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L-DOPA yield in plants of local *Vicia faba* varieties from Puebla, Mexico

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ABSTRACT: Significant quantities of 3,4-dihydroxy-L-phenylalanine (L-DOPA or levodopa) are present in the plant structures of *Vicia faba*, a compound utilized in the treatment of Parkinson's disease. In Mexico, the cultivation and per capita consumption of *V. faba* is low, suggesting that exploiting the species through its biomass could enhance the utilization of this phytogenetic resource. The objectives were: (1) to evaluate the morphological characteristics of seeds from 24 local *V. faba* varieties originating from production areas, and (2) to analyze the content and yield of L-DOPA in the young plants of these 24 local varieties to assess the variation of this compound. The local varieties were collected in seven growing localities in Puebla, Mexico. Seed local varieties were measured for length, width, and weight of 100 seeds to determine their size. Subsequently, the seeds were sown in the field, and plants were harvested 20 days after emergence. The plant material was processed, and L-DOPA content was quantified using UV-visible spectrophotometry, and the yield per plant was obtained. L-DOPA content and yield ranged from 127.91 - 179.52 mg g^{-1} DM and 223.27 - 584.21 mg $plant^{-1}$, respectively. Principal component and cluster analysis identified five groups differentiated by seed morphological variables, biomass and L-DOPA. It was shown that the L-DOPA content in the plant is not influenced by the place of origin of the seed, and that seed size can be an indicator of the amount and yield of L-DOPA. The production of this bioactive compound via *V. faba* biomass may be considered a natural resource with commercial potential.

1. INTRODUCTION

The faba bean (*Vicia faba* L.), a member of the legume family (Fabaceae or Leguminosae), is a valuable source of essential nutritional components. It has been documented to have a high protein content (27.6%) (Martineau-Côté et al., 2022), predominantly composed of globulins (69.5–78.1%), followed by glutelins (12.0–18.4%), prolamins (1.83–3.57%), and albumins (1.41–3.01%) (Alghamdi, 2009). The bean is also rich in carbohydrates (66%), including starch (40%) and dietary fiber (12.9%) (Martineau-Côté et al., 2022), and it contains significant amounts of minerals such as K (11,315 ppm), P (5,118 ppm), S (1,903 ppm), Mg (1,334 ppm), and Ca (971 ppm), as well as Fe (51 ppm) and Zn (42 ppm) (Khazaei & Vandenberg, 2020). Additionally, faba bean offers health benefits due to its content of antioxidants (Boukhanouf et al., 2016), bioactive peptides (Martineau-Côté et al., 2022), raffinose family oligosaccharides with prebiotic functions for gut microbiota (Labba et al., 2021), and 3,4-dihydroxy-L-phenylalanine (L-DOPA or levodopa), a precursor of dopamine, a critical neurotransmitter for the central nervous system (Oviedo-Silva et al., 2018).

Studies have documented that L-DOPA is found in outstanding amounts in plant organs or plant structures. For example, in germinated seed from day 1 to day 8 the values amounted from 0.43 to 11.11 mg g^{-1} DM (Duan et al., 2021), in the 8 - 9 day-old shoot the content is 125.87 mg g^{-1} DM (Oviedo-Silva et al., 2018), in seedlings 9 days after germination ~30 - 50 mg g^{-1} DM (Vered et al., 1994), in plants 20 days after emergence 67.30 - 88.04 mg g^{-1} DM (Fuentes-Herrera et al., 2023) and in flowers of different shades it is between 27.80 - 63.50 mg g^{-1} DM (Hu et al., 2015).

L-DOPA, due to its biological role as a precursor to the neurotransmitter dopamine, is the most widely used compound in the treatment of Parkinson's disease (PD). It helps restore dopamine levels that are no longer produced in the brain, leading to improvements in both motor and non-motor symptoms associated with the disease, which contributes to a better quality of life for patients (Gandhi & Saadabadi, 2023).

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PD is the second most common neurodegenerative disorder globally, primarily affecting older adults (Valadez-Barba et al., 2021). The prevalence of Parkinson's disease is estimated to be 1.51 cases per 1000 people (over 12 million people with Parkinson's disease worldwide), and was shown to have been increasing since 1980 with a steep rise in the last two decades; with 1.18 cases per 1000 people in the period 2000-09 and 3.81 cases per 1000 people in the period 2010-23, mainly in countries with high human development index or sociodemographic index (Zhu et al., 2024). The World Health Organization (WHO, 2023) recently reported that the most effective medication for PD is levodopa combined with carbidopa. However, access to this treatment is limited, particularly in low- and middle-income countries where it may not be affordable or readily available (WHO, 2023). Thus, exploring natural alternatives that can provide the necessary daily dose for patients, such as plant tissues from faba bean plants, is of significant interest. The consumption of *V. faba* as a natural source of L-DOPA has been supported by case studies involving patients with PD. For instance, the ingestion of 250 g of boiled (Apaydin et al., 2000) or cooked (Rabey et al., 1992) faba beans, or the consumption of 40 g of fresh seedlings (Vered et al., 1994), has shown substantial improvement in motor symptoms comparable to the effects observed with conventional drug administration.

Plants, through their biomass, can serve as valuable biological resources for the bioeconomy. In recent years, particularly in European countries, strategies have been developed to promote the bioeconomy, with a central focus on optimizing biomass utilization. This approach is leveraged by channeling biomass toward higher value-added applications, making it crucial to identify biological resources with potential for national and regional bioeconomic development (Rodríguez et al., 2017). The bioeconomy involves innovations through bioresources to develop solutions, services, and products that integrate sustainability with enhanced consumer benefits (Global Bioeconomy Summit, 2020). It also represents a new model for territorial development, generating a more sustainable and environmentally friendly economy by efficiently converting biomass into raw materials for various products, including pharmaceuticals (Cruz & Caballero, 2021). In Mexico, this model is still under-researched and is only just beginning to emerge (Cruz & Caballero, 2021).

