Vicia faba L. microgreens: effect of the variety and substrates on the morphological characteristics, photosynthetic pigment content, and soluble protein

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ABSTRACT

Objective: To evaluate the effect of variety and substrates on the morphological characteristics, photosynthetic pigment content, and soluble protein of fava bean (*Vicia faba* L.) microgreens cultivated in a prototype growth chamber.

Design/Methodology/Approach: Microgreens from two local fava bean varieties (T-5 and T-12) were cultivated in aluminum trays under 8/16 h light/dark cycles using 30 W LED bulbs. The substrates evaluated were sand and coconut fiber (CF). The microgreens were harvested eight days after emergence, and morphological variables (fresh weight, height, root length), photosynthetic pigment content, and soluble protein were determined.

Results: The chamber prototype maintained stable environmental conditions (temperature 20-25 °C, relative humidity 65-88%, luminosity 165-206 μmol m^{-2} s^{-1}). Variety T-5 showed higher fresh weight (2.44 g/plant) and height (19.86 cm) in CF compared to sand. Both varieties exhibited greater root length in CF. Only variety T-12 showed higher chlorophyll a (119.47 μg/g FW) and total chlorophyll (171.24 μg/g FW) content in CF. Soluble protein was higher in T-5 cultivated in sand (69.67 mg/g DM). Dry matter showed no significant differences between substrates (10.84-11.91%).

Limitations/Implications: The results are limited to the substrates and varieties evaluated; future studies could include more substrates and adjustments to the photoperiod.

Findings/Conclusions: The morphological and biochemical characteristics of fava bean microgreens were significantly influenced by the variety × substrate interaction, highlighting the importance of selecting the appropriate substrate according to the variety to optimize production and nutritional quality.

Keywords: sand, coconut fiber, fava bean, microgreens, growth chamber prototype.

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INTRODUCTION

The growing demand for alternative foods to combat chronic noncommunicable diseases, such as obesity, diabetes and cardiovascular disease, has motivated research into

functional foods (OMS, 2021). Among these, microgreens have gained relevance because of their enhanced organoleptic and nutritional profiles (Šamec *et al.*, 2018). These are harvested in early development stages, generally between 7 and 21 days after germination, which makes them ideal for urban and controlled production systems due to their short cycle and low space requirements (Xiao *et al.*, 2012; Yeargin *et al.*, 2023).

A defining factor in the production of microgreens is the selection of substrates, which directly influence the yield and the biochemical and physiological quality. Organic substrates, such as compost, have resulted in significant increases in biomass compared to inorganic substrates (Bilalis *et al.*, 2025). Mixtures such as coconut fiber (CF) and vermiculite (1:1) favor the germination and accumulation of bioactive compounds in Brassicaceae (Pant *et al.*, 2023). Likewise, commercial substrates with CF, perlite and peat improve the yield in species such as radish and mizuna, thanks to their high water retention and nutritional contribution (Alloggia *et al.*, 2025). Light regulated through LED systems is another key factor to optimize physiological processes in controlled environments (Pant *et al.*, 2023; Hamilton *et al.*, 2023).

Although species from the Brassicaceae family are the ones most used in the production of microgreens, legumes such as chickpeas, lentils and mung beans have gained importance due to their nutritional attributes (Gunjal *et al.*, 2024). In this context, fava bean (*Vicia faba* L.) emerges as a promising species to produce microgreens, not only because of its conventional nutritional value, but also because of the significant improvement in its bioactive profile during germination. Studies such as that from Wei *et al.* (2022) show that a short germination significantly increases the content of soluble proteins and essential amino acids, while reducing anti-nutrients such as phytic acid. This profile is enriched with the increase of antioxidant activity, associated with the biosynthesis of phenolic compounds during germination, as reported by Okumura *et al.* (2016), who identified phenols in fava bean sprouts with high capacity to neutralize free radicals.

