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LIMA BEAN POPULATIONS ASSESSMENTS VIA REML/BLUP METHODOLOGY[1]

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ABSTRACT – Based on its nutritional and economic value, the lima bean (*Phaseolus lunatus* L.) is the second most important species of the genus. It has high genetic diversity and potential for production and is considered an alternative food and income source. The objective of this study was to apply the restricted maximum likelihood/ best linear unbiased prediction (REML/ BLUP) methodology to estimate genetic parameters and predict genotypic values in F_3 populations of lima beans. Twelve characteristics were evaluated in five populations with indeterminate growth habits (H39, H72, H53, H90, and H56). Model 83 from the Selegen program was used for analysis. Considering the genetic parameters, the highest values of genetic variance were for plant height and number of pods per plant. Pod thickness and seed width are favorable for breeding programs. Seed width selection gain was significant for populations H56 and H90 at 11.26 mm and 10.50 mm, respectively. As for the length and thickness of seeds, the gains were less significant, with population H53 showing the greatest gain. The REML/ BLUP methodology proved efficient in estimating genetic parameters and predicting gains in lima bean populations. The estimated selection gains indicated that the highest gains were obtained for plant height, the number of pods per plant, pod thickness, seed width, and the number of days to maturity. Populations H53 and H56 stood out for having large and white seeds, thus being potential populations for species improvement.

Keywords: *Phaseolus lunatus*. Genetic parameters. Mixed models.

AVALIAÇÕES DE POPULAÇÕES DE FEIJÃO-FAVA VIA METODOLOGIA REML/BLUP

RESUMO – O feijão-fava (*Phaseolus lunatus* L.) é a segunda espécie mais importante do gênero, apresenta diversidade genética e potencial para produção bem como valor nutricional e econômico, sendo considerada alternativa alimentar e de renda. O objetivo do trabalho é aplicar o método REML/BLUP para estimar os parâmetros genéticos e predizer os valores genotípicos em populações F_3 de feijão-fava. Foram avaliados doze caracteres em cinco populações de hábito de crescimento indeterminado (H39, H72, H53, H90 e H56). Para as análises foi utilizado o modelo 83 do programa Selegen. Considerando os parâmetros genéticos, os maiores valores de variância genética foram para altura de planta e número de vagens por planta. Espessura de vagem, e largura de sementes, demostraram situação favorável ao programa de melhoramento. Os ganhos de seleção foram maiores e significativos em largura de sementes nas populações 56 e 90 com 11.26 mm e 10.50 mm, respectivamente. Quanto ao comprimento e espessura de sementes os ganhos foram menos significativos e a população 53 foi a que apresentou o maior ganho. A metodologia REML/BLUP mostrou-se eficiente na estimação dos parâmetros genéticos e predição de ganhos em populações de feijão-fava. Os ganhos de seleção estimados indicam que os maiores ganhos foram obtidos para os caracteres altura de plantas, número de vagens por planta, espessura de vagem, largura de semente e número de dias para a maturação. As populações H53 e H56 se destacaram por apresentar sementes grandes e brancas sendo assim populações promissoras no melhoramento da espécie.

Palavras-chave: *Phaseolus lunatus*. Parâmetros genéticos. Modelos mistos.

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INTRODUCTION

Based on its nutritional and economic value, the lima bean (*Phaseolus lunatus* L.) is the second most important species of the *Phaseolus* genus. It is characterized by its high genetic diversity and yield potential and is considered an alternative source of income and food (CHEL-GUERRERO et al., 2012; BARREIRO NETO et al., 2015). In addition, it can be utilized as green manure (PEGADO et al., 2008), adapt to the most diverse environmental conditions, and grow best in tropical and sub-tropical regions such as Africa and Southeast Asia (AGHKHANI et al., 2012; CHENG et al., 2019).

In Brazil, the lima bean is preferably cultivated in the northeast region, where it is widely consumed and has greater economic importance (ALVES et al., 2014). In this region, it is cultivated in almost all states, except for Bahia. According to research carried out by the Brazilian Institute of Geography and Statistics (IBGE), the Ceará state is the largest producer of the lima bean with 7,016 tons (t), followed by Paraíba (4,353 t), Pernambuco (1,932 t), Rio Grande do Norte (1,441 t), and Piauí states (910 t) (IBGE, 2021).