Recently, Fuentes-Herrera et al. (2023) documented that young faba bean plants of Mexican varieties have significant L-DOPA yields, ranging from 110 to 170 mg per plant. However, the L-DOPA content and yield of plants from local faba bean varieties grown in production areas remain unknown. Data from 2022 indicate that faba beans are cultivated in thirteen states across Mexico, with Puebla being one of the largest producers, contributing 21,889.89 t of green beans and 8,376 t of dry beans (SIAP & Servicio de Información Agroalimentaria y Pesquera, 2023). Therefore, it is essential to evaluate the germplasm of local varieties typically grown in these production areas to determine if their seeds are particularly suitable for the production of this metabolite. In Mexico, the area planted with faba beans (26,691.16 ha, green + dry) and per capita consumption is low (less than 0.5 kg) compared to common beans (*Phaseolus vulgaris* L.), which covers a much larger area (1,472,462.29 ha, 55.16 times more than faba beans) and has a higher per capita consumption (9.2 kg). Additionally, faba bean production has not seen a substantial increase over the years (71,000 t in 2000 vs. 85,000 t in 2022) (Fuentes-Herrera, 2021; SIAP & Servicio de Información Agroalimentaria y Pesquera, 2023). Consequently, utilizing this species for its biomass to obtain L-DOPA could promote its production and consumption, enhance the economy of rural families, and contribute to regional rural development. Thus, the objectives of this study were: (1) to evaluate the morphological characteristics of seeds from 24 faba bean local varieties from production areas in the state of Puebla, Mexico, and (2) to analyze the content and yield of L-DOPA in young plants 20 days after emergence to identify the variation in this bioactive compound among local varieties. The hypotheses are: (1) the place of origin of the seeds and their morphological characteristics will influence the L-DOPA content in the faba bean plant, and (2) the content and yield of L-DOPA in the plant will vary among local varieties.

2. MATERIALS AND METHODS

2.1. Reagents

All reagents and solvents used in the study were of analytical grade. Distilled H_2O, $NaNO_2$ (Sigma-Aldrich, 237213), HCl (HYCEL, 51349-2N19), CH_3COOH (J.T. Baker, 10433191), NaOH (Karal, 6080) and L-DOPA standard (Sigma-Aldrich, PHR1271, with a purity of 99.7 %) were used.

2.2. Plant material

During November - December 2022 twenty-four local faba bean varieties were collected in seven localities in the municipalities of Tlahuapan and Chiatzingo in Puebla (Figure 1 ,Table 1). Municipalities from the central-western part of the state of Puebla that had high production of green faba bean + grain faba bean consecutively during the period 2015 - 2019 (SIAP & Servicio de Información Agroalimentaria y Pesquera, 2023).

2.3. Environmental Variables of the Localities

The values of the environmental variables of the study localities—mean annual temperature (MAT), mean annual precipitation (MAP), and mean annual solar radiation (MASR)—were obtained using metadata from the 2023 Geoinformation Portal of the National Commission for the Knowledge and Use of Biodiversity CONABIO (2023). Subsequently, the necessary information for each locality was extracted using ArcMap software version 10.8. Altitude data were obtained from records provided by the National Institute of Statistics and Geography (INEGI, 2012) (Table 1).

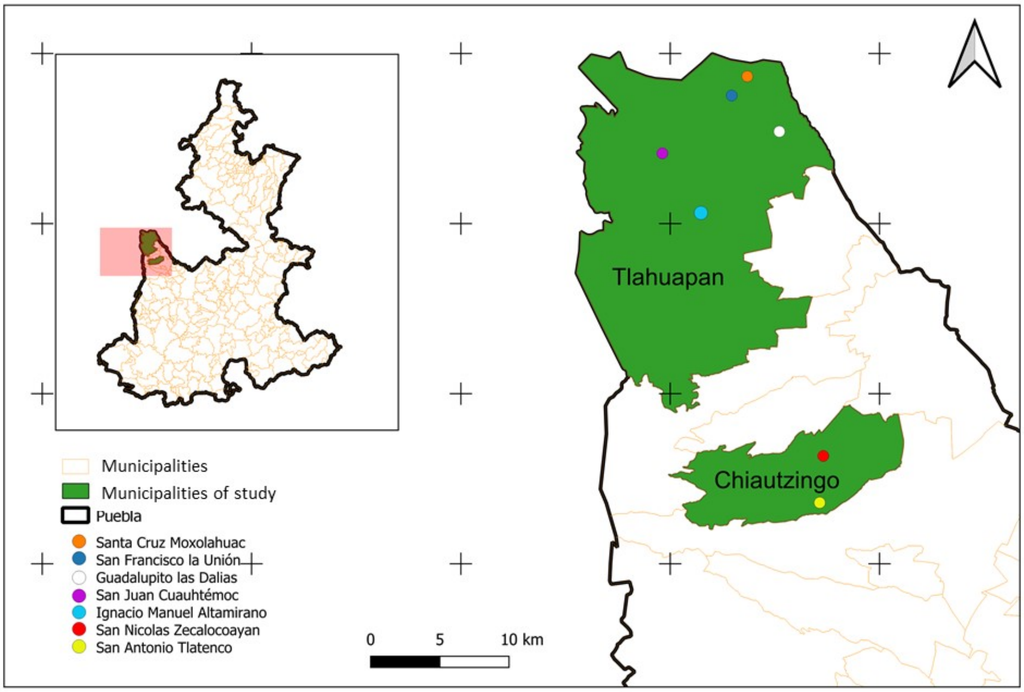

Figure 1. Faba bean-producing localities in the central-western part of the state of Puebla.

