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Article

# Genetic variability and heritability in broad bean collections from central Mexico

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## Abstract

This work was conducted with the aim of estimating variance components and broad sense heritabilities ($H^2$) in quantitative and qualitative traits in 25 cultivars of broad beans using an experimental design of randomized complete blocks with four replications in a series of trials. The differences between the two environments (A), the 25 genotypes (G), and their interaction were significant for most of the traits evaluated. The highest $H^2$ ranged from 51.73 to 96.68%. Principal component analysis accounted for 63.62% of the original total variation, yield was positively and significantly correlated with EFFI, NPP, PD, PL, WP, and YIE. The prediction with the GAM indicated that with NPP, PD, WP, and YIE, there would be greater genetic progress by selection. There was little phenotypic variability within each of the qualitative traits.

## Palabras clave:

*Vicia faba* L., green production, principal component analysis.

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# Introduction

Broad beans (*Vicia faba* L.) are grown in 64 countries and their grain is consumed fresh and dry. Due to their high protein value (23 to 43%), they are of great importance for the low-income population (Pérez *et al*., 2009; FAOSTAT, 2022). This is a diploid species (2n= 12) partially allogamous with crossbreeding of 4 to 60% (Suso and Maalouf, 2010), which has generated wide genetic variability but has also made it difficult to maintain various collections (Duc *et al*., 2010; Tadele *et al*., 2022).

The study of genetic variability based on agromorphological traits leads to the identification of varieties with higher yields and resistance to biotic and abiotic stresses (Arab *et al*., 2018; Tadele *et al*., 2021). Yield is influenced by genetic and environmental factors and by interactions between them (Annicchiarico and Iannicci, 2008) and has low heritability (Chaudhary *et al*., 2020).

Thus, it is sought to identify yield components with greater heritability, such as pods per plant (79.84%), seeds per plant (77.88%), seed weight per plant (87.61%) (Abo-Hegazy, 2022; Amjad and Alghamdi, 2023), and pod length (84.32%) (Kosev and Georgieva, 2023), to improve their response to selection.

In Mexico, there are few works on this subject. Peréz *et al*. (2015) and Bagink *et al*. (2013) considered seed size, health, and color relevant, characteristics that farmers in the High Valleys of Mexico use for their genetic improvement (Rojas *et al*., 2012). The State of Mexico is the largest producer of green broad beans and in the Municipality of Calimaya de Díaz González, 12 t ha$^{-1}$ are produced (SIAP, 2022). The present study aimed to estimate genetic variability and broad-sense heritability in quantitative and qualitative traits of broad beans collected in the center of the State of Mexico, Mexico.

# Materials and methods

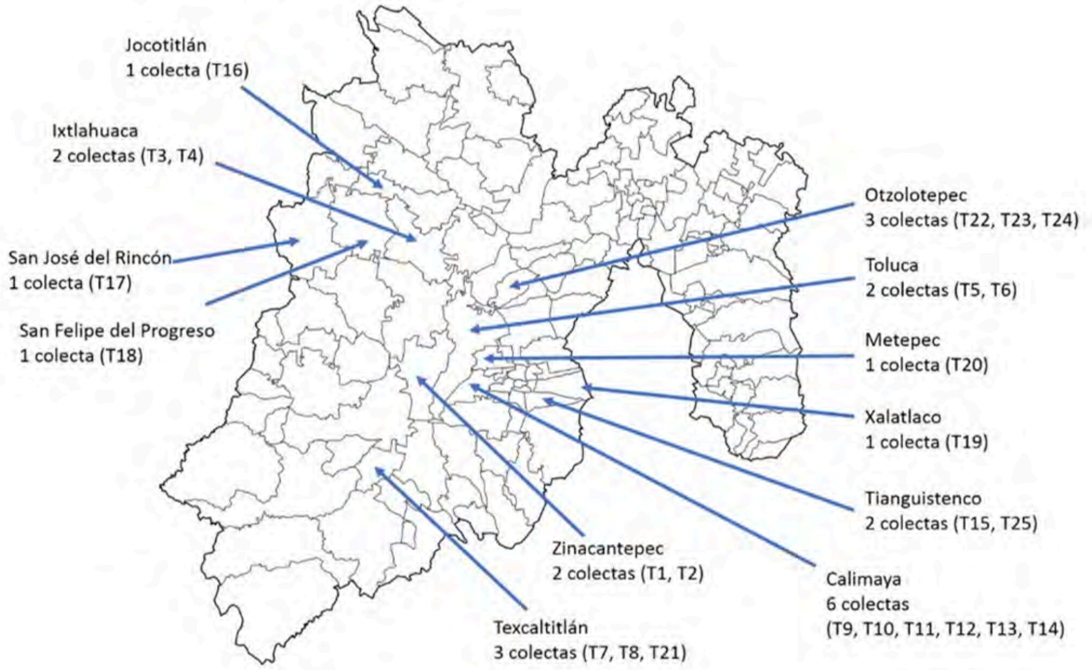
## Characteristics of the experimental site

