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Scientific Article

Lima bean responses to high temperatures in natural and controlled environments

Resposta do feijão-fava às altas temperaturas em ambiente natural e controlado

Rubens R. C. Silva[1], Gilmar M. de Carvalho Junior[1], Jefferson B. L. de Moura[1], Yasmin B. Diniz[1], Carlos H. A. Matos Filho[1], Ângela C. de A. Lopes[1], Regina L. F. Gomes[1], Verônica B. da Silva[1]*

[1]Department of Plant Science, Universidade Federal do Piauí, Teresina, PI, Brazil.

ABSTRACT - Effects of abiotic stresses, such as high temperature, on plants are exacerbated by climate change. Lima beans exhibit higher tolerance to high temperatures than the common beans. Understanding the tolerance of lima bean landrace germplasm to high temperatures is important to improve their breeding. Therefore, in this study, we aimed to examine the high temperature responses of lima bean landrace varieties obtained from the Phaseolus Germplasm Bank at Universidade Federal do Piauí (BGP-UFPI, Brazil) in two environments. Five landraces showing the best performance in emission of flowers and number of pods formed (UFPI-945, UFPI-1037, UFPI-876, UFPI-1036, and UFPI-1064) were evaluated in two cultivation environments, natural (29 ºC) and controlled (37 ºC), using a completely randomized design with four replications, with each plot consisting of a single plant. Analysis of variance and Tukey's test ($P < 0.05$) were performed for 12 quantitative traits, followed by Pearson's correlation analysis. Lima beans exhibited genetic variability in high temperature tolerance in both natural and controlled environments. Specifically, UFPI-1064 exhibited superior performance with higher pod thickness and width and number of flowers and lower flower and pod abortion than the other varieties in both natural and controlled environments. Pearson's correlation analysis revealed positive and strong correlations between the number of flowers and flower abortion in the natural environment and number of pods and seeds per pod in the controlled environment.

Keywords: *Phaseolus lunatus*. Abiotic stress. Floral abscission. High temperature tolerance. Pearson correlation.

RESUMO - Os estresses abióticos, como as altas temperaturas, são potencializados pelas mudanças climáticas. O feijão-fava apresenta maior tolerância às altas temperaturas em comparação ao feijão-comum. O conhecimento dessa tolerância as altas temperaturas de germoplasma landraces de feijão-fava é importante para o desenvolvimento de programas de melhoramento. Assim, objetivou-se com este trabalho verificar a resposta de variedades crioulas de feijão-fava do Banco de germoplasma de *Phaseolus* da Universidade Federal do Piauí (BGP-UFPI, Brasil) às altas temperaturas, em dois ambientes. Foram avaliadas as cinco variedades crioulas com maior desempenho emissão de flores e vagens formadas (UFPI-945, UFPI-1037, UFPI-876, UFPI-1036 e UFPI-1064) e dois ambientes de cultivo natural (29ºC) e controlado (37ºC), utilizando-se o delineamento inteiramente ao acaso, com quatro repetições, sendo uma planta por parcela. Realizou-se a análise de variância e o teste Turkey ($P < 0,05$) para as dozes variáveis quantitativas, seguidas pela correlação de Pearson. Há variabilidade genética em feijão-fava para tolerância à alta temperatura tanto em ambiente natural quanto controlado. Para os caracteres espessura e largura vagem, número de flores, abortamento de flor e de vagem destacaram-se tanto em ambiente natural quanto controlado o genótipo UFPI-1064. A correlação de Pearson aferiu que o ambiente natural, se sobresaiu com correlações positivas e de altas magnitudes em número e o abortamento de flores. Contudo, em ambiente controlado, o número de vagem e número de sementes por vagem também tiveram correlações de altas magnitudes.

Palavras-chave: *Phaseolus lunatus*. Estresse abiótico. Abscisão floral. Tolerância temperatura elevadas. Correlação de Pearson.

