

 Global Journal of Research in Humanities & Cultural Studies

 ISSN: 2583-2670 (Online) Volume 04 | Issue 06 | Nov.-Dec. | 2024 Journal homepage: https://gjrpublication.com/gjrhcs/

Original Research Article

Genetic Variability and Character Association for Advanced Faba bean (*Vicia faba* **L.) Genotypes in Southeastern Ethiopia**

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Abstract

Genetic variability refers to the tendency of genetic traits to differ within and among populations. In the 2023 growing season, 36 genetically distinct faba bean genotypes were evaluated across two locations using an alpha lattice design with two replications. The study aimed to assess genetic variation and identify parent materials with desirable traits. A combined analysis of variance indicated a highly significant (P < 0.001) difference among 36 faba bean genotypes for all studied traits, except for days to 50% flowering and reaction to rust disease. The phenotypic coefficient of variation is higher than the genotypic coefficient of variation for all traits except days to maturity and the number of seeds per pod. This suggests that the environment plays a significant role in the expression of genes for these traits. The genetic advance as a percentage of the mean ranged from 0.27% for days to flowering to 34.25% for thousand seed weights. The genotypic coefficients of variations were relatively higher in 1000-seed grain weight and number of pods per plant. The highest broad-sense heritability of 94% was recorded in days to maturity, and the lowest heritability of 16% was observed in plant height. The genotype-by-trait graph formed, the angles between the number of seeds per pod and rust were smaller than 90°, indicating a positive correlation between these traits. The genotypes that have been tested come from different breeding stages. The genetic variability of the traits becomes narrower or shifts towards more uniform characteristics due to selection. Therefore, unless a more diverse population is used and testing locations are increased, more genetic variance is not expected.

Keywords: Correlation, Faba bean, Genotype, Trait, Variation.

INTRODUCTION

Faba bean (*Vicia faba* L.) is a diploid plant with large chromosomes (2n = 2x = 12), exhibiting partial outcrossing from 20% to 80%. [1, 2, 3] based on [4] data, faba bean is the fourth most widely grown cool-season legume, following pea (*Pisum sativum*), chickpea (*Cicer arietinum*), and lentil (*Lens culinaris*). Faba bean is a staple legume crop in Ethiopia, providing protein for food and enhancing soil fertility through nitrogen fixation [5] and [6].

As compared to other pulse crops, faba bean has an advantage over soybeans in cool season environments because it is adapted to grow at low temperatures. As such, it is well suited to sustainable farming practices in temperate to cool environments [7]. However, faba bean yield remains unstable due to biotic and abiotic stresses, as is the case with many other major legumes. Genetic variability in a crop species for a specific trait suggests room for improvement. In today's modern world, with a rapidly growing population, improving crop production and the nutritional value of crops have become the primary goals of breeders and scientists.

The genetic variability of the faba bean is quite large; the variability could be caused by the presence of an intermediate crossing system between autogamy and allogamy. According to [1], the first study on genetic variability in faba beans was conducted in 1976. The report focused on natural variability and selection in local and exotic populations of faba beans. The success of a breeding program relies on the extent of genetic diversity within a population. This research aims to assess the field performance of 36 faba bean genotypes using agronomic measurements to identify key traits for future breeding works.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted at two locations in south-eastern Ethiopia, namely Bekoji and Assasa substations of Kulumsa Agricultural Research Center, during the main cropping season in 2023 under rain fed conditions. Bekoji is located at an altitude of 2780 m.a.s.l with a geographic coordinate of 07⁰ 3237"N latitude and 39⁰ 1521" E' longitude. The area receives a mean annual rainfall of 1020 mm. The mean annual maximum and minimum temperature of the site are about 18.6 °C and 7.9 °C, respectively. Similarly, Assasa sub-station is located at an altitude range of 2340 m.a.s.l with 0707'09"N and 3911'56"E of geographical positioning. The average annual temperature is around 15 $^{\circ}$ C, with an average rainfall of 620 mm.

