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Morpho-agronomic traits in *Phaseolus lunatus* L.: systematic review and meta-analysis as a tool in genotype selection

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ABSTRACT – This study estimated the mean values of morpho-agronomic traits of *Phaseolus lunatus* L. through a systematic review and meta-analysis. Seven studies were selected through the systematic review, which encompassed a total of 104 genotypes. The selected traits were pod length and width, number of seeds per pod, grain yield, and 100-seed weight. In the exploratory analysis, normality, descriptive parameters, box-plot graph, and quantile-quantile plot of the data were evaluated. In the meta-analysis, the Q test and I² statistic were applied, and then, the combined effect model and fixed effect model were tested, using the Genes and R software. The data showed a normal distribution, and the box-plot graph indicated the need to remove some outliers. The quantile-quantile plot showed no publication bias. Q values were high for most traits, indicating that the studies were not homogeneous, and the random effects model was adopted. The combined estimates of pod length, width, number of seeds, grain yield, and 100-seed weight were 69.80mm, 16.29mm, 2.69, 395.97kg/ha, and 48.61g, respectively. The values obtained were precise, due to their similarity with the mean values, indicating that the methodology is adequate for generating mean values of agronomic interest.

Keywords: lima bean, combined effect model, metafor package, plant breeding.

1. INTRODUCTION

Phaseolus lunatus, commonly known as lima bean, is an important crop worldwide. This crop has a central role in the human diet, as it is a rich source of protein, iron, manganese, phosphorus, isoleucine, tryptophan, valine, B-complex vitamins, vitamin C, and vitamin K, mainly for subsistence agriculture, in regions where animal protein is not available or is too expensive. Additionally, lima beans have a wide range of applications in the food industry, it used in the production of canned beans, soups, and stews, as well as it consumed as a fresh vegetable (Khan et al., 2018).

Lima bean is the second most important legume in the *Phaseolus* genus and is an economically significant species, being among the top twelve most utilized legumes in the world (Ogechukwu and Ikechukwu, 2017). Furthermore, this leguminous plant has high tolerance to adverse conditions such as elevated temperatures and prolonged periods of drought, showing better agronomic performance compared to common beans (*Phaseolus vulgaris*) under unfavorable edaphoclimatic conditions (Santos et al., 2021).

However, despite its economic importance, there is still a scarcity of literature on genetic improvement focused on this crop. Genetic improvement studies for agronomic species require a significant amount of time to obtain plants with desirable traits, making it essential to use techniques that optimize the use of available literature information. The ability to establish accurate diagnoses from known realities and use information databases is a promising perspective adopted by researchers (Lovatto et al., 2006).

The results of these evaluations can serve as guidance for decision-making regarding the best actions (Canellas, 2015). Additionally, they can identify areas where there is insufficient evidence to prove the effectiveness of an intervention or identify methodological flaws in available studies. Thus, they guide future research, avoiding unnecessary expenses and increasing study efficiency (Costa, 2018).

One alternative for this is meta-analysis, a statistical tool that, when combined with a systematic review, allows for the synthesis of a large volume of information to facilitate decision-making (Glass, 1976). The technique consists of combining different studies in the same database, which is subsequently subjected to statistical analysis to explain the variance of results using factors common to the studies (Roscoe and Jenkins, 2005). By synthesizing a certain number of conclusions in a specific research field, meta-analysis has the advantage of increasing the objectivity of literature reviews, minimizing biases, and allowing new conclusions to be drawn, different from those that can be made analyzing isolated studies (Fisher, 2015; Toler et al., 2019).

In the agricultural field, the number of published meta-analyses has increased substantially (Carvalho et al., 2012; Peluco, 2016; Martins, 2017; Vilela, 2017; Costa, 2018; Oliveira, 2018). This is a consequence of the benefits of the technique for studies in the agricultural area (Carvalho et al., 2012), as its application reduces costs and time with agronomic experimentation, besides increasing study efficiency (Costa, 2018). In plant breeding programs, meta-analysis can be applied in the combined analysis of specific and restricted experimental data, as it contributes to a greater precision of treatment effect evaluations and helps to consolidate isolated results (Eloy et al., 2018), especially with less studied crops, such as Lima bean.

