



RESEARCH ARTICLE

Unraveling genetic variation: Correlation insights and principal component analysis in F₃ generation of sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract

Understanding the significance of genetic variation in crop breeding programs is essential for improving agricultural productivity. This research delves into the genetic diversity within the F₃ generation of sorghum (*Sorghum bicolor* L. Moench) and investigates the variation, correlation and interrelationships among various traits. Through the utilization of a diverse panel of F₃ individuals, phenotypic data were collected for key agronomic traits, including plant height, days to 50 % flowering, number of leaves, flag leaf dimensions, panicle characteristics, seed weight and grain yield per plant. Genetic variability analysis revealed that panicle weight exhibited the highest genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), indicating significant genetic variability, while grain yield and number of leaves displayed moderate values of GCV and PCV, respectively. The highest genetic advance values were noted for panicle weight, number of leaves and grain yield, suggesting the presence of additive gene action. High heritability was detected for all the traits, except days to 50 % flowering, panicle length and 100-seed weight. The exploratory principal component analysis (PCA) retained PC1 to PC5 based on the scree plot. Correlation analysis revealed significant associations among traits, providing insights into potential pleiotropic effects and trait interdependencies. The findings of this study contribute valuable insights into the genetic basis of important agronomic traits in sorghum and lay a foundation for targeted breeding strategies aimed at developing improved varieties with enhanced resilience and productivity.

Keywords: correlation; genetic variation; principal component analysis; sorghum

Introduction

Sorghum (*Sorghum bicolor* L. Moench) stands out as one of the most resilient, adaptable and nutritionally significant crops globally, contributing significantly to food security and sustainable development. With a cultivated area of 40.1 million hectares and an annual yield of 61.36 million metric tons as of 2021, it ranks fifth in terms of global production, following wheat, maize, rice and barley (1). Originating from Africa, sorghum is an annual cereal crop with a diploid genetic makeup ($2n = 2x = 20$) and possesses a highly efficient C₄ photosynthetic system (2). India is also recognized as a secondary center of origin for sorghum, with a cultivated area of 4.38 million hectares and a production of approximately 4.81 million metric tons (3). In Tamil Nadu alone, sorghum covers 3.54 lakh hectares and a production of 2.88 lakh tonnes with a productivity of 809 kg ha⁻¹ (4).

Due to its gluten-free nature, sorghum serves as a viable alternative to wheat in addressing wheat allergies, gluten sensitivity and celiac disease, earning it the moniker of a "nutritious grain" owing to its exceptional nutritional profile and associated health benefits (5, 6). This study underscores the significance of genetic variability and correlations among traits in sorghum breeding programs. It highlights hybridization combined with selection as a promising practice for enhancing diverse traits, particularly grain

yield, while emphasizing the crucial role of genetic diversity in breeding programs, enhancing the potential of achieving desired trait improvements (7, 8). The study also discusses skewness and kurtosis as indicators of gene associations and regulatory mechanisms for particular traits. The degree of correlation among traits is deemed crucial, especially for complex and economically significant traits such as crop yield. Intergenerational correlation and narrow-sense heritability analyses are employed to evaluate the genetic potential and heritability of yield-related traits (9, 10).

The present research aims to capitalize on the benefits offered by these analyses and leverage analytical tools to facilitate the selection of desirable segregants and fixation of desired characteristics in sorghum breeding, with a specific focus on inter-varietal cross, notably CO 4 × CO 30.

Materials and Methods

This study aimed to investigate the genetic potential of the cross-combination CO 4 × CO 30 in the F₃ generation during the summer season of 2023, conducted at the Department of Millets, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The sorghum variety CO 30 is recognized as a promising type with yellow to white

grains, high grain yield and tolerance to shoot fly, grain mold and downy mildew. On the other hand, CO 4 is known for its fodder quality, attractive red-coloured grains and tolerance to drought, combined with lodging resistance and non-shattering characteristics. A total of 200 F₃ genotypes were evaluated in this study using a randomized complete block design (RCBD) with three replications. Quantitative traits, including plant height (PH), days to 50 % flowering (DFF), number of leaves (NL), flag leaf length (FLL), flag leaf width (FLW), panicle length (PL), panicle weight (PW), number of primaries (NP), 100-seed weight (SW) and grain yield (GY) per plant, were recorded in this experiment.