2.2 Design and Treatments

Thirty-six (34 breeding lines and 2 recently released variety utilizing for control) is used for this experiment. The experiment utilized a 6 x 6 simple lattice design, with each replication consisting of six incomplete blocks and each block comprising six genotypes ($6*6 = 36$) genotypes were involved in the experiment (Table 1). Each plot had two rows of 4 m in length, with a spacing of 40 cm between rows and 10 cm between plants. Each genotype was planted on a plot of 3.2 m2. Each plot was separated by a 1m spacing to distinguish between two genotypes. 121 kg ha of NPS fertilizer was applied, and other agronomic practices were done throughout the growing season. The grain yield from a 3.2 m2 plot was converted to hectares, and the moisture content was standardized to 10% for faba beans.

G-1 to G-36; Number of genotypes used in the experiment

2.3 Data Collection:

Data were collected on a single plant and plot basis. On a plant basis, data were collected from five randomly selected plants from each genotype in each replication, namely, plant height (PH) (cm), number of pods per plant (NPPP) (number), and number of seeds per pod (NSPP) (number), thousand seed weight (TSW) (gram), grain yield (GYH) (kg ha⁻¹). While the data on a plot basis were visually assessed in the entire plot, they included days to 50% flowering (DF), days to 90% maturity (DM), and rust (1to 9) scale, where 1 for nil: no visible disease symptom (Immune), 3 for a slight (resistant), 5 for a medium (moderately resistant), 7 for a severe (susceptible), and 9 for very severe (highly susceptible).

2.4 Data Analysis:

The study use of the alpha lattice design method as described by [8] to conduct an analysis of variance on all traits. This was done using the linear model (lm) function in the stats package of R software version 4.2 [9] to determine the significance of differences among genotypes. The genotypic variance $(\sigma^2 g)$ and environmental variance $(\sigma^2 e)$ were obtained using the lmer function of the lme4 mixed model package in R software version 4.1.2. This was achieved by treating the genotype in the linear mixed model as a random variable, while replication and block were considered as fixed variables. The Residual (restricted) Maximum Likelihood (REML) method was used to compute the variances from the variance component table generated by the software. Variance component estimation method:

Genotypic Variance $(\sigma^2 g) = \frac{MSG - MSE}{m}$ $\frac{-MSE}{r}$ Environmental variance ($\sigma^2 E$) = MSE Phenotypic Variance ($\sigma^2 p$) = $\sigma^2 G$ + σ^2 E Where; MSG = Mean square of genotypes, MSE = Mean square of error, r=Number of replications

Phenotypic and genotypic coefficients of variations of each trait were expressed as percentage of the corresponding phenotypic and genotypic standard deviations as described by [10] and expressed as follows:

$$
PCV = \frac{\sqrt{\sigma^2 p}}{\bar{x}} * 100
$$

$$
GCV = \frac{\sqrt{\sigma^2 g}}{\bar{x}} * 100
$$

Where: PCV = Phenotypic Coefficient of variation, GCV = Genotypic Coefficient of variation, $\sigma^2 p =$ Phenotypic variance, $\sigma^2 g =$ Genotypic Variance, \bar{x} = mean value of the trait

According to [11] the PCV and GCV estimates classified as low, <10%, Moderate, 10-20%, High, >20%. Broad sense heritability values for all parameters (H^2B) were estimated based on the formula given by [12]

$$
H^2 = \frac{\sigma 2g}{\sigma^2 p} * 100
$$

Where: H² = Heritability in broad sense, $\sigma^2 g =$ Genotypic Variance, $\sigma^2 p =$ Phenotypic variance

Heritability estimates in broad sense was categorized as high ($> 60\%$), medium (30 - 60%) and low ($< 30\%$). Genetic advance (GA) at 5% selection intensity was estimated as per formula given by [13]

$$
GA = K^* \sqrt{\sigma^2 p} * H^2
$$

Where: GA = genetic advance, K = Selection differential at 5% selection intensity which accounts to a constant value 2.063, $\sigma^2 p$ = Phenotypic variance, H² = Broad sense heritability

The genetic advance as percent of mean (GAM) was calculated using the following formula and was expressed in percentage [13].

$$
\text{GAM} = \frac{\text{GA}}{\bar{\mathbf{x}}} * 100
$$

Where: GAM = genetic advance as percent of mean, GA = Genetic advance at 5% selection intensity, \bar{x} = population Mean