Table 1
Environmental variables of faba bean-producing localities in Puebla, Mexico.

Municipality	Locality	MAT (°C)	MAP (mm)	MASR (MJ/m^2)	Altitude (msal)	Faba bean local varieties
Tlahuapan	Santa Cruz Moxolahuac	13	900	18.16	2,776	T1, T2
	San Francisco de la Unión	13	700	18.16	2,807	T3, T4, T17
	Guadalupito las Dalias	13	700	18	2,572	T5, T6
	San Juan Cuauhtémoc	13	700	17.5	2,782	T7, T8, T18
	Ignacio Manuel Altamirano	13	700	17.5	2,665	T9, T10, T 11, T12, T13, T14, T15, T16,
Chiautzingo	San Antonio Tlatenco	13	900	22.16	2,580	C1, C6
	San Nicolás Zecalacoayan	14	900	22.16	2,460	C2, C3, C4, C5

MAT = mean annual temperature, MAP= mean annual precipitation, MASR = mean annual solar radiation.

2.4. Morphological Characterization of Faba Bean Seeds

To measure seed dimensions, the methodology of Salamanca-Bautista et al. (2018) was used with modifications. From each local faba bean variety, 10 seeds were randomly selected, and their length and width were recorded using a digital caliper. This procedure was repeated three times for each set of 10 seeds. To determine the weight of 100 seeds (W100S), 100 seeds were randomly selected and weighed using a precision balance (OHAUS/H-5852), with the measurement performed in triplicate.

2.5. Sowing

Seeds from the 24 local varieties were sown on February 16, 2023, in the experimental field of the Center for Technological Innovation in Protected Agriculture (CITAP) (18° 55' 52" N, -98° 23' 59" W). The soil was prepared (plowing, harrowing and furrowing) and moistened three days before sowing. The sowing was conducted using a randomized complete block design, following the farmer's traditional tillage practices. Seeds were planted at a depth of 5 cm, with two seeds per hill and a spacing of 20 cm between hills. Each plot (120 x 60 cm) represented one replicate (28 seeds), and three replicates per local variety were established. During plant development, the field was irrigated by drip with potable water for 2 hours in the morning, three times per week (Figure 2). The species authenticity of the plant material used was based on the morphological descriptors of the International Board for Plant Genetic Resources IBPGR (1985) and Duc (1997).

2.6. Agro-Environmental Variables

Temperature (°C), relative humidity (%RH), and solar radiation (W/m^2) (Figure 3) were recorded every hour during

Figure 2. Sowing and development of the *V. faba* plant.

the cultivation of the faba bean plants using a weather station (ADCON TELEMETRY, addVANTAGE Pro 6.5) installed at the CITAP experimental field.

2.7. Plant Age Identification, Collection, and Processing of Plant Material

At the emergence stage, when the shoot breaks through the soil surface (stage 09) (Meier, 2018), the shoots were marked with colored raffia to facilitate the collection of plants 20 days after emergence (DAE ± 1). The plants were harvested approximately one month after sowing. Five plants per plot were harvested, with roots removed by a transverse cut. The plants were then weighed using a precision balance (OHAUS/H-5852) and dried in the shade at room temperature (a process lasting between 20 and 30 days, depending on plant size). Once dried, the plants were weighed again, and the moisture content (%H) was measured using a thermobalance (Adam, PMB 53, Milton Keynes, U.K). This process allowed for the determination of biomass per plant in terms of dry matter, with results reported as the mean value of five plants per plot. The dried plants were subsequently ground using a coffee grinder (Hamilton Beach, Model: 80393, U.S.A.), and the resulting powder was sieved with a No. 20 mesh (841 μm) and stored in food-grade polypropylene bags under refrigeration (4 ± 1 ºC) for further analysis.

2.8. L-DOPA Content

2.8.1 Extraction

L-DOPA was extracted following the methodology proposed by Polanowska et al. (2019) with some modifications. A 20 mg sample was placed in a glass tube, and 9 mL of an aqueous extractant solution acidified with 0.2% (v/v) acetic acid was added. The mixture was then stirred using a vortex mixer (Scientific Industries, G560, USA) at medium speed for 10 seconds. The tubes were subsequently placed in an ultrasonic bath (CREST, 275T, New York, USA) for 10 minutes (repeated twice), keeping the bath temperature below 30°C. The mixture was then centrifuged (HERMLE Labnet, Z326, Wehingen, Germany) at 5000 rpm for 6 minutes, and the supernatant was collected for analysis.

2.8.2 Analysis

The analysis was carried out based on the methodology of Rahmani-Nezhad et al. (2018), with modifications from Fuentes-Herrera et al. (2023) and adapted for a 10 mL volume. A 0.4 mL aliquot of the extract or standard was placed in a 10 mL volumetric flask. Subsequently, 0.8 mL of 3% (w/v) $NaNO_2$ and 0.4 mL of 1M HCl were added, mixed, and allowed to stand for 5 minutes until a yellow color developed. Then, 1.2 mL of 1M NaOH was added, the mixture was stirred, and it was allowed to stand for another 5 minutes until it turned orange-red. Finally, distilled water was added to bring the volume up to 10 mL, mixed, and the absorbance at λ 253 nm was recorded using a UV-visible spectrophotometer (JENWAY, Genova Nano, Staffordshire, UK) against a blank. All analyses were performed in triplicate.