Small-scale farmers produce Lima beans. Given that the northeast region is the poorest of the country and rural workers have a lower income per person and greater nutritional deficiency, the crop becomes more valuable because of the food and nutritional security it provides in rural communities (SILVA, 2019). Despite the favorable environmental conditions for grain production, few studies aim to select lima bean genotypes with stable production behavior in the region.

Knowledge of genetic parameters enables the breeder to define the best selection strategy for plant breeding (CRUZ; REGAZZI; CARNEIRO, 2012). Estimations of the genetic variance components reliably guide breeders in selecting superior individuals for segregating populations in a plant breeding program (BORÉM; MIRANDA, 2013). Parameters such as heritability and genetic correlations are fundamental for defining the most efficient breeding strategy (RESENDE, 2006).

Aiming to obtain the restricted maximum likelihood (REML) estimators and the best linear unbiased prediction (BLUP) predictors of genetic values, the use of mixed models in species like the lima bean has stood out mainly for the possibility of evaluating unbalanced experiments. The BLUP prediction presumes the knowledge of the true values of the variance components. As this is impossible, the solution is to estimate these components via mixed model equations (RESENDE, 2016).

The biggest advantage of the mixed model methodology is the comparison of individuals or varieties over time, correcting environmental effects, estimating variance components, and predicting genotypic values, allowing the analysis of data with complex structures (RESENDE, 2016).

Thus, the aim of this study was to apply the restricted maximum likelihood/ best linear unbiased prediction (REML/ BLUP) methodology in the Lima Bean Breeding Program at the Universidade Federal do Piauí to estimate genetic parameters and predict genotypic values in F_3 segregating populations of lima bean to evaluate traits related to precocity and grain quality.

MATERIAL AND METHODS

The experiment was conducted in the Department of Plant Science of the Center for Agricultural Sciences at Universidade Federal do Piauí, Teresina, PI (05º05’21’’S; 42º48’07’’W, and 72 m altitude) from February to July 2021.

Seeds from five lima bean populations with indeterminate plant growth were sown in plastic cups and later transplanted to the field for generation advance without an experimental design. The plot consisted of 5 lines with 10 plants each per population, i.e., 250 plants, with a spacing of 0.7 m between plants and 1 m between rows. The experiment was set up without an experimental design because of the low availability of seeds and areas. The evaluations were carried out on each plant of all populations individually, as performed by Sousa et al. (2017) in experiments with the common bean (*Phaseolus vulgaris*).

Populations were obtained from bi-parental crosses between parents from different countries that were advanced to the F_2 generation, sourced from the Active Germplasm Bank of *Phaseolus* at the Universidade Federal do Piauí (Table 1). The seeds were treated with a fungicide before sowing to prevent fungal attacks. Cultural treatments and fertilization were performed based on soil analysis and recommendations (LOPES; GOMES; ARAÚJO, 2010).

The traits were evaluated according to the following descriptors for *Phaseolus lunatus* L. (BIOVERSITY INTERNATIONAL, 2007): number of days to the beginning of flowering (NDF) – the period between emergence and the time when 50% of the plants showed flowers; the number of days to maturity (NDM) – comprising the period between emergence and the time when 50% of the pods are ripe; plant height (PH, cm) – measured in 10 random plants from the cotyledonary scar to the end of the plant; the number of pods per plant (NPP); pod length (PL, mm), pod width (PW, mm), pod thickness (PT, mm), number of locules per pod (NLP), and number of seeds per pod (NSP) using 10 randomly selected mature pods; and seed length (SL, mm), seed width (SW, mm), and seed thickness (ST, mm) using 10 randomly selected dry seeds.

Table 1. Identification of the lima bean (*Phaseolus lunatus* L.) F_3 populations obtained from bi-parental crossings, their respective parents, and seed color. These were sourced from the Active Germplasm Bank of *Phaseolus*, Universidade Federal do Piauí, Teresina, PI, Brazil.

Population	Female Parent	Male Parent	Seed color
H39	MEX PL 2	UC HASKELL	White
H53	UC 92	MEX PL 3	White
H56	UC 92	MEX PL 5	White
H72	MEX PL 3	UFPI 628	Light Brown
H90	UC 92	MEX PL 2	White

Pod length, pod width, pod thickness, seed length, seed width, and seed thickness were measured using a digital pachymeter. The data obtained from the populations were analyzed using model 83 from Selegen (RESENDE, 2016), which refers to the evaluation of a completely random design and evaluation in only one local and one harvest.

