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Response of *Spinacia oleracea* varieties cultivated in a plant factory with artificial lighting

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Abstract

The scarcity of water and arable land is driving the development of new farming technologies, such as Plant Factories with Artificial Lighting (PFAL). These systems help reduce water use, allow for year-round production, and eliminate the need for pesticides. These systems work well with leafy vegetables, especially baby leaves, as they provide a high amount of nutrients and are beneficial for health. Spinach is an excellent crop to grow in this way because it is abundant in vitamins, minerals, and bioactive compounds. Controlled environment agriculture is a promising solution for producing spinach in Nuevo León, where the climate hinders conventional techniques. In this experiment, three types of spinach (Virofly, Acadia F1, and Space F1) were grown in a controlled environment with a temperature of 22 ± 1 °C, a relative humidity of 53 ± 7%, and ambient CO2 levels. The plants were grown using a floating root hydroponic system with artificial RGB lighting (4.55:1:1 spectrum). The study assessed antioxidant activity, phenolic content, flavonoids, and photosynthetic pigments to determine the optimal variety for biomass production in a Plant Factory with Artificial Lighting. Virofly and Acadia F1 produced encouraging outcomes, whereas Space F1 was omitted due to seed non-viability. The majority of analyses showed no statistically significant differences between Acadia F1 and Virofly. This suggests that the common variety had comparable yields and nutraceutical properties to the hybrid when grown under the same conditions. Because Acadia F1 seeds cost ten times the amount, Virofly is the best choice for making the most savings without sacrificing quality.

Key words: PFAL, baby spinach leaves, biomass, antioxidant capacity, bioactive compounds.

Introduction

As the world grapples with water scarcity and a shortage of arable land, there is an urgent need for innovative agricultural technologies to secure a sustainable food supply (Shemer *et al.*, 2023). A shift in how we produce food is essential, focusing on the creation of high-quality products that enhance health, social well-being, and environmental sustainability through efficient resource use and minimal pollutant emissions (Pereira, 2017).

Controlled Environment Agriculture (CEA), particularly in systems known as Plant Factories with Artificial Lighting (PFAL), is advancing rapidly to tackle food shortages, optimize the use of natural resources, and address challenges faced by traditional agricultural methods (Kozai, 2018). This system offers several key advantages over conventional agriculture, including reduced water usage, year-round production, and the elimination of pesticides and herbicides (Rajaseger *et al.*, 2023). In addition to these benefits, which promote environmental sustainability and social well-being, Plant Factory systems hold significant potential to mitigate the impacts of urbanization and combat food insecurity.

Leafy vegetables are ideal for cultivation in Plant Factory systems with Artificial Lighting, owing to their high yield and nutritional quality, particularly those grown for baby leaf production. Baby leaves are gaining popularity due to their equal or higher concentrations of bioactive compounds compared to mature leaves (Bantis *et al.*, 2020; Miao *et al.*, 2023).

Hydroponic systems combined with LED artificial lighting represent advanced technology that ensures year-round production, delivering consistent, high-quality vegetables ideal for urban farming (Rajendran *et al.*, 2024).

Alterations in the light spectrum significantly influence plant growth, yield, and the nutritional quality and nutrient content of crops (Vaštakaitė-Kairienė *et al.*, 2023). Photosynthesis enables plants to convert photosynthetically active radiation (derived from light energy) into chemical energy, which they use to produce nutrients. This biochemical process is facilitated by various light receptors, each sensitive to specific wavelengths (Gutiérrez-Soto *et al.*, 2011). Among these photoreceptors, chlorophyll a and b play a key role, capturing light predominantly in the blue and red regions of the electromagnetic spectrum (Burattini *et al.*, 2017). Blue and red light have the most significant impact on plant growth. However, green light also contributes to the photosynthetic process through pigment photoreceptor proteins in phytochromes and cryptochromes, supporting the growth and development of green leafy vegetables. Studies have shown that

combining green light with blue light enhances plant growth (Bian *et al.*, 2018).