Despite the recognized nutraceutical potential of *Vicia faba* L. sprouts and the documented influence of substrates in microgreens from other species, little is known about the effect of alternative substrates on fava bean microgreens, particularly in their morphological characteristics, photosynthetic pigment concentration, and soluble protein content. Therefore, the objective of this study was to evaluate the effect of two substrates (sand and coconut fiber) on these parameters in two local varieties of *Vicia faba* L. cultivated in a growth chamber prototype, with the aim of optimizing their production and nutritional quality.

MATERIALS AND METHODS

Plant material and growth substrates

Seeds from two local varieties of fava bean (*Vicia faba* L.) were used: T-5 ("Cochinera") and T-12 ("Criolla Amarilla"), from the municipality of Tlahuapan, Puebla, Mexico. These varieties were selected due to their morphological variability and contrasts in biomass yield, according to what was previously reporte by Fuentes-Herrera *et al.* (2025).

The substrates evaluated were sand (obtained from San Antonio Cacalotepec, Puebla) and coconut fiber (CF; Eco Sustrato Orgánico®). The sand was sifted with a No. 12 sieve

(1.4 mm holes) to homogenize the particle size, and the impurities were eliminated. Both substrates were sterilized in autoclave at 125 °C during 30 minutes and saturated with tap water before sowing.

The seeds were disinfected through immersion in sodium hypochlorite solution at 100% during 3 minutes, followed by five rinses with distilled water.

Experimental design and cultivation conditions

Sowing was conducted in perforated aluminum trays (38×29×6.5 cm) which were divided into two sections (19×14.5 cm), each containing a different substrate (CF or sand). Three replicates per variety were used. The sowing density was 90 seeds/section for T-5 (15×6 arrangement) and 76 seeds/section for T-12 (11×6 arrangement + 1 row of 10). The treatments were randomized within each tray to eliminate potential effects from position.

Cultivation was carried out in a chamber prototype built with a transparent plastic box, made of virgin high-density polypropylene (HDPE), with measurements 52×34×18 cm, in which the aluminum tray was placed with the substrates and the seeds. To maintain the relative humidity inside the chamber, white cotton pads moistened were placed around the plastic box, which were regularly sprayed in the morning, at the beginning and end of the light period. The lighting conditioning in the growth chamber prototype was established with two wood structures. Each was built with four 68 cm high beams, with a 65 cm long by 20 cm wide board placed on the upper part, where three LED 30 W light bulbs were installed in a circuit (2700 lumens, 6500 K), placed in a series and equidistant (16.5 cm between them). Once the microgreens emerged, they were subjected to a photoperiod of 8/16 h (light/dark), which was controlled by a switch (Figure 1a).

The prototype with the growth trays was placed in a dark room, and the environmental conditions (temperature, relative humidity, and luminosity) were monitored every 30 minutes using a datalogger (HOBO, MX1105).

Harvest and morphological measurements

The days until emergence were recorded for each variety and replicate; the growth measurements of microgreens were taken on harvesting day. The harvest was conducted once the plants had passed the sprout stage and presented two true leaves emerging, which was 8 days after emergence (Figure 1b), taking as the first day of emergence when there were 50% of the seeds sprouted; this was recorded daily to determine the percentage of germination.

Measurements of morphological characteristics were recorded in 20 microgreens completely randomly from each variety, substrate and replicate at the time of harvest. The plant height was recorded in centimeters (cm) with a Vernier (Digimatic Caliper, Mitutuyo), taking as reference from the start of the epicotyl to the apical part of the sprout growth. The root length was measured in cm from the start of the epicotyl down, taking the longest root and the width of the epicotyl in millimeters (mm). The shoot fresh weight was measured in grams (g) on an analytical balance (A&D, GR-202), after removing any remaining seed coat and the roots.

Figure 1. Growth chamber prototype: a) Adaptation of the structure for illumination with tray and substrates, b) Fava bean microgreens during growth.

After recording the measurements and weight of the microgreens from each substrate and replicate, a portion was used for some fresh measurements such as percentage of humidity and photosynthetic pigments. The other portion of the microgreens was processed through freeze-drying for soluble protein analysis.

The percentage of humidity (%H) of each substrate and each repetition was determined by four replicates. The determination was made in an infrared radiation thermobalance (OHAUS, MB 45); based on the %H of each sample, the percentage of dry matter was obtained (%DM).

