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Genetic Variability, Heritability, Genetic Advance, Correlation, and Path-Coefficient Analysis for Yield-Attributing Traits in Chia (*Salvia hispanica* L.)

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Abstract

Chia (*Salvia hispanica* L.) is a promising multifunctional crop renowned for its numerous health benefits. It is essential to comprehend the genetic variability of chia and how various variables impact seed yield to increase its genetic improvement under the agro-climatic conditions of Bangladesh. A field experiment was conducted using eight chia genotypes to assess genetic diversity, heritability, and genetic advance (GA), as well as the correlation coefficients for eight factors and their level of association with yield. Analysis of variance results showed significant variation for all the traits, i.e., the number of branches per plant, the number of inflorescences per plant, the length of the main inflorescence, the number of seeds per floret, and seed yield per plant, which differed significantly from one another. GPBC 1, BAU Chia 2, BAU Chia 1, and GPBC 3 were found to be promising genotypes for yield-attributing traits. Higher genotypic and phenotypic coefficient of variation was observed for seed yield per plant and the number of inflorescences per plant. High heritability coupled with high GA% was recorded for the number of inflorescences per plant and seed yield per plant. Seed yield per plant was positively correlated with the number of inflorescences per plant, the length of the main inflorescence, and plant height, while being negatively correlated with days to first flowering and days to maturity. Plant height and the number of inflorescences per plant had the highest direct positive effect on seed yield per plant as revealed through path analysis. The first four principal components contributed 84% of the total variation. The results of the current research may help choose better genotypes and traits for chia breeding initiatives to increase yield.

Keywords: chia, genetic parameters, principal component analysis, yield

Introduction

Chia (*Salvia hispanica* L.) is a self-pollinating annual herbaceous plant belonging to the Lamiaceae family. It is renowned for having the highest concentration of omega-3 fatty acids among plant species and can thrive in a variety of climates, ranging from tropical to desert regions (Zare et al., 2024). The oil extracted from chia seeds has industrial applications and is highly valued for its nutritional properties. Chia was native to central Mexico and northern Guatemala (Ayerza, 1995) and was a staple in the diets of pre-Columbian civilizations, such as the Aztecs and Mayans, who also utilized it in traditional medicine (Rasha et al., 2020). It was domesticated 3,500 years BC (Ayerza and Coates, 2005). In 1991, a collaborative project initiated by Argentinian researchers and the US government facilitated the expansion of chia cultivation beyond Mexico to thirteen other countries. This initiative sparked a new era of scientific research into the plant's growth, characteristics, and potential applications (Sosa-Baldivia et al., 2018). Due to its exceptional nutritional qualities and resilience, chia is emerging as a promising crop to address food security and climate change challenges, with its cultivation rapidly spreading to new regions (Kirsch et al., 2024; Amer et al., 2025).

As food security and nutritional security are the primary challenges of humanity in today's world, the clamor for 'functional food' with several health benefits has been dramatically increasing day by day (John and Singla, 2021; Panghal et al., 2022). In recent years, chia seeds have gained significant popularity, driven by an increasing focus on health and well-being. Chia seed engenders a multitude of health benefits, including controlling type-II diabetes and blood pressure, role in weight loss, reduction of the risk of cardiovascular disease, anti-inflammatory properties (Cicero-Sarmiento et al., 2023; Saadh et

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al., 2024; Kamrul-Hasan et al., 2024; Karimi et al., 2024), reducing postprandial glycemia (Ho et al., 2013; Mihafu et al., 2020), prevention of cancer (Ali et al., 2024), maintaining a balanced serum lipid profile etc. (Rasha et al., 2020; Nikpayam et al., 2023). The word “chia” is derived from the Spanish term ‘Chian’, meaning oily (Motyka et al., 2023). Chia seeds possess remarkable potential as oilseeds, containing higher amounts of polyunsaturated fatty acids. Approximately 60% of these lipids are comprised of omega-3 fatty acids and 20% of omega-6 linoleic acid (Nahar, 2024) that are beyond the body’s synthesis and renowned for their positive impact on heart health, diabetes management, inflammatory diseases, dyslexia, depression and overall well-being (Huang and Zhao, 2022; Machaj et al., 2025; Xu et al., 2025). Thada et al. (2021) reported that chia seeds contain remarkable nutritional components, including 16.54% gluten-free proteins, 31% fat, 42.14% carbohydrates, 23-41% dietary fiber, as well as magnesium, zinc, iron, calcium, and phosphorus. It is regarded as a superfood with an abundance of vitamins, such as vitamin B, particularly niacin (59% DV), thiamine (54% DV), folate (12% DV), and riboflavin (14% DV). Furthermore, chia seeds contain antioxidants such as myricetin, quercetin, kaempferol, chlorogenic acids, and caffeic acid (Vera-Cespedes et al., 2023; Anwar et al., 2024; Ogunnowo et al., 2024), which protect against heart and neurological diseases by preventing fat peroxidation and fighting free radicals (Grancieri et al., 2019). This nutrient-rich profile highlights the importance of chia as a valuable dietary resource for promoting general health and well-being. Additionally, the European Parliament and the Council of Europe have designated chia as a novel food because it has no harmful, anti-nutritional, or toxic effects on the human body (Muñoz et al., 2013).

Considering the challenges posed by climate change to food systems, crops like chia that can withstand changing conditions are more important than ever. In Bangladesh, one of the world’s most climate-vulnerable countries, chia could be a game-changer. Its tolerance to acidic soil and drought stress makes it suitable for marginal lands affected by adverse climate conditions (Umesh et al., 2019; Fghire et al., 2022), which will help to increase cropping intensity and, in turn, boost the country’s GDP. The successful integration of chia in a new geographical area requires identifying the genetic differences among cultivars and evaluating their agronomic potential specific to that region. This is because the phenotypic expression of different germplasm can vary significantly with changes in environmental conditions (AlKhamisi et al., 2021).