This study was conducted in 2022 and 2023 during the spring-summer and autumn-winter agricultural cycles in San Nicolás Guadalupe (SNG; L1) and in San Francisco Oxtotilpan (SFO; L2), belonging to the municipalities of San Felipe del Progreso and Temascaltepec, respectively. SNG is located at 19° 36’ 30” north latitude and 100° 01’ 44” west longitude, at an altitude of 2 740 m, its average annual temperature is 15 °C and its average annual rainfall is 891.8 mm, the predominant soil type is andosol, and there is no irrigation infrastructure at this site. SFO is located at 19° 17’ 365” north latitude and 99° 89’ 956” west longitude, at an altitude of 2 648 m, its average annual temperature is 12 °C and its average annual rainfall is 1 200 mm, the predominant soil type is andosol, and supplemental irrigation was applied at this site (CONAGUA, 2014).

## Genetic material

A total of 25 collections from the following municipalities of Mexico were considered: Zinacantepec, Ixtlahuaca, Toluca, Texcaltitlán, Calimaya, Santiago Tianguistenco, Jocotitlán, San José del Rincón, San Felipe del Progreso, Xalatlaco, and Otzolotepec (Figure 1).

**Figure 1. Broad bean collections evaluated in 2022 and 2023.**



## Experimental design and plot size

The experimental design used was randomized complete blocks with four replications per locality in a series of experiments. The plot consisted of three 4 x 0.8 m furrows, but the central furrow was the useful plot. The distance between plants was 30 cm, placing two seeds per bush, thinning the seedling.

## Agronomic management of experiments

In SNG (L1), the preparation of the land was carried out mechanically, but in SFO (L2), this activity was done with animal traction. Sowing took place on April 22 (L1) and December 16, 2022 (L2). In SFO, two irrigations were applied (March 6 and April 20, 2023); 60N-60P-30K plus cow manure (240 kg in L1; 400 kg of this plus 500 kg of phosphorus in L2) were applied. In L1, the weeding was carried out on June 7 and July 7, 2022, and in L2, it was done on April 25 and March 21, 2023.

Weed control was manual in SNG, but in SFO, metribuzin 1 L ha$^{-1}$ was applied on December 29, 2022. The preventive phytosanitary control of chocolate spot disease (*Botrytis fabae*) in both localities was carried out by applying Mancozeb (1.0 kg ha$^{-1}$). The harvest was performed on September 10, 2022, in SNG, and on May 20, 2023, in SFO.

## Variables evaluated

Ten plants with full competence in each useful plot were considered and 13 quantitative and 10 qualitative traits were recorded applying the Technical Guide of the Varietal Description for broad beans of the National Seed Inspection and Certification Service (SNICS, 2001), in plant height (PH, cm), height to first pod (HFP, cm), total number of branches (TNB), branches with pods (NBP), efficiency (EFFI, NBP/TNB ratio), leaflets per leaf (NLL), pods per plant (NPP); seeds per pod (NSP), pod length (PL, cm), pod diameter (PD, cm), pod thickness (PT, cm), pod weight per plant (WP, g), and yield (YIE; green pod weight per plot was extrapolated to t ha$^{-1}$ ).

The qualitative traits were: growth habit (GH), plant size (PS), anthocyanin coloration in stem (ACS), intensity of green in foliage (IG), melanin spot on the standard (MSS), anthocyanin pigmentation on the standard (APS), extent of anthocyanin pigmentation on the standard (EAP), color of melanin spot on the wings (CMW), pod attitude (PA), and time of full pod development (TPD).