Spinach (*Spinacia oleracea*) is a widely cultivated leafy vegetable that generated over 240 million pesos in Mexico in 2020 (Axayacatl, 2022; Rocha & Rodríguez, 2022). This vegetable is a rich source of minerals, vitamins, phytochemicals, and bioactive compounds (Lu, 2021) with anti-inflammatory, anti-obesity, and anticancer properties that promote health (Roberts and Moreau, 2016). Due to its high nutritional value and fast growth, spinach has garnered significant attention in various communities (Vickers *et al.*, 2019). Spinach is primarily classified into three categories based on leaf texture: savoy, semi-savoy, and smooth-leaf varieties. Smooth-leaf varieties are favoured for processing due to their durability during transport and storage, while savoy and semi-savoy varieties are better suited for fresh consumption (Le Nguyen *et al.*, 2023). Spinach growth habits vary from horizontal to semi-erect to erect, directly influencing how plants receive photons and carry out photosynthesis. Growing horizontal or semi-erect varieties in Plant Factory systems can enhance light absorption, leading to higher yields and more efficient use of space (Ribera *et al.*, 2020).

In 2024, the main global exporters of spinach were the United States, Spain, Mexico, the Netherlands, and Italy. Canada was the leading importer, followed by Belgium as the second-largest importer, with the Netherlands, the United Kingdom, and Germany also showing significant interest (Team, 2024). In 2023, Mexico produced 40,924 tons of spinach, reflecting a 40.6% increase compared to 2022. The production came from 2,790 hectares, with an average yield of 15 tons per hectare. The states of Baja California (13,145 tons), Puebla (10,333 tons), and Guanajuato (5,670 tons) accounted for 71.2% of the national production (Bastida-Cañada, 2025). Growing this leafy green in Nuevo León presents challenges due to the region's climatic conditions, yet it also offers a market opportunity that can be capitalized on through the implementation of Plant Factory systems. Therefore, with the availability of spinach seeds in Mexico, this study aims to evaluate the biomass production (baby leaves) and nutraceutical quality of three Spinach varieties in a PFAL, to identify the most viable variety for this system.

Materials and methods

Controlled environmental agriculture system: A vertical hydroponic floating root system was implemented on a 121 cm long and 45.7 cm deep chrome-plated stainless steel Trinity rack. Flat trays were used as a base (55 cm x 28.5 cm each) and 136-cavity Styrofoam germination trays, which together constitute a seeding tray. The cavities contain volcanic rock as a substrate with a size of 0.5 cm. Three seeds were placed per cavity to reduce the probability of empty cavities. Each tray contains two experimental units of 68 replicates each, resulting in 8 experimental units and a total of 544 plants per treatment. Four trays were placed per level, and a different variety was planted per level. (Fig. 1). The *Spinacia oleracea* varieties used were: Virofly, Acadia F1 and Space F1. Each variety symbolizes treatment. The experiment was carried out in the municipality of General Escobedo, N.L., Mexico.

Environmental and operational conditions: The space was maintained at 22 ± 1 °C, 53 ± 7% relative humidity, and ambient CO_2 concentration (Fig. 2). The trays were monitored daily, with