Chia was first introduced to Bangladesh in 2010, as documented by Azad et al. (2017). Though initial research efforts, such as those by Karim et al. (2015) on optimal planting times, have provided valuable insights, this study represents only a fraction of what is needed. The primary challenges of chia cultivation in Bangladesh are the limited availability of high-yielding varieties and a lack of public awareness. Successful variety development requires breeders to evaluate a diverse range of cultivars and genotypes, understanding their variability to identify superior genotypes (Azam et al., 2023). Genotypic and phenotypic variances, along with their coefficients of variation, provide critical insights into how genetic makeup interacts with environmental factors to influence crop traits (Johnson et al., 1955; Azam et al., 2014; Sarker et al., 2022). This information enables breeders to prioritize traits for selection, laying the foundation for successful crop improvement programs. A key aspect of breeding programs is to understand the heritability and genetic advances of traits. Since the selection of parental lines is dependent on the degree to which desirable qualities are heritable, it is imperative to separate observed variability into heritable and non-heritable components (Robinson et al., 1949). Yield, being a complex trait, is influenced by multiple morphological characteristics. To determine the connections between yield and the factors that contribute to it, as well as the interactions between these traits, correlation analysis is essential (Risi and Galwey, 1989). This analysis helps breeders determine which relationships are most important for explaining yield potential. However, simple correlations often fail to fully explain the role of individual traits in determining yield. To address this, correlation coefficients are divided into direct and indirect effects using path coefficient analysis. This method provides a clearer understanding of how each trait directly influences yield and how indirect effects arise from associations among traits (del Moral et al., 2003). Additionally, multivariate analyses such as Principal Component Analysis (PCA) are invaluable for identifying key traits that contribute most to overall variation within the dataset. Considering the above facts, the primary objectives of this study were to examine the genetic divergence and heritability of seed yield and major agronomic traits in eight chia genotypes, to identify traits positively correlated with yield, and to select the best lines for variety development.

Materials and Methods

Experimental Materials and Sources

The experiment was carried out using eight genotypes, including two varieties: BAU Chia 1, a drought-tolerant variety, and BAU Chia 2. BAU Chia 1 and BAU Chia 2 were collected from the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh-2202. Genotypes GPBC 1, GPBC 2, GPBC 5, and GPBC 6 were obtained from the Department of Genetics and Plant Breeding of the same university. GPBC 3 and GPBC 4 were sourced from the Department of Agronomy, Bangladesh Agricultural University, Mymensingh-2202.

Experimental Site, Soil, and Season

Between November 2022 and January 2023, the field experiment was conducted at the Department of Genetics and Plant Breeding's Field Laboratory, located at Bangladesh Agricultural University in Mymensingh. The experimental location is situated in Agro-Ecological Zone 9 (Old Brahmaputra Floodplain) of the Sonatota series of gray floodplains. It is distinguished by sandy loam soil that has a pH between 6.5 and 6.7. There are noticeable seasonal differences in the area, with the Kharif (summer) season, which runs from April to October, bringing high temperatures and a lot of rainfall, and the Rabi (winter) season, which runs from November to March, bringing colder temperatures and less rainfall.

Design and Layout of the Experiment

Three replications of the experiment were carried out using a randomized complete block design. It had eight experimental units per block. Plots were 7.5 m² (3 m × 2.5 m), with eight rows per plot. The distance between plants inside each row was 2.5 cm, while the distance between rows was 45 cm.

Land Preparation and Sowing

A power tiller was used to plough and cross-plough the experimental field. The land was prepared by clearing the rubbish and weeds. The soil was then leveled and brought to an appropriate tilth through proper laddering. Seeds were sown using the line sowing method in moist soil on November 14, 2022. Seeds were sown shallowly (1–1.5 cm) and lightly covered with the fine soil. After sowing, the seedlings were allowed to grow under field conditions with proper care and management.

Fertilizer Application

Except for urea, all fertilizers were applied in full quantity during the soil preparation process. Half of the urea was applied at 25-30 days after sowing. The fertilizer doses for the experiment were urea-TSP-MoP-gypsum-zinc-boron at 70-100-40-40-2-2 kg per acre, respectively.

Only one irrigation was necessary for chia cultivation, applied 30 days after sowing, approximately at the flowering stage. Weeding and thinning were performed once, just before irrigation at 25 days after sowing, to ensure proper plant spacing of 2.5–5 cm.

Data Collection

Eight quantitative traits, such as days to first flowering, days to maturity, plant height (cm), number of branches per plant, number of inflorescences per plant, length of the main inflorescence (cm), number of seeds per floret, and seed yield per plant (g) were measured from ten randomly selected plants from each plot. To reduce border effects, data were collected from the central rows of each plot. This systematic random sampling ensured uniform representation of each genotype for trait evaluation.

Data Analysis

One-way analysis of variance (ANOVA) was used to identify differences across treatments, and Tukey's means comparison test was applied with a significance threshold of 5%. R Statistical Package version 4.3.1 was used for data analysis. One-way ANOVA was executed for eight quantitative traits to explore genotypic effects by using the following model, $Y_{ij} = g_i + r_j + \varepsilon_{ij}$, where Y_{ij}= observation of genotype i in replication j, g_i= effects of genotype i, r_j= effects of replication j, and ε_{ij}= the residual error of genotype i in replicate j.

Estimation of Genetic Parameters

The following genetic parameters were estimated to determine the genetic variability among genotypes and evaluate the genetic and environmental effects on the studied variables.

Genotypic and phenotypic variances

Genotypic and phenotypic variances were estimated using the formula provided by Johnson et al. (1955).

Genotypic variance, $\sigma^2_g = \frac{GMS-EMS}{r}$

Where, GMS = Genotypic mean square, EMS = Error mean square, r = Number of replications

Phenotypic variance, $\sigma^2_p = \sigma^2_g + EMS$
Where, σ^2_g = Genotypic variance, EMS = Error mean square

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Estimation of heritability

Heritability in the broad sense (h^2_b) was estimated according to the formula suggested by Johnson et al. (1955).