The statistical model used was as follows:

$y = Xu + Zg + e$

where y is the vector of the data; u is the scalar vector referring to the average mean (fixed effect); g is the vector of genotypic effectors, $g \sim N(0, Z\sigma_g^2)$, where σ_g^2 is the genetic variance; and e is the vector of experimental errors, $e \sim N(0, I\sigma_e^2)$, where σ_e^2 is the experimental variance. The uppercase letters represent the matrices of incidence for the referred effects.

The genetic parameters were estimated using REML, and the genotypic means were adjusted and estimated using the BLUP procedure. The following variance components were obtained from the model: σ_g^2 : genetic variance; σ_e^2: experimental variance; σ_f^2 : individual phenotypic variance; h^2g: heritability of individual plots in the broad sense, in other words, from the total genotypic effects; Ac: accuracy; CVgi%: genotypic coefficient of variation; CVe%: experimental coefficient of variation; and the average mean of the experiment.

Gains were estimated from the individual BLUPs of each population for each evaluated trait. The genotypic values for each genotype were obtained by adding each predicted genotypic effect to the overall average of the experiment. The genetic gain was calculated as the average of the predicted genetic effect vectors for the selected genotypes. Adding the general average to the genetic gain resulted in a new average genotype.

RESULTS AND DISCUSSION

The highest values for genetic variances were for plant height (PH) and the number of pods per plant (NPP) (Tables 2 and 3), indicating the possibility of successful transmission of the traits. Plant height is important for adaptation to mechanical harvesting (TEIXEIRA et al., 2017). The number of pods per plant is an important trait in lima bean breeding because it positively correlates with grain yield (ASSUNÇÃO FILHO et al., 2022).

As shown in Table 2, the highest values for genetic variances were for the traits pod thickness (PT), the number of locules per pod (NLP), and seed width (SW), indicating potential targets for the lima bean breeding program. The number of locules per pod is positively correlated with pod length and width, allowing an indirect selection for these traits (ASSUNÇÃO FILHO et al., 2022). However, the experimental variance values were higher than the genotypic variance for all traits, indicating a strong environmental influence in the experiment. Estimations of the variance components are useful tools for breeders because they enable conclusions about the genetic potential of the segregating population (BALDISSERA et al., 2014).

According to Resende (2015), individual heritability can be classified according to its magnitude as low ($0.01 \leq h^2 \leq 0.15$), medium ($0.15 < h^2 < 0.50$), and high ($h^2 \geq 0.50$). The number of days to maturity (NDM) and the number of pods per plant (NPP) showed heritability values of medium magnitude (20% and 17%, respectively) (Table 2). Pod length, pod width, the number of locules per pod, and seed width also showed values of heritability of medium magnitude (Table 3). The other traits presented low-magnitude values below 10% (Tables 2 and 3)

Table 2. Estimates of the genetic parameters for the number of days to the beginning of flowering (NDF), number of days to maturity (NDM), plant height (PH), and number of pods per plant (NPP) evaluated in five F_3 lima bean populations in Teresina, PI, Brazil.

Parameters	Traits			
	NDF (days)	NDM (days)	PH (cm)	NPP
σ_g^2	0.88	101.71	505.90	976.74
σ_e^2	111.90	415.68	3,024.19	4,899.88
σ_f^2	112.78	517.40	3,530.09	5,876.62
h^2g	0.01	0.20	0.14	0.17
Ac	0.42	0.83	0.82	0.82
CVgi%	1.75	8.86	23.79	50.89
CVe%	19.65	17.92	58.18	113.98
Mean	53.83	113.79	94.53	61.41

σ_g^2 : genetic variance; σ_e^2: experimental variance; σ_f^2: individual phenotypic variance; h^2g: broad sense heritability; Ac: accuracy; CVgi%: genotypic coefficient of variation; CVe%: experimental coefficient of variation; Mean: general mean of the experiment.

Table 3. Estimates of the genetic parameters for pod length (PL), pod width (PW), pod thickness (PT), number of locules per pod (NLP), number of seeds per pod (NSP), seed length (SL), seed width (SW), and seed thickness (ST) evaluated in five F_3 lima bean populations in Teresina, PI, Brazil.