Data collection

Individual plants were tagged and utilized for observing different biometrical traits. PH was measured from the base to the top of the plant. Similarly, the height from the base to the tip of the flag leaf was measured to determine FLL and the width along the side of the leaf was measured to determine FLW. PL was determined as the distance from the neck of node to the tip of the main panicle.

DFF were recorded by counting number of days from sowing to the stage when 50 % of the plant population at flowering for each genotype. NL was assessed by counting the number of leaves on each selected plant. PW and SW were measured by weighing the entire panicle and 100 filled grains, respectively, using an electronic balance with three replicates. GY in grams was measured by weighing the seeds of each randomly selected plant. Mean values of all measured traits were used for subsequent analyses.

Statistical analysis

The mean performance of the traits studied is provided in the Table 1, using MS-Excel software. Similarly, the GCV and PCV were calculated to quantify variability among genotypes for all studied traits, as outlined by the previously known methodology (11). Genetic advance (GA) and heritability (h^2) were determined (12). The above-mentioned genetic variability parameters are listed in Table 1. PCA was performed by using TNAU STAT software to simplify the complexity inherent in high-dimensional data while retaining key trends and patterns. Additionally, pie charts were generated using PAST software and Pearson's correlation coefficients were computed using IBM SPSS 20.

Results

Genetic variability, genetic advance and heritability

The results of genetic variability indicated that the highest GCV and PCV were observed for PW (23.66 % and 24.5 %), which exhibited the existence of large genetic variability and demonstrated the effective selection for the given traits. The moderate values of GCV and PCV were recorded for GY (11.01 % and 12.2 %), followed by NL (10.73 % and 11.5 %), respectively. Low GCV and high PCV were shown for SW (5.78 % and 10.9 %) and NP (9.35 % and 11.36 %). Low GCV and PCV were seen in PH (6.01 % and 7.14 %), DFF (3.93 % and 5.29 %), FL (5.78 % and 6.91 %), FW (6.45 % and 7.97 %) and PL (4.17 % and 7.80 %), which exhibited a huge impact of the environment on the trait (Table 1).

High heritability was witnessed for all traits, PW (93.17 %), NL (86.67 %), GY (81.42 %), PH (71.16 %), FLL (69.95 %), FLW (65.57 %), NP (64.37 %), DFF (55.32 %), PL (28.62 %) and SW (27.76 %) (Table 1). The highest value of genetic advance was recorded for PW (47.05 %), NL (20.57 %) and GY (20.48 %), indicating additive gene action, while moderate GA was detected for NP (15.45 %), FLW (10.76 %) and PH (10.46 %), which showed non-additive gene action. PL had the lowest GA (4.60 %), followed by DFF (6.02 %), SW (6.27 %) and FLL (9.97 %) (Table 1).

The mean values for all the traits were calculated in which the trait PH had the highest mean value of 240.00 and the trait, SW had the lowest mean value of 2.38. The positively skewed traits were PH (0.05), FLW (0.17), PL (0.54), PW (1.13) and SW (0.43), whereas the negatively skewed traits were DFF (-0.40), FLL (-0.01), NP (-0.51) and GY (-1.16). Similarly, the positive kurtosis was observed in PL (0.06), PW (0.52) and GY (2.02), whereas negative kurtosis was found in PH (-0.87), DFF (-0.69), NL (-1.20), FLL (-0.51), FLW (-0.83), NP (-0.13) and SW (-0.50) (Table 1; Fig. 1). Pie charts are utilized to display proportions of a whole, capturing proportions at a specific moment. However, they do not indicate changes over time. Pie charts depicting the distribution of PCV %, GCV %, h^2 % and GAM % in genotypes, displaying percentages at a particular moment in time (Fig. 2). The variation is split into several categories in a pie chart, which are shown as segments inside a circle.

Table 1. Estimation of genetic variability parameters of sorghum cross CO 4 × CO 30

Traits	Mean	Var	Skewness	Kurtosis	CV	PCV	GCV	h^2	GAM
PH	240.00	283.11	0.05	-0.87	7.01	7.14	6.01	71.16	10.46
DFF	59.25	18.69	-0.40	-0.69	7.30	5.29	3.93	55.32	6.02
NL	9.47	1.11	0.00	-1.20	11.11	11.52	10.73	86.67	20.57
FLL	30.99	4.42	-0.01	-0.51	6.78	6.91	5.78	69.95	9.97
FLW	5.15	0.12	0.17	-0.83	6.76	7.97	6.45	65.57	10.76
PL	24.48	10.86	0.54	0.06	13.46	7.80	4.17	28.62	4.60
PW	61.50	171.32	1.13	0.52	21.28	24.51	23.66	93.17	47.05
NP	25.08	8.84	-0.51	-0.13	11.86	11.65	9.35	64.37	15.45
SW	2.38	0.09	0.43	-0.50	12.71	10.97	5.78	27.76	6.27
GY	63.38	42.40	-1.16	2.02	10.27	12.21	11.01	81.42	20.48