According to (Johnson et al., 1955), the GAM was classified as low if <10%, Moderate, 10-20% and High, >20%

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance (ANOVA)

The analysis of variance showed the presence of a highly significant $(P < 0.001)$ difference among 36 faba bean genotypes for all studied traits except days to 50% flowering and reaction to rust disease (Table 2). The presence of a highly significant difference was an indication of the existence of considerable genetic variability in experimental materials for the studied traits, which justified carrying out further genetic analysis. This will provide an opportunity for a breeder to select superior and desired genotypes for their better seed yield and other yield related traits for further

improvement through exploiting the observed variations [14]. Different researchers reported significant differences for one or more of the studied traits [15]

Genotypes showed significant differences for the traits grain yield and days to maturity due to the mean square of genotype by environment interaction (G x E). As these results depicted, the genotype performance was different across the two locations for these particular traits. Conversely, the other traits showed relatively consistent performance; the expressions of phenotypic performance were not affected by different environments.

Table 2. Mean squares from Combined Analysis of Variance for 8 Traits of 36 Faba bean hybrid lines conducted in 2023

			Traits							
SV	Df	DF	DM	PН	PPP	SPP	Rust	TSW	GYH	
Genotype (G)	35	61.7	37.95***	$190***$	$25.62***$	$0.59***$	1.64 ns	114297***	1847338***	
Location (L)		64	33794.7***	18791.8***	$31.41**$	$3.36***$	364.2ns	$165039***$	136083345***	
Replication (Rep)	2	25.1	$21.69**$	$365.57**$	7 _{ns}	$1.05***$	2.3 _{ns}	9666	234133	
$G \times E$	35	56.6	$10.04**$	117.24	12.49	0.24	1.02 _{ns}	11106	1033366**	
Rep x L. x Block	20	35.4	$9.94**$	83.98	$9.62*$	0.19	0.56 ns	16841	664166	
Error	50	40.9	4.92	74.07	6.05	0.15	0.23	10645	465616	

 $SV =$ Source of Variation, $DF =$ Days to 50% flowering, $DM =$ Days to 90% maturity, $PH =$ Plant height, PPP = Number of pods per plant, $SPP =$ Number of seeds per pod, $TSW = 1000$ seeds weight and $GYH =$ Grain yield per hectare

3.2. Mean Performance of Faba bean Genotypes Across two Locations

The highest mean yield was recorded from genotype G16, while the lowest grain yield was obtained from genotype G7 (Table 3). A similar finding was reported by [16] and ([17]. The longest maturity days have been recorded for G-33, while the genotype G-1 took the shortest days to mature. An extensive range of genetic variability was found in the mean performance of 36 genotypes of faba beans for the trait days to maturity ranges (143.43 days to 154.2 days), plant height $(99.1cm$ to 133.7cm), number of pods per plant $(6.67 \text{ to } 16.25)$, thousand seed weight $(604.64 \text{ to } 1467 \text{ g})$ and grain yield (2208.45 kg/ha to 5330.39 kg/ha (Table 3). This indicates the presence of a genetic pool with a wide range of genetic variability that could be achieved through breeding.

Similarly, the maximum number of pods per plant was recorded for genotype G17, and the minimum one was recorded for genotype G10. In terms of thousand seed weights, the highest seed weight was measured from genotype G2, and genotype G7 was measured at the lowest (Table 3). These results generally indicated that the hybrid faba bean lines showed wide genetic variation to the trait 1000 seeds weight; this result will provide important genetic information for improving faba bean seed size from small (<500g per 1000 seeds weight) to large (>800g per 1000 seeds weight) seeds size through selection. This result is aligned with the report by [18].

Note: DF = Days to 50% flowering, DM = Days to 90% maturity, PH = Plant height, PPP = Number of pods per plant, $SPP =$ Number of seeds per pod, TSW = 1000 seeds weight and GYH = Grain yield per hectare, CV% = coefficient of variation.

