RESEARCH ARTICLE

Parent-offspring regression to accelerate nutritive traits in F_2 and F_3 generations of red sorghum (*Sorghum bicolor* L. Moench) genotypes

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Abstract

Addressing global micronutrient deficiencies through biofortification is essential for improving human health. Sorghum, as a drought-tolerant and resilient crop, offers significant potential in this regard. This study investigated the biochemical traits and genetic variability among red sorghum genotypes, focusing on breeding nutritionally enriched varieties. The cross derivatives of sorghum parental varieties *viz*., CO 32 × Paiyur 2 in F_2 and F_3 generation were analysed for key biochemical traits, including total carbohydrate, amylose content, total protein, total phenolic content, total flavonoid content, total tannin content, total anthocyanin content, iron and zinc, as well as antioxidant potential. The genotypes in F_2 generation exhibited considerable variation with iron content ranging from 772.15 mg/kg to 1094.84 mg/kg and zinc content between 59.05 mg/kg and 764.23 mg/kg, highlighting the potential for biofortification. Total antioxidant activity ranged from 10.63 % to 88.56 %, indicating diverse antioxidant profiles among the genotypes. The F_3 generation genotypes showed improved mean values for total carbohydrate averaging 63.54 g/100 g, amylose content of 38.89 % and Fe content of 958.65 mg/kg. Selection pressure led to reduced genetic variability, as evidenced by changes in skewness and kurtosis and significant improvements in micronutrient and antioxidant content in F_3 generation materials. High correlation coefficients (≥0.94) for traits such as amylose content, total protein, iron and zinc content suggested strong genetic control and inheritance of the traits. Heritability estimates above 0.50 indicated the potential for consistent genetic gains. These findings provided valuable insights for developing sorghum varieties with enhanced nutritional profiles, contributing to global food security and addressing micronutrient malnutrition.

Keywords: biochemical traits; kurtosis; red sorghum; regression; skewness

Introduction

The ability of sorghum (*Sorghum bicolor* L.) to thrive in diverse environments from semi-arid regions to areas with extreme heat has made it a unique and highly adaptable crop. The global sorghum production stood at approximately 60 million metric tons, with major producers including the United States, India and Nigeria (1). In many African and Asian countries, sorghum is a staple food for millions, particularly in regions prone to drought and food insecurity. Over 700 million people in Sub-Saharan Africa and South Asia relied on sorghum as a primary food source (2). In recent years, the focus on sorghum improvement has shifted from merely enhancing yield under stress conditions to improving its nutritional quality, particularly through biofortification. Traits such as grain iron and zinc content, total phenolics, flavonoids and antioxidant activity have emerged as critical indicators of dietary value of sorghum. These bioactive compounds not only contribute to human health but also serve as key targets in developing nutrient-dense sorghum genotypes. Integrating such traits into breeding programs aligns with global efforts to combat micronutrient malnutrition and enhance food security, especially in resource-limited regions (3).

The challenge of breeding high-yielding sorghum varieties that could perform well under such environmental stresses required innovative approaches in genetic selection. Parent-offspring regression analysis, a widely used tool in quantitative genetics, offers a robust method to estimate heritability of key traits such as grain yield, drought tolerance and stress resistance particularly in early segregating generations like F_2 and F_3, where accurate prediction of genetic gain is crucial for effective selection. By examining the relationship between parent and offspring performance, parent-offspring regression allowed breeders to identify genetic correlations and predict how traits would be passed down to the next generation. This method had been successfully applied in other crops to enhance breeding precision and accelerate the development of superior varieties (4). Quantitative genetics offered a powerful approach for exploring the complex genetic architecture underlying phenotypic traits (5, 6). While natural selection acted on an individual's phenotype, it was the genotype that was inherited and passed on to offspring. This led to a key question in evolutionary biology: how did genotype influence phenotype? The proportion of variation in a trait that could be attributed by genetic factors is referred to as broad-sense or true heritability (7).

This study was mainly focused on estimating the heritability of key agronomic and biochemical traits in red sorghum using parent-offspring (PO) regression analysis. This method involves calculating the slope of the linear regression between parental trait values and those of their progeny, providing an estimate of narrow-sense heritability. Parent-offspring regression was employed to assess the genetic transmission of traits such as grain yield, micronutrient content and antioxidant activity in early segregating generations. This method highlighted the relationship between the traits of the parents and those of their progeny (4). The objective of the study was to determine the potential of these traits for genetic improvement and to identify superior genotypes that could serve as parental lines in future breeding programs targeting nutritional enhancement.

