



RESEARCH ARTICLE

Gamma ray-induced genetic variability and multivariate trait analysis in M_2 and M_3 populations of sorghum (*Sorghum bicolor* (L.) Moench) genotype ICSV 18002

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Abstract

Induced mutagenesis is an effective strategy for generating novel genetic variability and accelerating crop improvement in sorghum. The present study was conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India, during 2023–25 to evaluate dose-dependent radiosensitivity, mutation induction and phenotypic variability in the sorghum genotype ICSV 18002 following gamma irradiation. Radiosensitivity assessment revealed a progressive reduction in seedling height with increasing radiation dose and the LD₅₀ was estimated at approximately 230 Gy, corresponding to a 50 % reduction in seedling height relative to the control. Screening of the M_2 population identified chlorophyll-deficient mutants with a mutation frequency of 1.0 %, confirming effective mutation induction. Frequency distribution and genetic parameter analyses indicated substantial induced variability for several agro-morphological and yield traits, such as plant height (PH), internode length (IL), stem girth (SG), panicle length (PL), panicle width (PW), disease score (DS), seed yield per plant (SYPP) and hundred-seed weight (HSW). High genotypic variability coupled with high heritability and genetic advance was observed for SYPP, DS, SG, PW and HSW, suggesting strong prospects for direct selection. Correlation analysis in the M_3 generation showed that yield was primarily associated with panicle architecture, particularly PW and PL, while reduced disease incidence contributed positively to productivity. Principal component and hierarchical cluster analyses revealed considerable phenotypic divergence among mutant families, indicating the generation of diverse trait combinations. Standardised deviation (Z-score) analysis identified several superior mutants exhibiting early flowering (74.00 days compared with 79.33 days in the control), reduced PH (278.33 cm compared with 360.56 cm in the control), higher SYPP (up to 63.90 g compared with 16.06 g in the control) and increased HSW (up to 3.49 g compared with 2.21 g in the control). Parent-progeny analysis indicated stronger transmission of structural vegetative traits than complex yield traits, suggesting the need for further generational stabilisation for reliable selection. Overall, the study demonstrates the effectiveness of gamma irradiation in generating useful variability and identifies promising mutant lines for sorghum improvement.

Keywords: gamma irradiation; mutation breeding; phenotypic variability; radiosensitivity; sorghum improvement

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a major cereal crop cultivated in arid and semi-arid regions, where irregular rainfall, high temperatures and marginal soils limit the productivity of other cereals. It serves as a staple food, livestock feed and industrial raw material, contributing significantly to food security and climate-resilient agriculture in South Asia and Sub-Saharan Africa (1, 2). Globally, sorghum is cultivated on approximately 40.76 million hectares, with a total production of about 57.58 million tonnes, mainly in Nigeria, Sudan, the United States, Mexico, Ethiopia and India. In India, the crop occupies nearly 3.80 million hectares, with an annual production of 4.15 million tonnes. Despite its adaptability to

diverse and stress-prone environments, sorghum productivity remains relatively low (approximately 1.41 t ha⁻¹ globally and 1.09 t ha⁻¹ in India, based on FAO statistics) and is constrained by recurrent drought, fungal diseases and the limited genetic diversity available for crop improvement (3, 4). Expanding the genetic base of sorghum is therefore essential for improving yield potential, stress tolerance and agronomic performance.

Induced mutagenesis has proven to be an effective approach for generating novel genetic variation and enhancing desirable traits in crop plants. It has played a fundamental role in crop improvement and has led to the development and release of numerous improved mutant varieties worldwide (5, 6). Among

physical mutagens, gamma rays are widely used because of their high penetrative power and ability to induce diverse mutations ranging from point mutations to chromosomal aberrations (6, 7). Numerous improved crop varieties developed through gamma irradiation demonstrate its utility in mutation breeding. According to FAO/IAEA Mutant Variety Database, more than 3400 mutant varieties have been released across 251 cultivated species in 78 countries. In sorghum, 22 mutant varieties have been released between 1970 and 2024 in countries including China, India, Mali, Indonesia and Russia. These varieties possess desirable traits such as enhanced grain yield, reduced plant height, early maturity and improved grain quality, including higher protein and starch content. Notably, in 2024, four new sorghum mutant varieties were released in Namibia through gamma ray-induced mutagenesis (8). This recent release highlights the continued global application of gamma ray-induced mutation breeding in sorghum improvement. The success of mutagenesis, however, depends on identifying optimal radiation doses that balance mutation frequency with plant survival, as well as on efficiently characterising induced variability using appropriate statistical and multivariate approaches.