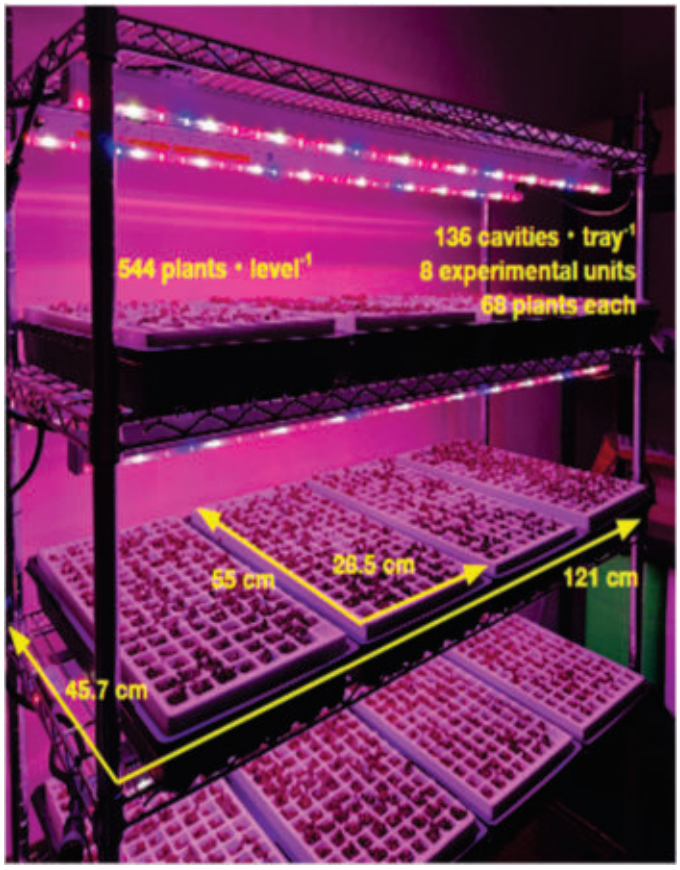

Fig. 1. Plant factory prototype based on a vertical hydroponic floating root system.

a spray of nutrient solution applied every 24 hours (Le Nguyen *et al.*, 2023) during the first 7 days (germination stage). A 1 L bed of nutrient solution was added to the base of the tray, serving as a reservoir. Two Arize Life 2 General Electric LED light strips with a PKB spectrum were installed on each level, positioned 15 cm apart between the LED emission source and the cultivation tray. The light intensity was set at 325 µmol m^{-2} s^{-1} with a photoperiod of 12 hours per day and a spectrum equivalent to 4.55:1:1 (RGB) or 69.5% red (R) + 15% green (G) + 15.5% blue (B) (Fig. 3). The trays were irradiated, achieving a DLI of ~14 mol m^{-2} d^{-1} (Semenova *et al.*, 2023; Nguyen *et al.*, 2019).

Nutrient Solution: The components of the nutrient solution are detailed in Table 1.

Table 1. Composition of the nutrient solution used in the experiment

Element	Unit (ppm)	Source
N	200	Present in same mineral salts
P	60	$NH_4H_2PO_4$
K	250	KNO_3
Ca	200	$Ca(NO_3)_2 \cdot 4H_2O$
Mg	50	$Mg(NO_3)_2$
S	100	H_2SO_4
Fe	0.5	$FeSO_4 \cdot 7H_2O$
Mn	0.25	$MnSO_4 \cdot H_2O$
B	0.25	H_3BO_3
Cu	0.03	$CuSO_4 \cdot 5H_2O$
Zn	0.25	$ZnSO_4 \cdot H_2O$
Mo	0.01	$Na_2MoO_4 \cdot 2H_2O$

The nutrient solution was prepared with a pH of 5.5 and an electrical conductivity (EC) of 1.5 mS cm^{-1} (Avendaño-Abarca *et al.*, 2020). After germination, the nutrient solution was gradually