## Statistical analysis

An individual and combined analysis of variance (Anava) were performed assuming that localities (A), cultivars (G) and their interaction are random factors. Heritability by environment was estimated as follows: $H^2 = (\sigma^2_{G}/\sigma^2_{F})100$, but across environments, it was calculated with the following formula: $H^2 = 100 \{\sigma^2_{G} / [\sigma^2_{G} + (\sigma^2_{GA}/a) + (\sigma^2_{E}/ra)]\}$. Where: $\sigma^2_{G}$ is the genetic variance between cultivars; $\sigma^2_{F}$ is the phenotypic variance, $\sigma^2_{GA}$ is the variance of the interaction of cultivars by environments; $\sigma^2_{E}$ is the variance of the combined error, a and r are the number of environments and replications (Holland *et al*., 2003; Pérez *et al*., 2007).

The statistical package used was InfoStat version 2017 (Di Rienzo *et al*., 2008). The principal component analysis (Sánchez, 1995) was carried out with the Statistical Analysis System (SAS, 1988) but the biplot was created with Microsoft Excel. Genetic advance (GA) was calculated in absolute units and genetic gain was calculated as a percentage of the grand mean (GAM) by applying a selection pressure of 5% based on: $GA = (i\, H^2\, \sigma^{f} /_{mean})\, 100$. Where: i is the intensity of selection at 5% and $\sigma_f$ is the phenotypic standard deviation (Johnson *et al*., 1955).

# Results and discussion

## Analysis of variance

For environments (A) and cultivars (G), the F-values were significant (*p*# 0.01) in all variables, except leaflets per leaf (NLL). On the other hand, in the GxA interaction, there were no significant differences ($p < 0.05$) in total number of branches (TNB), leaflets per leaf (NLL), seeds per pod (NSP), pod length (PL), pod diameter (PD), and pod thickness (PT).

The results shown in Table 1 indicate that there was environmental heterogeneity in both localities, there is genetic variability between cultivars, and there is a different classification of broad beans in both sites, as reported by Pérez *et al*. (2015); Tekalign *et al*. (2020); Ton *et al*. (2021); Abo-Hegazy (2022); Kosev and Georgieva (2023), who found significant differences in the three previously indicated sources of variation for plant height (PH), total number of branches (TNB), pods per plant (NPP), seeds per pod (NSP), pod weight per plant (WP), and yield ha$^{-1}$ (YIE). On the other hand, Baginsky *et al*. (2013) reported significant differences only for YIE and pod weight per plant.

**Table 1. Mean squares and statistical significance of the F-values, grand mean (X), and coefficient of variation (CV %) in 13 quantitative traits measured in 25 broad bean cultivars.**

| Trait | Environment (A) | Rep (A) | G | G x A | Error | X | CV (%) |
|---|---|---|---|---|---|---|---|
| PH | 0.12* | 0.13 | 0.06$^{**}$ | 0.03$^{*}$ | 0.02 | 1.39 | 10.36 |
| HFP | 3757.84$^{**}$ | 140.66 | 172.39$^{**}$ | 61.92$^{**}$ | 20.27 | 35.86 | 12.55 |
| NBP | 345.58$^{**}$ | 4.01 | 3.69$^{**}$ | 1.2$^{**}$ | 0.58 | 5.56 | 13.66 |
| TNB | 173.72$^{**}$ | 5.55 | 3.82$^{**}$ | 0.77ns | 0.7 | 6.38 | 13.15 |
| EFFI | 0.88$^{**}$ | 0.01 | 0.03$^{**}$ | 0.03$^{**}$ | 0.01 | 0.87 | 8.85 |
| NLL | 0.27ns | 0.22 | 0.11ns | 0.09ns | 0.07 | 6.41 | 4.16 |
| NPP | 21201.94$^{**}$ | 867.96 | 313.96$^{**}$ | 141.39$^{**}$ | 48.72 | 28.2 | 24.75 |
| NSP | 0.68$^{**}$ | 0.22 | 0.15$^{**}$ | 0.07ns | 0.06 | 2.04 | 11.67 |
| PL | 3.73$^{*}$ | 5.73 | 7.39$^{**}$ | 0.77ns | 0.63 | 10.25 | 7.76 |
| PD | 0.93$^{**}$ | 0.02 | 0.57$^{**}$ | 0.01ns | 0.02 | 2.39 | 5.94 |