Materials and methods

The study investigated the genetic potential of the cross between CO 32 × Paiyur 2 (White × Red cross) in the F_2 and F_3 generations during the summer season (January-April 2023) and summer season (January-April 2024) at the Millet Breeding Station, Coimbatore, Tamil Nadu, India. Regular cultural practices and plant protection measures were followed to maintain a uniform crop stand. The experiment was conducted at Tamil Nadu Agricultural University, Coimbatore (11°00′N, 76°57′E, 427 m MSL), under red loamy soil (pH 7.2). During the cropping period, the mean temperature ranged from 24.5 °C to 36.8 °C, with total rainfall of 410 mm. The samples were collected at physiological maturity for biochemical analysis. The female parent CO 32 is a popular and high-yielding dual-purpose sorghum variety released in 2020 by Department of Millets, Tamil Nadu Agricultural University (TNAU), Coimbatore. Paiyur 2, the male parent, is a dual-purpose, short-duration red sorghum variety released from the Regional Research Station, Paiyur, TNAU, Coimbatore. Additionally, its grains are rich in nutrients with many uses, including its high iron (Fe) ranging from 4.5 to 7.2 mg/100 g, zinc (Zn) from 2.0 to 4.5 mg/100 g and B-complex vitamins such as thiamine (B_1) at 0.28 mg/100 g, riboflavin (B_2) at 0.15 mg/100 g and niacin (B_3) at 2.9 mg/100 g. The high nutrient content of this variety has made it a grain of choice for optimum health. With this background the main aim of the study to investigate the nutritional content of red sorghum genotypes across generations.

A total of 185 individuals in the F_2 generation of a cross were biochemically analysed and subjected to selection (Supplementary file). The experiment was laid out in a randomized complete block design with three replications to ensure statistical accuracy. Among them, 17 entries with better mean performance in terms of high single-plant yield and presence of red coloured seeds were forwarded to the next generation (F_3). In both the segregating generations, observations for ten biochemical traits *viz*., Total Carbohydrate (TC), Amylose Content (AC), Total Protein (TP), Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Tannin Content (TTC), Total Anthocyanin Content (TAC), Iron (Fe), Zinc (Zn) and Total Antioxidant Activity (DPPH) were recorded on randomly selected five plants and the mean values of these traits were utilized for further analysis in F_3 generation. The complete analysis procedure is given in the Supplementary file. Using Origin software version 10, intergenerational correlation and parent-progeny regression studies were carried out. To interpret gene interactions, skewness and kurtosis were derived from the mean data using Microsoft Excel and the frequency distribution graphs were created with the R software (package - corrplot).

Narrow sense heritability (8)

$$h^2 = b_{yx}/2r_{xy} \times 100$$

b_{yx}-Regression coefficient of F_3 progeny means on F_2 parental values for respective characters.

ryx - Intergenerational correlation coefficient between the parent "x" and its offspring "y".

Results and Discussion

In order to find appropriate parents for developing hybrids and commercial varieties and enhance desired traits of interest, it is essential to choose appropriate parental lines (9, 10). In this study the superior plants were selected based on diversity among the segregating generations. The mean and range values of the biochemical characters were recorded in F_2 (Table 1) and F_3 (Table 2) generation materials of the cross CO 32 × Paiyur 2. The analysis revealed significant variability among the genotypes for all traits studied at probability 1 %, underscoring the genetic diversity within the segregating populations. The parental lines, CO 32 and Paiyur 2, exhibited contrasting biochemical profiles, providing a strong basis for genetic recombination. In the F_2 generation, the mean performance for TC was 60.48 g/100 g, with the highest level in genotype III-140 (99.14

Table 1. Biochemical content present in different individuals of F$_2$ generation