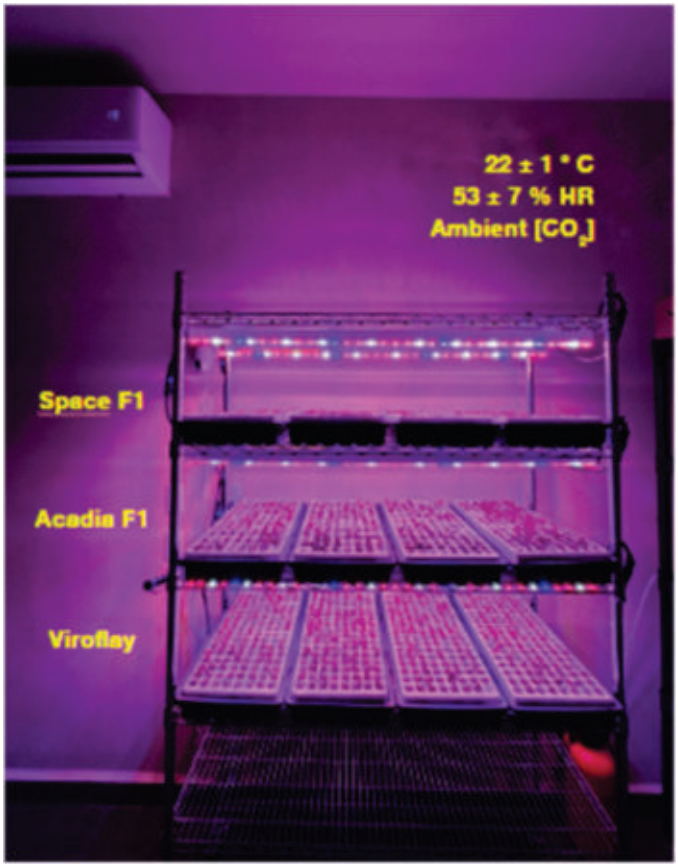

Fig. 2. Experiment overview with environmental conditions.

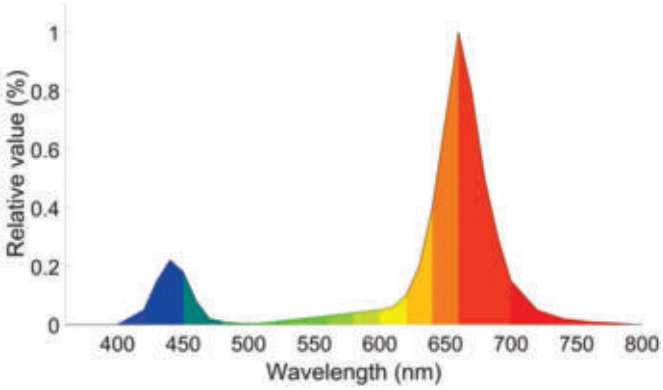

Fig. 3. General Electric Arize Life 2 LED Lamp PKB Spectrum.

added to the tray reservoir based on crop growth and demand, starting with a standard feeding of 1 L per tray.

Biomass (Fresh weight and dry weight): The harvest of baby leaves was carried out 28 days after planting (Fig. 4). They were weighed using an analytical balance to determine the fresh weight (FW). Subsequently, the samples were placed in a dehydrator at 40°C for 48 hours, allowing the dry weight (DW) of the experimental units to be obtained.

Extraction: A total of 100 g of dry spinach leaf biomass was weighed and homogenized with 80% acetone (1:2 w/v) using a Waring blender cooled for 5 minutes. The sample was further homogenized with a Polytron homogenizer for 3 minutes to obtain a completely homogenized mixture. The homogenates were filtered through Whatman No. 2 filter paper using a Büchner funnel under vacuum. The filtrate was recovered, and the acetone was evaporated using a rotary evaporator at 45 °C until approximately 90% of the filtrate was evaporated. The spinach extract was frozen at -40 °C until the time of analysis.


Fig. 4. Harvest of baby leaves of *Spinacia oleracea* 28 days after sowing.

Antioxidant activity

DPPH• Method (2,2-diphenyl-1-picrylhydrazyl): The antioxidant activity was evaluated following the protocol by Fukumoto and Mazza (2000). An 80% methanol solution of 150 µM DPPH• was prepared, and the assay was performed in 96-well microplates (ICN Biomedicals Inc.) by adding 22 µL of sample and 200 µL of DPPH• solution to each well. Samples were prepared in triplicate for each concentration (0-500 µM), using at least seven different concentrations. The microplate was kept covered and in darkness at room temperature (~22 °C), with readings taken at 30, 180, and 360 min using an MRX microplate reader with a 520 nm filter. For caffeic acid, the incubation time was extended to 48 hours to ensure complete reaction. The antiradical activity was determined by calculating the initial slope of the linear regression curve ($r^2 > 0.800$) and was expressed as µmol Trolox equivalents per gram of dry weight (µmol TE/g DW), presented as mean ± standard deviation for eight replicates.

ABTS•$^+$ Method (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid): The ABTS method was adapted for dried spinach biomass. The ABTS•+ radical was generated and diluted in ethanol to an absorbance of 0.700 at 734 nm before mixing with the samples. A total of 20 µL of extract and 200 µL of ABTS•+ were added to a 96-well microplate, and the absorbance was read after 6 minutes. The results were expressed as $\mu \pm \sigma$ for eight replicates, in µmol Trolox equivalents per gram of dry weight (µmol TE/g dry weight).