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***Corresponding author:**
<verabritosl@hotmail.com>

INTRODUCTION

Lima bean (*Phaseolus lunatus* L.), a legume of *Fabaceae* family, is a key species of genus *Phaseolus* that serves as an alternative source of plant protein (VIEIRA, 1992; CARVALHO et al., 2022). It contains fiber, iron, folic acid, vitamins, lipids, potassium, magnesium, calcium, and phosphorus (MORAES, 2017). It is consumed in the form of green and dry beans and green pods by humans (GUIMARÃES et al., 2007). Additionally, it is used as animal feed (VIEIRA, 1992) to promote food and nutritional security, especially in farming (BARBOSA; ARRIEL, 2018).

In Brazil, lima beans are cultivated in almost all regions and have special economic importance in some states, particularly those in the Northeast region (MEDEIROS et al., 2015; ASSUNÇÃO NETO et al., 2022). According to the Instituto Brasileiro de Geografia e Estatística – IBGE (2022), the country exhibits a productivity of 315 kg/ha, with 9.554 t of dry beans and 30.316 ha of planted area. Specifically, Ceará and Paraíba states are the largest producers of lima beans in the country, yielding 4.362 and 3.885 t of lima beans, respectively. During the same period, Piauí state produced 696 t of lima beans.

Lima beans exhibit high genetic diversity and productivity (ALMEIDA et al., 2021). They mainly grow in medium-to-high altitude regions and are sensitive to high temperatures (LOPES; ARAÚJO; GOMES, 2015). Climate changes and rising global temperatures due to greenhouse gas emissions in most cultivation areas have impacted its food security. Therefore, exploring the negative effects of high temperatures on crop productivity is necessary to enhance their yield (HOFFMANN JÚNIOR et al., 2007).

Machado et al. (2022) investigated the effects of temperature on the phenological traits and development of lima bean plants under natural conditions (average annual temperature: 26.5 °C and average relative humidity: 70%) and revealed promising accessions for flower and pod emissions at high temperatures. Considering the socioeconomic importance of lima beans and impact of high temperatures during flowering and seed development on its crop yield (HOFFMANN JÚNIOR et al., 2007), identification of high temperature-tolerant genotypes is necessary for efficient lima bean breeding.

In addition to univariate methods, multivariate techniques are widely used to evaluate the morphoagronomic and phenological traits of plants as they can simultaneously assess various desired traits (CRUZ; CARNEIRO; REGAZZI, 2014). For example, they reveal simple correlations among traits of interest, facilitating the assessment of the magnitude and direction of association among the traits. Moreover, these techniques are extensively used in breeding programs for indirect selection of the desirable trait, which is faster than direct selection in some cases (CRUZ; REGAZZI; CARNEIRO, 2012).

In this study, we aimed to investigate the responses of lima bean landrace varieties obtained from the Phaseolus Germplasm Bank at Universidade Federal do Piauí (BGP-UFPI, Brazil) to high temperatures in both natural and controlled environments.

MATERIAL AND METHODS

Five genotypes of lima beans from the *P. lunatus* Core Collection of the Phaseolus Germplasm Bank (BGP – UFPI) were used in this study (Table 1). Particularly, UFPI-945 genotype exhibited superior performance with higher numbers of flowers (NF) and pods (NP) and lower number of aborted pods (NAP) than the other varieties at high temperatures (MACHADO et al., 2022). Additionally, UFPI-1037, UFPI-876, UFPI-1036, and UFPI-1064 genotypes also exhibited high productivity with more flowers and pods (MACHADO et al., 2022).

Table 1. Names and origins of five lima bean genotypes obtained from the Phaseolus Germplasm Bank at Universidade Federal do Piauí (UFPI).