S.No	Genotypes	SPY (g)	TC (g/100 g)	AC (%)	TP (g/100 g)	TPC (mg/g)	TFC (mg/g)	TTC (mg/g)	TAC (mg/g)	Fe (mg/kg)	Zn (mg/kg)	DPPH (%)
1	III-1	44.2	65.73	35.95	3.42	26.54	8.43	17.23	3.01	934.76	623.55	84.77
2	III-4	49.8	59.92	40.39	4.97	24.43	11.67	15.17	1.08	772.15	59.05	80.27
3	III-16	38.6	64.61	28.45	3.65	21.72	5.7	11.85	0.20	1067.07	95.60	76.13
4	III-28	52.3	64.01	38.11	4.02	19.47	8.94	12.43	1.23	1048.89	74.85	46.56
5	III-46	40.1	73.8	39.70	6.57	8.27	6.63	4.71	0.86	796.39	153.85	45.59
6	III-50	45.8	64.61	24.02	8.08	12.31	7.57	8.33	1.71	1013.71	140.35	72.25
7	III-53	46.9	58.90	38.80	2.71	20.57	10.81	12.92	0.86	1004.4	764.23	74.77
8	III-55	58.5	72.78	44.82	2.97	29.89	8.72	18.84	0.53	858.00	90.75	88.56
9	III-56	50.7	20.12	19.02	4.08	43.62	11.31	16.88	0.98	859.75	200.43	84.77
10	III-67	53.6	64.92	45.61	8.34	10.29	9.87	6.91	1.99	1007.48	652.85	49.82
11	III-85	56.9	63.80	28.8	10.48	10.52	8.08	5.05	0.98	1094.84	308.75	77.93
12	III-115	41.7	30.94	15.16	6.62	13.22	7.57	7.35	2.14	787.3	140.7	34.14
13	III-140	56.3	99.14	55.63	11.05	13.18	8.36	4.75	0.15	792.35	98.1	41.17
14	III-149	42.8	39.20	37.66	9.37	9.92	6.20	5.39	0.08	972.13	234.05	80.18
15	III-167	47.9	72.98	49.82	4.31	14.37	11.24	16.25	0.08	1011.01	115.2	74.70
16	III-171	45.5	57.43	28.29	9.91	3.61	3.41	1.53	1.23	953.44	79.75	10.63
17	III-174	50.4	55.22	36.98	3.77	14.51	7.35	8.81	0.88	866.58	62.60	64.86
	Mean	**51.92**	**60.48**	**35.72**	**6.14**	**17.44**	**8.34**	**10.26**	**1.06**	**931.78**	**229.10**	**63.95**
	CO 32	**115.24**	**78.23**	**47.48**	**6.97**	**7.68**	**5.03**	**2.16**	**1.15**	**827.7**	**143.8**	**15.17**
	Paiyur 2	**76.34**	**99.05**	**39.48**	**7.47**	**23.79**	**14.26**	**14.19**	**2.24**	**1245.32**	**863.21**	**91.43**

SPY: Single Plant Yield; TC: Total Carbohydrate; AC: Amylose Content; TP: Total Protein; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TTC: Total Tannin Content; TAC: Total Anthocyanin Content; Fe: Iron; Zn: Zinc; DPPH: Total antioxidant activity.

Table 2. Biochemical content present in different individuals of F$_3$ generation

S.No	Genotypes	SPY (g)	TC (g/100 g)	AC (%)	TP (g/100 g)	TPC (mg/g)	TFC (mg/g)	TTC (mg/g)	TAC (mg/g)	Fe (mg/kg)	Zn (mg/kg)	DPPH (%)
1	III-1	42.6	72.12	41.54	5.68	30.60	10.53	20.47	4.17	952.57	653.23	86.90
2	III-4	50.2	60.70	41.83	5.37	26.23	12.27	16.53	2.40	789.90	81.73	83.23
3	III-16	38.4	66.73	26.10	4.47	22.17	10.73	12.23	1.30	1117.83	112.20	81.37
4	III-28	47.1	65.20	41.00	4.63	22.27	12.43	14.60	2.47	1068.00	82.63	51.33
5	III-46	44.7	76.43	46.63	7.07	10.80	10.80	5.30	1.63	808.23	181.93	51.43
6	III-50	42.6	67.57	27.30	7.57	14.10	8.33	10.20	3.53	1062.30	164.50	76.37
7	III-53	48.3	64.97	42.43	3.43	26.67	12.33	14.47	1.50	1016.77	782.67	80.67
8	III-55	59.4	77.60	41.93	3.27	35.47	10.47	20.30	2.40	909.70	123.70	92.43
9	III-56	46.2	36.63	26.50	4.80	40.83	12.50	18.37	0.90	868.93	213.37	87.63
10	III-67	47.9	72.20	47.23	8.62	11.80	10.40	8.33	2.33	1025.33	710.77	53.30
11	III-85	55.9	67.23	31.27	11.67	15.27	14.60	6.27	1.53	1128.07	315.10	80.93
12	III-115	46.5	44.13	20.07	7.87	18.93	8.50	10.23	0.23	805.27	151.97	41.40
13	III-140	59.8	61.63	61.97	10.93	10.30	10.10	6.37	0.28	806.00	101.60	51.90
14	III-149	49.2	42.67	41.97	10.30	10.70	8.30	7.47	0.15	1014.20	251.57	83.63
15	III-167	53.7	76.63	50.97	5.53	19.93	14.67	18.37	0.24	1024.73	122.10	80.77
16	III-171	52.6	66.40	31.33	12.50	8.43	7.63	2.60	0.63	1014.37	81.97	13.10
17	III-174	54.3	61.30	41.03	4.27	17.60	10.30	10.23	1.40	884.93	72.67	81.90
	Mean	**42.6**	**63.54**	**38.89**	**6.94**	**20.12**	**10.88**	**11.90**	**1.59**	**958.65**	**247.28**	**69.31**
	CO 32	**50.2**	**80.4**	**45.3**	**6.01**	**7.2**	**5.1**	**1.15**	**1.02**	**802.4**	**125.6**	**14.9**
	Paiyur 2	**38.4**	**97.20**	**40.2**	**8.2**	**20.3**	**13.2**	**13.5**	**1.56**	**1026.4**	**625.1**	**75.6**