FRAP Method (Ferric Reducing Antioxidant Power): The FRAP reagent was prepared by mixing acetate buffer (pH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine), and iron sulfate (Fe_2SO_4). The dry biomass sample was extracted and added to the FRAP reagent, then maintained at 37 °C for 30 minutes. Absorbance was measured at 593 nm, and the results were reported as $\mu \pm \sigma$ for eight replicates, in µmol equivalents of Fe^{2+} per gram of dry weight (µmol eq. Fe^{2+}/g dry weight).

Determination of total phenolic content: The total phenolic content was determined using a colorimetric method described by Dewanto *et al.* (2002). Samples were analyzed spectrophotometrically to determine the total phenolic content using a modified Folin-Ciocalteu colorimetric method. All spinach extracts were diluted 1:5 with distilled water to obtain readings within the range of the standard curve (0.0–600.0 µg gallic acid/mL). Then, 125 µL of the gallic acid standard solution or diluted spinach extract was mixed with 0.5 mL of distilled water in a test tube, followed by the addition of 125 µL of Folin-Ciocalteu reagent (FCR). The samples were mixed well and allowed to stand for 6 minutes before adding 1.25 mL of a 7% aqueous sodium carbonate solution. The final volume was adjusted to 3 mL with distilled water. The samples were left to stand for 90 minutes at room temperature before measuring the absorbance at 760 nm against a blank using a Lamda XLS PerkinElmer spectrophotometer in comparison with similarly prepared standards of known gallic acid concentrations. The results were expressed as $\mu \pm \sigma$ for eight replicates, in mg gallic acid equivalents per gram of dry weight (mg GAE/g dry weight).

Determination of total flavonoid content: The total flavonoid content was determined using a colorimetric method described by Dewanto *et al.* (2002). Briefly, 0.25 mL of the spinach extract or the (+)-catechin standard solution was mixed with 1.25 mL of distilled water in a test tube, followed by the addition of 75 µL of a 5% $NaNO_2$ solution. After 6 minutes, 150 µL of a 10% $AlCl_3 \cdot 6H_2O$ solution was added, and the mixture was left to stand for another 5 minutes before adding 0.5 mL of 1 M NaOH. The mixture was brought to a final volume of 2.5 mL with distilled water and mixed well. Absorbance was measured immediately at 510 nm against a blank using Lamda XLS PerkinElmer spectrophotometer in comparison with similarly prepared standards of known (+)-catechin concentrations. The results were expressed as $\mu \pm \sigma$ for eight replicates, in mg catechin equivalents per gram of dry weight (mg QE/g dry weight).

Photosynthetic pigments quantification: The extraction of chlorophyll a, chlorophyll b, and carotenoids was performed according to Masoudi-Sadaghiani *et al.* (2011). Briefly, 0.25 g of dry spinach leaf biomass was homogenized with 80% acetone. The optical density (O.D.) of the extracted pigments was measured at 645, 663, and 470 nm using a Lamda XLS PerkinElmer spectrophotometer. Chlorophyll A (CHLa), chlorophyll B (CHLb), and carotenoids (CAR) were calculated using the following equations:

$$CHLa = (0.0127 \times O.D.663) - (0.00269 \times O.D.645)$$

$$CHLb = (0.0229 \times O.D.645) - (0.00468 \times O.D.663)$$

$$CAR = (0.0202 \times O.D.645) + (0.00802 \times O.D.663) - (0.00129 \times O.D.470)$$

where O.D.470 is the absorbance at 470 nm. These equations are commonly used to quantify pigment concentrations in plant extracts based on spectrophotometric measurements.The results were expressed in µg of pigment per gram of dry weight. The sum of the absorbances allowed the calculation of the total content of chlorophylls and photosynthetic pigments.

Statistical analysis: The experiment was conducted under a completely randomized design. To evaluate the results of antioxidant activity (ABTS, DPPH, and FRAP), phenolic and flavonoid concentrations, as well as photosynthetic pigment content, an independent samples *t*-test was applied, assuming equal variances. Additionally, a mean comparison was performed using Tukey's test ($P \leq 0.05$) with Minitab Statistical 21 software to identify significant differences between the evaluated varieties.