To freeze-dry the microgreens, they were cut into small pieces (0.5 cm^2 approximately), wrapped in aluminum foil and frozen at −50 °C. The samples were kept frozen for at least 24-48 h before starting the freeze-drying process. A Labconco freezer was used (FreeZone Triad) with the following parameters: collector temperature of −80 °C and vacuum pressure of 0.220 mbar; at −80 °C, the process lasted 36 to 48 hours until reaching a humidity of 5-10%. Later, the freeze-dried samples were ground with an electrical mill (Krups GX4100) and sifted with a number 35 sieve with particle size 420 μm. Once the samples were ground, they were stored in sealed amber containers and kept frozen at −20 °C.

Determination of photosynthetic pigments

The quantification of chlorophyll a and b, carotenoids, and xanthophylls was carried out according to the method described by Lichtenthaler (1987) with some adaptations according to Viveros (2024). For the extraction, six samples of 400 mg of fresh microgreens of the two varieties were randomly used from each substrate and each replicate, cut into small slices (3-5 mm), which were placed in 2 mL Eppendorf tubes with 1600 μL of acetone at 80%. During this process, the tubes with the samples were kept in ice. Then, the samples were placed in an ultrasonic bath (Ultrasonic Cleaner, model AS5150B) at 4 °C with ice, for 3 minutes, with 6 pulses of 30 seconds and 10 seconds of rest between each pulse (at power 7 and 6 of degasification). Later, the extracts were kept under refrigeration at 4 °C for 24 h. Next, the tubes were placed again un the ultrasonic bath for 30 s (at power 7 and 6 of degasification) and centrifuged at 2350 *g* at 4 °C

for 5 minutes. Finally, absorbance at 470 nm, 646 nm and 663 nm was read on the supernatant with a spectrophotometer UV/VIS (Evolution 300, Thermo-Scientific). With the absorbance data, the concentration of chlorophylls (chl a, chl b), xanthophylls and carotenoids (Cx + c) was calculated according to the equations from Lichtenthaler (1987). The concentrations were expressed in μg/g FW.

Determination of soluble protein

To elaborate extracts, 10 mg of freeze-dried sample were weighed in Eppendorf tubes and 1 mL of deionized water was added; during this process, the tubes were kept in ice. Then, the tubes were agitated in Vortex (Genie 2 Digital de Scientific Industries) for 10 seconds, to later centrifuge them in a microcentrifuge (Hettich, MIKRO 200R) for 10 minutes at 1960 *g* at 4 °C. The supernatant was used as extract.

The quantification of soluble protein was carried out according to the Bradford (1976) method. On a 96-well plate (Costar), 50 μL of diluted or standard extract and 200 μL of Bradford reagent were placed (Sigma Aldrich. Catalogue B6916); the plate was shaken for one minute, and it was left resting for 5 min in the dark. Deionized water was used as control. Then, the absorbance readings were conducted in a multimode microplate reader (Varioskan Flash, Thermo Scientifc) at a wavelength of 595 nm. The calibration curve was prepared with a stock solution of 1mg/mL Bovine Serum Albumin (BSA) in a range of 0 to 250 μg/mL. The concentration of soluble protein was calculated based on the curve equation and expressed in mg/g DM.

Statistical analysis

Prior to the analysis of variance, the assumptions of normality and homogeneity of variances were verified. The normality of the residues was evaluated through the Shapiro-Wilk test, and the homogeneity of variances through the Levene test. All the variables analyzed (fresh weight, height, root length, sprout width, dry matter, photosynthetic pigments, and soluble protein) fulfilled both assumptions ($p \geq 0.05$), except for the variables fresh weight and carotenoids + xanthophylls, which do not fulfill the assumption of homogeneity of variances ($p \leq 0.05$). However, given that the ANOVA is robust in the presence of mild violations of these assumptions and given the balanced design of the experiment, the parametric analysis was started.

To determine the effect of the variety × substrate interaction, analysis of variance (ANOVA) was conducted as well as Tukey's means comparison with a level of significance $p \leq 0.05$ (PROC GLM; SAS Institute Inc., 2002).

