the physical properties of these materials. Denser substrates exhibit greater water retention and structural support, whereas lighter substrates provide enhanced aeration and drainage, which are beneficial for crops with smaller roots (Albano et al., 2017; Mendonça et al., 2021).

The findings of the PCA and the clustering of substrates serve to reinforce the influence of physicochemical properties on seedling performance. The formation of groups, such as the G3 group for tomato and the G5 group for lettuce, demonstrated that substrates with specific physical characteristics, including particle size composition and density, exert a direct influence on crop growth. The larger particles and greater density of carnauba bagasse favored group G3, which exhibited higher root growth and total dry mass parameters in tomato. Conversely, group G5, comprising substrates of lower density and a greater proportion of fine particles, was observed to facilitate enhanced leaf development in lettuce, as evidenced by elevated emergence rates and leaf area growth (Mendonça et al., 2021; Griebeler et al., 2023).

Conclusions

The findings of this study corroborate the hypothesis that particular organic substrates, comprising varying proportions of carnauba bagasse and acerola bagasse, can facilitate the optimal growth of diverse crops. These substrates offer a sustainable and cost-effective alternative to conventional commercial substrates. The formulations in question enhance both seedling performance and sustainability by leveraging agro-industrial waste and reducing reliance on traditional substrates. Nevertheless, future research should prioritize elucidating the interactions between diverse substrate components and the plant's specific physiological requirements.

Supplementary material: Figure S1 accompanies the paper on SJAR's website.

Competing interests: The authors have declared that no competing interests exist.

Statement about use of generative AI: The author(s) employed Translator DeepL in order to translate the title, abstract and key-words from English to Spanish, while preparing this work. Following their use, the author(s) assumed full responsibility for the publication's content and reviewed and edited it as necessary.

Authors' contributions: **Ricardo S. de Sousa:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Larissa M. de P. Barbosa:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Luís A. P. L. Nunes:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Valber M. Ferreira:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Carlos J. G. de S. Lima:** Formal analysis, Investigation, Writing – review & editing. **Herbert M. M. Ramos:** Formal analysis, Investigation, Writing – review & editing. **Carlos H. A. Matos Filho:** Visualization, Writing – original draft, Writing – review & editing.

Funding: The authors received no specific funding for this work.

References

Albano FG, Cavalcante IHL, Machado JS, Lacerda CF, Silva ER, Sousa HG, 2017. New substrate containing agroindustrial

- carnauba residue for production of papaya under foliar fertilization. *Rev. Bras. de Eng. Agríc. Ambient.* 21(2):128-133. <https://doi.org/10.1590/1807-1929/agriambi.v21n2p128-133>
- Alvares CA, Staple JL, Sentelhas PC, Gonçalves JLM, Sparovek G, 2013. Köppen's Climate Classification Map for Brazil. *Meteorol. Z.* 22: 711-728. <https://doi.org/10.1127/0941-2948/2013/0507>
- Arnhold E, 2013. Package in the R environment for analysis of variance and complementary analyses. *Braz. J. Vet. Res. Anim. Sci.* 50: 488-492. <https://doi.org/10.11606/issn.1678-4456.v50i6p488-492>
- Basso SMSC, 1999. Caracterização morfológica e fixação biológica de nitrogênio de espécies de *Adesmia* DC. e *Lotus* L. Tese de Doutorado. Universidade Federal do Rio Grande do Sul, Porto Alegre. 268 pp.
- Bernal MP, Albuquerque JA, Moral R, 2009. Composting of animal manures and chemical criteria for compost maturity assessment: A review. *Bioresour. Technol.* 100: 5444–5453. <https://doi.org/10.1016/j.biortech.2008.11.027>
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento, 2007. Instrução Normativa nº 17, de 7 de abril de 2017. Brasília, DF: MAPA. <https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-agricolas/fertilizantes/legislacao/in-17-de-21-05-2007-aprova-metodo-substrato.pdf>.
- Castro VC, Fernandes MM, Fernandes MRM, Nóbrega RSA, 2016. Avaliação de diferentes substratos orgânicos para mudas de *Enterolobium contortisiliquum* em uma área desertificada. *Rev. Agrogeoambiental* 8(3):101-109. <https://doi.org/10.18406/2316-1817v8n32016884>
- Funes-Pinter I, Salomón MV, Martín JN, Uliarte EM, Hidalgo A, 2022. Effect of bioslurries on tomato *Solanum lycopersicum* L and lettuce *Lactuca sativa* development. *Rev. Fac. Cienc. Agrar.* 54: 48–60. <https://doi.org/10.48162/rev.39.082>
- Giuffrida F, Consoli S, 2016. Reusing perlite substrates in soilless cultivation: analysis of particle size, hydraulic properties, and solarization effects. *J. Irrig. Drain. Eng.* 142: 04015047. [https://doi.org/10.1061/\(ASCE\)IR.1943-4774.0000968](https://doi.org/10.1061/(ASCE)IR.1943-4774.0000968)
- Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C, 2010. Food security: the challenge of feeding 9 billion people. *Science* 327: 812–818. <https://doi.org/10.1126/science.1185383>
- Griebeler AM, Araujo MM, Turchetto F, Berghetti ÁLP, Rorato DG, Fermino MH, Aimi SC, Costella C, 2023. Organic waste and controlled-release fertilizer maximize the growth of *Citharexylum montevidense* in nursery and in the field. *CERNE* 29:e–103003. <https://doi.org/10.1590/01047760202329013003>
- Gutiérrez-Castorena MDC, Escobar JH, Ortiz-Solorio CA, Sánchez RA, Lara EH, 2011. Relación porosidad-retención de humedad en mezclas de sustratos y su efecto sobre variables respuesta en plántulas de lechuga. *Rev. Chapingo Ser. Horticultura* 17: 183-196.
- Karsinah, Prajanti SDW, Amelia DR, 2023. Organic mulch innovation from straw waste in the context of realizing climate smart agriculture as an acceleration of green economy achievement. *J. Law Sustain. Dev.* 11: e2281. <https://doi.org/10.55908/sdgs.v11i12.2281>
- Kassambara A, Mundt F, 2020. factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7.
- Kassambara A, 2020. ggpubr: 'ggplot2' based publication ready plots. R package version 0.4.0.
- Kiehl EJ, 2004. Manual de Compostagem: maturação e qualidade do composto. 4 ed. Piracicaba: E. J. Kiehl.
- Leite MRL, Costa RM, Matos SS, Andrade HAF, Silva-Matos RRS, 2023. Production of pre-sprouted sugarcane seedlings using carnauba bagana as substrate. *Pesqui. Agropecu. Trop.* 53:e74082. <https://doi.org/10.1590/1983-40632023v5374082>
- Maechler M, Rousseeuw P, Struyf A, Hubert M, Hornik K, 2021. Cluster: cluster analysis basics and extensions. R package version 2.1.2.
- Mahmoud AMA, Mohamed Y, El-Helaly MA, Afifi MMI, El-Tawashy MKF, 2024. Potential of producing organic lettuce seedlings without peat using agricultural and agro-industrial compost. *J. of Plant Nutr.* 47(19):3316–3342. <https://doi.org/10.1080/01904167.2024.2378927>