Heritability, $h^2_b = \frac{\sigma^2 g}{\sigma^2 p} \times 100$

Where, σ^2_g = Genotypic variance, σ^2_p = Phenotypic variance

Estimation of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV)

Genotypic and phenotypic coefficients of variations were estimated according to Hill (1978).

Genotypic coefficient of variation, GCV = $\frac{\sqrt{\sigma^2 g}}{\overline{X}} \times 100$

Where, σ^2_g = Genotypic variance, $\overline{X}$ = Population mean

Phenotypic coefficient of variation, PCV = $\frac{\sqrt{\sigma^2 p}}{\overline{X}} \times 100$

Where, σ^2_p= Phenotypic variance, $\overline{X}$ = Population mean

Estimation of genetic advance

Estimation of genetic advance was done following the formula given by Johnson et al. (1955).
Genetic advance, GA=h^2_b. K. σ_p

Where, h^2_b = Heritability in the broad sense, K = Selection differential, the value of which is 2.06 at 5% selection intensity, σ_p = Phenotypic standard deviation

Estimation of genetic advance in percentage of the mean

Genetic advance in percentage of mean was calculated by the formula of Comstock et al. (1952) as follows:

Genetic advance in percentage of mean, GA (%) = $\frac{GA}{\overline{X}} \times 100$

Where, GA = Genetic advance, $\overline{X}$ = Population means

Estimation of the correlation coefficient

The phenotypic and genotypic correlation coefficients were estimated by the formula suggested by Miller et al. (1958).

Phenotypic correlation, $r_{p1.2} = \frac{\mathrm{CoV.\,p1.2}}{\sqrt{\sigma^2 p_1 x \sigma^2 p_2}} x100$

Where, CoV.$p_{1.2}$=Phenotypic covariance between the trait x_1 and x_2, $\sigma^2 p_1$=Phenotypic variance of the trait x_1, $\sigma^2 p_2$ =Phenotypic variance of the trait x_2.

Genotypic correlation, $r_{g1.2} = \frac{CoV.g_{1.2}}{\sqrt{\sigma^2 g_1 \times \sigma^2 g_2}} \times 100$

Where, CoV.$g_{1.2}$= Genotypic covariance between the trait x_1 and x_2, $\sigma^2 g_1$= Genotypic variance of the trait x_1, $\sigma^2 p_2$ = Genotypic variance of the trait x_2.

Path coefficient analysis

Path analysis was made by using the following formula given by Dewey and Lu (1959):

$$rij = Pij + \Sigma rikpkj$$

where, rij = mutual association between the independent character (i) and dependent character (j) as measured by the correlation coefficients
Pij = direct effects of the independent character (i) on the dependent variable (j) as measured by the path coefficients
Σrikpkj = summation of components of indirect effects of a given independent character (i) on a given dependent character (j) via all other independent traits (k)
Residual factors (R), which represent the unexplained variance in a dependent variable, was estimated by using Chaudhary and Singh (1985) method:

$$R = \sqrt{1} - \Sigma pijrij$$

Principal component analysis (PCA)

PCA analysis was conducted using the R Statistical Package version 4.3.1.

Results

Analysis of Variance

The results of the analysis of variance for eight quantitative traits studied across eight chia genotypes are presented in Table 1. The mean square values for genotypes indicated highly significant variation ($p<0.001$) for five traits (days to maturity, plant height, number of branches per plant, number of inflorescences per plant, and seed yield). Additionally, two traits (days to first flowering and number of seeds per floret) showed significant variation at the $p<0.01$ level, and only one trait, length of the main inflorescence, showed significant variation at the $p<0.05$ level. All the characters under study showed highly significant variation.

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Mean Performance of Different Traits of Chia Genotypes

The mean performance of eight traits across eight chia genotypes is presented in Table 2. Among the studied genotypes, GPBC 2 and GPBC 1 emerged as the earliest flowering genotypes, requiring the fewest days to flower (56.66 days and 58.67 days, respectively). In contrast, GPBC 5 and GPBC 6 required the highest days to flower, with durations of 64 and 63.33 days, respectively. Regarding maturity, GPBC 2, GPBC 1, BAU Chia 1, and BAU Chia 2 were the earliest maturing genotypes, requiring 107.33, 108.33, 109.66, and 110.66 days, respectively. On the other hand, GPBC 6, GPBC 5, and GPBC 3 exhibited the longest times to maturity, taking 115.66, 114.66, and 114.33 days, respectively; however, the differences among the genotypes were non-significant. In terms of plant height, the tallest plants were observed in BAU Chia 2 (144.47 cm), followed by GPBC 3 (141.85 cm), while the shortest plants were recorded in GPBC 5 (124.33 cm), followed by GPBC 4 (131.19 cm) and GPBC 1 (132.09 cm), which are statistically similar. The highest number of branches per plant was observed in GPBC 6 (14.10), followed by GPBC 1 (13.13) and GPBC 2 (12.66). In contrast, the lowest were recorded in GPBC 4 (10.76) and BAU Chia 1 (10.99), where the differences were statistically non-significant. For the number of inflorescences per plant (NIP), GPBC 1 recorded the highest value (57.09), followed by GPBC 2 (48.76). On the contrary, the lowest number of inflorescences per plant values were found in GPBC 4 (37.35), GPBC 5 (38.33), and BAU Chia 1 (38.42), which are statistically similar. Regarding the length of the main inflorescence (LMI), GPBC 1 exhibited the highest value (21.46 cm), while the lowest values were recorded in BAU Chia 1 (16.68 cm) and GPBC 6 (17.00 cm). The highest number of seeds per floret (NSF) was found in BAU Chia 1 (3.56), whereas GPBC 2 and GPBC 3 exhibited the lowest value (2.66). A significant variation was observed for the trait seed yield per plant, where the genotype GPBC 1 and BAU Chia 2 showed the best performance (11.00 g and 10.28 g, respectively), followed by BAU Chia 1 (9.24 g) and GPBC 3 (9.10 g); however, the differences among the genotypes are non-significant. On the contrary, the lowest seed yield per plant was recorded in GPBC 5 (5.72 g) and GPBC 6 (6.66 g).