2.8.3 Calibration Curve

Quantitative analysis was performed using an L-DOPA standard. A stock solution of 208 μg mL^{-1} prepared with the standard and water acidified with 0.2% (v/v) acetic acid. A series of ten 10 mL volumetric flasks was prepared, each containing 100 to 1000 μL of the stock solution, along with the other reagents as per the methodology described above. The volume of distilled water added was adjusted according to the corresponding volumetric capacity of the flask. This process yielded a calibration curve with concentrations ranging from 2.08 to 20.8 μg mL^{-1}.

2.9. Statistical Analysis

Twenty-four treatments with three replications each were evaluated. The morphological variables of *V. faba* seeds, along with the results of biomass, L-DOPA content, and yield in *V. faba* plants, were analyzed using one-way ANOVA (PROC ANOVA) with the SAS statistical package (SAS version 9.4, SAS Institute, Cary, NC, USA). Significant differences between treatments were determined using Tukey's Honestly Significant Difference (HSD) test ($p \leq 0.05$). Additionally, multivariate principal component analysis (PCA) and cluster analysis (applying Ward's method and squared Euclidean distance) were performed using IBM SPSS Statistics for Windows, version 27 (IBM Corp., Armonk, NY, USA) and JMP (Pro version 16), utilizing the mean values of environmental variables (Table 1), morphological variables, faba bean plant biomass, L-

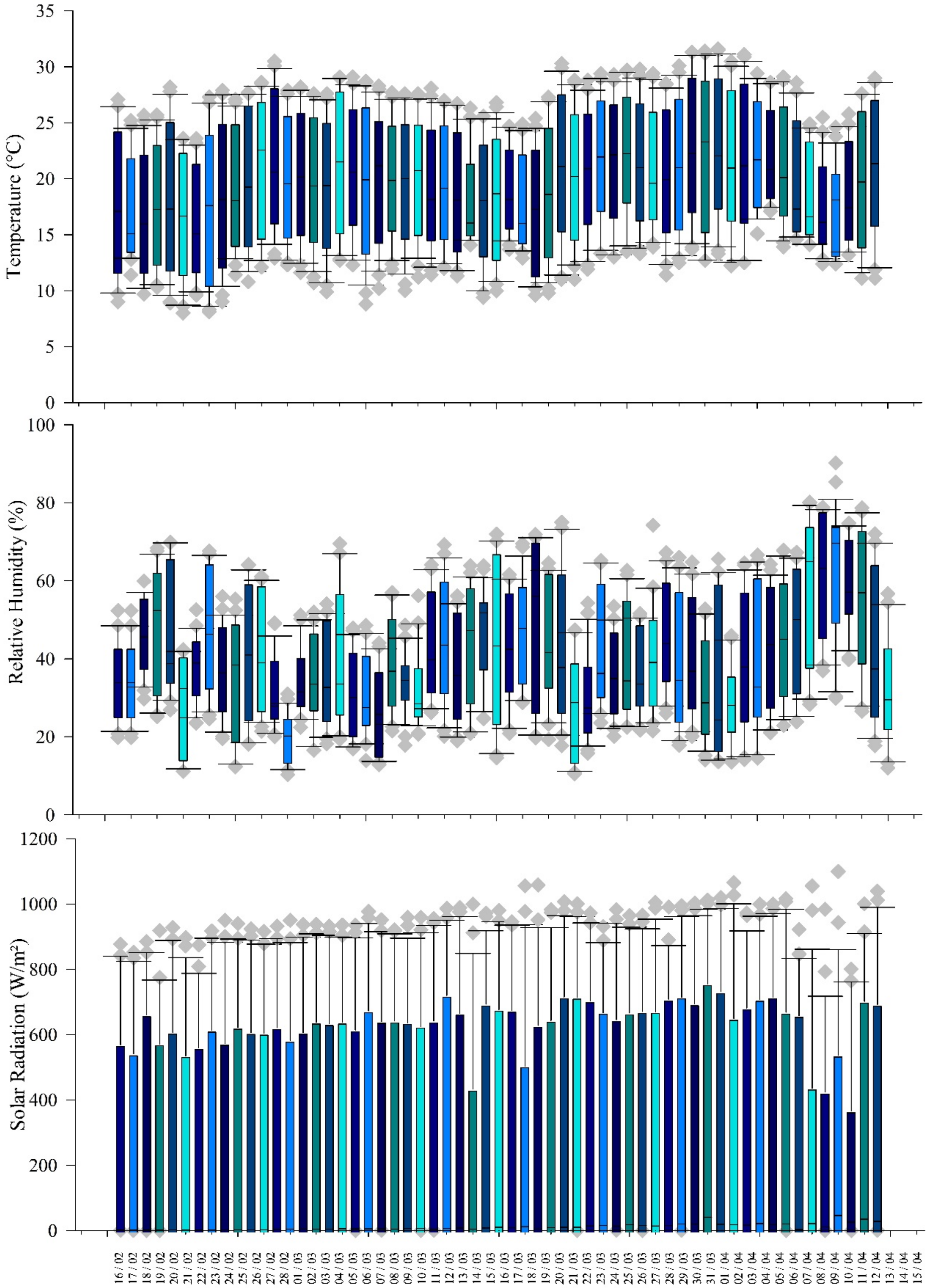

Figure 3. Data of agro-environmental variables during the cultivation of *V. faba* plants 20 days after emergence. Each box represents 24 data per day. The first and third quartiles represent the largest distribution of values, while the second quartile indicates the median of values. The arms represent the minimum and maximum values and the outer points represent the outliers.

DOPA content, and yields. Subsequently, the most common characteristics in each group were identified through means testing and bidirectional clustering.