- Maguire JD, 1962. Speed of germination—aid in selection and evaluation for seedling emergence and vigor. *Crop Sci.* 2: 176-177. <https://doi.org/10.2135/cropsci1962.0011183X000200020033x>
- Mendonça AM, Natale W, Sousa GG, Silva Junior FB, 2021. Morphophysiology and nutrition of yellow passion fruit seedlings grown in substrates based on carnauba palm bagana, *Rev. Bras. de Cien. Agra.* 16(3):e132. <https://doi.org/10.5039/agraria.v16i3a132>
- MirseyedHosseini H, Alavipoor E, Delshad M, 2023. Evaluation of different growth media for tomato seedlings to optimize production and water use. *Iran Agric. Res.* 36(2):61-70. <https://doi.org/10.22099/iar.2017.4138>
- Mo J, Yang Q, Zhang N, Zhang W, Zheng Y, Zhang Z, 2018. A review on agro-industrial waste (AIW) derived adsorbents for water and wastewater treatment. *J. Environ. Manage.* 227: 395–405. <https://doi.org/10.1016/j.jenvman.2018.08.069>
- Monteiro AS, Barbosa MM, Silva FFM, Bezerra RF, Maia KS, 2020. Preparation, phytochemical and bromatological evaluation of flour obtained from the acerola (*Malpighia puniceifolia*) agroindustrial residue with potential use as fiber source. *LWT* 134:110142–110142. <https://doi.org/10.1016/j.lwt.2020.110142>
- Olaria M, Nebot JF, Molina H, Troncho P, Lapeña L, Llorens E, 2016. Effect of different substrates for organic agriculture in seedling development of traditional species of Solanaceae. *Span. J. Agric. Res.* 14: e0801. <https://doi.org/10.5424/sjar/2016141-8013>
- Parada F, Ercilla-Montserrat M, Arcas-Pilz V, Lopez-Capel E, Carazo N, Montero JI, Gabarrell X, Villalba G, Rieradevall J, Pere Muñoz, 2021. Comparison of organic substrates in urban rooftop agriculture, toward improving crop production resilience to temporary drought in Mediterranean cities. *J. Sci. Food Agric.* 101: 5888–5897. <https://doi.org/10.1002/jsfa.11241>
- R Core Team, 2022. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.
- Rasband WS, 2004. ImageJ. National Institutes of Health, Bethesda, Maryland, USA. <http://rsb.info.nih.gov/ij/>.
- Ripp PG, Gusmão AP, Lorin HEF, Costa MSS de M, Edwiges T, 2020. Composting process in the production of lettuce seedling substrates: effect of covering and turning frequency. *Eng. Agric.* 40: 562-570. <https://doi.org/10.1590/1809-4430-eng.agric.v40n5p562-570/2020>
- Silva NC da, Dourado PLA, Tosi MM, 2023. Uma revisão sobre os subprodutos da acerola (*Malpighia emarginata* DC.) como uma matéria-prima promissora para a geração de produtos verdes. *Rev. Bras. Tecnol. Alimentar* 26: e2023039. <https://doi.org/10.1590/1981-6723.03923>
- Silva TV, Melo HC, Campos LFC, Tarazi MFA, Cunha Junior LC, Nascimento AR, 2021. Growth and productivity of tomato under use of biostimulant and ages of seedling transplantation. *Colloq. Agrariae* 17: 23-33. <https://doi.org/10.5747/ca.2021.v17.n4.a446>
- Sousa MO, Matos RRSS, Cardoso JPS, Cordeiro KV, Barbosa LMP, Santos JF, Oliveira ARF, Machado FGA, Costa NA, Oliveira MMT, 2020. Bagana de carnaúba como substrato na produção de mudas de açaí cultivar BRS-Pará. *Rev. Ibero-Am. Cien. Amb.* 11(5):113-122. <https://doi.org/10.6008/CBPC2179-6858.2020.005.0012>
- Zuffo AM, González-Aguilera J, Lima RE, Zaratín Alves C, 2020. Substrates for the production of lettuce seedlings. *Eur. J. Hortic. Sci.* 85: 372-379. <https://doi.org/10.17660/eJHS.2020/85.5.9>