Genetic Parameter Analysis

Genotypic and phenotypic variance, genotypic and phenotypic coefficient of variance (GCV and PCV), heritability, genetic advance (GA), and GA% are shown in Table 3. In this study, the highest magnitudes of genotypic and phenotypic variance were observed for the trait plant height (44.82 and 46.27, respectively) and number of inflorescences per plant (44.33 and 45.95, respectively). At the same time, the lowest were recorded for the number of seeds per floret (0.07 and 0.10, respectively) and the number of branches per plant (NBP) (1.17 and 1.51, respectively). The coefficient of variation analysis indicated that the phenotypic coefficient of variation (PCV) values exceeded the genotypic coefficient of variation (GCV) values for all traits, suggesting an environmental influence. Among the characteristics, high GCV and PCV values were recorded for seed yield per plant (21.50 and 23.41, respectively), and moderate values were recorded for the number of inflorescences per plant (14.86 and 15.13, respectively). Conversely, the lowest genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) values were found for days to maturity (2.78 and 2.97, respectively), days to first flowering (2.79 and 4.50, respectively), and plant height (4.91 and 4.99, respectively). Notably, plant height showed the smallest difference between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), but the length of the main inflorescence showed the largest. The majority of the characteristics under study had high broad-sense heritability (h^2_b), while the length of the main inflorescence had a moderate level of heritability (h^2_b = 0.43). Genetic advance as a percentage of mean was high for the traits seed yield per plant (40.67%) and number of inflorescences per plant (30.07%), moderate for number of branches per plant (16.20%) and number of seeds per floret (14.58%), and low for days to maturity (5.36%), days to first flowering

Table 1. Analysis of variance (mean square) for different growth and yield contributing traits of eight chia genotypes

Sources of variation	Days to first flowering	Days to maturity	Plant height	Number of branches per plant	Number of inflorescences per plant	Length of main inflorescence	Number of seeds per floret	Seed yield per plant
Replication	16.66	30.37	135.92	3.859	134.61	6.991	0.251	10.24
Genotype	1.167**	3.042***	0.146***	0.283***	0.904***	0.005*	0.045**	0.756***
Error	2.881	1.375	1.447	0.345	1.625	2.099	0.037	0.598

Notes: *, **, and *** indicate significant differences at 5%, 1% and 0.1% level of probability, respectively.

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(5.70%), length of main inflorescence (9.38%), and plant height (9.96%). Importantly, the number of inflorescences per plant and seed yield per plant demonstrated both high heritability and high genetic advance percentage (GA%).

Phenotypic and Genotypic Correlation Coefficients of Yield and Yield-Attributing Traits

The results of phenotypic and genotypic correlation coefficients of yield and yield-attributing traits of chia are presented in Table 4. According to the correlation study, for the majority of the traits, the genotypic correlation coefficients were greater than the phenotypic correlation coefficients. From the result, it is found that seed yield per plant (SYP) has a significant positive correlation with length of main inflorescence (LMI) (r_p=0.5005* and r_g=0.7559*) both at the phenotypic and genotypic level, and with plant height (PH) (r_p=0.463*) and number of inflorescences per plant (NIP) (r_p=0.6127**) only at the phenotypic level. On the contrary, it showed significant negative correlation with days to first flowering (DFF) (r_p=-0.4151*) and days to maturity (DM) (r_p=0.502*) only at the phenotypic level. Length of main inflorescence (LMI) exhibited a significant positive correlation with number of inflorescences per plant (NIP) (r_p=0.6719** and r_g=1.0152**), both at phenotypic and genotypic levels, and a negative correlation with days to first flowering (DFF) (r_g=-0.7176*) only at the genotypic level. Number of inflorescences per plant (NIP) had a significant positive correlation with number of branches per plant (NBP) (r_p=0.6153**) and a negative correlation with days to first flowering (DFF) (r_p=-0.448*) and days to maturity (DM) (r_p=-0.455*) only at the phenotypic level. There was a significant

Table 2. Mean performance of eight different traits of eight chia genotypes

Genotypes	Days to first flowering	Days to maturity	Plant height	Number of branches per plant	Number of inflorescences per plant	Length of main inflorescence	Number of seeds per floret	Seed yield per plant
BAU Chia1	60.33 ABC	109.66C	140.99B	10.99C	38.42D	16.68B	3.56A	9.24AB
BAU Chia2	60.67 ABC	110.66BC	144.473A	11.80BC	45.947BC	19.13AB	3.12AB	10.28A
GPBC 1	58.67 BC	108.33C	132.09D	13.13AB	57.09A	21.46A	3.12AB	11.00A
GPBC 2	56.66C	107.33C	135.76C	12.66AB	48.76B	18.92AB	2.66B	7.42BC
GPBC 3	61.33 AB	114.33A	141.85AB	11.71BC	47.62BC	19.39AB	2.66B	9.10 AB
GPBC 4	60.33 ABC	113.66AB	131.19D	10.76C	37.35D	18.02AB	3.04AB	7.26BC
GPBC 5	64.00A	114.66A	124.33E	11.52BC	38.33D	17.84AB	3.16AB	5.72C
GPBC 6	63.33A	115.66A	139.47B	14.10A	44.75C	17.00B	3.04AB	6.66C

Notes: Values are means of three replications (n=10). Different letters in each column indicate significant difference at $p<0.05$ according to Tukey's test.