The scientific importance of statistical validation through meta-analysis for Lima beans has been highlighted by recent studies in the field. Authors such as Oliveira (2018), Santos et al. (2021) and de Jesus Dantas et al. (2023) have emphasized the need for a more comprehensive and accurate understanding of genetic diversity in *P. lunatus*. Using meta-analytic techniques, it is possible to combine data from multiple studies and obtain more precise and reliable estimates of mean values for important morpho-agronomic characteristics. This approach not only helps to guide decision-making in plant breeding programs, but also allows for the identification of knowledge gaps and methodological flaws in existing research. Thus, the application of statistical validation through meta-analysis represents a valuable tool for advancing our understanding of Lima beans and other agronomic crops.

Therefore, the compilation of these studies, through a systematic review and statistical validation through meta-analysis, is an interesting alternative for understanding the genetic diversity involving Lima bean. In this context, the objective of this study was to estimate the combined effects of data, through meta-analytic statistics, to produce precise results regarding the real mean values of morpho-agronomic characteristics of *P. lunatus*.

2. MATERIAL AND METHODS

2.1. Systematic review: compilation and structuring of the database.

For the selection of articles used in the composition of the database, a systematic review of scientific papers was carried out on the platforms "Periódico CAPES", Scopus, Scielo, and "Google Scholar" between the years 2002 and 2022. All searches were conducted until July 2022, using the keywords "Morpho-agronomic characterization", "Agronomic characterization", "Morphometry", "Breeding", "Genetic diversity", "*Phaseolus lunatus* L.", "Fava", "Lima bean", and "Fava bean". Studies that contained these keywords anywhere in the text were evaluated.

The search on the platforms resulted in a total of 183 publications, with those that did not deal with plant breeding being discarded. Subsequently, fifty-one were qualified to pass through the selection filters (Fig. 1).

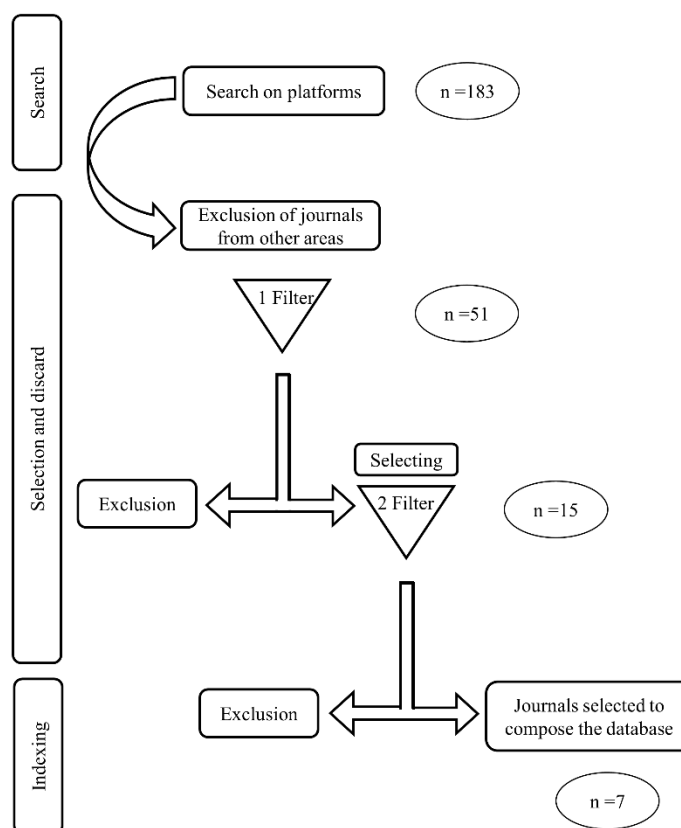


Fig. 1. Flowchart of the article selection process found through systematic search using keywords on scientific research platforms for the composition of the database.