3. RESULTS AND DISCUSSION

3.1. Characteristics of Faba Bean Seeds

The morphological variables—width, length, and hundred-seed weight—showed significant differences ($p \leq 0.01$) among the local varieties. Local variety T1 exhibited the highest values for width (19.76 mm), length (30 mm), and hundred-seed weight (W100S) (295.82 g). Conversely, local variety C4 had the lowest values for width (13.89 mm) and length (20.92 mm), while local variety T12 had the lowest W100S (122.37 g) (Table 2). In terms of seed dimensions and hundred-seed weight, and considering seed size characteristics from other studies on Mexican bean cultivars (Herrera-Cabrera et al., 2010; Salamanca-Bautista et al., 2018), the seeds were classified into small, medium, and large categories (Table 2).

3.2. Biomass, L-DOPA Content, and Yield

Plant biomass exhibited significant differences ($p \leq 0.05$) among local varieties, while L-DOPA content and yield showed highly significant differences ($p \leq 0.01$) among local varieties. Plant biomass ranged widely from 1.56 to 3.76 g DM, with local variety T1 having the highest value, though it was statistically different only from local variety C5 (Table 3). The L-DOPA content in the local varieties ranged from 127.91 to 179.52 mg g^{-1} DM, with a mean of 154.51 mg g^{-1} DM (Figure S1, Appendix A). Local variety T7 had the highest L-DOPA content, but it was not significantly different ($p \geq 0.05$) from eighteen other local varieties (T1, T2, T3, T4, T6, T9, T10, T11, T12, T14, T15, T16, T17, T18, C1, C4, C5, C6).

Initial trials of L-DOPA for PD in the 1960s revealed transient improvements in rigidity and tremor but were limited by side effects such as nausea and vomiting. The addition of a DOPA decarboxylase inhibitor (carbidopa or benserazide) in 1967 was a breakthrough, reducing peripheral metabolism of L-DOPA to dopamine and minimizing side effects, thereby enhancing its ability to cross the blood-brain barrier (Riederer & Horowski, 2023). It remains uncertain whether L-DOPA from plants would require co-administration with carbidopa or if it could be effective on its own, given that plant tissue also contains other bioactive compounds that might provide additional benefits for PD (Fuentes-Herrera et al., 2022). It is important to consider that each patient requires specific doses, so it is necessary to know the contents of this bioactive in the plant, given that, patients on existing medication might experience dopaminergic overstimulation if consuming natural L-DOPA sources (Ramírez-Moreno et al., 2015).

The L-DOPA content in the faba bean plants, with some local varieties showing up to twice the content reported by Fuentes-Herrera et al. (2023) (67.30 - 88.04 mg g^{-1} DM). This discrepancy may be attributed to differences in the varieties used and the drying methods employed. In this study, plants were dried in the shade at room temperature (22 - 25°C), whereas Fuentes-Herrera et al. (2023) used a drying temperature of 38°C. L-DOPA is known to be thermolabile and can degrade with increased temperature. For example, Kai-Bin et al. (2016) found that drying at temperatures of 40 - 100°C reduced L-DOPA content by 60% in *V. faba* flowers, likely due to oxidative decomposition or accelerated degradation of the metabolite. Zhou et al. (2012) also demonstrated that L-DOPA undergoes oxidation through thermal action (37 - 80°C), following first-order kinetics.

Regarding L-DOPA yield, a wide range was observed among the local varieties (223.27 - 584.21 mg $plant^{-1}$), with local variety T1 showing the highest yield per plant and local variety C5 the lowest (Table 3).

Both metabolite content and plant biomass are essential parameters for evaluating the yields of bioactive compounds (Fuentes-Herrera et al., 2023). In this study, plant biomass was found to be a determinant of L-DOPA yield. For instance, local variety T1, despite not having the highest L-DOPA content (155.80 mg g^{-1} DM), exhibited the highest L-DOPA yield (584.21 mg $plant^{-1}$) due to its superior biomass (3.76 g DM). Conversely, local variety T7, which had the highest L-DOPA content (179.52 mg g^{-1} DM), had a lower biomass (2.09 g DM), resulting in a lower L-DOPA yield (363.86 mg $plant^{-1}$) (Table 3). This underscores the critical role of biomass in determining L-DOPA yield. Similarly, Etemadi et al. (2018) reported that while drought stress increased L-DOPA concentrations in faba bean seedlings, it compromised metabolite yield due to reduced biomass production. Thus, higher biomass correlates with higher L-DOPA yields (Table S1, Appendix A).

Although the study identified local varieties with high L-DOPA content (e.g., T7) and yield (e.g., T1), no statistically significant differences were found among several local varieties (Table 3). Consequently, local faba bean varieties from these production localities could potentially be cultivated to produce L-DOPA for human consumption or to develop supplements or nutraceuticals for PD treatment or other ailments, since it has also been documented that L-DOPA has also been shown to improve male sexual behavior and reproductive functions (Tangsrisakda et al., 2022). Given the complexity and expense of synthetic L-DOPA production, deriving L-DOPA from natural sources such as plant tissue is more feasible, providing an economical and enantiometrically pure product (Patil et al., 2013; Tesoro et al., 2022). Cultivating faba bean plants to produce biomass for L-DOPA could also enhance economic opportunities for bean producers.

3.3. Principal Component Analysis and Cluster Analysis

The dispersion of the 24 local *V. faba* varieties represented in the space by the first three principal components explained 87.25% of the cumulative variation of the 11 variables analyzed (Table 4). The first principal component (PC1) explained 62.47% of the total variation and was primarily associated with seed morphological variables, environmental

Table 2
Mean values of morphological variables of seeds from twenty-four local varieties of *V. faba*.