Although gamma irradiation has been widely used to induce genetic variability in sorghum, comprehensive information on genotype-specific radiosensitivity and the extent of induced phenotypic variability remains limited. Furthermore, studies integrating genetic variability parameters, trait associations, multivariate analyses and parent-progeny relationships within a single mutant population are scarce. Therefore, the present study was undertaken to evaluate the radiosensitivity of four sorghum genotypes (ICSV 15014, S 35, IS 33871 and ICSV 18002) and to determine an effective irradiation dose. Among these, ICSV 18002 was selected for bulk irradiation because it exhibited moderate radiosensitivity ($LD_{50} \approx 230$ Gy), providing a desirable balance between mutation induction and plant survival. The study further characterised induced phenotypic variability, genetic parameters, trait relationships and selection potential among mutant lines using correlation analysis, principal component analysis, hierarchical cluster analysis and parent-progeny evaluation. By integrating genotype-specific radiosensitivity assessment with comprehensive phenotypic, genetic and multivariate analyses within a single mutant population, this study provides a systematic framework for identifying superior mutant lines and demonstrates the effectiveness of gamma irradiation for enhancing breeding efficiency and broadening the genetic base of sorghum.

Materials and Methods

Experimental site

The field experiments were conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, India during 2023–25. The experimental site is located at 17°32' N latitude and 78°16' E longitude, at an elevation of 545 m above mean sea level. The region has a semi-arid tropical climate with an average annual rainfall of approximately 750 mm, most of which is received during the southwest monsoon (June–September). The experimental field is characterised by deep black clay soil.

Gamma irradiation treatment and LD_{50} estimation

Four sorghum genotypes, ICSV 15014, S 35, IS 33871 and ICSV 18002, representing major market segments, were used for initial

radiosensitivity assessment. Uniform, self-pollinated seeds were exposed to gamma irradiation at doses of 0 (control), 75, 150, 300, 450 and 600 Gy at the International Atomic Energy Agency (IAEA), Vienna, Austria. Mutagenised (M_0) and control seeds were evaluated under glasshouse conditions at the ICRISAT, Patancheru, Hyderabad, using a completely randomised design (CRD) with 3 replications per treatment. Each replication consisted of three pots, with five plants maintained per plot, resulting in 15 plants per replication and 45 plants per treatment. Seedling emergence and height were recorded and percentage height reduction relative to the control was calculated using mean seedling height of treated and control plants. Height reduction (%) was used to generate dose-response curves to estimate LD_{50} .

Height reduction (%) =

$$\frac{\text{Mean height of control} - \text{Mean height of treated plants}}{\text{Mean height of control}} \times 100$$

Raising of mutant populations

Based on the estimated LD_{50} value, seeds of the sorghum genotype ICSV 18002 were bulk irradiated with 230 Gy to develop mutant populations. The bulk irradiated seeds (M_0) were sown under field conditions at the ICRISAT, with a spacing of 45 cm between rows and 15 cm between plants (six rows of 100 m each) to generate large M_1 population. Standard agronomic practices were followed to ensure optimal plant growth. The mutagenised population was maintained under isolation and individual M_1 plants were self-pollinated using selfing bags to prevent foreign pollen contamination. Seeds from individual selfed M_1 plants were harvested separately and a single seed from each M_1 plant was bulked to constitute the M_2 population. A total of 1825 M_2 seeds were sown to raise M_2 population following the same recommended agronomic practices. Germination percentage was calculated based on the number of seedlings established relative to the total number of seeds sown. The M_2 population was carefully screened from seedling emergence onward to identify chlorophyll-deficient mutants, while the parental genotype was grown in border rows for comparison. Agro-morphological and yield traits were recorded on selected M_2 plants, were harvested separately and their progenies were subsequently evaluated as M_3 families.

Estimation of mutation frequency, mutagenic effectiveness and efficiency

Chlorophyll-deficient mutants, including albino, chlorina, xantha and viridis types, were identified at the seedling stage based on deviations in leaf pigmentation from the normal green phenotype. Mutation frequency (MF) was calculated as the proportion of mutant seedlings relative to the total number of plants screened using the following formula:

$$MF = \frac{\text{Number of mutant plants}}{\text{Total number of plants screened}}$$

Mutagenic effectiveness and mutagenic efficiency were estimated to evaluate the effectiveness of gamma irradiation in inducing mutations relative to the applied dose and the associated biological damage, following the method of Konzak et al. (9).