PH- Plant height (cm), DFF- Days to 50 % flowering (days), NL- Number of leaves, FLL- Flag leaf length (cm), FLW- Flag leaf width (cm), PL- Panicle length (cm), PW- Panicle width (cm), NP- Number of primaries (No.), SW-100-Seed weight (g), GY- Grain yield per plant (g).

CV- Coefficient of variance (%); PCV- Phenotypic coefficient of variation (%); GCV- Genotypic coefficient of variation (%); h^2 - Heritability (%); GAM- Genetic advance as percentage of mean (%).

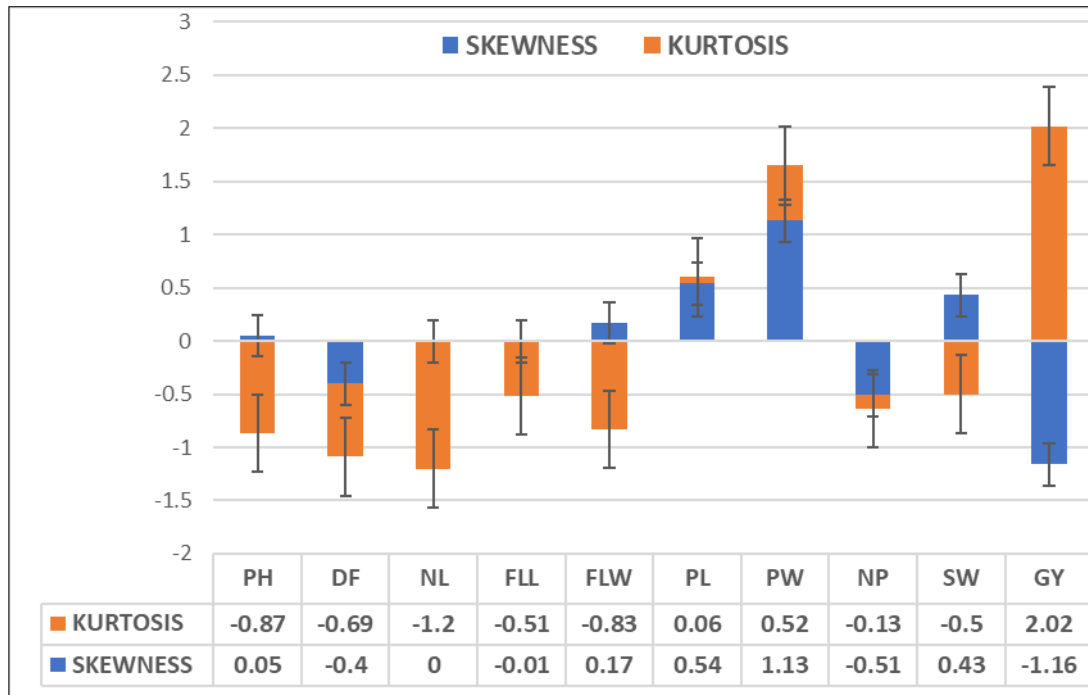


Fig. 1. Skewness and kurtosis of sorghum cross CO 4 × CO 30.