Genotype	Origin
UFPI-945	Brazil
UFPI-1064	Azerbaijan
UFPI-1036	United States
UFPI-1037	United States
UFPI-876	Brazil

A greenhouse (50% shading rate) at the Department of Plant Science, Center for Agricultural Sciences, UFPI (Teresina-PI municipality; 05°05’ S; 42º48’ W; altitude: 74.4 m) was set as the natural environment. This region has an average annual temperature of 26.5 °C, average relative humidity of 70%, and average annual precipitation of 1,448 mm. From February to July, 2022, the climate in this region was dry or megathermal, according to Köppen’s classification (KÖPPEN, 1948). The maximum, minimum, and average temperature and humidity in this region are shown in Figure 1.

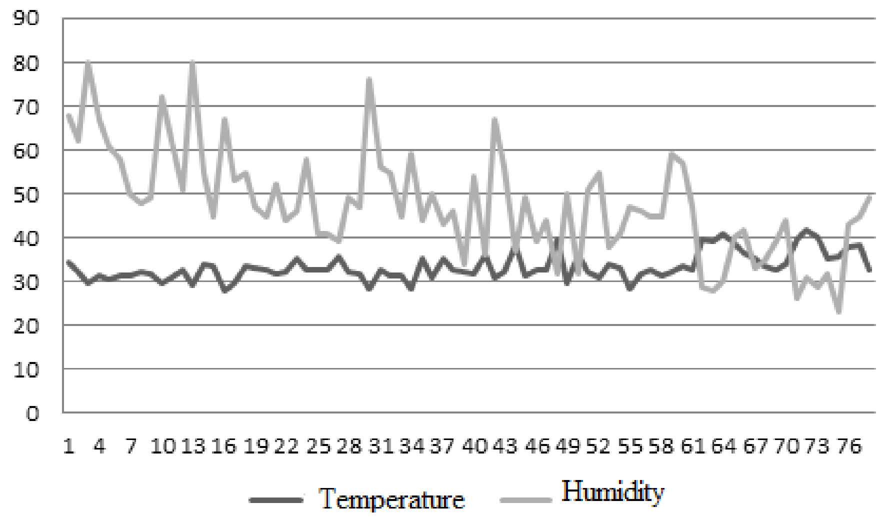

Figure 1. Temperature and humidity recorded throughout this study. Source: Instituto Nacional de Meteorologia – INMET (2022).

In the natural environment, the experimental design was completely randomized with four replicates. Each plot consisted of a polyethylene pot (14 L) containing two plants. Immediately after sowing and throughout the crop development cycle, manual irrigation was performed as required. Plants with indeterminate growth were supported by bamboo stakes. The experiments involved seed treatment with fungicides, with a 1:3 soil and plant compost mixture and base fertilization of 05N-30P-15K, as per previous recommendations (GOMES; LOPES, 2006). Integrated pest management was performed using insecticides (imidacloprid and beta-cyfluthrin) in an active gradient to control aphids.

A room with a temperature of 37 °C and 12 h of light over a 72-h period in the Soil Analysis Laboratory was set as the controlled environment. Pots from replicates 1 and 2 of each genotype were moved to a controlled environment after the appearance of the first floral buds and pods, where they remained for 72 h before being returned to the greenhouse, as previously described (BARLOW et al., 2015).

For agronomic characterization, the following descriptors recommended by Bioversity International (2007) were used in both studied environments (natural and controlled): pod length (PL; mm), pod width (PW; mm), pod thickness (PT; mm), seed length (SL; mm), seed width (SW; mm), seed thickness (ST; mm), number of locules per pod (NLP), number of seeds per pod (NSP). Additionally, the following phenological descriptors were assessed (HOFFMANN JÚNIOR et al., 2007): NF, average number of aborted flowers (ABF, total), average number of pods formed (NP, total), and average number of aborted pods (ABP, total). To determine the dimensions of pods and seeds (mm), a digital caliper was used, and floral abortion was estimated as the difference between the number of emerging flowers and number of pods produced.