3.3. Genetic Parameters and Heritability

The traits under consideration were analyzed to determine the genotypic (σ^2 g) and phenotypic (σ^2 p) variances, as well as the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV). Additionally, the heritability (broad sense), genetic advance, and genetic advance as a percentage of the mean were estimated (Table 4). The component of phenotypic variance for the measured traits was the function of genetic and environmental variance; however, we are focusing only on the genetic variance component to understand the extent of genetic contribution to improving faba bean desirable traits.

The current investigation showed that the highest GCV and PCV were obtained from the trait thousand seed weight (TSW) and number of pods per plant (PPP), respectively. However, the lowest GCV and PCV were recorded from 50% to flowering (DF) and days to maturity (Table 4). According to [19], the phenotypic coefficient of variation and genotypic coefficient of variation reveal the extent of variability present for different characters and are used to measure the amount of genetic variation that exists in a given population but not the heritable portion of variability. All traits except days to maturity and the number of seeds per pod have a higher PCV than GCV; this indicates the role of environment on the expression of genes for a particular trait. In plant breeding, effective parent or trait selection is a crucial step; hence, evaluating genotypic and phenotypic coefficients of variation with heritability helps to assess the role of genes in the expression of each trait phenotypic character.

The GCV and PCV values delineate small (<10%), medium (10%–20%), and high (>20%) levels of variation. Analysis of the data reveals that days to flower, days to maturity, and rust disease exhibited lower GCV, whereas the number of seeds per pod and rust displayed the highest phenotypic variation (PCV). Seed weight and number of pods per plant had intermediate GCV, while the other traits showed the lowest PCV (Table 4). This suggests that the traits exhibit variation,

which can only be improved through hybridization or mutagenesis. The medium-to-high PCV and GCV values indicate the presence of genetic variability and good opportunities for selection, while the lowest PCV and GCV results indicate that traits may not improve through direct selection.

The heritability in the broad sense $(H²B)$ spans from 7.3% for traits such as rust to 99% for traits like grain yield per hectare or 1000-seed weight (Table 4). Heritability, in a broad sense, holds significance for plant breeders as it delineates the potential and scope of genetic enhancement through selection. According to [20] the highest heritability (>60%) was discerned in traits such as days to heading and grain yield, while the lowest heritability was documented for plant height. This signifies the feasibility of selection based on phenotypic performance, given the minimal impact of the environment. High heritability estimates along with high genetic advance are more helpful in predicting gain under selection than heritability alone. In the present investigation, genetic advance as a percent of the mean varied from 0.27% for days to flowering to 34.25% for thousand seed weights (Table 4). These results indicate that whenever the best 5% of accessions are selected for a given trait, the genotypic value of the new population for the trait will improve from 0.27% to 34.25%. A high genetic advance was recorded for seed weight and grain yield. These traits also had high and medium broad sense heritability, respectively; consequently, the traits of 1000 seed weight could be improved through direct selection. [21], stated that traits with high broad sense heritability and high genetic advance are more influenced by genetic factors.

The finding agrees with that of [22] and [23], who report high heritability and high genetic advance for the number of pods per plant. Similar results were reported by [17] for the number of pods per plant, biomass weight per plot, and thousand-seed weight. A low estimate of heritability and a low genetic advance were observed for days for flowering and rust disease, which may be due to a lack of genetic variability for that trait. In such a situation, advancement in this trait through usual selection may not be effective. However, recombination breeding and recurrent selection advocate for the improvement of traits. A similar finding was reported by [3] with a low estimate of heritability and genetic advance and disease reaction. Medium GAM values were obtained for traits like pod length and days to flowering. On the contrary, the lowest GAM was recorded for plant height. The result indicates that selection of accessions based on this trait might result in a new response in a new population. In other ways, the expression of this trait is governed by additive gene effects.