Faba bean local varieties	Length (mm)	width (mm)	W100S (g)	Size
T1	30	19.76	295.82	L
T2	28.62	18.92	249.08	L
T3	27.96	18.35	215.93	M
T4	28.91	18.72	258.83	L
T5	21.53	14.7	130.31	S
T6	24.75	16.53	191.67	M
T7	29.35	18.59	231.41	L
T8	27.6	17.6	222.77	M
T9	27.55	17.83	257.52	M
T10	29.06	18.09	291.00	L
T11	25.9	16.78	231.93	M
T12	22.39	14	122.37	S
T13	26.18	16.53	211.77	M
T14	29.08	17.33	257.60	L
T15	26.98	17.29	205.86	M
T16	29.28	18.35	263.48	L
T17	25.88	16.9	236.33	M
T18	23.92	16.12	160.64	M
C1	21.73	14.13	169.01	S
C2	23.45	15.38	165.99	M
C3	21.62	14.09	139.23	S
C4	20.92	13.89	146.40	S
C5	22.44	15.12	168.45	S
C6	22.96	15.02	157.82	S

W100S = hundred-seed weight; S = Small; M = Medium; L = Large.

Table 3
Biomass, content, and yield of L-DOPA in plants of twenty-four local varieties of *V. faba*.

Local faba bean varieties	Biomass (g MS)	L-DOPA content (mg g^{-1} MS)	L-DOPA yield (mg $plant^{-1}$)
T1	3.76a	155.80abcdef	584.21a
T2	1.74ab	169.70abcde	295.39ab
T3	2.88ab	157.88abcdef	450.84ab
T4	2.33ab	175.22ab	411.98ab
T5	2.57ab	139.24bcdef	356.78ab
T6	2.38ab	168.31abcde	393.77ab
T7	2.09ab	179.52a	363.86ab
T8	2.75ab	127.91f	351.97ab
T9	2.38ab	160.43abcdef	380.42ab
T10	2.55ab	174.37abc	427.90ab
T11	3.42ab	150.42abcdef	514.67ab
T12	1.98ab	146.56abcdef	276.96c
T13	3.21ab	136.57cdef	426.25ab
T14	2.93ab	149.92abcdef	428.10ab
T15	1.88ab	158.85abcdef	299.54ab
T16	2.16ab	165.92abcdef	348.84ab
T17	2.38ab	170.62abcd	407.36ab
T18	1.99ab	142.57abcdef	278.05ab
C1	2.14ab	154.58abcdef	329.39ab
C2	2.20ab	131.78ef	261.72c
C3	1.71ab	133.73def	229.82c
C4	1.58ab	154.24abcdef	243.48c
C5	1.56c	152.30abcdef	223.27c
C6	2.12ab	151.86abcdef	320.08ab

variables, and L-DOPA yield. The second principal component (PC2) accounted for 13.88% of the explained variation and was associated with L-DOPA content. The third principal component (PC3) explained 10.90% of the total variation and was mainly associated with plant biomass. For each component, this association was considered by taking as a selection threshold the eigenvectors, positive or negative, greater than 0.570 (Table 4).

Table 4
Component matrix of each variable, eigenvalues, variance, and cumulative proportion of variance explained in the first three dimensions.

	CP1	CP2	CP3
MAT	-0.754	0.360	0.293
MAP	-0.596	0.482	0.512
MASR	-0.826	0.334	0.339
Altitud	0.860	-0.050	-0.135
Width	0.914	0.297	0.094
Length	0.929	0.262	0.073
W100S	0.884	0.321	0.226
Size	0.856	0.367	0.067
Levodopa	0.529	0.571	-0.362
Biomass	0.627	-0.506	0.580
Yield	0.799	-0.275	0.425
Eigenvalues	6.87	1.53	1.20
Variance (%)	62.47	13.88	10.90
Cumulative proportion (%)	62.47	76.35	87.25

MAT = Mean annual temperature, MAP = Mean annual precipitation, MASR = mean annual solar radiation, W100S = weight of one hundred seeds.

Based on the configuration obtained from the first three principal components, five groups were differentiated (Figure 4). Group one (G I) consisted of local variety T1. Group two (G II) was composed of local varieties T2, T4, T7, T10, and T16. Group three (G III) comprised local varieties T3, T6, T8, T9, T11, T13, T14, T15, and T17. Group four (G IV) included local varieties T5, T12, T18, and C1, while group five (G V) consisted of local varieties C2, C3, C4, and C5 (Figure 4).

The groups located on the positive axis of PC1 are characterized by having large-sized local varieties with high L-DOPA yields (G I and G II). In contrast, groups on the negative axis consist of local varieties from environments with a mean annual temperature higher than 13°C, mean annual precipitation around 900 mm, and mean annual solar radiation (MASR) of 22 MJ/m^2 (G V). The groups located on the positive axis of PC2 include local varieties that produce a high amount of L-DOPA (G II), whereas those on the negative side produce less (G I). In PC3, the groups located on the positive axis consist of cultivars that develop a high amount of plant biomass (G I and G III) (Figure 4).

Cluster analysis shows that, with a squared Euclidean distance of 3.98 in the dendrogram, four clusters were observed, in which the local varieties of Groups G I and G II from the principal component analysis were united into a single group. At a squared Euclidean distance of 3.60, the five clusters previously defined by the principal component analysis were identified, corroborating the results obtained (Figure 5).

Using the test of means (Table S2, Appendix A) and bidirectional clustering of the dendrogram (Figure 5), the specific characteristics of each group were identified. Group G I was primarily characterized by local varieties from higher altitude areas (2,776 masl) and higher mean annual rainfall (900 mm). This group had large seeds, developed plants with high biomass, and presented high L-DOPA yields. This group is represented by local variety T1 from the locality of Santa Cruz Moxolahuac.