The data were subjected to individual variance analysis using the statistical model in Equation 1:

$$y_{ij} = \mu + t_i + e_{ij} \;\{i/j = 1, 2, \ldots, k\} \quad (1)$$

yij is the response of treatment i;
eij is the error associated with the j observation of the i treatment;
μ is the mean effect;
ti is the treatment effect with $\Sigma_{ti}=0_{ki}=1$;
k is the number of treatments;
ni is the number of repetitions of the i treatment with $\Sigma_{ni}=N_{ki}=1$.

where, *yij* is the response of treatment i, *eij* is the error associated with j observation in i treatment, *μ* is the mean effect, *ti* is the treatment effect with $\Sigma_{ti}=0_{ki}=1$, *k* is the number of treatments, and *ni* is the number of repetitions of i treatment with $\Sigma_{ni}=N_{ki}=1$.

Next, the data were subjected to Tukey's test, and the Pearson's correlation matrix was estimated based on the observed data for the morphoagronomic and phenological traits. The significance of r was assessed using the corrplot package in R software (WEI; SIMKO, 2021). Associations between the explanatory variables and responses were determined using Equation 2:

$$r_{xy} = \frac{Cov(X,Y)}{\sqrt{V(X).V(Y)}} = \frac{\delta(X,Y)}{\delta(X)\delta(Y)} \quad (2)$$

where, r (XY) is the Pearson's correlation coefficient for variables X (response) and Y (explanatory), δ (X, Y) is the covariance between variables X and Y, and δ(X) and δ(Y) are the standard deviations of variables X and Y, respectively.

Next, the direction and intensity of the linear relationships between the variables were assessed using the Pearson's linear correlation coefficient (r), which ranges from –1 to +1. Values closer to 1 indicate a strong linear relationship, whereas those closer to zero indicate a weak linear correlation between the variables. Statistical analyses were conducted using the R software (R DEVELOPMENT CORE TEAM, 2020) with the CCA (GONZÁLEZ; DÉJEAN; DÉJEAN, 2023), CCP (MENZEL, 2022), and yacca (BUTTS, 2021) packages.

RESULTS AND DISCUSSION

Analysis of variance revealed significant differences in all traits, except NF and ABP, among the tested lima bean landrace varieties (Table 2). The varieties exhibiting superior performance in terms of NP, NF, and ABP under high temperature conditions were selected in this study, as per previous recommendations (MACHADO et al., 2022).

Due to variations in both environments, significant differences were observed in NSP, SL, NF, NAF, and NP, indicating that variations impacted the traits in both controlled and natural environments (Table 2). G × E interactions also demonstrated significant differences in the relative performances of lima bean landraces for the following descriptors in both environments: PT, PW, total NP, total ABF, and total ABP (Table 2). Among various factors, high temperatures significantly affect seed development and plant floral structures (PEREIRA et al., 2014). At very high temperatures, plants initiate the abscission of reproductive organs, and no pod formation is observed at temperatures over 35 °C, thus significantly compromising the final yield (VIEIRA; PAULA JÚNIOR; BORÉM, 2006). Moreover, abscission of reproductive organs at very high temperatures is associated with the increased synthesis of ethylene, a growth-regulating hormone.

Here, UFPI-945 demonstrated superior performance in terms of ST, SW, SL, and PL, whereas UFPI-1064 exhibited better NSP and NLP than the other genotypes. The other genotypes were statistically similar across all traits according to Tukey's test at the 5% significance level (Table 3). During the reproductive period, lima beans are extremely sensitive to high temperatures, which impact the pod and seed formation in these plants (HOFFMANN JÚNIOR et al., 2007). Machado et al. (2022) reported that lima bean accessions UFPI-922 and UFPI-945 performed well at high temperatures with high NF and NP and low NAP.

Table 2. Summary of analysis of variance for 12 traits observed in five landraces of lima beans, evaluated in two growing environments (natural and controlled).