In the first filter, all articles that contained the following conditions were excluded: studies conducted in greenhouse, consortium, stress conditions, response to treatments such as fertilization, exposure to pathogens, pests, among others. In the second filter, all publications that had the highest number of variables in common among Lima bean breeding studies were selected to compose the database.

At the end of the selection process, a total of seven publications were gathered and indexed into the database in an electronic spreadsheet. The collected data set contains information regarding five morphoagronomic characteristics: pod length (PL), pod width (PW), number of seeds per pod (NSP), grain yield (GY), and one hundred (100) seed weight (WHS) of 104 *P. lunatus* genotypes, conducted in 10 distinct environments (Table 1).

Table 1. Studies indexed in the database through search and selection in research platforms.

Reference	Source	Local of study
Assunção Neto et al. (2022)	Revista Caatinga	São Domingos -MA, Brazil Teresina – PI, Brazil Bom Jesus – PI, Brazil Tianguá - CE
Silva et al. (2019)	Revista de Ciências Agrárias	Areia – PB, Brazil
Gonçalves et al. (2019)	Revista Caatinga	Teresina – PI, Brazil
Oliveira et al. (2011)	Revista Caatinga	Mossoró – RN, Brazil
Akande e Balogun, 2007	Revista Científica UDO Agrícola	Lle-lfe -Nigéria
Guimarães et al. (2007)	Revista Brasileira de Engenharia Agrícola e Ambiental	Recife – PE, Brazil
Santos et al. 2002	Pesquisa Agropecuária Brasileira	Bananeiras – PB, Brazil

2.2. Statistical procedure of meta-analysis.

Initially, an exploratory analysis of the dataset was performed for each variable to assess normality, main parameters of descriptive statistics, box-plot and quantile-quantile plot to check for outliers and publication bias, respectively (Larson and Farber 2016).

After the initial analyses, the heterogeneity of the meta-analysis was verified using the Q test proposed by Cochran (1954), given by:

$$Q = \sum_{j=1}^J W_j(Y_j - \theta_m)^2$$

Where:

W_j is the weight of study j

Y_j is the effect size of study j

θ_m is the estimate for the meta-analytical measure.

To quantify the proportion of the total variation due to heterogeneity, the I^2 statistic (Higgins and Thompson 2002) was used. It is obtained through the Q statistic of the Cochran test and the number J of studies involved in the meta-analysis, and is given by:

$$I^2 = \frac{Q - (J-1)}{Q} \times 100\%$$

The fixed-effects model and the random-effects model were evaluated to determine which was more appropriate for the meta-analytic estimation of the database.

Fixed-effects models aim to evaluate the combined estimate θ_m . In other words, in a meta-analytic study, consisting of a certain number (n) of experiments, its effect measure for the j -nth study is given by θ_j , responsible for summarizing the results of the number of experiments involved (Borenstein, 2022). The fixed-effects model is given by:

$$\theta_j = \theta_m + \varepsilon_j$$

Where:

$j = 1, 2, \dots, n$;

θ_j is the effect size of the j -nth study, assumed to be normally distributed with mean θ_m and variance $\tau^2 + \sigma^2_j$;

θ_m is the weighted average effect size across studies, with weights proportional to the inverse variance of each study;

τ^2 represents the between-study variance, reflecting the heterogeneity of effect sizes;

σ^2_j is the within-study variance, assumed to be known for each study;

W_j is the weight assigned to the j -nth study, which depends on the inverse variance of the effect size and the estimated between-study variance.

$$\theta_j = \theta_m + \delta_j + \varepsilon_j$$

Where:

δ_j : are the random effects

ε_j : are the residuals, with $j = 1, 2, \dots, k$.

The software programs Genes (Cruz 2016) and R (Version 4.1.3) were used to assist in the analyses.