Group G II also consisted of seeds from higher altitude zones (2,665 - 2,807 masl) with similar environmental characteristics to those of G I. Seeds in this group were medium to large, with high L-DOPA content, and medium to high plant biomass content and yield of this bioactive compound. Local varieties in this group are from the localities of Santa Cruz Moxolahuac (T2), San Francisco de la Unión (T4), San Juan Cuauhtémoc (T7), and Ignacio Manuel Altamirano (T10, T16).

Group G III was characterized by seeds primarily from areas of medium to higher altitude (2,572 - 2,807 masl), with a mean annual temperature (MAT) of 13°C, mean annual precipitation (MAP) of 700 mm, and mean annual solar radiation (MASR) of 17.5 - 18.6 MJ/m^2. This group had medium-sized seeds and average values for the other variables analyzed. It includes local varieties from the localities of San Francisco de la Unión (T3, T17), Guadalupito las Dalias (T6), San Juan Cuauhtémoc (T8), and Ignacio Manuel Altamirano (T9, T11, T13, T14).

Group G IV was identified by local varieties from areas with an altitude range of 2,572 - 2,782 masl, MAT of 13°C, MAP of 700 - 900 mm, and MASR of 17.5 - 22.16 MJ/m^2 (Table 1). This group was characterized by small seeds, and low to medium values for plant biomass and L-DOPA content and yields. Local varieties in this group are from the localities of both municipalities, Tlahuapan (T5, T12, T18) and Chiautzingo (C1, C6) (Table 1).

Group G V was identified by local varieties from environments with MAT values of 14°C, MAP of 900 mm, and high MASR. These local varieties had small seeds, developed small plants, and exhibited low L-DOPA content and yields (Figure 5). This group consists solely of local varieties from the municipality of Chiautzingo in the locality of San Nicolás Zecalacoayan (C2, C3, C4, C5).

Principal component analysis illustrated the relationships between environmental, morphological, biomass, and L-DOPA content and yields variables with respect to the variability found in the local faba bean varieties. The PCA results, which identified five groupings consistent with those from cluster analysis, indicated that L-DOPA content present in the plant is not defined by the place of origin of the seed, since the groupings obtained in the study revealed that some groups presented local varieties from different localities (e.g., G II and G III), even from different municipalities (e.g., G IV), suggested that L-DOPA content variability possibly is attributed to genetic differences

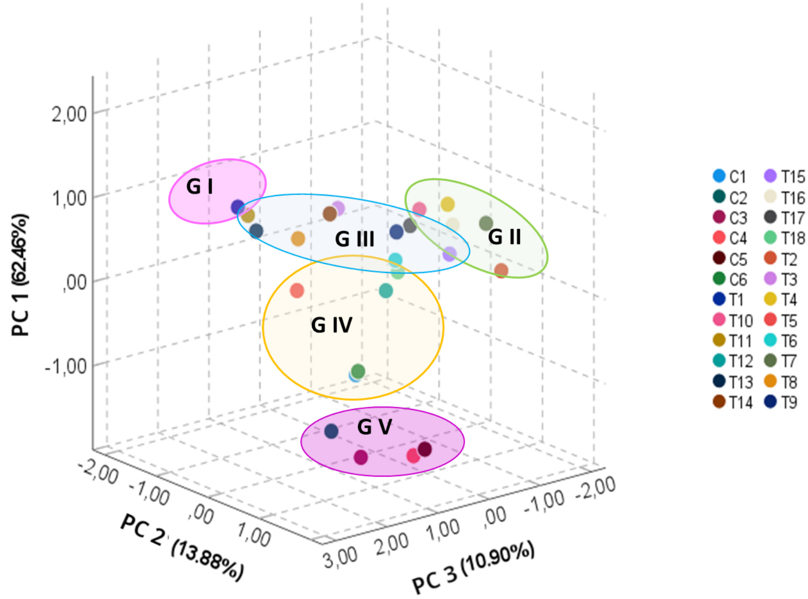

Figure 4. Dispersion of twenty-four local *V. faba* varieties defined by the first three principal components (PC) from seven bean-producing localities in the state of Puebla, Mexico.

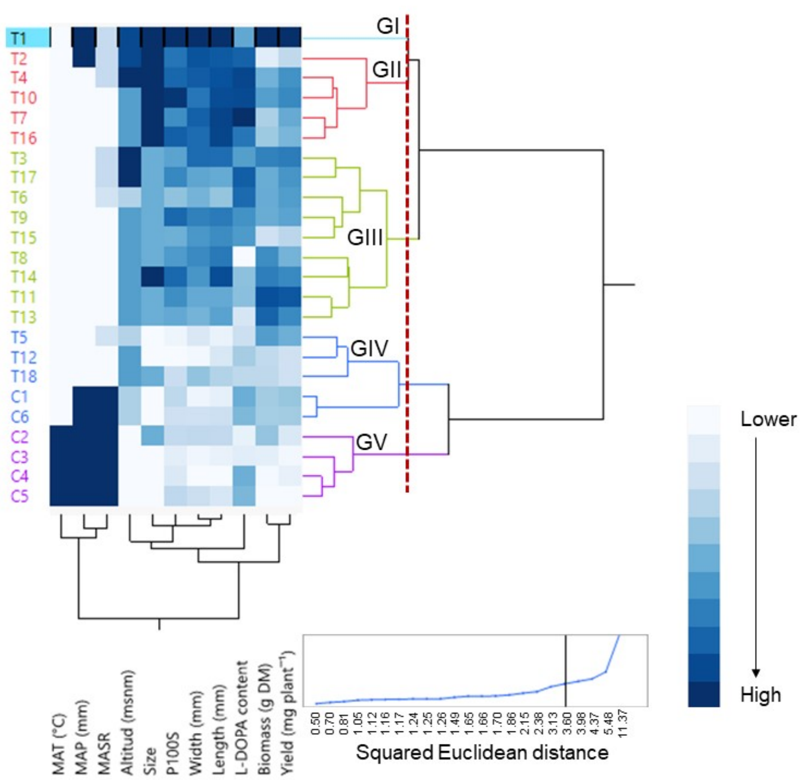

Figure 5. Bidirectional hierarchical clustering constructed using Ward's method of twenty-four local *V. faba* varieties from seven producing localities in the state of Puebla, Mexico.