| Trait | Environment (A) | Rep (A) | G | G x A | Error | X | CV (%) |
|---|---|---|---|---|---|---|---|
| PT | 0.39$^{**}$ | 0.02 | 0.19$^{**}$ | 0.01ns | 0.01 | 1.74 | 6.32 |
| WP | 13297324.5$^{**}$ | 212521.17 | 79596.88$^{**}$ | 36935.96$^{**}$ | 9369.08 | 457.25 | 21.17 |
| YIE | 11976.57$^{**}$ | 235.87 | 78.95$^{**}$ | 28.99$^{**}$ | 8.87 | 13.63 | 21.84 |

Rep= replication; G= genotype; A= environment; GxA= genotype-by-environment interaction; $^{**}$= significant at 0.01; $^{*}$= significant at 0.05; ns$^{=}$ not significant.

## Variance components (VC) and heritability (H$^2$)

VCs are evaluated in terms of responses to selection and the use of the moment method, calculated with its linear functions, allows estimating the genetic variability (H$^2$) that exists between cultivars when the mean squares of the analysis of variance are used (Holland *et al*., 2003; Pérez *et al*., 2007; Shimelis and Shringani, 2010).

In most quantitative traits, H$^2$ ranged from 53.84 to 92.59% in SNG and from 37.5 to 90.9% in SFO (Table 2); these results were similar to those by Kumar *et al*. (2020), who used inbred lines (40.01% for PL, 88.43% for NPP and 56.59% for PD).

**Table 2. Arithmetic mean (X), coefficient of variation, and heritability (H$^2$) in 13 traits assessed in 25 cultivars of broad beans.**

| Variable | X | | CV (%) | | H$^2$ | |
|---|---|---|---|---|---|---|
| | SNG | SFO | SNG | SFO | SNG | SFO |
| PH | 1.41 a | 1.36 a | 8.55 | 12 | 75 | 40 |
| HFP | 40.2 a | 31.53 b | 10.34 | 15.29 | 87.58 | 75.55 |
| NBP | 4.25 b | 6.87 a | 17.94 | 11.02 | 74.44 | 78.24 |
| TNB | 5.45 b | 7.31 a | 14.95 | 11.8 | 64.51 | 72.52 |
| EFFI | 0.8 b | 0.93 a | 11.57 | 6.07 | 80 | 68 |
| NLL | 6.44 a | 6.37 a | 3.73 | 4.56 | 0 | 38.46 |
| NPP | 17.91 b | 38.5 a | 28.18 | 22.04 | 72.41 | 80.17 |
| NSP | 2.1 a | 1.99 b | 11.61 | 11.73 | 53.84 | 37.5 |
| PL | 10.12 a | 10.39 a | 7.36 | 8.13 | 80.82 | 86.45 |
| PD | 2.32 b | 2.46 a | 5.54 | 6.28 | 92.59 | 0 |
| PT | 1.7 b | 1.78 a | 6.09 | 6.52 | 87.5 | 90.9 |
| WP | 199.4 b | 715.1 a | 27.44 | 17.55 | 78.29 | 84.67 |
| YIE | 5.89 b | 21.37 a | 27.58 | 18.18 | 83.24 | 83.63 |

The codes to identify the statistical significance of the F-values, as well as those corresponding to the variables recorded, were previously described.

In the combined analysis, it was observed that the variance of the IGA was slightly greater than or equal to the variance between genotypes (G); similar estimates were observed by Pérez *et al*. (2015); Tadele *et al*. (2021).

In the present study, it was observed that in most traits, H$^2$ ranged from 41.39 to 96.69% (Table 3). These results indicate that there is wide genetic variability between broad bean cultivars but that this can also be masked by the IGA. An important strategy to counteract the negative effects of IGA would be to stratify the heterogeneity caused by contracting localities by forming groups with those that are similar.