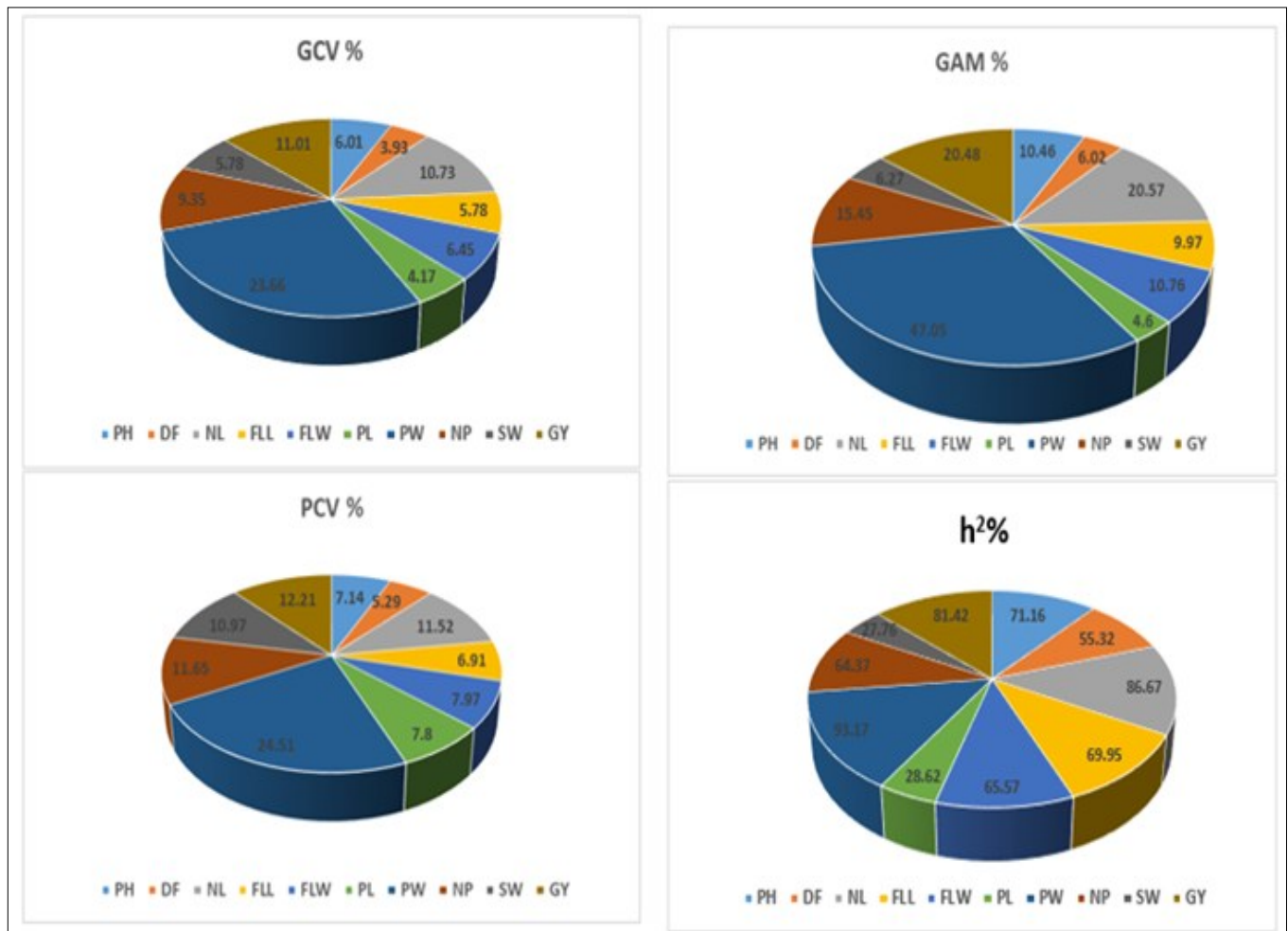


Fig. 2. PCV, GCV, genetic advance as percentage of mean and heritability of sorghum cross CO 4 × CO 30.

Principal component analysis (PCA)

PCA is a statistical technique utilized to decrease the complexity of datasets, enhancing interpretability while minimizing loss of information. In scree plot analysis, eigenvalues were plotted on the y-axis against the number of components on the x-axis, resulting in a descending curve. The "elbow" point, where the curve's slope

noticeably flattens, indicated the optimal number of factors to be generated by the analysis. There present scree plot, PC1, PC2, PC3, PC4 and PC5 should be retained in exploratory analysis to keep in PCA and the rest behind the first three components are disregarded (Fig. 3). The eigenvalues of PC1 (3.771), PC2 (1.756), PC3 (1.007), PC4 (0.927) and PC5 (0.701) are shown in Table 2. The loadings (eigenvectors) of correlation matrix are given in Table 3.