Results and discussion

The results regarding the biomass produced (yield) and the laboratory analyses of antioxidant capacity, bioactive compound content, and photosynthetic pigments are presented in Tables 2-5.

The results obtained in this study indicate that, in general, most assays did not reveal statistically significant differences in yield, antioxidant capacity, bioactive compound content, and photosynthetic pigments between the Acadia F1 and Virofly varieties. This lack of significant differences suggests that both varieties could offer similar benefits in terms of nutritional composition and yield when cultivated under controlled environment, such as in Plant Factory systems. No relevant data were obtained for the Space F1 variety, which limits the comparison and analysis among the three varieties. Therefore, the laboratory results will focus on the two varieties that prevailed in the system.

Biomass yield: Based on the results presented in Table 2, it is observed that the Acadia F1 and Virofly varieties produced higher amounts of biomass, both in fresh weight and dry weight, compared to the Space F1 variety. The Acadia F1 and Virofly varieties did not show significant differences in either fresh weight or dry weight, suggesting that, under controlled cultivation conditions, either of these two varieties is viable in terms of biomass yield. However, since the objective is to identify the most cost-effective variety, the choice of Virofly could be preferable due to its lower production cost, as Acadia seeds are 10 times more expensive. This highlights the importance of this study from an operational perspective, as it identifies an economically viable option without compromising biomass yield.

Table 2. Biomass produced per experimental unit at 28 days after sowing

Variety	FW (g)	DW (g)
Acadia F1	57.13 ± 2.54^a	5.92 ± 1.23^a
Virofly	64.32 ± 12.12^a	5.29 ± 1.27^a
Space F1	5.96^b	0.565^b

These findings are relevant as they contribute to the optimization of resources in intensive cultivation systems such as Plant Factories. Commercially, selecting the Virofly variety can reduce production costs, thereby increasing profit margins for producers seeking efficiency without sacrificing the quality of the final product.

Antioxidant activity (ABTS, DPPH, FRAP): The antioxidant capacity of the varieties was evaluated using the ABTS, DPPH, and FRAP assays (Table 3). The values obtained for ABTS were 3063 ± 306 µmol TE/g DW for Acadia F1 and 3300 ± 314 µmol TE/g DW for Virofly, with no statistically significant differences between the two. This suggests that both varieties have a similar capacity to neutralize the ABTS radical, which is relevant for the antioxidant profile, as a higher neutralization capacity indicates a more effective response against oxidative stress.

For DPPH, Virofly showed a significantly higher value (231.8

Table 3. Results of antioxidant activity (ABTS, DPPH and FRAP)

Variety	ABTS•$^{+}$ µmol TE/g DW)	DPPH• (µmol TE/g DW)	FRAP (µmol eq. Fe_2SO_4/g DW)
Acadia F1	3063 ± 306^{a}	117.81 ± 19.5^{b}	938.5 ± 148.4^{a}
Virofly	3300 ± 314^{a}	231.8 ± 31^{a}	1054.1 ± 130.2^{a}
Space F1	n/a	n/a	n/a

± 31 µmol TE/g DW) compared to Acadia F1 (117.81 ± 19.5 µmol TE/g DW), which could suggest a higher concentration of compounds capable of neutralizing the DPPH radical in Virofly (Carey and Nair, 2022). This difference indicates that, under the conditions of this study, Virofly may offer a greater capacity to inhibit free radicals than Acadia F1, benefiting its overall antioxidant profile.

For FRAP, both varieties also showed similar values, with 938.5 ± 148.4 µmol eq. Fe_2SO_4/g DW for Acadia F1 and 1054.1 ± 130.2 µmol eq. Fe_2SO_4/g DW for Virofly. The similarity in FRAP implies that both varieties can reduce ferric ions to ferrous ions with comparable efficiency, which is beneficial for human health, as reducing activity is associated with the prevention of chronic diseases (Bian *et al.*, 2018).