Table 3. Estimation of genetic parameters for morphological traits related to yield in eight chia genotypes

Characters	Genotypic variance (σ^2_g)	Phenotypic variance (σ^2_p)	GCV (%)	PCV (%)	Broad sense heritability (h^2_b) (%)	GA	GA (%)
Days to first flowering	4.59	7.47	2.79	4.50	61	3.46	5.70
Days to maturity	9.66	11.04	2.78	2.97	87	5.99	5.36
Plant height	44.82	46.27	4.91	4.99	96	13.57	9.96
Number of branches per plant	1.17	1.51	8.95	10.18	77	1.95	16.20
Number of inflorescences per plant	44.33	45.95	14.86	15.13	96	13.47	30.07
Length of main inflorescence	1.63	3.73	6.88	10.40	43	1.74	9.38
Number of seeds per flower	0.07	0.10	8.74	10.80	65	0.44	14.58
Seed yield per plant	3.21	3.81	21.50	23.41	84	3.39	40.67

Notes: GCV= genotypic coefficient of variance; PCV= phenotypic coefficient of variance; GA=Genetic advance; GA (%)= genetic advance as percentage of mean.

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positive correlation between days to first flowering (DFF) and days to maturity (DM) (r_p=0.8071** and r_g=0.9431**), both at phenotypic and genotypic levels, whereas days to first flowering (DFF) showed a significant negative correlation with number of inflorescences per plant (NIP) (r_p=-0.448*) and seed yield per plant (SYP) (r_p=-0.4151*) at phenotypic level and with length of the main inflorescence (LMI) (r_g=-0.7176*) at genotypic level.

Phenotypic and Genotypic Path Coefficient Analysis

The results of phenotypic and genotypic path coefficient analysis, indicating the relationship between yield and yield-attributing traits, are presented in Table 5. From the partitioning of phenotypic and genotypic correlations into direct and indirect effects of traits related to yield using path analysis, it is revealed that the number of inflorescences per plant (r_{yp}= 1.0485 and r_{yg}= 2.0574) exhibits a higher positive direct effect on seed yield per plant (SYP). This trait also showed a significant positive correlation with seed yield per plant (SYP). The days to maturity (DM) (r_{yp}=0.1454 and r_{yg}=0.4290), plant height (r_{yp}=0.3391 and r_{yg}=0.3514), and number of seeds per floret (r_{yp}=0.4137 and r_{yg}=0.6689) demonstrated a direct positive effect on seed yield per plant. On the contrary, days to first flowering (DFF) (r_{yp}=-0.2053 and r_{yg}=-0.0493), number of branches per plant (NBP) (r_{yp}=-0.5140 and r_{yg}=-1.1466), and length of main inflorescence (r_{yp}=-0.0839 and r_{yg}=-0.2584) had a direct negative effect on seed yield per plant.

Principal Component Analysis

The results of the principal component analysis (PCA) for eight quantitative traits across eight chia genotypes are presented in Table 6. The analysis revealed that the first four principal components (PCs) had eigenvalues greater than one, cumulatively explaining 84% of the total variation among the studied genotypes. The first PC accounted for 39.80% of the total variation, where the number of inflorescences per plant had the highest positive loading (0.49), followed by the seed yield per plant (0.42), length of the main inflorescence (0.36), number of branches per plant (0.23), and plant height (0.18). The second PC accounted for 18.2% of the total variation, mostly by number of seeds per floret (NSF), number of branches per plant (NBP), seed yield per plant (SYP), days to maturity (DM), number of inflorescences per plant (NIP), and plant height (PH). The third and fourth components of the PCA accounted for only 13.9% and 12.7% of the total variation, respectively. According to the biplot (Figure 1), it is evident that genotype 5 (GPBC 3) is the most stable, as it is the closest to the origin, while genotypes 3 (GPBC 1), 1 (BAU Chia 1), and 7 (GPBC 5) are the least stable. From the biplot, it appears that genotypes 3 (GPBC 1), 4 (GPBC 2), and 5 (GPBC 3) had the highest values for the number of branches

Table 4. Correlation coefficients of yield and yield contributing traits

Characters		Days to maturity	Plant height	Number of branches per plant	Number of inflorescences per plant	Length of main inflorescence	Number of seeds per floret	Seed yield per plant
Days to first flowering	r_p	0.8071**	-0.1436	-0.1381	-0.448*	-0.267	0.2373	-0.4151*
	r_g	0.9431**	-0.1611	0.0667	-0.5442	-0.7176*	0.3065	-0.507
Days to maturity	r_p		-0.1208	-0.0431	-0.455*	-0.2958	-0.0257	-0.502*
	r_g		-0.1382	-0.0253	-0.513	-0.5857	-0.0942	-0.6042
Plant height	r_p			0.0918	0.1658	-0.0513	-0.0463	0.463*
	r_g			0.1161	0.1862	-0.0984	-0.0396	0.5489
Number of branches per plant	r_p				0.6153**	0.1234	-0.2753	0.0602
	r_g				0.6619	0.3315	-0.2955	-0.0416
Number of inflorescences per plant	r_p					0.6719**	-0.3513	0.6127**
	r_g					1.0152**	-0.4523	0.6058
Length of main inflorescence	r_p						-0.125	0.5005*
	r_g						-0.6635	0.7559*
Number of seeds per floret	r_p							0.1278
	r_g							0.1793

Notes: * and ** indicate significant differences at 5% and 1% level of probability, respectively.

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per plant, the number of inflorescences per plant, and the length of the main inflorescence. Genotype 2 (BAU Chia 2) had the highest value for seed yield per plant and plant height. The number of seeds per floret was higher in genotype 1 (BAU Chia 1) and 6 (GPBC 4). Values for days to first flowering and days to maturity were higher in genotypes 7 (GPBC 5) and 8 (GPBC 6).

Discussion

The growing demand for chia to meet the high protein requirements of the increasing population indicates the urgency of developing new chia varieties. Genetic variability among existing genotypes is a prerequisite for effective genetic improvement. In the present study, the analysis of variance revealed significant differences among the studied genotypes for all the evaluated traits (Table 1). This indicates the presence of substantial genetic variability, providing a promising foundation for yield improvement through breeding programs. Other researchers have also reported consistently with our findings, significant variability in yield-contributing traits (Grimes et al., 2020; Thada et al., 2021).