SPY: Single Plant Yield; TC: Total Carbohydrate; AC: Amylose Content; TP: Total Protein; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TTC: Total Tannin Content; TAC: Total Anthocyanin Content; Fe: Iron; Zn: Zinc; DPPH: Total antioxidant activity.

g/100 g) and the lowest quantity in III-56 (20.12 g/100 g). Amylose content (AC) varied significantly, ranging from 15.16 % in III-115 to 55.63 % in III-140, with a mean of 35.72 %. Total protein (TP) exhibited variability, with III-140 recording the highest value of 11.05 g/100 g and III-53 with the lowest level of 2.71 g/100 g, averaging 6.14 g/100 g. The high variability in protein content among sorghum cultivars, attributed to genetic diversity and agronomic conditions, this align was noted and reported in previous study (11). TPC displayed a wide range, with the highest being 43.62 mg/g in III-56 and the lowest 3.61 mg/g in III-171, indicating significant variation in phenolic potential. Similarly, TFC ranged from 3.41 mg/g in III-171 to 11.67 mg/g in III-4, averaging 8.34 mg/g, while TTC spanned from 1.53 mg/g in III-171 to 18.84 mg/g in III-55 with the mean of 10.26 mg/g; whereas TAC ranged from 0.08 mg/g for III-149 and III-167 to 3.01 mg/g in III-1 with the mean of 1.06 mg/g. Iron content ranged widely, from 772.15 mg/kg in III-4 to 1094.84 mg/kg in III-85, with a mean of 931.78 mg/kg. Zinc content also showed considerable variation, from 764.23 mg/kg in III-53 to 59.05 mg/kg in III-4, with an average of 229.10 mg/kg. DPPH was highest in III-55 (88.56 %) and lowest in III-171 (10.63 %), reflecting the diverse antioxidant profiles among the genotypes in F_2 generation. Similar findings were also reported in the previous study which aimed to scrutinize the inheritance patterns of agronomical traits and amylose content in the sorghum population (12). Here the three F_2 populations, Pulut 3 × Kawali (372 plants), Pulut 3 × Soraya 3 IPB (340 plants) and Pulut 3 × PI150-20A (363 plants) and their parental genotypes showed significant variations.

In the F_3 generation, the mean values for most traits improved, reflecting selection pressure and potential genetic gain. Total carbohydrate (TC) increased to an average of 63.54 g/100 g, with the highest level of 77.60 g/100 g in III-55 and the lowest level of 36.63 g/100 g in III-56. Amylose content (AC) showed a slight increase, averaging 38.89 %, with genotype III-140 recording the highest value of 61.97 % and lowest value of 20.07 % in III-115. Similar findings were also reported in the previous study (13, 14). They reported the highest value (18.59 %) of amylose content followed by 17.86 % in accessions 228739 and 69128, respectively. The lowest amylose content of 3.51 % was found in accession 223525. Total protein (TP) averaged 6.94 g/100 g, with III-171 reaching 12.50 g/100 g and III-55 showing the lowest level of 3.27 g/100 g similar findings were observed in the previous study (15). TPC decreased slightly to a mean value of 20.12 mg/g, with III-