RESULTS AND DISCUSSION

Environmental conditions for growth

Recent studies have documented the different ways in which microgreens can be cultivated, and under quite varied conditions, such as open or closed spaces, like greenhouses, whether in a hydroponics system with inert substrates, on soil, or in a soil-less medium (Arya *et al.*, 2022; Seth *et al.*, 2025). Although growing microgreens is a relatively simple and quick process, it is important to control and evaluate the environmental conditions for

each species, to improve their growth in terms of productivity, nutritional and functional quality (Franks and Richardson, 2009).

For this study, a prototype of growth chamber was designed and tested, through which there was a constant supply of light in a light/dark cycle of 8/16 h. The recording of the environmental variables within the growth chamber of microgreens of the two fava bean varieties since sowing on July 23 until October 11, showed that, in general, the temperature, relative humidity and luminosity showed low variation throughout the period and between replicates. For example, in variety T-5, cultivated from July 23 to August 21, 2024, the temperature was kept at 22.9 °C on average, the range per day in the replicates varied between 20.4 and 25.7 °C, with a coefficient of variation (CV) under 1% (Figure 2a). The relative humidity showed an average of 77.2%, and the variation per day within the replicates was 60.9 to 88.4%, with a CV under 2% (Figure 2b). The luminosity showed an average of 9841 lux, with a variation in the replicates per day of 8915 to 11281 with a CV below 5% (Figure 2c).

In variety T-12, the environmental conditions also remained without much variation. Since sowing on September 1 until harvesting on October 11, 2024, the temperature was kept at 21.9 °C on average, with a variation per day in the replicates between 20 and 25.3 °C, and CV under 8% (Figure 2a'). The average relative humidity was 78.2%, where the CV was under 1%, with a variation per day in the replicates between 67.4 and 88.2% (Figure 2b'). The average luminosity was 9742 lux with a variation per day in the replicates of 8999 to 10975 lux and a CV lower than 3% (Figure 2c').

The light regime during cultivation of microgreens is an important parameter, given that in addition to supplying the energy for photosynthesis (Delian *et al.*, 2015), it performs a fundamental role in various physiological and biochemical processes. It is known that light conditions, in terms of spectral quality (wavelength) and light intensity (quantity), can influence both the morpho physiology and the biosynthesis of phytochemicals of the sprouts and micro-vegetables (Santin *et al.*, 2022). In this study, the prevailing light intensity ranged between 8957 lux (165 μmol m^{-2} s^{-1}) and 11128 lux (206 μmol m^{-2} s^{-1}). In this regard, some authors suggest preferably using artificial light, such as light from light emitting diodes (LEDs) to cultivate microgreens in controlled environments (Seth *et al.*, 2025), such as the type of light that was used in this study.

Percentage of germination

Microgreens of the two varieties cultivated in the CF and sand substrates presented percentages of germination greater than 90%. Variety T-5 showed 96% in the sand substrate, the plants emerged at 5 days, and the time from sowing to harvesting was 13 days (Figure 3). This same variety in the CF substrate showed 95% germination, 7 days until seed emergence, and 15 days from sowing to harvesting of the microgreens. Meanwhile, variety T-12 exhibited a slightly lower germination percentage; for example, in the sand substrate they showed 93% and in CF 92%, and the seeds in both substrates showed on average the same days until emergence (4 days) and the same time from sowing until harvesting of the microgreens (14 days) (Figure 3).