3. RESULTS AND DISCUSSION

During the exploratory analysis to remove outliers before the meta-analysis, it was observed that only the variable "pod width" did not present any outliers (Fig. 2A). The outliers present in the other variables were removed, because they can cause greater errors in the residuals, as they are observations that are at an atypical distance from the other values in the random sample of the population (Larson and Farber, 2016). The presence of such data can mask relevant results and distort final inferences (Auer et al., 2016).

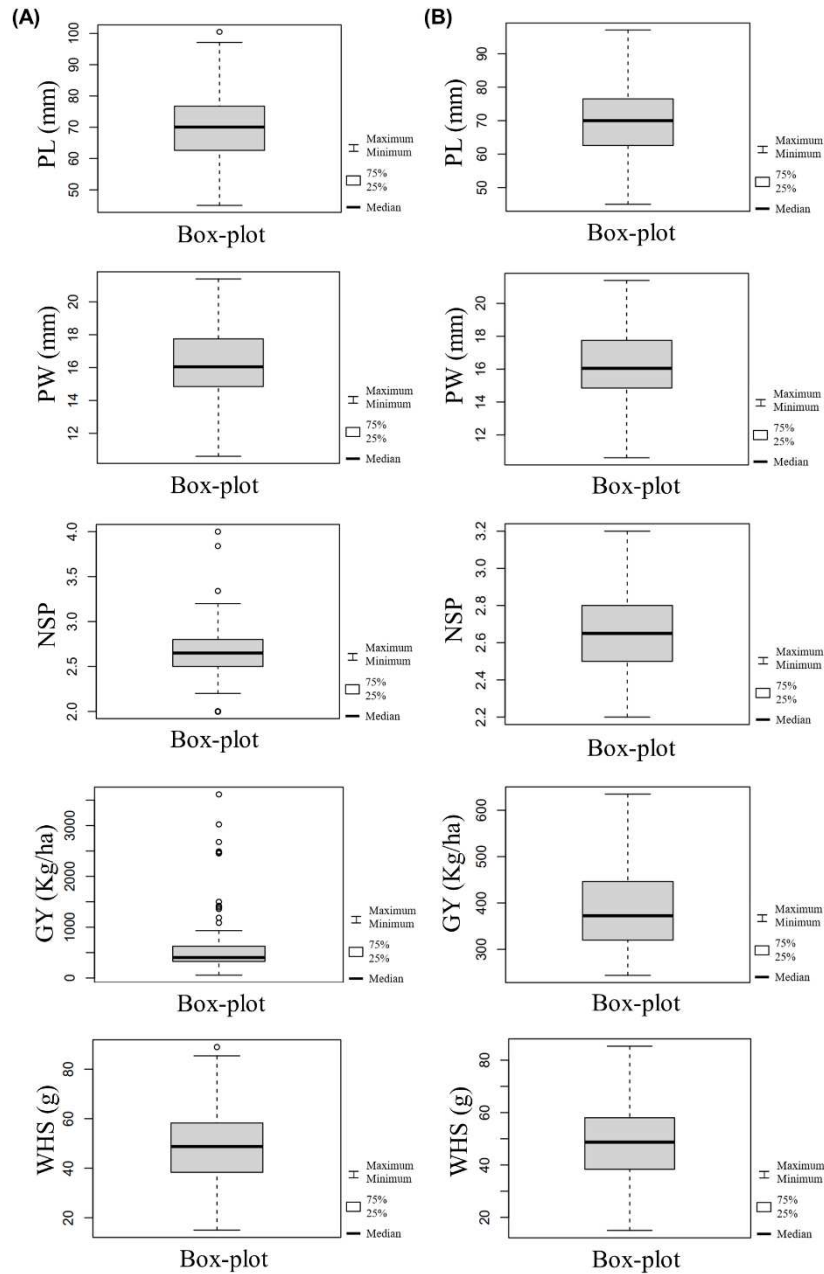


Fig. 2. A - Box-plot of original data of the variables pod length (PL), pod width (PW), number of seeds per pod (NSP), grain yield (GY), and 100 seed weight (WHS). B - Box-plot of variables with outliers removed.