Phenolic and flavonoid content: The content of phenols and flavonoids (Table 4) is crucial for the antioxidant capacity and anti-inflammatory properties of spinach. In this study, Acadia F1 had a phenolic content of 1.15 ± 0.1023 mg GAE/g DW, slightly higher than Virofly (1.06 ± 0.1852 mg GAE/g DW), although without significant differences. This suggests that both varieties contain similar levels of phenolic compounds, contributing to their overall antioxidant capacity and health benefits.

Table 4. Content of bioactive compounds

Variety	Total Phenolic Content (mg GAE/g DW)	Total Flavonoid Content (mg QE/g DW)
Acadia F1	1.15 ± 0.1023^{a}	0.3004 ± 0.0467^{b}
Virofly	1.06 ± 0.1852^{a}	0.39 ± 0.0317^{a}
Space F1	n/a	n/a

For flavonoids, Acadia F1 had a content of 0.3004 ± 0.0467 mg QE/g DW, while Virofly reached 0.39 ± 0.0317 mg QE/g DW. Flavonoids, being potent antioxidant compounds, directly contribute to the plant's ability to mitigate oxidative damage. The results show that Virofly has a higher flavonoid concentration, which may partly explain its greater activity in the DPPH assay.

Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophylls and carotenoids): The analysis of photosynthetic pigments (Table 5) revealed variations between the varieties, though without significant differences. For chlorophyll A, Acadia F1 showed a slightly higher content (4837 ± 312 µg/g DW) compared to Virofly (4500 ± 383 µg/g DW). Chlorophyll A is essential for photosynthesis, as it enables light capture and energy conversion (Ebrahimi *et al.*, 2023).

Table 5. Photosynthetic pigments quantification

Variety	CHLa (µg/g DW)	CHLb (µg/g DW)	Total CHLs (µg/g DW)	CAR (µg/g DW)	CHLs and CAR (µg/g
Acadia F1	4837 ± 312^{a}	1810 ± 317^{a}	6623 ± 553^{a}	1511.6 ± 118.2^{a}	8159 ± 703^{a}
Virofly	4500 ± 383^{a}	1482 ± 320^{a}	5982 ± 702^{a}	1423.3 ± 115.9^{a}	7405 ± 741^{a}
Space F1	n/a	n/a	n/a	n/a	n/a

For chlorophyll B, Acadia F1 also showed higher values (1810 ± 317 µg/g DW compared to 1482 ± 320 µg/g DW in Virofly). Chlorophyll B facilitates light capture in a different spectrum than chlorophyll A, improving the plant's adaptability to varying light conditions. The sum of both chlorophylls provides a total value of 6623 ± 553 µg/g DW for Acadia F1 and 5982 ± 702 µg/g DW for Virofly, indicating that Acadia F1 has a higher total chlorophyll content, which could confer an advantage in photosynthetic efficiency and growth capacity, especially under controlled light conditions like those in a Plant Factory.

The carotenoid content also showed differences, with Acadia F1 at 1511.6 ± 118.2 µg/g DW and Virofly at 1423.3 ± 115.9 µg/g DW. Carotenoids are important antioxidants that contribute to human health by protecting against cellular damage, in addition to acting as protective pigments in the plant by reducing damage from excessive light (Elvira-Torales *et al.*, 2020). The sum of chlorophylls and carotenoids yielded a value of 8159 ± 703 µg/g DW for Acadia F1 and 7405 ± 741 µg/g DW for Virofly, reaffirming Acadia F1's ability to capture and utilize light efficiently, making it potentially more adaptable in controlled cultivation systems (Nguyen *et al.*, 2019).

Genetic-environmental interpretation: The observed differences in biomass, bioactive compounds, photosynthetic pigments, and antioxidant activity among varieties can be attributed to genetic effects, environmental factors and interaction. This underscores the importance of considering both genetic selection and environmental conditions to optimize yield and nutritional quality in controlled cultivation systems.

From the perspective of quantitative genetics, the phenotypic equation describes the observed variability in a population as a function of genotype (G), environment (A), and their interaction (G×A). In this study, although the Virofly and Acadia F1 varieties showed numerical differences in biomass and the laboratory analyses performed, the statistical analyses revealed no significant differences.