56 maintaining the highest value of 40.83 mg/g. TFC exhibited a marginal increase to an average of 10.88 mg/g, with III-167 displaying the highest value of 14.67 mg/g. Total Tannin Content (TTC) recorded a notable improvement in genotypes like III-1 (20.47 mg/g) and III-55 (20.30 mg/g), reflecting successful selection for this trait. Similarly, the TAC content also showed improvement for the genotypes III-1 (4.17 mg/g) and III-55 (2.40 mg/g). Fe and Zn contents improved slightly, averaging 958.65 mg/kg and 247.28 mg/kg respectively, with III-85 continuing to show superior performance. Similar results were also reported in the previous studies in which the tested genotypes showed significant ($p < 0.05$) variation in protein, amino acid, iron and zinc contents (16). They reported that the genotype Macia exhibited high iron (156.32 mg/kg) and zinc (44.64 mg/kg) concentrations and high crude protein content were observed for the genotypes Macia, AS1 and 3484 × 424, with 13.60 %, 13.37 % and 12.77 % thereby these genotypes could be considered for sorghum nutritional improvement. Similarly significant positive correlations between Zn and Fe were observed in the findings of previous study (11). The antioxidant activity increased on average with many genotypes thereby maintaining higher values. These findings emphasize the intricate genetic control of biochemical traits in red sorghum, revealing substantial genetic variation among segregating generations. The observed variability suggested that these traits are likely governed by multiple genes with significant interactions, highlighting the complexity of their inheritance patterns. The improvement in mean values from the F_2 to the F_3 generation indicated the effectiveness of selection in stabilizing desirable traits, thereby confirming the heritability of key biochemical components.

Skewness and kurtosis values in the F_2 and F_3 generations provided insights into the distribution and nature of gene interactions (Table 3). Kurtosis, on the other hand, indicated the peakedness of the distribution and provided clues about the genetic control of traits. In this study a negative kurtosis value was observed in the biochemical traits like total carbohydrate (-0.294), amylose content (-0.358) and antioxidant activity using DPPH (-0.897) in F_2 generation, indicated a longer left tail, suggesting a predominance of high-performing alleles for this trait in the population (Fig. 1). Conversely, positive kurtosis values, such as those for total protein (0.361), TPC (1.162), total flavonoid content (0.241), total tannin content (0.068), total anthocyanin content (0.691), Fe content (0.517) and zinc

Table 3. Skewness and kurtosis for biochemical traits in F_2 and F_3 progenies of sorghum cross CO 32 × Paiyur 2

Traits	F_2		F_3	
	Skewness	Kurtosis	Skewness	Kurtosis
TC (g/100 g)	1.054	−0.294	0.728	−0.296
AC (%)	-0.132	−0.358	−0.013	0.001
TP (g/100 g)	-1.250	0.361	-0.532	0.767
TPC (mg/g)	1.772	1.162	0.013	0.712
TFC (mg/g)	0.159	0.241	0.073	−0.074
TTC (mg/g)	-1.337	0.068	−1.074	0.083
TAC (mg/g)	0.223	0.691	−0.067	0.479
Fe (mg/kg)	0.021	0.517	−0.840	0.196
Zn (mg/kg)	0.703	1.465	0.424	1.413
DPPH (%)	-0.251	-0.897	0.443	−1.191

SPY: Single Plant Yield; TC: Total Carbohydrate; AC: Amylose Content; TP: Total Protein; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TTC: Total Tannin Content; TAC: Total Anthocyanin Content; Fe: Iron; Zn: Zinc; DPPH: Total antioxidant activity.

content (1.465) in F_2 generation, recorded a longer right tail, reflecting the presence of fewer genotypes with exceptionally high content of those traits. In the F_3 generation, skewness patterns shifted slightly, reflecting changes due to selection pressure. For example, total protein (TP) kurtosis became more pronounced (0.767) highlighting the directional selection for higher protein content (Fig. 2). Similar results were also observed in the findings (17, 18).

Skewness measures the asymmetry of the frequency distribution (19) (Table 3). A positive skewness value for total carbohydrate (1.054), TPC (1.772) total flavonoid content (0.159), total anthocyanin content (0.223), iron content (0.021) and zinc content (0.703) in F_2 generation, suggested platykurtic (flatter) or mesokurtic (normal-like), with some showing bimodal or skewed distributions, indicating that most genotypes clustered closely around the mean with fewer extreme values (Fig. 1). This pattern often signified polygenic inheritance with additive effects. In contrast, negative skewness values were observed for amylose content (-0.132), total phenol (-1.250), total tannin content (-1.337) and antioxidant activity (-0.251) in F_2 generation, reflected a platykurtic distribution, characterized by a broader spread and a flatter peak, implying greater environmental influence or non-additive gene effects. The shift in kurtosis values from F_2 to F_3 for all traits like TFC and TTC highlighted changes in genetic variability and the impact of selection. In F_3 generation, traits such as total carbohydrates (TC) and amylose content (AC) showed slightly reduced kurtosis values compared to F_2 generation materials, suggesting a stabilization of genetic variability due to the selection of superior genotypes (Fig. 2). Thus, kurtosis dropped from F_2 to F_3, reflecting a narrowing of variability as favourable alleles were fixed. Similarly, the negative kurtosis for DPPH in both generations (-0.897 in F_2 and -1.191 in F_3) suggested a strong directional selection toward higher antioxidant activity, a key objective in breeding for health-promoting properties (12). Additionally, traits exhibiting high kurtosis may require more refined selection strategies to avoid extreme variations in future generations. Understanding the nature of these distributions allows breeders to design effective strategies for selecting high-performing genotypes while maintaining genetic diversity.