Note that the grain yield variable showed the highest range of values among the other morpho-agronomic traits, especially in the data set including outliers, while the pod length did not present outliers (Fig. 2A). Therefore, there is a high genetic variability among varieties regarding grain yield, indicating potential for selecting more productive materials, which is of great interest for plant breeding programs. Removing the outliers reduced the amplitude of the estimates (Fig. 2B), eliminating possible biases.

The exclusion of outliers from the grain yield variable reduced the range of variation by about 80% (Fig. 1). However, without outliers, the database remained representative for all selected traits, due to the range of values and the number of genotypes included (Silva et al., 2015; Medeiros et al., 2016; Soares, 2019; Penha, 2018; Costa et al., 2022). This demonstrates that there is a high genetic variability among the studied genotypes, highlighting the need for meta-analyses to determine combined estimates of variables.

Costa et al. (2022) working with rice (*Oryza sativa* L.) found outliers in the review of heritability estimates, coefficient of variation, and mean for grain yield, plant height, and days to flowering. However, Custódio et al. (2012) and Carvalho et al. (2012) in studies with *Coffea canephora* and *Coffea arabica* L. respectively, did not find outliers in the review of heritability estimates for several studied traits, and therefore, did not need to exclude discrepant data from the analysis.

For the traits PL(a), PW (b), NSP (c), GY (d), and WHS (e), in the quantile-quantile plot, between the intervals of -1 and 1, 71.07%, 72.12%, 73.93%, 71.83%, and 71.30% of the estimates fell, respectively, distributed along the line passing through the origin.