Mutagenic effectiveness (%) =

$$\frac{\text{Mutation frequency (\%)}}{\text{Dose (Gy)}} \times 100$$

Mutagenic efficiency (%)=

$$\frac{\text{Mutation frequency (\%)}}{\text{Lethality (\%)}} \times 100$$

where Lethality (%) was calculated as the percentage reduction in seedling survival relative to the untreated control.

Observations recorded

In the M_2 generation, the following agro-morphological traits were recorded on individual plants: plant height (PH, cm), number of leaves per plant (NL), leaf length (LL, cm), leaf width (LW, cm), internode length (IL, cm), stem girth (SG, cm), panicle length (PL, cm), panicle width (PW, cm), disease score (DS), seed yield per plant (SYPP, g), and hundred-seed weight (HSW, g). In the M_3 generation, all the above traits except SG were recorded, along with days to 50 % flowering (DFF).

Statistical analysis

Frequency distributions of agro-morphological and yield-related traits in the M_2 population were generated using R software (R Core Team, 2024) to examine the pattern of phenotypic variation. Genetic variability parameters, including phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), environmental coefficient of variation (ECV), broad-sense heritability (h^2) and genetic advance as percent of mean (GAM), were estimated following Burton (10), Lush (11) and Johnson et al. (12). Pearson's correlation analysis was performed to assess relationships among traits in the M_3 population following Falconer (13). Principal component analysis (PCA) was conducted using the prcomp function in R based on standardised trait values according to Rao (14) and hierarchical cluster analysis was performed using Euclidean distance and Ward's minimum variance method (15). Superior mutants were identified using the standardised deviation (Z-score) approach. The Z-score for each mutant was calculated as:

$$Z = \frac{X_i - \mu}{\sigma}$$

where X_i is the observed value of the i^{th} mutant and μ and σ are the mean and standard deviation of the corresponding control, respectively. Mutants were evaluated following the principles described by Gaul (16) and Konzak et al. (9). Parent-progeny relationships between M_2 plants and their corresponding M_3 families were evaluated using Pearson's correlation and simple linear regression following Falconer (13) and Singh and Chaudhary (17). All statistical analyses were performed using R software (R Core Team, 2024).

Results

Radiosensitivity assessment and LD₅₀ determination in ICSV 18002

Gamma irradiation caused a progressive reduction in seedling height in genotype ICSV 18002, indicating a clear dose-dependent inhibition of early growth (Fig. 1). Growth reduction was minimal at lower doses but became pronounced beyond 150 Gy, with a sharp decline observed at 300 Gy and higher doses. Based on the fitted regression curve, the LD₅₀ value for ICSV 18002 was estimated at approximately 230 Gy. This dose was subsequently selected for bulk irradiation to develop the mutant population.

Mutation frequency, mutagenic effectiveness, efficiency and spectrum of chlorophyll mutants in the M_2 population

A total of 1825 seeds of sorghum genotype ICSV 18002 were sown in the M_2 generation, of which 1500 seedlings established successfully, resulting in a germination rate of 82.19 %. Screening at the seedling stage identified 15 chlorophyll-deficient mutants, corresponding to a mutation frequency of 1.00 %. Based on the observed mutation frequency at the selected irradiation dose of 230 Gy, the mutagenic effectiveness and efficiency were estimated to be 0.43 % and 1.32 %, respectively. The chlorophyll-deficient mutants included albino, xantha, chlorina and viridis types (Fig. 2).