This would indicate that the contribution of G and G×A to phenotypic variability in this system may have been minimal, likely due to the stability of the controlled environment. It is important to note that this study did not directly quantify this interaction. The analyses focused on comparing phenotypic responses under fixed environmental conditions, where the absence of significant differences between varieties indirectly suggests a low relative contribution of the G×A component, thus requiring additional research to quantify this interaction.

However, the absence of statistically significant differences should not be interpreted as an absence of biological differences between varieties. It is possible that the controlled environment of the system attenuated or neutralized certain phenotypic expressions, such as resistance to abiotic stress or pests that might manifest under field conditions where environmental variability is greater.

Under the homogeneous conditions of this experiment, it is inferred that phenotypic variability was minimal. This uniformity suggests that the controlled environment tends to standardize crop response, decreasing the phenotypic expression that might normally be attributed to genotype. From a practical perspective, this reinforces the idea that in agricultural systems with controlled environments, such as Plant Factories, varietal selection can be oriented toward criteria such as seed cost, ease of management, and operational efficiency.

Commercial aspects, strategies, and sustainability: This approach is scalable to other high-value commercial crops, facilitating the selection of more profitable varieties adapted to controlled environment production systems. If genetic variability does not significantly affect yield within these environments, it becomes feasible to choose agronomically fewer demanding varieties with lower seed costs and greater management and productivity efficiency. These findings are particularly relevant for environmentally sensitive species such as strawberries and various aromatic or medicinal herbs, where environmental uniformity can stabilize production. Furthermore, the ability to precisely modulate growing conditions could induce specific phenotypic responses, adjusting quality parameters according to market demands or production objectives, creating new opportunities for product specialization and differentiation in systems like Plant Factories.

Additionally, this strategy would enable the implementation of Plant Factories in areas with low food security, where open-field horticultural production is unviable due to extreme climate conditions, water scarcity, and land use limitations. By establishing production units in urban or peri-urban environments, it promotes agricultural system decentralization, reducing dependence on imports and the need for long-distance transportation, with logistical, economic and environmental benefits.

In this study, baby leaves of two spinach varieties were successfully grown in a controlled environment in the state of Nuevo León, Mexico, despite the extreme climatic conditions in the region, which is considered a semi-arid zone.

Considering the study's objective to determine the most viable variety for commercial production, it is crucial to evaluate the costs associated with each variety. The lack of significant differences in most evaluated parameters implies that choosing the Virofly variety would be a more economically rational decision, particularly if the production system aims to maximize profitability without sacrificing product quality.

This choice carries significant operational and commercial implications, as it reduces initial costs in intensive cultivation systems like Plant Factories. Scientifically, the study reinforces that investing in hybrid varieties is not always necessary to obtain high-quality products, offering accessible alternatives to optimize resources. Commercially, Virofly enables production of competitive, high-quality spinach, favoring the crop's economic sustainability and market accessibility of the product.

Since no significant differences were found in most evaluated parameters, such as antioxidant capacity, bioactive compounds, and photosynthetic pigments, the Virofly variety is recommended as a more viable and cost-effective option for controlled cultivation systems. While Acadia F1 showed a slight increase in chlorophyll and carotenoid content, its seed cost ten times higher than that of Virofly does not justify the difference, especially considering both varieties offer the same nutraceutical quality.

The apparent low Genotype × Environment (G × E) interaction suggests that the controlled environment of the Plant Factory may have attenuated the expected genetic differences. This phenotypic stability allows varietal selection to focus primarily on criteria such as cost and operational efficiency, rather than on yield variations.

These results can apply to other sensitive crops, such as strawberries, arugula, and herbs. By adjusting environmental conditions, desired phenotypic variability could be induced, optimizing quality and productivity.

Overall, this study supports the viability of Plant Factories as a sustainable, predictable alternative for horticultural production, where environmental stability enhances cost-effectiveness over genetic selection.

Author contribution statement: AILM: Conceptualization, methodology, formal analysis and writing the original draft. EDRA: Conceptualization, methodology, data collection, investigation, formal analysis, and writing the original draft. JFGL: Laboratory analysis, review and editing. HRF, SRSG, JAV: Review and editing.

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