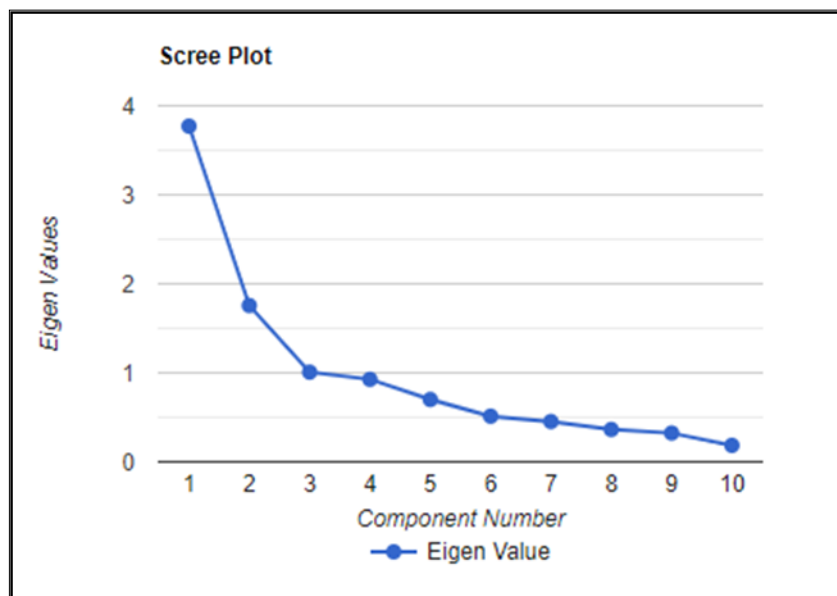


Fig. 3. Scree plot of PCA analysis of sorghum cross CO 4 × CO 30.

Table 2. Eigenvalues of correlation matrix for the principal components in sorghum cross CO 4 × CO 30

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Eigenvalues	3.771	1.756	1.007	0.927	0.701	0.513	0.453	0.364	0.323	0.185
Proportion	0.377	0.176	0.101	0.093	0.070	0.051	0.045	0.036	0.032	0.019
Cumulative Proportion	0.377	0.553	0.653	0.746	0.816	0.867	0.913	0.949	0.981	1.000

Table 3. Loadings (eigenvectors) of correlation matrix for the principal components in sorghum cross CO 4 × CO 30

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
PH	-0.053	0.600	0.240	0.377	-0.095	0.200	0.443	-0.299	-0.017	0.320
DFF	0.225	0.459	-0.120	-0.202	-0.550	0.329	-0.508	0.056	0.030	-0.109
NL	-0.074	0.597	0.169	-0.058	0.500	-0.350	-0.197	0.408	-0.006	-0.169
FLL	0.432	0.131	-0.184	-0.148	0.176	0.071	0.363	-0.316	-0.194	-0.660
FLW	0.420	0.070	-0.299	-0.194	0.068	-0.313	-0.039	-0.074	-0.513	0.565
PL	0.306	-0.021	-0.173	0.628	-0.365	-0.495	0.052	0.228	0.141	-0.170
PW	0.333	-0.111	0.071	0.486	0.456	0.280	-0.514	-0.280	0.059	0.053
NP	0.411	-0.105	0.082	0.011	0.103	0.475	0.307	0.682	0.024	0.136
SW	0.203	-0.163	0.832	-0.053	-0.223	-0.152	-0.089	-0.031	-0.377	-0.119
GY	0.405	0.017	0.208	-0.349	0.034	-0.234	0.075	-0.201	0.729	0.195

PH- Plant height (cm), DFF- Days to 50 % flowering (days), NL- Number of leaves, FLL- Flag leaf length (cm), FLW- Flag leaf width (cm), PL- Panicle length (cm), PW- Panicle width (cm), NP- number of primaries, SW-100-Seed weight (g), GY- Grain yield per plant (g).

Pearson correlation coefficient

Correlation represents the inter-relationship between two traits. The correlation coefficient analysis, PH showed a highly significant positive correlation with NL (0.502**) and DFF (0.295**). DFF exhibited a highly significant positive association with PH (0.295**), NL (0.215**), FLL (0.387**), FLW (0.397**) and PL (0.200). NL exhibited significant positive correlation with PH (0.502**), DFF (0.215**), showed significant negative correlation with PL (-0.176) and NP (-0.191). FLL showed significant positive correlation with days to DFF (0.387**), FLW (0.744**), PL (0.370**), PW (0.441**), NP (0.611**) and GY (0.634**). FLW expressed significant positive correlation with DFF (0.397**), FLL (0.744**), PL (0.435**), PW (0.394**), NP (0.527**) and GY (0.587**). PL showed significant positive correlation with DFF (0.200), FLL (0.370**), FLW (0.435**), PW (0.436**),