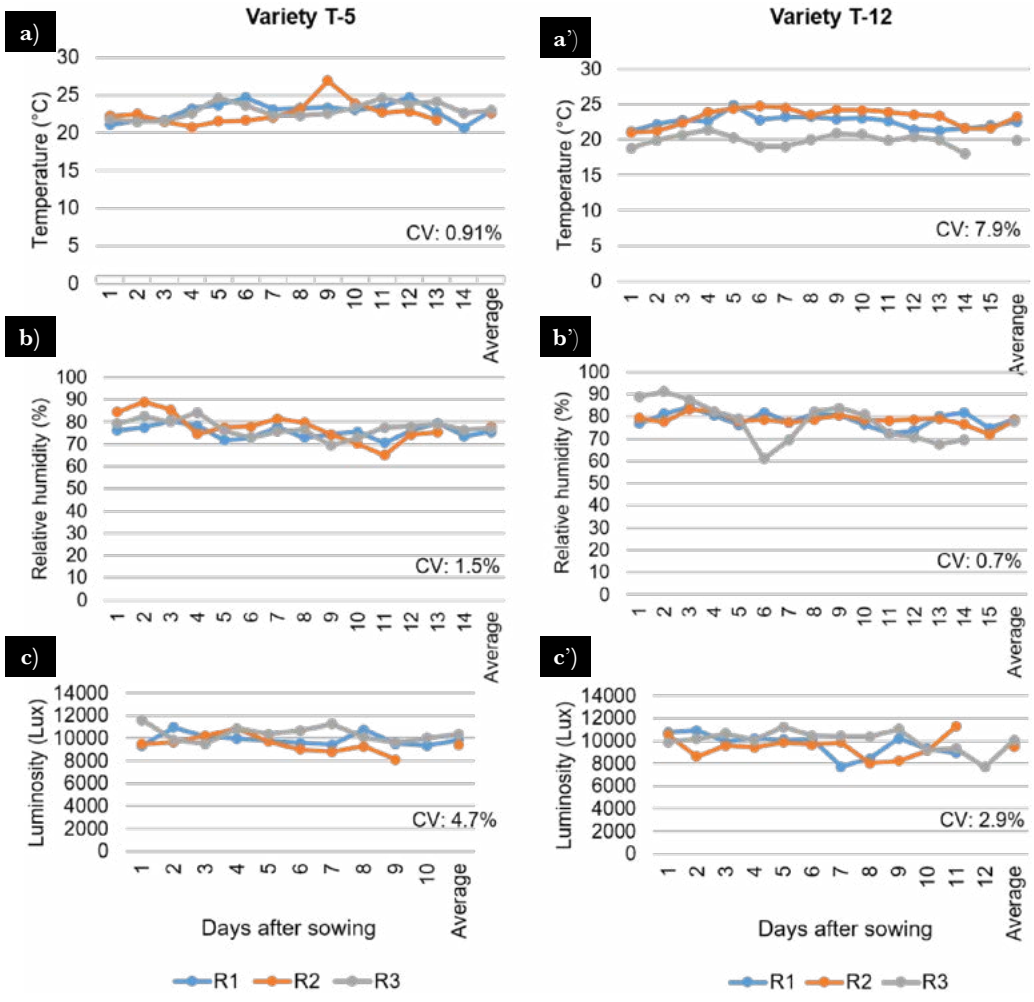

Figure 2. Environmental conditions found in the growth chamber during the growth of microgreens of variety T-5 (a-c) and variety T-12 (a'-c') with a light/dark cycle of 8/16 h per day. a) and a') Temperature; b) and b') Relative humidity; and c) and c') Luminosity per day of the replicates, and average at the end of the period when the microgreens harvest was conducted.

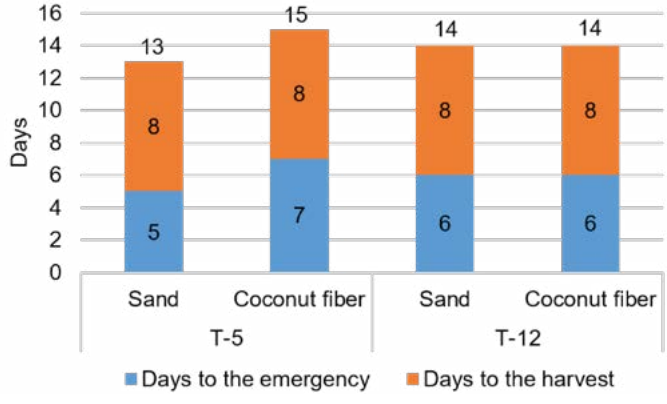

Figure 3. Days until emergence and days until harvest of fava bean microgreens, varieties T-5 and T-12, cultivated in a sand and coconut fiber substrate.