Parameters	Traits							
	PL (mm)	PW (mm)	PT (mm)	NLP	NSP	SL (mm)	SW (mm)	ST (mm)
σ_g^2	0.72	0.23	158.20	112.65	17.04	104.60	0.47	2.02
σ_e^2	1.86	311.31	594.84	569.07	176.91	382.58	7.83	71.73
σ_f^2	2.58	311.53	753.04	681.72	193.95	487.19	8.30	73.76
h^2g	0.28	0.00	0.21	0.17	0.09	0.21	0.06	0.03
Ac	0.86	0.77	0.81	0.75	0.88	0.55	0.47	0.75
CVgi%	5.78	0.88	42.77	59.72	50.05	73.10	17.68	16.17
CVe%	9.30	32.50	82.93	134.22	161.26	139.81	71.87	96.25
Mean	14.67	54.28	29.41	17.77	8.25	13.99	3.89	8.80

σ_g^2 : genetic variance; σ_e^2: experimental variance; σ_f^2: individual phenotypic variance; h^2g: broad sense heritability; Ac: accuracy; CVgi%: genotypic coefficient of variation; CVe%: experimental coefficient of variation; Mean: general mean of the experiment.

The selection of traits that presented low heritability values in the F_3 generation should be carried out in a more advanced generation when the genetic value of the population can be more accurately assessed (BARROSO NETO et al., 2017). Thus, the low values of heritability observed for certain traits can be explained by the early generation (i.e., F_3), having a low degree of inbreeding, which is reflected in the obtained values.

According to Pimentel et al. (2014), these low -magnitude values suggest that the conditions are not favorable for selecting individual plants. The high values of experimental variance indicated that the traits have a strong environmental influence on the phenotype. This may have been caused by the lack of an appropriate experimental design, lack of repetitions in the experiment, or evaluations performed only in a single environment, influencing the low heritability values obtained. These results indicated that a stricter selection method and evaluation must be applied to obtain better results (RAMOS et al., 2014). In addition, the lima bean has a high percentage of cross-fertilization (38.1%) and is classified as a mixed species (PENHA et al., 2017), potentially influencing heritability values.

Accuracy refers to the correlation between the true genotypic value and that estimated from the experimental information; thus, the greater the selective accuracy in the evaluation, the greater the confidence in its evaluation and the predicted value (RESENDE; DUARTE 2007). According to Resende and Duarte (2007), the values of accuracy may vary from 0 to 1 and are classified as very high (≥ 0.90), high (≥ 0.70 and < 0.90), moderate (≥ 0.50 and < 0.70), and low (< 0.50).

Thus, the accuracy values were classified as high for most of the traits, except for the number of days to the beginning of flowering and seed length, which were classified as low (0.42 and 0.47, respectively), and for seed width, which was moderate (0.55). The values higher than 0.70 are considered suitable for providing a precise inference about the genetic value, which is a measure associated with precision in selection (RESENDE; DUARTE, 2007). Therefore, the obtained accuracy values guarantee greater reliability in the evaluation of most traits. For traits with moderate and low values, adequate experimentation can increase accuracy (RESENDE, 2015).

Considering that the experimental coefficient of variation (CVe) is one of the most used measures to assess the precision of experimental agricultural trials (STORCK et al., 2010), this coefficient was also used to infer the experimental precision.

There are no good referential values for the lima bean. This study used the values proposed by Gomes (2009) that considered CVe values below 10% as low, between 10 and 20% as medium, between 20 and 30% as high, and above 30% as very high. Thus, in this study, the CVe values were low for pod width (Table 3) and medium for the number of days to the beginning of flowering and maturity (Table 2). However, the other traits showed high coefficients. These values showed that a good experimental design could increase the precision of the evaluations.

According to Cruz, Regazzi, and Carneiro (2012), these estimations provide the breeder with very useful information regarding the different traits evaluated in the accessions, providing guidance on the most appropriate selection strategy and the prediction of the outcomes of breeding programs.

The selection gains estimated via BLUP (Tables 4, 5, and 6) revealed that, in general, significant gains were obtained in the evaluated generation. The number of days to maturity, plant height, number of pods per plant, pod thickness, and seed width had the highest genetic gains, indicating that these populations could be used as the basis for breeding programs aimed at larger seeds and greater grain yield, which is of interest to producers and consumers (ASSUNÇÃO FILHO et al., 2022).

Table 4. Ranking, selection gains, and averages of the number of days to maturity (NDM), plant height (PH), and the number of pods per plant (NPP) evaluated in five F_3 lima bean populations in Teresina, PI, Brazil.