Table 3. Variance components (VC) and heritability ($H^2$) in the experiment series.

| Variables | Combined | | | | | | |
|---|---|---|---|---|---|---|---|
| | $\sigma^2_g$ | $\sigma^2_f$ | $\sigma^2_{gxa}$ | $\sigma^2_e$ | $H^2$ | GA | GAM |
| PH | 0.002 | 0.007 | 0.003 | 0.02 | 41.39 | 0.07 | 5.3 |
| HFP | 13.808 | 21.549 | 10.414 | 20.265 | 64.08 | 6.13 | 17.09 |
| NBP | 0.31 | 0.46 | 0.156 | 0.576 | 67.32 | 0.94 | 16.93 |
| TNB | 0.381 | 0.477 | 0.015 | 0.704 | 79.93 | 1.14 | 17.84 |
| EFFI | 0 | 0.003 | 0.005 | 0.01 | 0 | 0 | 0 |
| NLL | 0.003 | 0.013 | 0.005 | 0.07 | 18.18 | 0.04 | 0.68 |
| NPP | 21.57 | 39.244 | 23.16 | 48.72 | 54.96 | 7.09 | 25.15 |
| NSP | 0.009 | 0.018 | 0.003 | 0.056 | 51.73 | 0.14 | 7.05 |
| PL | 0.827 | 0.923 | 0.033 | 0.633 | 89.58 | 1.77 | 17.3 |
| PD | 0.068 | 0.071 | 0.009 | 0.018 | 96.68 | 0.53 | 22.24 |
| PT | 0.022 | 0.023 | 0 | 0.011 | 94.06 | 0.3 | 17.14 |
| WP | 5332.615 | 9949.61 | 6891.717 | 9369.08 | 53.59 | 110.12 | 24.08 |
| YIE | 6.244 | 9.868 | 5.031 | 8.866 | 63.27 | 4.09 | 30.04 |

The codes that are reported in this table were previously described.

It would also be of great help to practice family selection to expand adaptability in the best cultivars, especially considering primary components of yield that show high positive correlations between them and with productivity, both in fresh and dry grains.

Nevertheless, these results contrast with those published by Abo-Hegazy (2022), who, when using lines and varieties, recorded values of 79.8, 80.9 and 87.6% in pods per plant, seeds per plant, and seed weight per plant, respectively. On the other hand, Chaudhary *et al*. (2020) worked on lines, where they obtained 62.8% for TNB, 70.3% for NPP, and 82.9% for yield.

## Expected genetic advance

The genetic gain, expressed as a percentage of the grand mean (GAM) and applying a selection pressure of 5% to determine the response to selection, is presented in Table 3; in it, GAM showed the lowest values in PH (5.3%), NLL (0.68%), and NSP (7.05%) and their $H^2$ were less than 50%. A moderate genetic gain (GΔ) was also observed for TNB (17.8%), YIE (30.04%), NPP (25.15%), WP (24.08%), and PD (22.24%), and their $H^2$ estimates were greater than 50%; Chaudhary *et al*. (2020) reported values of 25.11% for TNB, 23.2% for NPP and 27. 43% for YIE. Tadele *et al*. (2021); Ton *et al*. (2021); Abo-Hegazy (2022); Kosev and Georgieva (2023) concluded similarly. These results imply that, the greater the heritability, the greater the genetic gain and, therefore, these traits are a criterion for selecting outstanding genotypes.

## Principal component (PC analysis

Principal components 1 (41.26%) and 2 (22.36%) explained 63.62% of the total variation (Figure 2), desirable percentages to reliably interpret the approximate correlations observed in the biplot, as suggested by Sánchez (1995); Pérez *et al*. (2009). Quadrant 1 showed a high positive correlation between yield (YIE) and efficiency (EFFI), pod weight per plant (WP), pod diameter (PD), pod thickness (PT), and pod length (PL). These characteristics will serve as an indirect selection criterion to increase the production in green pods or dry grains.