In both varieties, the days until harvesting to obtain microgreens were short. In this regard, Bewley *et al.* (2013) mention that, in cultivating sprouts or microgreens, where the production cycle is short and high productivity is sought in a short time, achieving quick and homogeneous germination becomes essential to obtain quality products and reduce losses.

Effect of the variety and the substrate on the growth and accumulation of biomass in fava bean microgreens

Although some variables deviated from normality, the ANOVA is considered robust to such deviations given the sample size (>30 per group). The interaction between variety × substrate showed a significant effect ($p \leq 0.05$) on the morphological variables and the fresh weight of fava bean microgreens (Table 1). In variety T-5, the CF substrate promoted a higher fresh weight (2.44 g/plant), sprout height (19.86 cm), and root length (12.65 cm) compared to sand (1.96 g, 16.37 cm and 10.17 cm, respectively). On the contrary, in variety T-12, only the root length was significantly higher in CF (12.13 cm *vs.* 10.46 cm in sand), while the fresh weight and height did not differ between substrates. These results suggest a differential response of the varieties to the type of substrate, possibly related to their capacity for morphological adaptation and efficiency in resource absorption.

The longer root length observed in CF for both varieties is consistent with what was reported by Di Gioia *et al.* (2017), who emphasize that a well-developed root system improves the absorption of water and nutrients, which can translate into better aerial growth. In addition, the presence of longer and less ramified roots in CF (Figure 4a) compared to those in sand (Figure 4b), suggests differences in the root architecture influenced by the physical properties of the substrate, such as porosity and humidity retention.

The absence of differences in the sprout width between substrates suggests that this variable is more influenced by genetics than by the growth medium. Köpke and Nemecek (2010) describe that, in legumes, characteristics such as stem thickness is associated with the production of aerial biomass and can be indicative of the productive potential of the plant.

Regarding dry matter (DM) content, no significant differences were observed between substrates for any variety, with values between 10.84% and 11.91%. These

Table 1. Weight, morphological characteristics, and dry matter of fava bean microgreens of varieties T-5 and T-12 cultivated in two substrates.

Variable	T-5		T-12		HDS
	Coconut fiber	Sand	Coconut fiber	Sand	
Weight (g)	2.44a±0.29	1.96b±0.20	2.52a±0.35	2.41a±0.37	0.15
Sprout height (cm)	19.86a±3.19	16.37b±2.48	17.70b±3.58	16.80b±3.22	1.49
Sprout width (mm)	3.93b±0.47	4.00ab±0.40	4.17a±0.49	4.10ab±0.56	0.23
Root length (cm)	12.65a±3.01	10.17b±2.33	12.13a±2.31	10.46b±2.36	1.19
Dry matter (%)	10.84a±1.08	11.89a±1.33	11.30a±1.81	11.91a±1.04	2.03

Mean values±standard deviation. MSD=minimum significant difference; a different letter in the same line marks significant differences according to Tukey's test ($p \leq 0.05$).

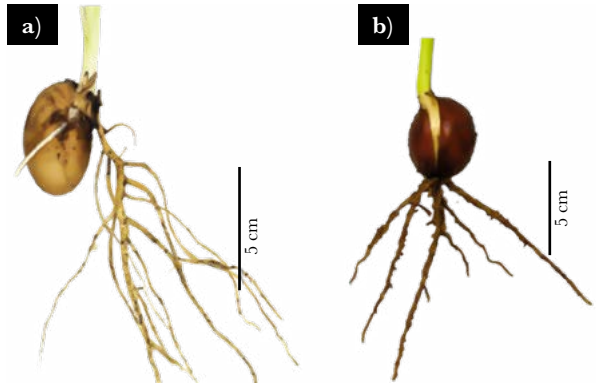

Figure 4. Roots of fava bean microgreens 8 days after emergence. a) Roots of seeds cultivated in coconut fiber substrate, b) Roots of seeds cultivated in sand substrate.

values are notably high in comparison to those reported for other microgreens, such as those from Brassicaceae, which tend to fluctuate between 4.5% and 7.4% (Bafumo *et al.*, 2024). This reflects the potential of legumes, such as fava beans, to accumulate dry biomass and compounds of nutritional value in early stages of development (Mangla *et al.*, 2025).