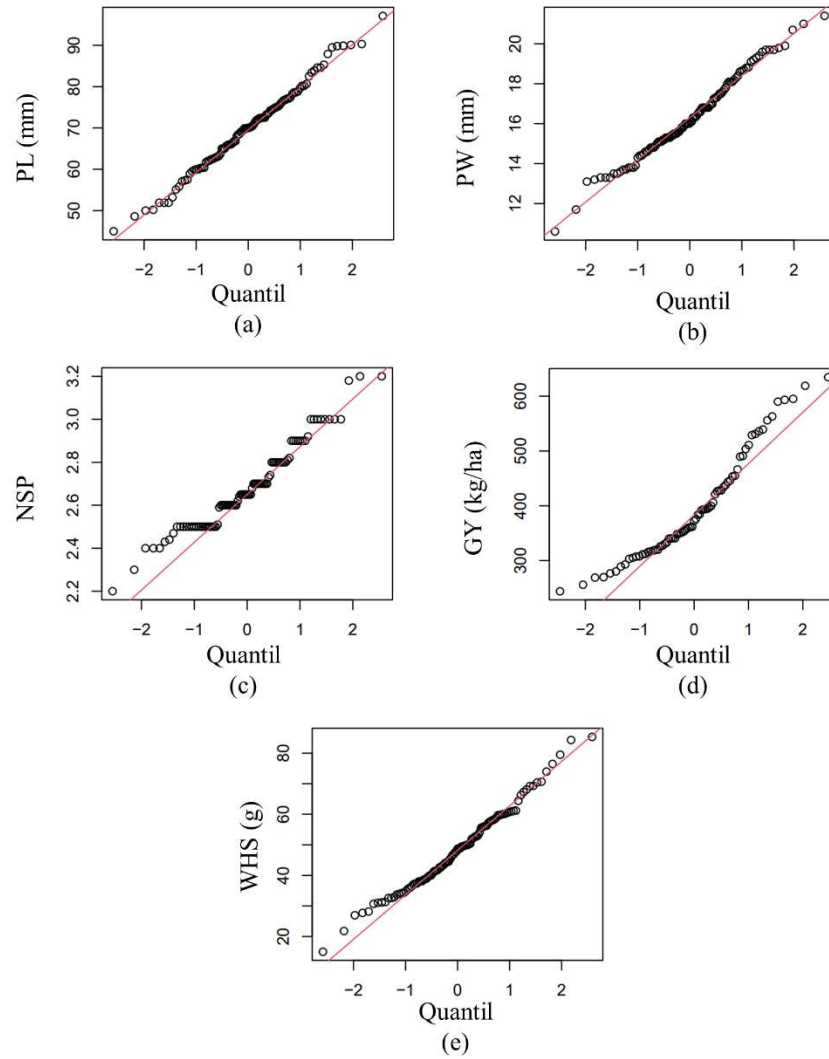


Fig. 3. Quantile-quantile of the variables: (a) pod length (PL), (b) pod width (PW), (c) number of seeds per pod (NSP), (d) grain yield (GY), and (e) 100-seed weight (WHS).

Given the above, it is possible to affirm that the data did not present publication bias. The absence of bias is considered when the points are distributed along the line passing through the origin and approximately 68% of them are between 1 and -1 (Wang and Bushman, 1999).

Independence between random variables is a condition for analysis, as the data come from different experiments. The values of PL, PW, NSP, GY, and WHS did not reject the null hypothesis of the Shapiro-Wilk and Kolmogorov-Smirnov (D) tests for normality, indicating that they have a normal probability distribution of their data (Table 2).

Table 2. Statistics values of Shapiro-Wilk (W) and Kolmogorov-Smirnov (D) tests for normality, heterogeneity (Q), percentage of heterogeneity (I^2), and sampling variability (H^2). Pod length (PL), pod width (PW), number of seeds per pod (NSP), grain yield (GY), and 100-seed weight (WHS).

Variables	W	D	Q	(I^2)	(H^2)
PL	0.99	1	3940.11	97.13	34.86
PW	0.98	1	702.09	91.78	12.17
NSP	0.96	0.99	20.32	0.00	1.00
GY	0.93	1	83531.26	99.92	1183.50
WHS	0.99	1	5219.61	97.89	47.42

The meta-analysis of combined effects obtains the combined estimate of variables through a dataset formed by several studies that have no chronological relation. However, they were executed under common experimental conditions for the crop of interest (Costa et al., 2022). Therefore, during the systematic review, the difference in acquired values related to a measure of precision reflecting experimental conditions, utilized designs, experiment sizing, among others, was taken into consideration.

As a result, Q statistics were high for the most part of traits: PL, PW, GY, and WHS, including NSP, despite presenting the lowest value (Table 2). Therefore, it is assumed that the characteristics are not homogeneous and that there is variation between them (Borenstein, 2022). Thus, the adoption of the random-effect model is justified for using combined estimates, and the need for using the fixed-effect model is discarded.

It is essential to note that the fixed-effect model assumes that there is homogeneity between the estimates of the parameters. This model is appropriate when the treatment effect is identical among the studies, given that its purpose is to evaluate a treatment effect for a specific population, not extending it to other populations (Rodrigues and Ziegelmann, 2010).

In contrast, the random-effect model considers that there is variability between the estimates. This model is indicated to be applied when there is the combination of several studies that have the same purpose but were carried out independently. Thus, it is possible to extend it to other populations, making the analysis more comprehensive (Roever, 2020).

Regarding the proportion of total variation due to heterogeneity (I^2), it can be observed that GY obtained the highest percentage (99.92%) and NSP the lowest (0.00%). This measure

is responsible for quantifying the observed variance proportion that reflects real differences in effect size, expressed as a percentage with an interval from 0 to 100% (Estrela, 2018).

In plant breeding, there are few studies related to meta-analysis, which is why a scale for I^2 in this area has not yet been established. However, for studies in the medical field, it is suggested that values close to 0% are not heterogeneous, as can be observed in the NSP variable. In the case of values close to or above 75%, it indicates high heterogeneity among the studies, as observed for the other variables (PL, PW, GY, and WHS) (Higgins and Thompson, 2002).