NP (0.384**) and GY (0.290**). PW exhibited significant positive correlation with FLL (0.441**), FLW (0.394**), PL (0.436**), NP (0.509**), SW (0.244**) and GY (0.360**). NP exhibited significant positive correlation with DFF (0.233**), FLL (0.611**), FLW (0.527**), PL (0.384**), NP (0.509**), SW (0.333**) and GY (0.554**), whereas significant negative correlation with NL (-0.191). SW showed significant positive correlation with PW (0.244**), number flag leaf width of primaries (0.333**) and GY (0.415**). The trait grain yield exhibited significant and positive correlation with DFF (0.326**), FLL (0.634**), FLW (0.587**), PL (0.290**), PW (0.360**), NP (0.554**) and SW (0.415**) (Table 4). The positive correlation of DFF, FLL, FLW, PL, PW, NP and SW with GY could be improved by directly selecting these traits.

Table 4. Pearson correlation matrix of sorghum cross CO 4 × CO 30

	PH	DFF	NL	FLL	FLW	PL	PW	NP	SW	GY
PH	1.000	0.295**	0.502**	0.020 ^{NS}	-0.151 ^{NS}	0.042 ^{NS}	-0.069 ^{NS}	-0.132 ^{NS}	-0.050 ^{NS}	-0.117 ^{NS}
DFF	0.295**	1.000	0.215**	0.387**	0.397**	0.200 [*]	0.077 ^{NS}	0.233**	0.029 ^{NS}	0.326**
NL	0.502**	0.215**	1.000	-0.015 ^{NS}	-0.028 ^{NS}	-0.176 [*]	-0.112 ^{NS}	-0.191 [*]	-0.126 ^{NS}	-0.031 ^{NS}
FLL	0.020 ^{NS}	0.387**	-0.015 ^{NS}	1.000	0.744**	0.370**	0.441**	0.611**	0.140 ^{NS}	0.634**
FLW	-0.151 ^{NS}	0.397**	-0.028 ^{NS}	0.744**	1.000	0.435**	0.394**	0.527**	0.127 ^{NS}	0.587**
PL	0.042 ^{NS}	0.200 [*]	-0.176 [*]	0.370**	0.435**	1.000	0.436**	0.384**	0.141 ^{NS}	0.290**
PW	-0.069 ^{NS}	0.077 ^{NS}	-0.112 ^{NS}	0.441**	0.394**	0.436**	1.000	0.509**	0.244**	0.360**
NP	-0.132 ^{NS}	0.233**	-0.191 [*]	0.611**	0.527**	0.384**	0.509**	1.000	0.333**	0.554**
SW	-0.050 ^{NS}	0.029 ^{NS}	-0.126 ^{NS}	0.140 ^{NS}	0.127 ^{NS}	0.141 ^{NS}	0.244**	0.333**	1.000	0.415**
GY	-0.117 ^{NS}	0.326**	-0.031 ^{NS}	0.634**	0.587**	0.290**	0.360**	0.554**	0.415**	1.000

PH- Plant height (cm), DFF- Days to 50 % flowering (days), NL- Number of leaves, FLL- Flag leaf length (cm), FLW- Flag leaf width (cm), PL- Panicle length (cm), PW- Panicle width (cm), NP- number of primaries (No.), SW-100-Seed weight (g), GY- Grain yield per plant (g).

Discussion

Genetic advance, heritability and genetic diversity are crucial factors in selecting parents and hybrids, thereby enhancing breeding decisions. All examined traits exhibited considerable values of h^2 , indicating their high heritability (Table 2). Prior research, such as a study, examined 64 sorghum genotypes across 17 quantitative traits and recorded a spectrum of PCV ranging from 4.74 % for days to flowering to 24.74 % for PW and GCV ranging from 3.58 % for leaf length to 20.33 % for PW (13). PW, head weight and harvest index demonstrated the highest PCV and GCV values, whereas traits such as grain filling period, PH, panicle length and grain yield exhibited moderate PCV and GCV values, highlighting the efficacy of genotype-based selection.

Genetic variability serves as the foundation for any selection strategy, as greater genetic variability within the population allows for greater potential in genotype improvement for particular traits (14, 15). Higher values of PCV and GCV indicate a wider scope for selection, as more variation enhances the efficacy of the selection process (16). In general, PCV values were slightly higher than GCV values for the analysed traits, emphasizing the impact of the environment on trait manifestation. In our investigation, PW demonstrated the highest GCV and PCV values. Nine quantitative traits were assessed in a cross between Paiyur 2 and Kottathur local 5 (17). Elevated PCV values were observed for characteristics such as PI (20.36 %), test weight (23.59 %) and single plant yield (44.52 %), whereas lower PCV values were noted for DFF (9.80 %), days to maturity (6.54 %) and NL (7.14 %).