Order	NDM (days)			PH (cm)			NPP		
	Population	Gain	Mean	Population	Gain	Mean	Population	Gain	Mean
1	H90	11.04	124.82	H39	26.67	121.20	H72	24.44	85.86
2	H56	7.93	121.72	H72	21.79	116.32	H39	23.73	85.14
3	H53	6.50	120.28	H53	11.99	106.52	H53	20.78	82.19
4	H72	2.86	116.65	H56	5.23	99.76	H56	8.57	69.98
5	H39	0.00	113.78	H90	0.00	94.52	H90	0.00	61.41

Plant height and the number of pods per plant showed the highest gains with selection, with populations H39 and H72 being the best performers with higher averages than the other populations (Table 4). However, the number of days to maturity in these populations did not result in significant gains. This can facilitate the selection of earlier genotypes, considering the decrease in the means of this trait.

Given the scarcity of reports on early accessions with indeterminate growth habit, early accessions are important sources of genes to be included in lima bean breeding programs. In addition, precocity has advantages such as escape from climate stress and diseases, lower water consumption, and shorter cultivation periods, favoring crop rotation (BURATTO et al., 2007).

Concerning pod traits (Table 5), populations H53 and H56 presented the highest gains, and the most significant gain occurred in pod thickness. Gains in these traits are desirable because larger pods favor manual harvesting. In addition, these traits positively correlated with grain yield, which is of great importance for improving this species (ASSUNÇÃO FILHO et al., 2022).

Table 5. Ranking, selection gains, and averages of pod length (PL), pod width (PW), and pod thickness (PT) evaluated in five F_3 lima bean populations in Teresina, PI, Brazil.

Order	PL (mm)			PW (mm)			PT (mm)		
	Population	Gain	Mean	Population	Gain	Mean	Population	Gain	Mean
1	H53	0.75	15.42	H53	0.034	54.315	H56	15.69	45.10
2	H56	0.69	15.36	H56	0.033	54.314	H90	12.52	41.94
3	H90	0.57	15.25	H72	0.022	54.303	H72	6.27	35.68
4	H39	0.27	14.94	H39	0.009	54.290	H53	2.84	32.25
5	H72	0.00	14.67	H90	0.000	54.281	H39	0.00	29.41

Table 6. Ranking, selection gains, and averages of seed length (SL), seed width (SW), and seed thickness (ST) evaluated in five F_3 lima bean populations in Teresina, PI, Brazil.

Order	SL (mm)			SW (mm)			ST (mm)		
	Population	Gain	Mean	Population	Gain	Mean	Population	Gain	Mean
1	H56	11.26	25.25	H39	0.65	4.54	H53	1.01	9.81
2	H90	10.50	24.49	H53	0.48	4.37	H90	0.96	9.75
3	H53	4.87	18.86	H90	0.33	4.22	H56	0.60	9.40
4	H39	1.97	15.96	H72	0.20	4.10	H39	0.26	9.06
5	H72	0.00	13.99	H56	0.00	3.89	H72	0.00	8.80

Concerning seed traits (Table 6), the selection gains were higher and more significant in seed width for the H56 and H90 populations. The gains were less significant for seed length and thickness, with the populations presenting similar values. This may be due to the low genetic variance directly impacting the selection gain. The gain in these traits is desirable because seed size is an important trait in the physiological development of crops, where larger seeds tend to produce a greater number of nodules and dry matter (DOBERT; BLEVINS, 1993).

Seed traits are important for the commercialization of lima beans, where selection should be aimed at improving these traits. There is a high possibility of genetic gains and selection at the population level.

In plant breeding programs, it is necessary to use specific methodologies that translate the heritability of selected parents reliably, resulting in productive offspring with good grain quality. Therefore, using genetic values predicted by the REML/BLUP methodology is an alternative for selecting plants with mixed reproductive systems, such as the lima bean (TORRES FILHO et al., 2017).

CONCLUSION

The REML/ BLUP methodology proved efficient in estimating genetic parameters and predicting gains in the evaluated lima bean populations. The estimations proved genetic variability and selection potential in the lima bean populations.

The estimated selection gains indicated that the greatest gains were obtained for plant height, the number of pods per plant, pod thickness, seed length, and the number of days to maturity. Populations H53 and H56 presented larger white seeds, and are potential populations for the improvement of the species.

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