The intergenerational correlation analysis between F_2 and F_3 generations provided crucial insights into the genetic architecture and inheritance of biochemical traits in the sorghum population derived from the CO 32 × Paiyur 2 cross (Table 4). The correlation coefficients, regression coefficients and heritability estimate collectively highlighted the extent to which traits were influenced by genetic versus environmental factors and their potential for improvement through selection. Traits such as amylose content (AC), total protein (TP), total tannin content (TTC), iron (Fe), zinc (Zn) and DPPH displayed exceptionally high correlation coefficients (≥0.94) and regression coefficients near or equal to 1, reflecting a linear relationship and strong predictability of F_3 progeny performance based on F_2 parental values. suggested minimal environmental interference and underscored the dominance of additive genetic effects, as confirmed by heritability values of 0.50 or higher, ensuring consistent genetic gain through successive generations as reported (20). From the previous author findings reported that TAC showed a lower correlation coefficient of 0.45 but an impressively high heritability of 0.72, indicating a more complex inheritance pattern likely influenced by non-additive genetic interactions or genotype-environment interactions (21). This suggested that while direct prediction of TAC inheritance might be less reliable and the trait still held significant potential for improvement through selection due to the substantial genetic control over its expression. TFC and TC exhibited moderate correlation coefficients (0.78 and 0.84, respectively) and heritability values around 0.50 and 0.37 indicating that environmental factors played a partial role in their expression. Nonetheless, the genetic component is strong enough to ensure measurable progress in those traits with proper breeding strategies (22). Trait such as TPC displayed high correlation and regression coefficients (0.95 and 0.92, respectively) with moderate heritability values (0.48), suggested that while these traits was moderately heritable and their improvement might require more intensive selection pressure or larger population sizes to isolate superior genotypes effectively. The uniformly high heritability values observed for most traits indicated a substantial genetic contribution, with additive gene effects playing a predominant role in the inheritance of key biochemical traits. However, the research indicates the traits such as grain micronutrient content and antioxidant activity, the potential influence of genotype × environment (G×E) interactions must also be considered, as environmental factors like soil type, temperature and rainfall can significantly modulate trait expression (18, 23).

Table 4. Intergenerational correlation and regression values of biochemical traits in F_2 and F_3 progenies of sorghum cross CO 32 × Paiyur

Traits	Correlation Coefficient	Regression Coefficient	Heritability (%)
TC (g/100 g)	0.84**	0.62**	0.37
AC (%)	0.97**	0.96**	0.50
TP (g/100 g)	0.94**	1.00**	0.53
TPC (mg/g)	0.95**	0.92**	0.48
TFC (mg/g)	0.78**	0.78**	0.50
TTC (mg/g)	0.99**	1.00**	0.51
TAC (mg/g)	0.45**	0.65**	0.72
Fe (mg/kg)	0.99**	1.01**	0.51
Zn (mg/kg)	1.00**	1.00**	0.50
DPPH (%)	0.98**	0.98**	0.50

*5% significance level.