				MQ			
FV	DF	NSP	NLP	ST(mm)	SW(mm)	SL(mm)	PT(mm)
Environment(A)	1	2.23*	0.65	0.77	1.20	14.94***	4.70
Genotype(G)	4	2.10***	2.28***	4.95***	15.13***	77.24***	4.02*
G x A	4	0.38	0.56	0.40	0.57	2.38	8.69***
Error	168	0.38	0.40	0.70	0.49	1.17	1.41
FV	DF	PW(mm)	PL(mm)	NF(total)	NAF(total)	NP(total)	NAV(total)
Environment(A)	1	2.57	23.24	15401.2***	12005.0***	211.25***	0.12
Genotype(G)	4	31.43***	1791.50***	5554.2	6884.1**	101.05***	0.24
G x A	4	14.03***	134.37	6404.1***	6008.9**	15.00	0.22***
Error	168	2.24	88.23	550.00	547.40	12.50	0.04***

FV = source of variation, DF = degrees of freedom, MQ = mean square, NF = total number of flowers produced, NP = number of pods formed, NAF = number of aborted flowers, NAP = number of aborted pods, PL = pod length, PW = pod width, PT = pod thickness, SL = seed length, SW = seed width, ST = seed thickness, NLP = number of locules per pod, NSP = number of seeds per pod. * significant at 5%, ** significant at 1%, and *** significant at 0.1%, ns – not significant.

Table 3. Tukey test for the traits Number of seeds per pod (NSP), Number of loci per pod (NLP), Seed thickness (ST), Seed width (SW), Seed length (SL) and Pod length (PL) observed in five landraces of lima beans.

Genotype	NSP	NLP	ST(mm)	SW(mm)	SL(mm)	PL(mm)
UFPI-945	1.65ab	1.65bc	5.01a	10.08a	15.11a	72.08 a
UFPI-876	2.025a	2.02ab	4.15b	9.25b	12.87b	60.25bc
UFPI-1036	1.75ab	1.82abc	4.08b	8.87bc	11.55c	55.05d
UFPI-1037	1.43b	1.59c	4.26b	8.18d	11.36c	52.04d
UFPI-1064	2.05a	2.22a	3.93b	8.58cd	11.15c	63.07b

Table 4 shows that the genotypes excelled separately in each environment in terms of PT, PW, NF, NAF, and NAP. In the controlled environment, UFPI-945 exhibited the highest PT, whereas the NF and NAF were superior in UFPI-945, UFPI-876, UFPI-1036, and UFPI-1037. UFPI-1064 showed lower averages than the other genotypes for NF, NAF, and NAP in the control environment. This indicates that, at high temperatures, lima bean genotypes with higher averages of floral abortion produce greater number of flowers. Therefore, lima beans exhibit a natural adjustment mechanism for flower loss during thermal stress periods, similar to other crops, such as the common bean (*Phaseolus vulgaris* L.) (ORSI et al., 2021).

Table 4. Mean values for pod thickness (PT), pod width (PW), number of flowers emitted (NF), number of aborted flowers (NAF) and number of aborted pods (NAV) observed in five landraces of lima beans, evaluated in two cultivation environments (controlled and natural).

	PT(mm)		PW(mm)	
Genotype	C. E.	N. E.	C. E.	N. E.
UFPI-945	4.83bA	6.46aA	14.50bA	14.72abA
UFPI-876	5.44bA	6.06abcA	14.7bA	14.36bA
UFPI-1036	5.88abA	5.15cA	16.001bA	15.94aA
UFPI-1037	5.63abA	5.37bcA	15.63bA	15.74aA
UFPI-1064	6.86aA	6.36abA	19.64aA	16.08aA

	NF(total)		NAF(total)		NAV(total)	
Genotype	C. E.	N. E.	C. E.	N. E.	C. E.	N. E.
UFPI-945	47.5 aA	93 bA	28 aA	69.5 bA	1.5731 aA	1.5731abA
UFPI-876	59.5 aA	75.5 bA	50.5 aA	56.5 bA	1 aA	1 aA
UFPI-1036	42.5 aA	71 bA	28 aA	51 bA	1.2071 aA	1.2071 aA
UFPI-1037	61 aA	54 bA	43 aA	34 bA	1.2071 aA	1 aA
UFPI-1064	48 aB	242.5 aA	45 aB	228.5 aA	1 aB	1.9840 aA

E: Environment, C: Controlled, N: Natural, A: Environment, a: Genotype.