The observed variability highlights the potential to exploit these traits for selection and hybridization, thereby accelerating the development of improved chia cultivars with higher yield and adaptability. On the other hand, the present study was conducted with a limited number of genotypes and under specific environmental conditions, which may restrict the generalization of results. Further multi-location and multi-season evaluations, along with molecular characterization, are necessary to validate these findings and better capture the full extent of genetic diversity in chia.

Mean Performance of Different Traits of Chia Genotypes

Chia is a short-day plant that typically flowers around 66 to 77 days after sowing, as reported by Njoka et al. (2024). From the mean performance presented in Table 2, it was observed that days to first flowering ranged significantly from 56.66 to 64 days, which is comparatively shorter than the findings of Rodríguez et al. (2022). A similar significant variation in days to first flowering was observed by Thada et al. (2021) while evaluating 26 genotypes. The days to maturity (DM) in our study ranged from 109.66 to 115.66 days, aligning with the results of Karim et al. (2016) and Rasha et al. (2020). Gravé et al. (2019) noted that chia seeds generally reach maturity within 27 to 35 days after flowering. Environmental conditions largely govern flowering and maturity in chia. Photoperiod is the key factor, as chia is a short-day plant that flowers under decreasing day length (Rodríguez-Abello et al., 2018; Hassani et al., 2022). Temperature strongly influences phenology, with optimum flowering observed at 30–31°C, while higher temperatures adversely affect development (Harisha et al., 2025). Additionally, relative humidity of 67%–72% and rainfall between 200–350 mm favor flowering, whereas prolonged bright sunshine hours exert negative effects (Harisha et al., 2025). Plant height is a critical growth parameter as it directly influences traits that contribute to overall production (Miao et al., 2024). In this study, the plant height of the chia genotypes varied from 124.33 to 144.47 cm. These findings are consistent with Karim et al. (2016), who reported average heights of 135.9 cm and 140 cm, respectively, for their cultivated chia genotypes. In contrast, Grimes et al. (2000) observed a comparatively shorter plant height, ranging from 103 to 115.6 cm. Recent studies suggest that plant height is influenced by sowing time, where early sowing results in taller plants and higher seed yields due to prolonged vegetative growth under favorable photoperiod and temperature conditions (Rodríguez-Abello et al., 2018). The number of branches per plant showed significant variability in this study, ranging from 10.76 to 14.10. Singh et al. (2023) concluded that the variation of the number of branches per plant (NBP) largely depends on plant spacing. The number of inflorescences per plant (NIP) exhibited significant variation among the studied genotypes, ranging from 38.33 to 57.09. The length of the main inflorescence also significantly varied, ranging from 16.68 to 21.46 cm. This agrees with the findings of Grimes et al. (2020), who reported length of main inflorescence (LMI) values between 16.0 and 19.5 cm. For yield-determining traits, the number of seeds per floret (NSF) ranged from 2.66 to 3.56, which aligns with Karim et al. (2016), who recorded number of seeds per floret (NSF) values ranging from 2.16 to 3.45 for their cultivated chia genotypes. The seed yield per plant (SYP) showed significant differences across genotypes, as supported by Cahill and Ehdaie (2005) and Thada et al. (2021). In this study, seed yield per plant (SYP) ranged from 5.72 to 11.00 g, which is notably higher than the findings of Karim et al. (2016), who reported a range of 0.61 to 4.71 g. The genotypes GPBC 1 and GPBC 3 had the highest yield per plant; however, they showed a non-significant difference with the cultivated varieties BAU Chia 1 and BAU Chia 2. This highlights the potential of the genotype to be used in developing a high-yielding chia variety.

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Table 5. Estimates of direct (bold diagonal) and indirect effects at both phenotypic and genotypic levels for different traits on seed per plant on chia genotypes

Characters		Days to first flowering	Days to maturity	Plant height	Number of branches per plant	Number of inflorescences per plant	Length of main inflorescence	Number of seeds per floret	Correlation coefficient
Days to first flowering	r_{yp}	**-0.2053**	0.1174	-0.0487	0.0709	-0.4697	0.0224	0.0979	-0.4151*
	r_{yg}	**-0.0493**	0.4046	-0.0566	-0.0764	-1.1197	0.1854	0.2050	-0.507
Days to maturity	r_{yp}	-0.1657	**0.1454**	-0.0409	0.0221	-0.4771	0.0248	-0.0105	-0.502*
	r_{yg}	-0.0465	**0.4290**	-0.0485	0.0290	-1.0555	0.1513	-0.0630	-0.6042
Plant height	r_{yp}	0.0295	-0.0175	**0.3391**	-0.0471	0.1738	0.0043	-0.0191	0.463*
	r_{yg}	0.0079	-0.0592	**0.3514**	-0.1331	0.3830	0.0254	-0.0265	0.5489
Number of branches per plant	r_{yp}	0.0283	-0.0062	0.0311	**-0.5140**	0.6451	-0.0103	-0.1137	0.0602
	r_{yg}	-0.0032	-0.0108	0.0408	**-1.1466**	1.3617	-0.0856	-0.1976	-0.0416
Number of inflorescence per plant	r_{yp}	0.0920	-0.0661	0.0562	-0.3163	**1.0485**	-0.0564	-0.1450	0.612**
	r_{yg}	0.0268	-0.2201	0.0654	-0.7589	**2.0574**	-0.2623	-0.3025	0.6058
Length of main inflorescence	r_{yp}	0.0548	-0.0432	-0.0174	-0.0632	0.7045	**-0.0839**	-0.0511	0.5005 *
	r_{yg}	0.0354	-0.2512	-0.0345	-0.3800	2.0887	**-0.2584**	-0.4438	0.7559
Number of seeds per floret	r_{yp}	-0.0487	-0.0037	-0.0157	0.1417	-0.3687	0.0104	**0.4137**	0.1278
	r_{yg}	-0.0151	-0.0404	-0.0139	0.3388	-0.9305	0.1714	**0.6689**	0.1793

Notes: * and ** indicate significant differences at 5% and 1% level of probability, respectively. Residual effect for phenotypic path-coefficient = 0.2081 & Residual effect for genotypic path-coefficient = 0.1773. r_{yp}= phenotypic path-coefficient and r_{yg}= genotypic path-coefficient.