These findings emphasize the importance of selecting substrates that favor root development and productivity, especially in controlled production systems where efficiency in the use of resources is critical (Rouphael *et al.*, 2018). Future studies could explore the combination of substrates or the use of conditioners to further optimize the growth and nutritional quality of fava bean microgreens.

Effect of the interaction variety x substrate in photosynthetic pigments and soluble protein

Results from this study show a significant effect ($p \leq 0.05$) of the variety × substrate interaction on the content of photosynthetic pigments and soluble protein in fava bean microgreens (Table 2, Figure 5). This interaction highlights the importance of considering both the genotype and the growth medium to optimize the nutritional and functional quality of microgreens.

Table 2. Photosynthetic pigment content of fava bean microgreens of the varieties T-5 and T-12 cultivated in coconut fiber and sand.

	T-5		T-12		
Variable	**Coconut fiber**	**Sand**	**Coconut fiber**	**Sand**	**MSD**
Total Chlorophyll (μg/g FW)	176.92a±14.44	180.28a±25.81	171.24a±30.01	112.86b±17.89	23.48
Chlorophyll a (μg/g FW)	125.05a±8.41	125.38a±11.10	119.47a±13.99	85.42b±13.91	11.96
Chlorophyll b (μg/g FW)	51.86a±11.04	54.90a±15.99	51.77a±18.05	27.43b±6.83	14.32
Chlorophyll a/b	2.51b±0.50	2.44b±0.63	2.46b±0.50	3.25a±0.75	0.59
C+x (μg/g FW)	34.66a±2.82	32.41ab±6.27	34.24a±3.65	28.31b±7.65	5.01

Mean values±standard deviation. C+x=: carotenoids + xhanthophylls. MSD=Minimum significant difference. Means with the same letter per line are not significantly different between substrates according to Tukey's test ($p \leq 0.05$).

Variability in the photosynthetic pigment content

The photosynthetic pigment content showed a differential response between varieties and substrates. While variety T-5 did not show significant differences in the concentration of chlorophylls between substrates, variety T-12 cultivated in CF showed significantly higher values of chlorophyll a (119.47 μg/g FW) and total chlorophyll (171.24 μg/g FW), compared to cultivation in sand (85.42 and 112.86 μg/g FW, respectively) (Table 2). This difference represents an increase of 39.6% in chlorophyll a, and 51.6% in total chlorophyll for T-12 in CF. The a/b chlorophyll rate, which ranged between 2.42 and 3.45, suggests a photosynthetic adaptation to the light intensity used, consistent with what was reported by Wojdyło *et al.* (2020) and Lichtenthaler (2007), who mention that this proportion reflects the cultivation conditions, where high values indicate exposure to high light intensity.

The absence of significant differences in carotenoids and xanthophylls between substrates agree with studies such as Amitrano *et al.* (2024), who observed that these secondary pigments are less variable in the presence of changes in the substrate, possibly due to their stabilizing role in the photosynthetic apparatus under stress conditions.

Influence of the substrate in the accumulation of soluble protein

The soluble protein content showed a marked influence of the substrate depending on the variety. Variety T-5 cultivated in sand presented a significantly higher content (69.67 mg/g DM) than in CF (49.9 mg/g DM), which represents a reduction of 28.4 % in the latter substrate (Figure 5). On the contrary, variety T-12 did not show significant differences between substrates, although a slightly higher trend was observed in CF (62.65 mg/g DM vs. 58.36 mg/g DM in sand).