Machado et al. (2022) identified that the combined selection of traits, such as NF, NAF, PL, PW, SL, SW, and ST, should be prioritized in lima bean cultivation under high-temperature conditions. Therefore, high temperatures can reduce the grain yield of lima beans grown in tropical regions because of the abortion of their reproductive structures.

Pearson's correlation analysis revealed strong positive correlations between SL and SW (0.87) and between NLP and NSP (0.91; Figure 2). Previous studies have also shown the association between NLP and NSP, which are important components of grain production. This study demonstrated strong correlations between these traits in both environments, indicating that pods with more locules exhibit high number of seeds (COSTA et al., 2022). However, NSP was negatively correlated with PW, SL, and SW, suggesting a trend towards higher production of smaller-sized seeds. In such cases, seed and pod traits can be used for the indirect selection of lima bean genotypes adapted to high temperatures (ASSUNÇÃO FILHO et al., 2022; ASSUNÇÃO NETO et al., 2022).

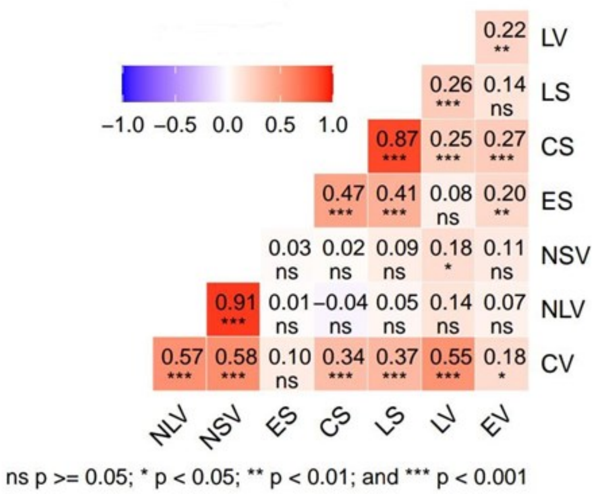

Figure 2. Pearson correlation analysis between the traits: pod length (PL), pod width (PW), pod thickness (PT), seed length (SL), seed width (SW), seed thickness (ST), number of locules per pod (NLP), and number of seeds per pod (NSP) in both environments.

Pearson's correlation analysis revealed strong positive correlations between NAF and NF and between NAF and NAP (0.82), NSP (0.81), and NP (0.77; Figure 3). However, NF exhibited a positive correlation with NAV (0.83) and negative correlations with NSP (–0.79) and NP (–0.75). NAP exhibited negative correlations with NP (–0.45) and NSP (–0.61), whereas NSP and NP showed a strong positive correlation (0.88). Therefore, selection programs should aim to reduce the NAP and NAF during growth to enhance the lima bean traits of commercial value, such as those related to pods and seeds.

In most crops, productivity is influenced by thermal stress from the onset of flowering to pod formation, affecting traits, such as the NP, NSP, and SW (PEREIRA et al., 2014). NS and NAF exhibit strong positive correlations with the total number of seeds produced and NP in the natural environment (MACHADO et al., 2022).