**1% significance level.

SPY: Single Plant Yield; TC: Total Carbohydrate; AC: Amylose Content; TP: Total Protein; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TTC: Total Tannin Content; TAC: Total Anthocyanin Content; Fe: Iron; Zn: Zinc; DPPH: Total antioxidant activity.
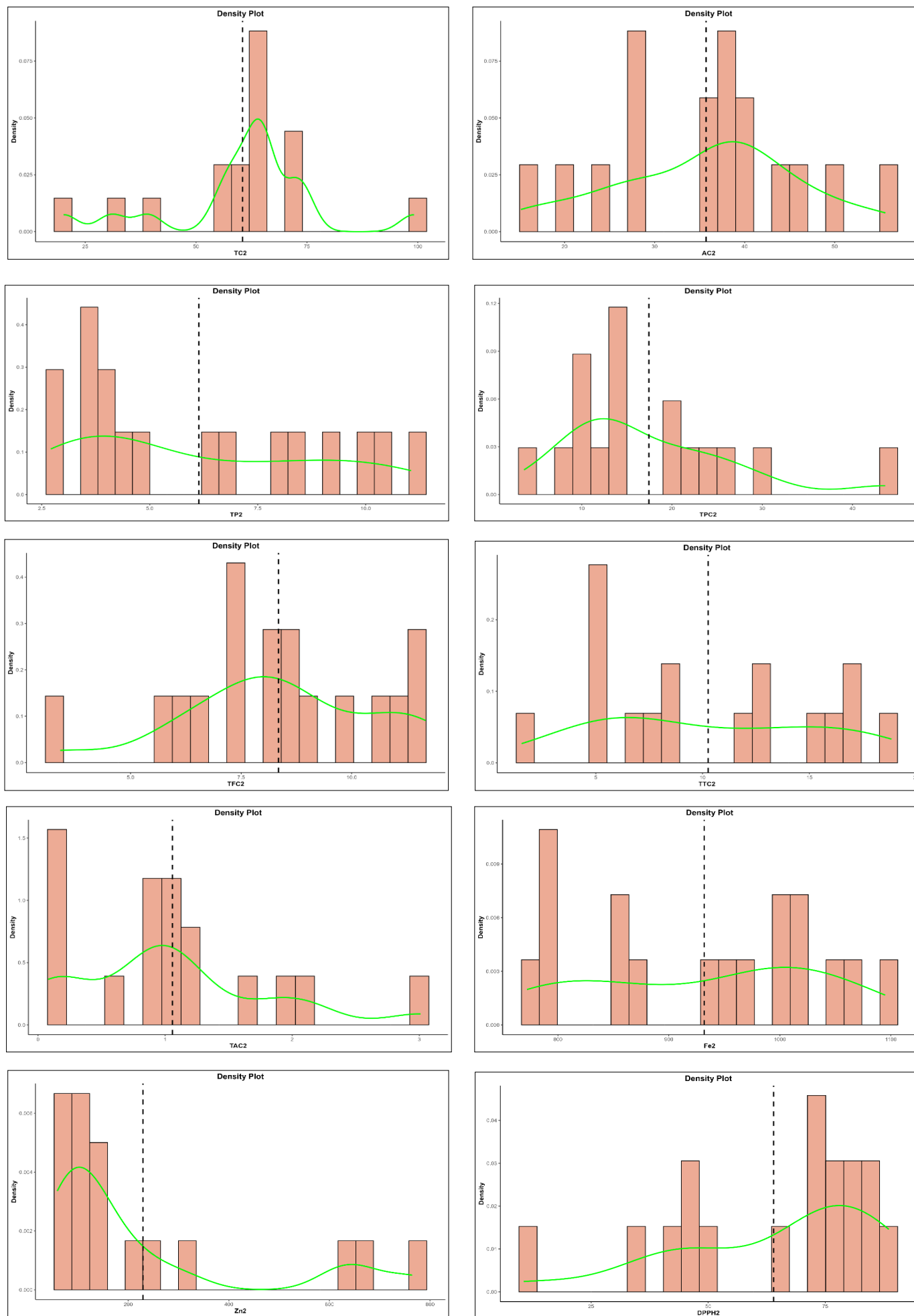
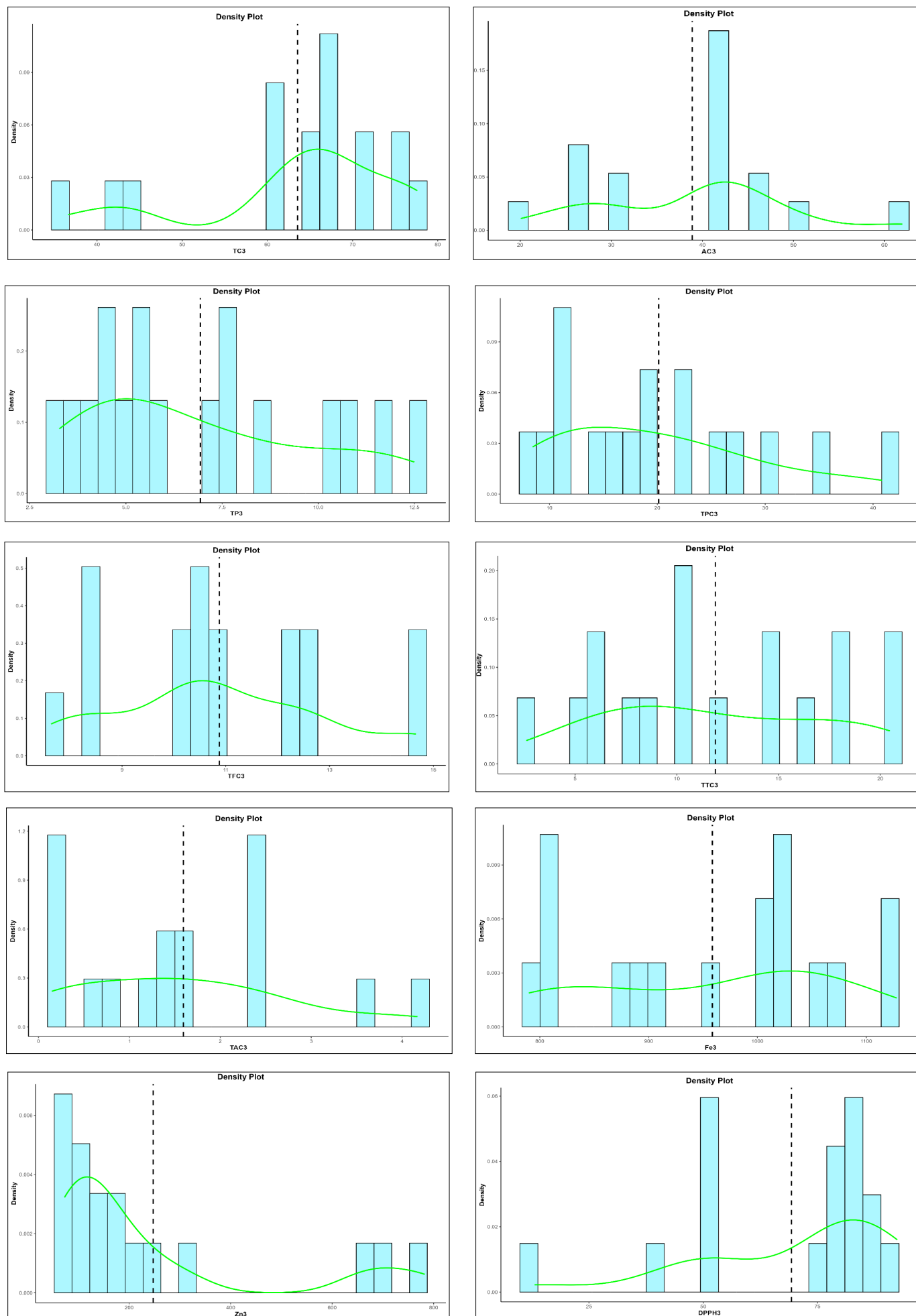Fig. 1. Frequency distribution patterns of traits studied in F₂ progenies of sorghum cross CO 32 × Paiyur 2.