These results can be attributed to differences in the absorption efficiency of nutrients and water between substrates, as well as the capacity of each variety to modulate its nitrogenated metabolism in response to the medium. Mangla *et al.* (2025) point out that legumes, such as fava bean, have a high potential to accumulate proteins in early stages,

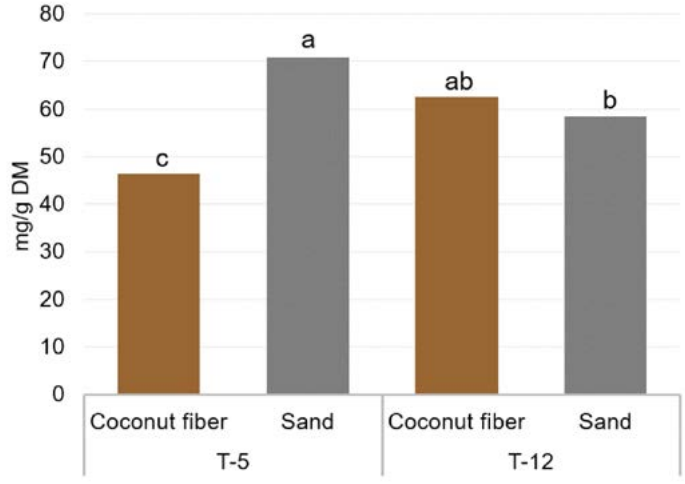

Figure 5. Soluble protein content of fava bean microgreens of varieties T-5 and T-12 cultivated in coconut fiber and sand substrates. Bars with the same letter are equally significant according to Tukey's test ($p \leq 0.05$).

although this accumulation is subject to abiotic factors such as availability of nutrients and water in the substrate. Since it has lower water retention capacity, sand could induce a slight water stress that favors the synthesis of soluble proteins as a mechanism for osmotic adjustment, especially in sensitive genotypes such as T-5.

Substrate selection is crucial to maximize the nutritional quality of microgreens, particularly in legume species such as fava bean, which show a high genetic variability in response to the growth medium. This study's findings agree with Kyriacou *et al.* (2019), who reported wide variation in the content of chlorophylls and other bioactive compounds in microgreens of different species and cultivation conditions.

The 8/16 h light/dark cycle used in this study may not be optimal for the synthesis of soluble proteins. Silva *et al.* (2025) showed that longer light cycles (12/12 or 16/8) significantly increase the protein content in kale microgreens, which suggests that adjustments in the photoperiod could improve the protein quality of fava bean microgreens.

The interaction variety × substrate is a determinant factor in the accumulation of photosynthetic pigments and soluble protein in fava bean microgreens. While CF favored the content of chlorophylls in variety T-12, sand promoted a higher accumulation of soluble protein in variety T-5. These findings underline the need to select specific combinations of variety and substrate, to optimize the production of microgreens with high nutritional and functional value.

CONCLUSIONS

The growth chamber prototype proved to be an effective tool to keep the environmental conditions stable (temperature, relative humidity, and luminosity), providing a viable controlled environment for the production of fava bean microgreens, which validates its use for future studies and applications for urban or precision agriculture.

A significant interaction was confirmed between the fava bean variety and the type of substrate, which determined the growth, development and biochemical profile of the microgreens. This emphasizes that the plant's response is not independent from the substrate, but rather that it critically depends on the genotype; thus, generalizations should be avoided, highlighting the need to select specific combinations.

For variety T-5, the CF substrate was better to promote morphological characteristics such as fresh weight, height, and root length. However, although the sand substrate induced a higher content of soluble protein, this suggests that the selection of the substrate for this variety should prioritize the objective of production: biomass (CF) or protein quality (sand).

For variety T-12, CF significantly favored the biosynthesis of chlorophyll a, and total chlorophyll, as well as a greater root length. The soluble protein did not show differences between substrates. Therefore, for this variety, CF presents as the most adequate substrate to improve both the development and the photosynthetic pigment content.

The high percentage of dry matter (10.84-11.91%) compared to other microgreens, together with the high contents of soluble protein, place *Vicia faba* L. microgreens as a promising functional food, rich in compounds of nutritional value.

The main contribution of this study lies in proving, with scientific evidence, that the optimization of the production of fava microgreens requires a joint selection of genotypes and substrates. These findings are relevant for farmers, plant breeders, and the food industry, by providing specific criteria to maximize the yield and the nutritional quality in controlled agriculture systems, thus contributing to the diversification of nutritious and sustainable food sources.

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