Table 6. Principal components (PCs) for yield and yield-contributing traits in eight chia genotypes from PCA with Eigenvectors (loadings) of the first four principal components

Variable	PC1	PC2	PC3	PC4
Days to first flowering	-0.40	0.16	0.33	0.45
Days to maturity	-0.40	0.31	0.33	0.27
Plant height	0.18	-0.22	0.76	-0.31
Number of branches per plant	0.23	0.54	0.26	-0.02
Number of inflorescences per plant	0.49	0.28	0.06	0.19
Length of main inflorescence	0.36	0.08	-0.18	0.59
Number of seeds per floret	-0.14	-0.57	0.07	0.41
Seed yield per plant	0.42	-0.34	0.27	0.23
Eigenvalue	3.18	1.45	1.10	1.01
%Variation explained	39.80%	18.20%	13.90%	12.70%
Cumulative variance (%)	39%	58%	71%	84%

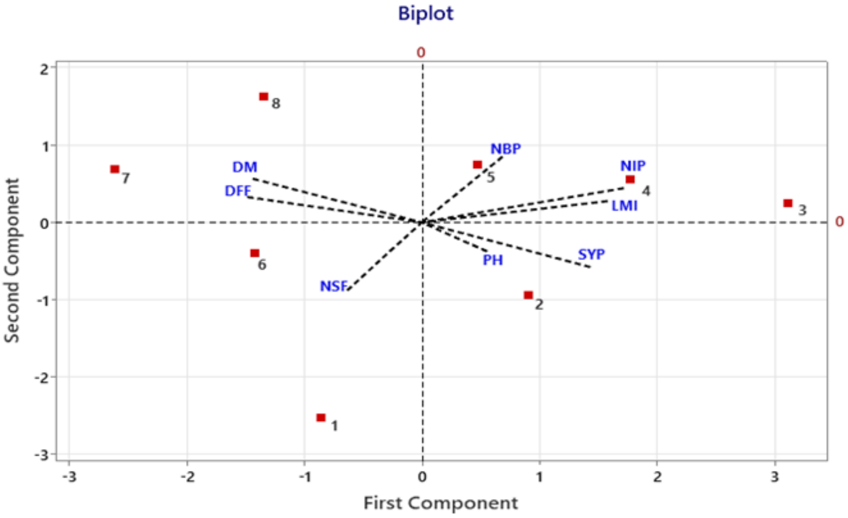

Figure 1. Biplot from Principal component analysis on eight chia genotypes. DFF= days to first flowering, DM= days to maturity, PH= plant height (cm), NBP= number of branches per plant, NIP= number of inflorescences per plant, LMI= length of main inflorescence (cm), NSF= number of seeds per floret, SYP= seed yield per plant (g).

Genetic Variability

The availability of genetic diversity and the inheritance patterns of the desired traits are key factors in crop breeding success. Breeders must analyze genetic variation to determine suitable breeding plans and create efficient selection criteria for enhancing desired features. In this study, the evaluated chia genotypes exhibited a wide range of variations, offering a broad genetic base for breeders to select superior and desirable genotypes. Phenotypic variance (σ^2p) is typically greater than genotypic variance (σ^2g) since it represents the combined effect of genotypic variance (σ^2g) and environmental variance (σ^2e) (Al-Naggar et al., 2017). In the present study, phenotypic coefficient of variation (PCV) was marginally greater than genotypic coefficient of variation (GCV) for every feature (Table 3), suggesting that the environment had a minimal effect on the expression of these traits (Meena et al., 2014). GCV and PCV values are classified as low (<10%), moderate (10–20%), and high (>20%) by Deshmukh et al. (1986). According to this category, seed yield per plant (SYP) had high genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) values in this study, whereas the number of inflorescences per plant had moderate values, indicating a substantial potential for selection-based genetic improvement. Similar findings were reported by Thada et al. (2021) and Cahill and Ehdaie (2005), who observed higher genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) values for NIP in their studied genotypes.

The heritability estimates (h^2_b) and genetic advance (GA) for eight quantitative traits are presented in Table 3. For breeders, heritability is a crucial factor because it allows genotypes to be chosen according to their phenotypic performance. Johnson et al. (1955) distinguished three levels of heritability: low (0%–30%), moderate (31%–60%), and high (>60%). High heritability indicates strong genetic control of a trait with minimal environmental influence, allowing for more effective selection (Islam et al., 2015; Roka et al., 2024). In this study, all traits exhibited high heritability except days to first flowering (DFF) and length of main inflorescence (LMI), which showed moderate heritability (Table 3). This indicates that selection and genetic improvement for these qualities will be very successful in the subsequent breeding efforts. These findings align with those of Thada et al. (2021) for most of the evaluated traits.

However, high heritability alone does not guarantee significant genetic gains. Johnson et al. (1955) emphasized that high heritability combined with high genetic advance as a percentage of the mean (GA%) is a more reliable indicator of genetic improvement, as it suggests that additive genetic factors primarily govern the trait and will respond well to selection. GA% can also be categorized as low (<10%), moderate (10%-20%), and high (>20%), as described by Johnson et al. (1955). In this study, the number of inflorescences per plant and seed yield per plant showed high GA%, while the number of branches per plant and the number of seeds per floret showed moderate GA%. Traits such as number of inflorescences per plant and seed yield per plant exhibited both high heritability and high GA%. These results align with the findings

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of Thada et al. (2021) and underscore the importance of additive genetic effects in these traits. Therefore, breeders can achieve significant genetic gains by actively selecting genotypes based on these traits, making them valuable targets for future improvement programs.