among local faba bean varieties rather than the place from which the seed was collected. In addition, bidirectional hierarchical clustering clearly showed that seed size can be an indicator of the amount and yield that can be obtained from L-DOPA, regardless of where the seed comes from, since large seeds were clustered together found to provide higher L-DOPA content and yield (e.g., G I and G II) than small seeds (e.g., G IV and G V) (Figure 5). Kai-Bin et al. (2016) also demonstrated this behavior, but in flowers of faba bean plants, where it was observed that L-DOPA content in flowers was strongly associated with seed size. Moreover, also in this study it was found a positive correlation between seed size and biomass content ($p \leq 0.05$) (Table S1, Appendix A), indicating that larger seeds generally produce higher biomass, which in turn influences L-DOPA yield, regardless of seed origin.

The study identified significant variability in the morphological characteristics of faba bean seeds across different localities. In the PCA and cluster analysis the clustered shown that the higher altitude localities (2,665 - 2,807 masl), seeds were predominantly medium to large in size with substantial 100-seed weights (249.08 - 295.82 g), as observed in the G I and G II. Conversely, smaller seeds with lower 100-seed weights (122.37 - 169.01 g) were more common in lower altitude areas (2,460 - 2,665 masl), such as in G IV with local varieties C1 and C6 from San Antonio Tlatenco and GV with local varieties C2, C3, C4 and C5 from San Nicolás Zecalacoayan (Figure 4 and 5). This finding aligns with Herrera-Cabrera et al. (2010), who also reported larger seeds in higher altitudes (2,700 - 3,400 masl) and smaller seeds in lower altitudes (2,200 - 2,500 masl), regardless of local variety.

The influence of mean annual solar radiation (MASR) on seed size was also noted; local varieties in areas with higher MASR had smaller seeds (e.g., G V), while those in areas with lower MASR had larger seeds (e.g., G I and G II) (Figure 5). However, this study did not find conclusive evidence of the effects of temperature and precipitation on seed characteristics. This may be due to the proximity of the localities, which could limit the range of agro-environmental variation necessary to discern their impact on faba bean seed morphology. Previous research has indicated that temperature can affect seed characteristics in other species. For instance, Razzaque et al. (2023) demonstrated that increasing mean annual temperature negatively impacted seed mass in *Panicum hallii*.

According to the WHO (2023), levodopa with carbidopa remains the most effective PD treatment, but accessibility, affordability, and availability issues persist, particularly in low- and middle-income countries. *V. faba* plant tissue could offer a biologically and economically viable source of L-DOPA. Nonetheless, studies on microbiological safety and potential toxicity, as well as comprehensive *in vivo* assays, are needed to confirm the safety and efficacy of L-DOPA derived from faba bean plants.

Current PD treatments alleviate symptoms but do not halt disease progression (Valadez-Barba et al., 2021). Recent findings suggest that faba bean plants at 20 DAE contain L-DOPA along with other compounds such as flavonoids, rutin, and isoorientins. These compounds, in conjunction with L-DOPA, might not only alleviate PD symptoms but also offer potential neuroprotective effects (Fuentes-Herrera et al., 2023).

The utilization of *V. faba* for L-DOPA production exemplifies a bioeconomic approach, leveraging plant biomass to produce vital bioactive compounds for PD. The bioeconomy emphasizes the sustainable use of biological resources, integrating knowledge, science, and technology to deliver innovative and sustainable solutions. It fosters synergies across agriculture, medical research, and food science (IACGB & International Advisory Council on Global Bioeconomy, 2020), promoting new value chains and rural development through the circular economy approach (IICA, 2019). The present research is the first to assess L-DOPA content and yield in plants of local faba bean varieties from Puebla, Mexico, highlighting their potential to obtain L-DOPA. The use of the species for this proposal could help increase the *V. faba* production and consumption in México, benefit farming communities, and, in turn, contribute to regional rural development.

4. CONCLUSION

This study evaluated twenty-four local faba bean varieties from producing localities in Puebla, Mexico, assessing their morphological characteristics and analyzing the content and yield of L-DOPA in plants harvested twenty days after emergence. The results revealed that the plants had outstanding L-DOPA contents and yields higher than those obtained in other studies. Notably, the seed's place of origin did not influence L-DOPA content present in the plant; however, seed size was a significant factor influencing these parameters. Furthermore, variability in L-DOPA content and plant L-DOPA yield among local varieties was evident. Given that L-DOPA is still the most widely used compound in Parkinson's disease, utilizing biomass from faba bean plant for its production presents a promising natural alternative to help reduce or eliminate the use of the synthetic drugs, then faba bean plant biomass production could serve as a bioeconomic proposal for rural development. However, further research is necessary to explore the consumption and health benefits of faba bean plant-derived L-DOPA for Parkinson's patients.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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A. APPENDIX A. SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTIONS

PBFH, ADA, BPA - Research concept and design, PBFH, BPA - Collection and/or assembly of data, PBFH - Data analysis and interpretation, PBFH - Writing the article, ADA, BPA - Critical revision of the article, BPA - Final approval of the article.

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