Fig. 2. Frequency distribution patterns of traits studied in F$_3$ progenies of sorghum cross CO 32 × Paiyur 2.

The integration of molecular tools, such as genomic selection and marker-assisted breeding, could enhance selection accuracy and accelerate breeding progress for these traits. The strong heritability estimates for Fe and Zn highlighted their potential in biofortification efforts, contributing to the development of nutritionally superior sorghum varieties (14, 23).

Conclusion

This study on the segregating populations of the red sorghum cross CO 32 × Paiyur 2 demonstrated significant genetic variability in all biochemical traits. The contrasting parental lines and subsequent selection pressure contributed to marked improvements in the mean performance of most traits from F_2 to F_3 generations. Skewness and kurtosis analyses provided insights into trait distribution patterns and gene interactions, indicating both additive and non-additive effects. Traits such as amylose content, total protein, tannins, Fe, Zn and antioxidant activity showed high intergenerational correlations and heritability, highlighting their reliability for selection and breeding. The moderate to high heritability estimates for most traits emphasized the genetic potential for trait improvement. These findings underscored the effectiveness of early-generation selection and suggested the utility of the identified genotypes, such as III-55, III-85 and III-140, as promising candidates for biofortified and health-promoting sorghum varieties. Future research should incorporate molecular tools like QTL mapping, genomic selection and functional markers to dissect the complex inheritance of bioactive traits and enhance breeding precision.

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Authors' contributions

Conceptualization and writing were carried out by SJ, with review and editing contributions from RC, SS, KC, VVAG, NMB and TN. All the authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest.

Ethical issues: None

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Additional information