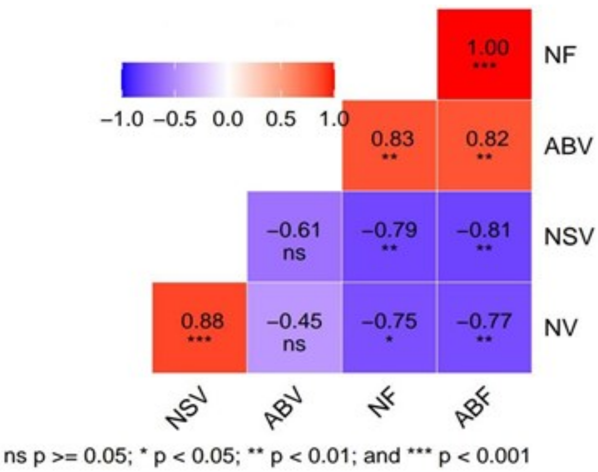

Figure 3. Pearson correlation analysis, between the traits, number of seeds per pod (NSp), pod abortion (NAP), flower abortion (NAF), number of flowers (NF), number of pods (NP), subjected to a natural environment.

In the controlled environment, Pearson's correlation between NSP and NP was 0.92, indicating that high NP is associated with high NSP. NF and NAF also exhibited a correlation of 0.92, indicating that high NF corresponds to high NAF (Figure 4). In both environments (natural and controlled), positive correlations were observed between NF and NAF and NAP and negative correlations between NF and NP and NSP. However, in the natural environment, a strong positive correlation was observed between NF and NAF. In contrast, NP and NSP exhibited a strong positive correlation in the controlled environment. Understanding these correlations and their magnitudes can aid in indirect selection of the desired trait. However, phenotypic correlations are affected by both genetic and environmental values, and only genetic values are passed on to the next generation (COSTA et al., 2022). These results suggest that external environmental factors exhibit strong correlations with plant traits in the natural environment.

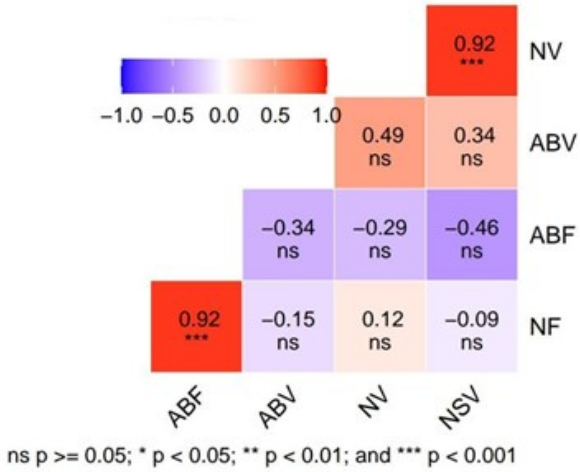

Figure 4. Pearson correlation analysis, between the traits, number of seeds per pod (NSP), pod abortion (NAV), flower abortion (NAF), number of flowers (NF), number of pod (NP), subjected to a controlled environment.

Among the climate factors affecting crop development, temperature exhibits the strongest effects on pod development, flowering, and fruiting. Both genotype and environmental conditions affect floral abortion. The lima bean genotypes analyzed in this study and other *Phaseolus* species exhibit variable tolerance to high temperatures in controlled environments (HOFFMANN JÚNIOR et al., 2007; ORSI et al., 2021; MACHADO et al., 2022). Even under optimal conditions, reproductive organs may undergo abscission, leading to different patterns among genotypes. These patterns may represent the regulatory responses specific to each genotype. Therefore, to achieve a satisfactory yield, when floral abortion occurs, the remaining flowers should be provided more assimilates, which can maintain or even increase the yield through other yield components (ASCOUGH et al., 2005). These results can be used in genetic improvement programs to improve the temperature tolerance and select the best parental lines.

CONCLUSION

Overall, this study revealed the genetic variability in the high temperature tolerance of lima beans in both natural and controlled environments.

In the natural environment, UFPI-945, UFPI-876, UFPI-1036, and UFPI-1037 exhibited highest temperature tolerance, with the lowest NF, ABF, and ABP. UFPI-1064 exhibited superior performance in terms of PT, PW, NF, ABF, and ABP at high temperatures in both natural and controlled environments.

Pearson's correlation analysis further confirmed that selecting the lima bean varieties with low ABF and ABP for cultivation increased the NP and NSP.

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