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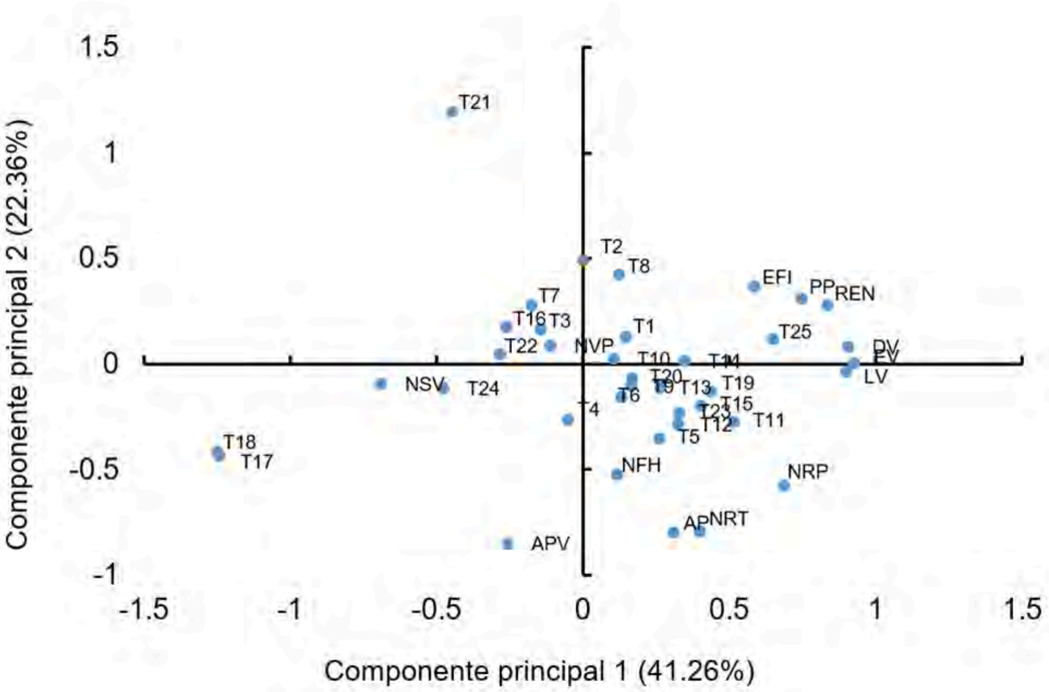
**Figure 2. Interrelationships between 25 broad bean cultivars (in numbers) and 13 agronomic variables (in letters).**



The dispersion of the 25 cultivars in the four quadrants of the biplot confirms the existence of wide genetic variability among broad bean cultivars, which is available to begin a new and better breeding program in the central region of Mexico. The phenotypic variability detected in the 25 broad bean collections was greater in PH, HFP, NBP, TNB, NPP, PL, PD, PT and WP. On the other hand, Arab *et al*. (2018), when carrying out an evaluation during two cycles and working on 42 local collections and two commercial varieties in Egypt, reported greater variability in NPP, NSP and PH.

The most outstanding collection was the one identified as T25, from San Bartolo Apostol, municipality of Santiago Tianguistenco, which was the one that obtained the highest values in PH (1.48 cm), PL (11.26 cm), PD (2.65 cm), PT (1.89 cm) and WP (656.25 g), followed by T11 (Zaragoza de Guadalupe), the genotypes T22 and T24 genotypes stood out for their bigger number of pods per plant.

These results are similar to those reported by Pérez *et al*. (2015); Kumar *et al*. (2020), who suggested the diameter, thickness, and length of the pod (Kosev and Georgieva, 2023) and number of seeds per plant (Arab *et al*., 2018; Abo-Hegazy, 2022) as the main components of green broad bean yield.

On the other hand, Kumar *et al*. (2020); Chaudhary *et al*. (2020) indicated a similar situation for the number of pods per plant and the weight of green pods per plant (Yahia *et al*., 2012; Mohammed *et al*., 2013; Baginsky *et al*., 2013). In this study, the highest yields were observed in T25 (20.18 t ha$^{-1}$) and T19 (17.56 t ha$^{-1}$) due to the larger pod diameter (2.65 and 2.59 cm, respectively) and pod weight per plant (656.25 and 602.5 g, respectively).

## Qualitative traits

The identification of genetic variability (GV) between and within cultivars allows the selection of varieties with desirable agronomic characteristics (Salazar *et al*., 2019) and contributes to the partial increase in production and seed quality in broad beans (Díaz *et al*., 2008). The varietal description allows for quick and easy discrimination between phenotypes (SNICS, 2001), in highly heritable characteristics easily detected with the naked eye and with little or no variation with environments (Yahia et al., 2012; Pérez et al., 2015).