In studies conducted with coffee (Custódio et al., 2012; Carvalho et al., 2012) and rice (Costa et al., 2022) to evaluate combined estimates of random effects regarding the proportion of total variation due to heterogeneity (I^2) and total variability over sampling variability (H^2). It was observed that productivity presented the highest heterogeneity among the other evaluated characteristics. This corroborates with the results found in this study where GY values had the highest heterogeneity for I^2 (99.90%) and H^2 (1,183.50).

This high heterogeneity, in most characteristics, can be explained by the large genotypic diversity of the species (de Jesus Dantas et al., 2023). In a study developed from chloroplast DNA of 109 Lima bean accesses, the formation of two large domestication centers was identified. The Andean germplasm, located in Ecuador and the northern region of Peru, with varieties that have large seeds and are adapted to high altitudes, and the Mesoamerican germplasm, ranging from central-west Mexico to Honduras, with varieties that have small seeds in low-altitude locations (Motta-Aldana et al., 2010).

This variation in morpho-agronomic characteristics and its relation to the geographic distribution of the *P. lunatus* germplasm is also confirmed by research conducted on 262 accessions using chloroplast DNA (Andrueza-Noh et al., 2013). Therefore, the high values of heterogeneity are justified, as these characteristics are directly influenced by edaphoclimatic, genetic, and morpho-physiological conditions of the varieties, such as their development cycle (annual, biennial, or perennial) and growth habit (Souza et al., 2019; Lopes et al., 2015).

The combined estimates of the variables (τ^2) (Figure 4A) pod length (69.80 mm), pod width (16.29 mm), number of seeds per pod (2.69), grain yield (395.97 kg/ha) and 100-seed weight (48.61 g), were similar to those obtained by the arithmetic mean: 69.84 mm, 16.31 mm, 2.68 seeds per pod, 395.97 kg/ha, and 48.65g, respectively.

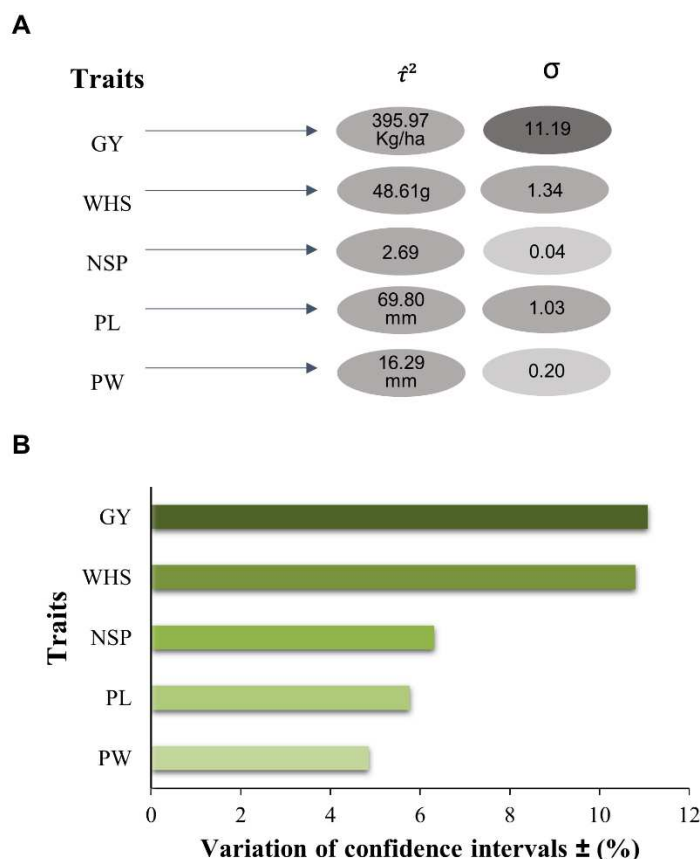


Figure 4. **A** - Combined estimates of the variables (τ^2) and the standard deviation (σ). **B** – Variation of Confidence Intervals (95%), in proportion. Pod length (PL), pod width (PW), number of seeds per pod (NSP), grain yield (GY) and 100-seed weight (WHS).