Correlation Analysis

Table 4 displays the phenotypic and genotypic correlation coefficients between seed yield and the characteristics that contribute to it. Yield is a complex quantitative trait that is dependent on the cumulative effects of several genes. It is influenced by various morphological characteristics, both directly and indirectly. Some of these traits are also related with themselves. Correlation analysis helps quantify the degree of association between different traits and yield, as well as among themselves. Genotypic correlation indicates the genetic relationships between traits. In contrast, phenotypic correlation reflects the overall observed association among the expression of traits, which both genetic and environmental factors can influence. Almost all the traits in the current study exhibited genotypic correlation coefficients that were marginally larger than their corresponding phenotypic correlation coefficients, indicating that genes were primarily responsible for trait expression and that the environment had very little influence.

In this study, seed yield per plant has shown a significant positive correlation with length of main inflorescence (LMI) both at genotypic and phenotypic levels. This result suggests that selecting plants with longer main inflorescences could be an effective strategy for enhancing seed yield. Additionally, seed yield per plant demonstrated a strong positive correlation with the number of inflorescences per plant and plant height, indicating that taller plants with a higher number of inflorescences contributed to increased seed yield. This aligns with the observations made by Thada et al. (2021), particularly regarding plant height. Nevertheless, their study found no significant correlation between seed yield per plant and either the number of inflorescences per plant or the length of main inflorescence. Instead, they found a strong positive correlation between seed yield per plant and number of branches per plant. These differences could be attributed to variations in genetic material and environmental factors. On the contrary, the negative correlation of seed yield per plant with days to first flowering and days to maturity suggests that early-maturing varieties tend to yield better, possibly due to reduced exposure to diseases. Similarly, the negative correlation of the number of inflorescences per plant with days to first flowering and days to maturity reinforces this finding. Saroha et al. (2022) also showed similar results in the case of linseed.

Path Analysis

Grain yield and yield-contributing features were found to be related by both phenotypic and genotypic path coefficient analysis, and the results are shown in Table 5. Simple correlations may not offer a comprehensive understanding of the significance of individual traits in influencing yield (Bhargava et al., 2007). The use of path coefficient analysis, which considers both the causal linkages between features and measures their strength, becomes essential in these situations. This method of analysis makes it possible to separate the direct impacts of each characteristic on yield from the indirect effects brought about by the relationships between the traits. A direct effect occurs when one variable directly influences another without the mediation of any other variables. In contrast, indirect effects involve a chain of influences through one or more intermediate variables in the path analysis model. In this study, the number of inflorescences per plant, the number of seeds per floret, days to maturity, and plant height had a direct positive effect on seed yield. In contrast, days to first flowering, the length of the main inflorescence, and the number of branches per plant showed a negative direct influence. Interestingly, seed yield per plant showed a strong positive correlation with the number of inflorescences per plant and plant height and a strong negative correlation with days to first flowering. Therefore, by considering both the result of path analysis and correlation analysis, days to maturity, plant height, and number of inflorescences per plant can be relied on to increase seed output. The residual effect was calculated 0.2081 for phenotypic level and 0.1773 for genotypic level (Table 5), indicating that the variation contributed by the eight yield-attributing traits in seed yield per plant was 79.19% phenotypically and 82.27% genotypically. It represents the percentage of the dependent variable’s volatility that the independent variables in the model are unable to account for. These findings suggest that additional traits should be considered in future studies to elucidate further the factors influencing seed yield per plant.

Principal Component Analysis

PCA analysis has been made to identify the key traits or variables that contribute the most to the overall variation in the dataset. It is a multivariate analysis that reduces the dimensionality of complex data by considering multiple traits simultaneously and guide decisions on parental selection, breeding strategies, and the development of improved cultivars. In our study, we found that the first four PC explained 84%

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of the total variation, where the first two components contribute 58% of the variability (Table 6). seed yield per plant (SYP), length of main inflorescence (LMI), number of inflorescences per plant (NIP), number of branches per plant (NBP), number of seeds per floret (NSF), and plant height (PH) were the most significant traits that caused variation, according to PC1 and PC2, which showed that the genotypes under investigation have considerable genetic variability for the majority of the assessed traits. Xingú López et al. (2022) also revealed a similar result in chia, where yield, grain weight per plant, number of fruits per spike, number of spikes per plant, and spike length have a positive and significant contribution, which allows specifying the contribution of the variables to the principal components and their relationship with the explained variation. Singh and Tewari (2015) reported that the traits that contributed more to the overall variation should be given more importance for selection and hybridization in the breeding program for further improvement. From the PCA biplot in Figure 1, it was observed that genotype 2 (BAU Chia 2) had the highest values for seed yield per plant and plant height, which justified its mean performance in these two traits and the correlation between them. BAU chia 2 yields the highest mean performance for seed yield per plant and plant height, and a strong positive correlation exists between seed yield per plant and plant height. Therefore, yield and yield-related traits, especially seed yield per plant and plant height, are the major contributors to genetic variation in chia. This means that selecting these traits—particularly in genotypes like BAU Chia 2—will be the most effective strategy for breeding high-yielding cultivars.

Conclusions

This study reveals significant genetic diversity among chia genotypes for yield-related traits, indicating potential for future genetic improvement. GPBC 1, BAU Chia 2, BAU Chia 1, and GPBC 3 were identified as the most promising genotypes for yield and yield-attributing traits. GPBC 2 was the earliest maturing variety. The minimal gap between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) suggests that genetic factors primarily drive diversity. High genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were found for the trait seed yield per plant. High heritability was recorded in days to maturity, plant height, number of branches per plant, number of inflorescences per plant, number of seeds per floret, and seed yield per plant. In contrast, high GA% was found in the number of inflorescences per plant (NIP) and seed yield per plant. High heritability, coupled with high GA%, was recorded for the traits number of inflorescences per plant (NIP) and seed yield per plant, which should be considered for simple selection. To increase the production of chia, seed yield per plant should be given priority, which is positively correlated with the number of inflorescences per plant, plant height, and the length of the main inflorescence. Among these factors, the number of inflorescences per plant and plant height have the strongest direct effect on yield.

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