Traits such as plant height, leaf length, stem diameter, test weight and single plant yield showed high heritability accompanied by genetic advance, indicating additive gene action and suitability for selection. These findings highlight the inherent differences among parental varieties, offering ample scope for selection to develop potential cultivars adaptable to environmental changes and maintaining high-yielding characteristics. In our research, the majority of traits displayed moderate to high values of both GCV and PCV. Moreover, several crucial traits exhibited higher PCV values than GCV values, underscoring the notable influence of environmental factors on trait manifestation (18, 19). This observation aligns with previous research findings, whom similarly reported moderate GCV and PCV values for the number of branches per plant (20).

The heritability (h^2) determines the extent to which a trait is inherited (21). High heritability suggests that genetics accounts for a significant portion of the variation in a trait among different parents, while low heritability, approaching zero, indicates that the majority of the variance is not attributable to genetic factors. Simply having a high heritability is not considered a critical criterion for selection. Nevertheless, the effectiveness of selection is enhanced when there is a considerable genetic advance (22). Reliable heritability estimation is essential for an efficient breeding program to improve quantitative traits (15).

Genetic advance serves as another crucial biometric indicator for selection decisions, demonstrating the extent of selection possibilities (22). Increased genetic advance and higher heritability magnitude offer a more reliable prediction of genetic gain through selection (23). When there is higher heritability along with a moderate genetic advance, it suggests the necessity for single-plant selection to enhance genotypes. When both components are low for certain traits, additional crossing becomes necessary to generate desired variations (24).

PW, NL and GY demonstrated elevated values across all variability components. The combination of increased genetic advance and higher heritability observed for PW, NL and GY strongly suggests the presence of additive gene action. This implies that selecting genotypes for improvement would likely produce highly effective results (25). Additive gene effects indicate that each gene contributes equally to the phenotype, without one gene exerting dominance over the others. With an increase in the number of genes, the phenotype tends to become more robust (26).

Opting for superior genotypes could significantly bolster this trait. A moderate genetic advance coupled with higher heritability was noted for NP, FLW and PH, indicating the potential for individual plant selection to further improve these traits. It is crucial to recognize that high heritability does not always correlate with higher genetic advance (27). Choosing parents with both greater genetic advance and higher heritability for yield-related traits is essential (28). In this study, higher heritability was generally observed alongside moderate to low genetic advance for most traits, highlighting the necessity for further selection to enhance these traits. Conversely, PW, NL and GY demonstrated high heritability along with a high genetic advance as a percentage of the mean, indicating that these traits could be prioritized for the development of superior genotypes.

The conjunction of a higher genetic advance as a percentage of the mean with high heritability holds more significance than heritability alone in forecasting the outcomes during genotype selection (29). Likewise, increased heritability and genetic advance as a percentage of the mean (GAM) for yield per plant (YP), which is consistent with our findings (30). These traits indicate the occurrence of additive gene action, given their high heritability. When there is higher heritability alongside low to moderate genetic advance as a percentage of the mean, it suggests the influence of environmental factors on specific trait expressions (29).

Investigating these traits may involve the manifestation of heterosis, which includes dominance and epistatic components. In contrast, the differences in genetic advance as a percentage of the mean, ranging from 4.8 % for biomass yield to 41.95 % for panicle width (13). Traits such as grain filling rate, panicle length, panicle width, head weight, grain yield and harvest index displayed both high heritability and genetic advance as a percentage of the mean, suggesting their regulation by additive gene action and implying the effectiveness of phenotypic selection for these traits. On the other hand, the study, revealed low moderate genetic advances as a percentage of the mean for traits such as number of branches per plant, days to flowering and number of grains per panicle (31). High genetic advance combined with high heritability suggests minimal environmental influence on trait expression, indicating the potential for enhancement through natural selection (32, 33).

Utilizing biplot and scree plot analyses is essential for understanding the similarity and divergence patterns of genotypes and traits (34). PCA pinpointed PC1, PC2, PC3 and PC4 as pivotal factors for further exploration, as determined by the scree plot analysis (35) (Fig. 3). Correlation analysis conducted in this study revealed positive correlations among DFF, FLL, FLW, PL, PW, NP and SW, indicating the potential enhancement of GY through direct selection (Fig. 4).