Frequency distribution and genetic variability in the M_2 population

Frequency distribution analysis of eleven agro-morphological and

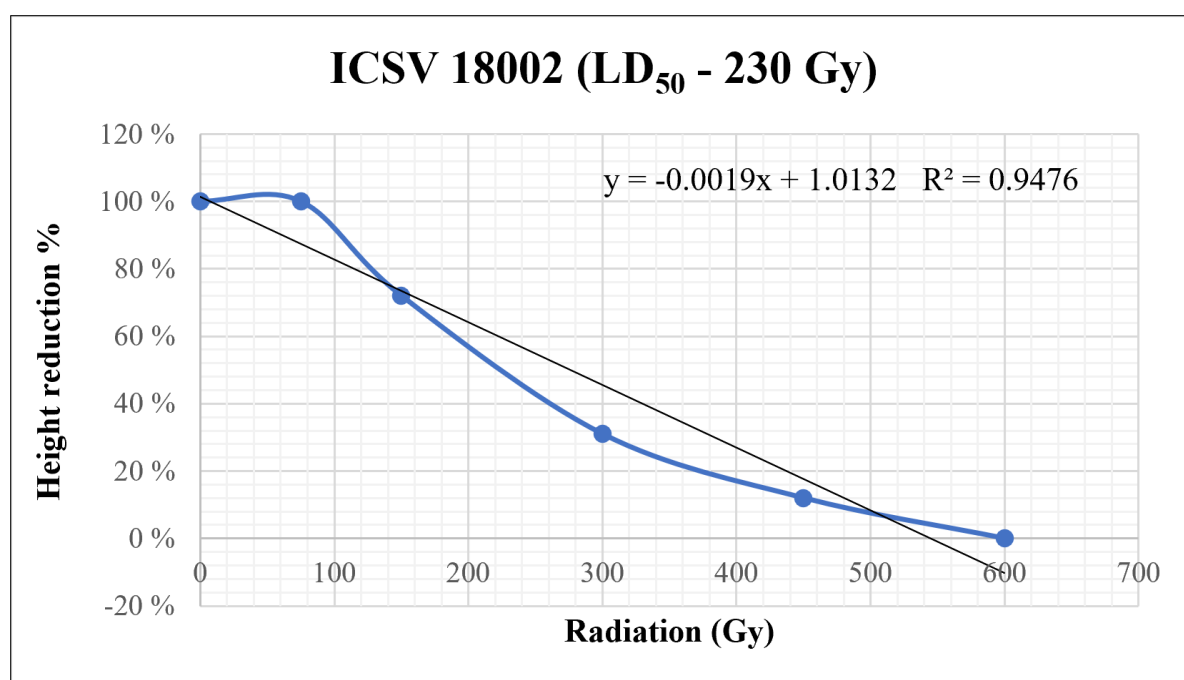


Fig. 1. Dose-response relationship between gamma irradiation and seedling height reduction in sorghum genotype ICSV 18002.



Fig. 2. Spectrum of chlorophyll-deficient mutants observed in the M_2 generation of sorghum genotype ICSV 18002.

yield-related traits in the M_2 population of sorghum genotype ICSV 18002 revealed substantial phenotypic variation induced by gamma irradiation (Fig. 3). Most traits exhibited continuous distributions with near-normal patterns, indicating quantitative inheritance. Traits such as PH, IL, LL, PW and HSW showed relatively symmetrical distributions, whereas SYPP, DS and SG displayed wider dispersion. The PCV was consistently higher than the corresponding GCV for all traits (Table 1). High PCV and GCV were observed for SYPP (61.45 % and 44.79 %) and DS (37.78 % and 29.98 %). Moderate variability was recorded for SG, PL and PW, while relatively low variability was observed for NL, IL, PH and HSW. Broad-sense heritability ranged from 17.41 % for LW to 95.01 % for NL. High heritability coupled with high GAM was observed for SYPP (53.12 % and 67.24 %), DS (58.82 % and 45.79 %), SG (90.37 % and 37.51 %), PW (62.55 % and 25.83 %) and HSW (93.65 % and 24.69 %). In contrast, LL and LW exhibited comparatively lower heritability and GAM.

Correlation analysis of yield and associated traits in the M_3 population

Correlation analysis among agro-morphological and yield-related traits in the M_3 population of sorghum genotype ICSV 18002 revealed several significant relationships (Fig. 4). The PH showed strong positive associations with NL ($r = 0.81^{***}$) and IL ($r = 0.51^{***}$) and a weak but significant negative correlation with DFF ($r = -0.19^{**}$). The LL exhibited positive correlations with LW ($r = 0.59^{***}$), PL ($r = 0.23^{***}$) and PW ($r = 0.14^{*}$). Among yield components, PW exhibited a strong positive correlation with SYPP ($r = 0.71^{***}$), while PL ($r = 0.46^{***}$) and HSW ($r = 0.30^{***}$) were also positively associated with SYPP. The DS showed a significant negative correlation with yield ($r = -0.36^{***}$).

Principal component analysis of the M_3 population

The PCA revealed that the first four principal components (PCs) accounted for a substantial proportion of the total phenotypic variation (Fig. 5A). The PC1 explained 23.39 % of the total variance, followed by PC2 (19.65 %), PC3 (14.05 %) and PC4 (10.69 %), together contributing 67.78 % of the total phenotypic variability. Trait loadings indicated that PC1 was primarily associated with yield and panicle-related traits, whereas PC2 reflected vegetative and phenological attributes. The PCA biplot showed clear trait grouping

and wide dispersion among mutant families (Fig. 5B).