Traits	σ^2 g	$H^2B\%$	$\sigma^2 \mathbf{p}$	GA	$GAM\%$	$\mathbf{GCV}\%$	$PCV\%$	$CV\%$	SE
DF	1.37	35.1	39.08	3.39	0.27	1.24	1.86	2.90	3.80
DM	6.97	31.2	22.37	3.04	2.03	1.76	3.16	2.62	15.40
PH	19.44	60.9	31.94	7.10	6.19	3.85	4.93	3.09	12.51
PPP	3.16	63.2	5.00	2.91	25.64	15.64	19.67	11.94	1.84
SPP	0.09	24	0.38	0.30	10.34	10.33	21.29	18.62	0.29
Rust	0.13	7.3	1.78	0.19	4.92	8.98	33.86	32.64	1.66
TSW	24905.74	99	25025.58	324.79	34.25	16.64	16.68	1.15	119.84
GYH	220619.26	99	221706.85	966.61	26.81	13.03	13.06	0.91	1087.59

Table 4: Genetic parameters for eight traits for 36 Faba bean advanced genotypes conducted across two locations in 2023

σ ²g = genetic variance, σ²p = Phenotypic variance, GCV% = Genotypic coefficient of variance, PCV% = Phenotypic Coefficient of variance, H²B% = Heritability in broad sense, GA = Genetic Advance, GAM% = genetic advance as percent of mean, GYH = Grain yield per hectare, SE = Standard error, CV % = Coefficient of Variation

3.4 Genotype by Trait Biplot Analysis

A genotype-by-trait biplot is a valuable tool for analyzing the relationships among different traits that are considered breeding objectives. This analysis helps in identifying traits that are positively or negatively associated with each other, pinpointing traits that are redundantly measured, and determining which traits can be used for indirect selection of another trait.

Genotype by trait (GT) biplot analysis can be applied to assess the superiority genotypes based on multiple traits, and identify the superior genotypes by recognizing the improvement of target traits in the breeding programs [24]. Genotype by Trait biplot of 36 Faba bean genotypes is presented in Figure 1. GT biplot is constructed from Genotype by Trait mean data in Table 3. The vectors of the GT biplot were drawn based on the original biplot data for every trait, to

investigate the relationship between traits and used to visualize the associations among traits and the trait profiles of the genotypes [24, 25].

The correlation between traits can be determined by examining the angle between their vectors. A smaller angle than 90° indicates a positive correlation, while a greater angle than 90° indicates a negative correlation. The vectors of TSW and DM, PH and TSW, Seed Yield per Hectare (SYPH) and Plant Height (PH), between PPL vs. SPP and SPP vs. Rust, formed angles smaller than 90°, suggesting a positive correlation between these traits (Figure 1). These findings align with similar results reported by previous researchers [26]. Conversely, the vectors of Rust and SYPH, and DM and SYPH traits formed angles greater than 90°, indicating a negative correlation between these traits.

The magnitude of the vector length for each trait indicates the level of representativeness or association with the variation in genotypes located in the quadrant or their respective sectors. In accordance with the findings of this study, genotypes G21, G14, and G7 exhibited notable variation with respect to faba bean rust, as depicted in figure 1b. Similarly, the traits of seed yield per hectare (SYPH), Number of pods per plant (PPP), and number of seeds per pod (SPP) were more effectively represented by the respective genotypes in the sector. However, seven genotypes (G22, G2, G13, G15, G23, G10, and G11) were not fully accounted for by all traits.

Figure 1 The contribution of seven traits of faba bean for PC1 and PC2 variation figure (a), and genotype by traits biplot (GT) graphical display figure (b)

Conclusion

Knowing genetic variability is crucial for comprehending the genetic background and inheritance of various traits for effective crossing in crop improvement. The slightly higher phenotypic coefficient of variation (PCV) compared to the genotypic coefficient of variation (GCV) suggests the presence of environmental effects that may limit the association of trait inheritance. The findings highlight the valuable traits or characters used as a parent material for faba bean breeding program in Ethiopia.

Authors Contribution

All authors contributed to the study's conception, design, material preparation, data collection, and analysis. Gebeyaw Achenef wrote the first draft of the manuscript, and all authors approved the final version.

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CITATION

Gebeyaw A., Temesgen A., & Deressa T. (2024). Genetic Variability and Character Association for Advanced Faba bean (Vicia faba L.) Genotypes in Southeastern Ethiopia. In Global Journal of Research in Humanities & Cultural Studies (Vol. 4, Number 6, pp. 76–83). https://doi.org/10.5281/zenodo.14286726