In this study, phenotypic differences were observed in the 25 collections (Table 4) in determinate growth habit (GH); in plant size, two were low, 17 intermediate and six high; anthocyanin coloration in stem (ACS) was present (P); for intensity of green color in foliage (IG), three cultivars were light and 22 medium; melanin spot in the standard (MSS) was present in all collections; anthocyanin pigmentation in the standard (APS) was present.

**Table 4. Characteristics of plant, foliage, flower, and pod in 25 cultivars of broad beans in two environments of the State of Mexico.**

| Cultivar | Plant | | | Foliage | | | Flower | | | Green pod |
|---|---|---|---|---|---|---|---|---|---|---|
| | GH | PS | ACS | IG | MSS | APS | EAP | CMW | PA | TPD |
| T1 | D | M | P | M | P | P | L | Bl | S | E |
| T2 | D | L | P | M | P | P | L | Bl | S | M |
| T3 | D | M | P | M | P | P | L | Bl | S | M |
| T4 | D | M | P | M | P | P | L | Br | S | E |
| T5 | D | H | P | M | P | P | L | Bl | S | M |
| T6 | D | M | P | M | P | P | L | Br | S | M |
| T7 | D | M | P | M | P | P | L | Bl | S | M |
| T8 | D | M | P | M | P | P | L | Bl | S | E |
| T9 | D | M | P | M | P | P | L | Bl | E | E |
| T10 | D | M | P | M | P | P | L | Br | S | M |
| T11 | D | H | P | M | P | P | L | Bl | S | M |
| T12 | D | H | P | L | P | P | L | Bl | S | E |
| T13 | D | M | P | M | P | P | L | Bl | S | M |
| T14 | D | M | P | M | P | P | L | Bl | S | M |
| T15 | D | H | P | M | P | P | L | Br | E | M |
| T16 | D | M | P | M | P | P | L | Bl | E | E |
| T17 | D | M | P | M | P | P | L | Bl | E | E |
| T18 | D | M | P | M | P | P | L | Br | S | E |
| T19 | D | M | P | L | P | P | L | Bl | S | E |
| T20 | D | M | P | M | P | P | L | Bl | E | M |
| T21 | D | L | P | L | P | P | L | Br | S | M |
| T22 | D | M | P | M | P | P | L | Bl | E | M |
| T23 | D | H | P | M | P | P | L | Bl | S | E |
| T24 | D | M | P | M | P | P | L | Br | S | M |
| T25 | D | H | P | M | P | P | L | Bl | S | M |

Collection (T); GH= growth habit; D= determined; I= indeterminate; PS= plant size; L= low; M=medium; H= high; ACS= anthocyanin coloration in stem; P= present; A= absent; IG= intensity of the green color in foliage; L= light; M= medium, D= dark; MSS= melanin spot on the standard; P= present; A= absent; APS= anthocyanin pigmentation on the standard; P= present; A= absent; EAP= extent of anthocyanin pigmentation in the standard; S= small; M= medium; L= large; CMS= color of the melanin spot on the wings; GY= greenish yellow; Br= brown; Bl= black; PA= pod attitude; E= erect; S= semi-erect; H= horizontal; SP= semi-pendulous; P= pendulous; TPD= time of full pod development (first fully developed pods); E= early; M= medium; L= late.

The extent of anthocyanin pigmentation in the standard (EAP) was large; for the color of the melanin spot on the wings (CMW), seven cultivars were brown and 18 black; regarding the pod attitude (PA), six collections were erect and 16 semi-erect; for the time of full pod development (TPD), 15 were recorded with medium development and 10 as late. These results contrast with those reported by Arab *et al*. (2018); Amjad and Alghamdj (2023), who estimated wide phenotypic variability in quantitative and qualitative traits during two evaluation cycles. In this study, little phenotypic variability was reported.

## Conclusions

Heritability values greater than 50% indicated that there is phenotypic variability in most of the traits evaluated. The principal component analysis explained 63.62% of the original total variation and showed that the collection identified as T25 was positively and significantly correlated with EFFI, NPP, PD, PL, WP and YIE, so it is possible to practice selection to increase yield based on these yield components.

Based on the GAM, it can also be predicted that, considering NPP, PD, WP and YIE, greater genetic progress will be achieved through selection. Regarding the qualitative traits, there was little phenotypic and therefore little genetic variability, but these simple inheritance traits could be reliably used to standardize homogeneity within the broad bean cultivars assessed in the present research.

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