Hierarchical cluster analysis of the M_3 population

Hierarchical cluster analysis using Ward's minimum variance method grouped the mutants based on standardised agro-morphological and yield-related traits, revealing clear phenotypic divergence within the ICSV 18002 M_3 population (Fig. 6). At the selected dissimilarity level, the mutants were separated into three major clusters. Cluster I (blue) comprised the largest proportion of genotypes and exhibited gradual internal branching. Cluster II (green) formed a relatively compact group with shorter linkage distances. Cluster III (red) was distinctly separated from the other clusters by longer branch lengths and displayed noticeable internal branching.

Identification of superior mutants in the ICSV 18002 M_3 population based on standardised deviation (Z-score) analysis

Standardised deviation (Z-score) analysis identified several M_3 mutant families that exhibited significant deviations from the control for important agro-morphological and yield-related traits (Table 2; Fig. 7). Four mutants (ICSV 18002- M_1 -700, -617, -649 and -607) exhibited significantly reduced DFF. The PH showed bidirectional variation, with mutants ICSV 18002- M_1 -1165, -879, -891, -1015 and -1114 exhibiting significantly reduced PH. Substantial improvements were observed for yield-related traits, with mutants ICSV 18002- M_1 -1232, -888, -1094 and -700 exhibiting significant increases in PL, PW and SYPP. Several mutants, including ICSV 18002- M_1 -1173, -1285, -1063, -700 and -933, recorded significantly higher HSW. Lower disease scores were observed in mutants ICSV 18002- M_1 -1232, -1094, -1173 and -1007.

Parent-progeny relationships between M_2 plants and derived M_3 families

Parent-progeny analysis indicated that only a few traits showed consistent transmission from M_2 plants to their corresponding M_3 families (Table 3). The LL and IL exhibited moderate and highly significant positive correlations along with relatively higher regression estimates. PH showed a weak but significant positive association. In contrast, NL, LW, PL, PW, DS and SYPP exhibited non-significant regression and correlation values. The HSW showed a