Direct selection of the traits correlating with the yield enhanced the reliability of yield improvement (36). Likewise, a notable positive correlation between plant height and single plant yield, indicating that selecting for plant height directly impacts yield (17). Moreover, plant height and test weight showed a positive direct influence on single plant yield, highlighting the potential to enhance yield through selection for these attributes. Previous researchers have documented genetic variability components for various yield and yield-related traits. The inclusion of PW represents a novel aspect of our study, indicating that maximizing YP (kg) could be attained by augmenting this trait. Increased PW correlates with higher yield. Further research is warranted to enrich genetic variability for improved selection programs. Therefore, breeders should employ suitable breeding strategies to harness both additive and non-additive gene effects concurrently, as both varietal and hybrid development will significantly contribute to breeding programs, particularly for sorghum.

Conclusion

This study delved into the complex realm of genetic variation within the F_3 generation of sorghum, offering detailed insights into parameters of genetic variability, correlations among traits and PCA. Correlation analysis revealed significant associations among traits, providing insights into pleiotropic effects and interdependencies, which are crucial for informed breeding decisions. Additionally, PCA elucidated the underlying structure of genetic variation, aiding in identification of key factors driving phenotypic diversity within the population. Overall, these findings contribute to ongoing sorghum breeding efforts by providing a comprehensive understanding of genetic variation, which could be harnessed to develop improved cultivars with enhanced resilience and productivity to help address global food security challenges. Further research on the practical relevance of identified genetic variants and their interactions with environmental factors is essential for their successful application in breeding strategies.

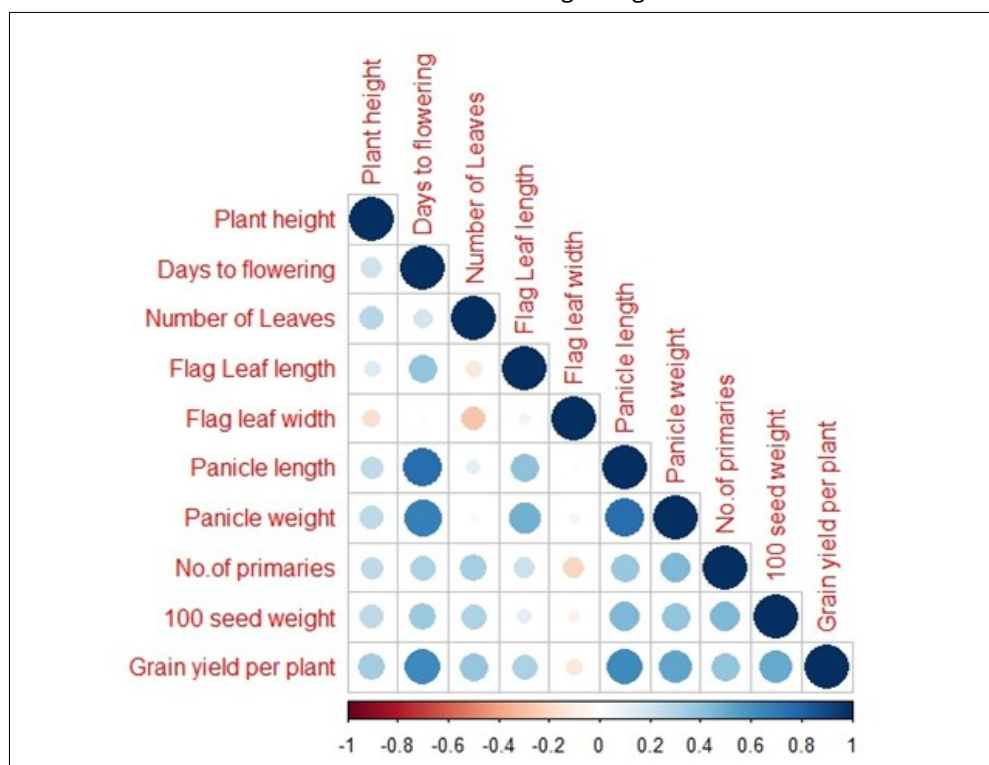


Fig. 4. Heat map and correlation among the traits of sorghum cross CO 4 × CO 30.

Authors' contributions

KD and CR conceived and designed the research experiments. They also provided guidance on the experimental design and data analysis. KD conducted the experiments and documented the biometrical data. KD, CR and SJ analysed the data and SJ wrote the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

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