According to the results observed earlier, it is possible to infer that the similarity of the means (estimate and arithmetic) demonstrates that the values obtained in the meta-analysis are precise, as there were no major divergences between the means (Borenstein, 2022). This result is important as it contributes to avoiding biases and thus verifying whether the methodology and execution of the analysis were appropriate. Therefore, it can be stated that the use of box-plot, quantile-quantile, and normality tests (Shapiro-Wilk and Kolmogorov-Smirnov) are indispensable for this type of analysis as they ensure a better representation of the dataset.

The results obtained from the combined estimates of the variables are a highly representative synthesis of the values present in the database produced through a systematic review. This information can be used as comparative values in breeding programs of *P. lunatus* for selecting more productive varieties, as there are few commercial materials to employ as comparatives (de Jesus Dantas et al., 2023; Assunção Neto et al., 2022; Gonçalves et al., 2020; Souza et al., 2019).

According to data from IBGE - SIDRA (2021), over the last 19 years (2002-2021), the average productivity of Lima bean grains in Brazil has been around 359.9 kg/ha. This average value, when compared to the one obtained by using the meta-analysis (395.97 kg/ha), presents an approximate result. Therefore, it can be inferred that this statistical tool assists breeders in obtaining comparative information that can guide decision-making on the best alternatives to be adopted.

Thus, it is possible to point out which activities do not have enough evidence to demonstrate the effectiveness of a particular treatment. Moreover, it also contributes to identifying methodological flaws in research. This allows future research to avoid onerous work and increase the efficiency of their studies (Roever, 2020; Costa et al., 2018).

The standard deviation of the estimates was low, including for the GY characteristic, which frequently presents high values (Akanke and Balogun, 2007; Gonçalves et al., 2020; Silva et al. 2019). Thus, the estimate obtained by the meta-analysis is precise and well reflects the average value of genotypes concerning the evaluated studies. This occurs due to the increase in the number of events, which increases statistical power and the possibility of analyzing variability between studies, which does not happen when performed only with the mean of published results (Weir et al., 2018).

The confidence interval limits (95%) calculated for each variable were: PL 69.80 ± 2.02 ; PW 16.29 ± 0.4 ; NSP 2.69 ± 0.09 ; GY 395.97 ± 21.93 , and WHS 48.61 ± 2.62 . These results indicate that the characteristics with the highest percentage regarding the amplitude of confidence interval values were GY and WHS, respectively, while NSP, PL, and PW had the smallest amplitudes (Fig. 4B).

Therefore, it can be noted that the characteristics with the highest amplitude should receive more attention during the selection of genotypes, as they present greater variation. This allows genetic diversity to be better exploited in *P. lunatus* breeding programs, especially grain productivity, which showed high variation among varieties. However, this variable, when compared to the average (Kg/ha) of other bean species, such as common bean (*P. vulgaris*), is considered extremely low (Mambrin et al., 2015; Dique, 2019), so more work is needed for improving the culture.

4. CONCLUSION

The systematic review generated a representative database. Through meta-analytic statistics, precise results were obtained that reflect the real average values of the selected morpho-agronomic characteristics of *P. lunatus*.

Grain productivity, 100-seed weight, and pod length showed better fit with the analysis, as they had the highest variations in values, with grain productivity being the variable with the highest potential for selection gain. However, it is important to emphasize that each trait must be analyzed individually, with prior knowledge of the study being conducted, to avoid biased interpretations. In addition, more studies focused on this type of analysis are necessary to form an adequate database to use as a comparative basis among plant breeding programs.

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Ethics declarations

Conflict of interest

The authors of this manuscript declare no conflicts of interest.

Ethical approval

This article does not report any studies involving human participants or animals conducted by any of the authors.

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