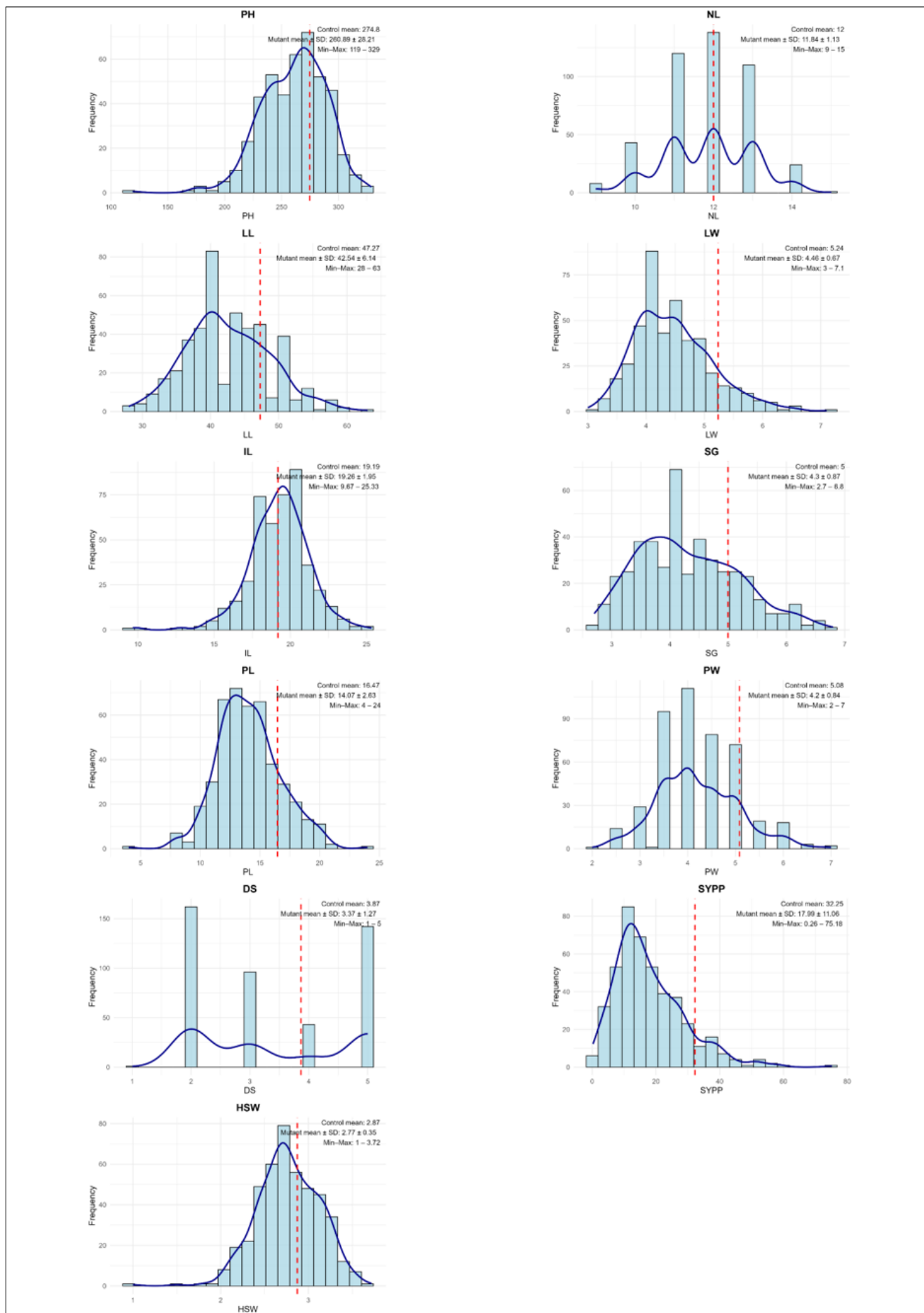


Fig. 3. Frequency distribution of agro-morphological and yield traits in the M_2 population of sorghum genotype ICSV 18002. The vertical dashed line represents the control mean, while the continuous curve indicates the fitted density distribution, illustrating the extent and pattern of phenotypic variability generated by mutagenesis. PH: plant height; NL: number of leaves; LL: leaf length; LW: leaf width; IL: internode length; SG: stem girth; PL: panicle length; PW: panicle width; DS: disease score; SYPP: seed yield per plant; HSW: hundred seed weight.

Table 1. Estimates of genetic variability parameters for agro-morphological and yield traits in the M₂ population of sorghum genotype ICSV 18002

Characters	Control Mean	Mutant		PCV (%)	GCV (%)	ECV (%)	h ² (bs)	GAM (%)
		Mean	Range					
Plant height (cm)	274.80	260.89	119 – 329	10.81 ^b	10.13 ^b	3.79 ^c	87.71 ^a	19.53 ^b
Number of leaves	12.00	11.84	9 – 15	9.56 ^c	9.32 ^c	2.14 ^c	95.01 ^a	18.72 ^b
Leaf length (cm)	47.27	42.54	28 – 63	14.42 ^b	11.62 ^b	8.55 ^c	64.87 ^a	19.28 ^b
Leaf width (cm)	5.24	4.46	3 – 7.1	14.95 ^b	6.24 ^c	13.58 ^b	17.41 ^c	5.36 ^c
Internode length (cm)	19.19	19.26	9.7 – 25.3	10.13 ^b	9.25 ^c	4.14 ^c	83.32 ^a	17.39 ^b
Stem girth (cm)	5.00	4.30	2.7 – 6.8	20.15 ^a	19.15 ^b	6.25 ^c	90.37 ^a	37.51 ^a
Panicle length (cm)	16.47	14.07	4 – 24	18.72 ^b	6.33 ^c	17.61 ^b	11.43 ^c	4.41 ^c
Panicle width (cm)	5.08	4.20	2 – 7	20.05 ^a	15.85 ^b	12.27 ^b	62.55 ^a	25.83 ^a
Disease score	3.87	3.37	1 – 5	37.78 ^a	29.98 ^a	24.25 ^a	58.82 ^b	45.79 ^a
Seed yield per plant (g)	32.25	17.99	0.26 – 75.18	61.45 ^a	44.79 ^a	42.07 ^a	53.12 ^b	67.24 ^a
Hundred seed weight (g)	2.87	2.77	1 – 3.72	12.80 ^b	12.39 ^b	3.23 ^c	93.65 ^a	24.69 ^a

PCV: phenotypic coefficient of variation (%); GCV: genotypic coefficient of variation (%); ECV: environmental coefficient of variation (%); h² (bs): heritability (broad sense); and GAM: genetic advance as percentage of the mean (%); a: high; b: moderate; c: low.

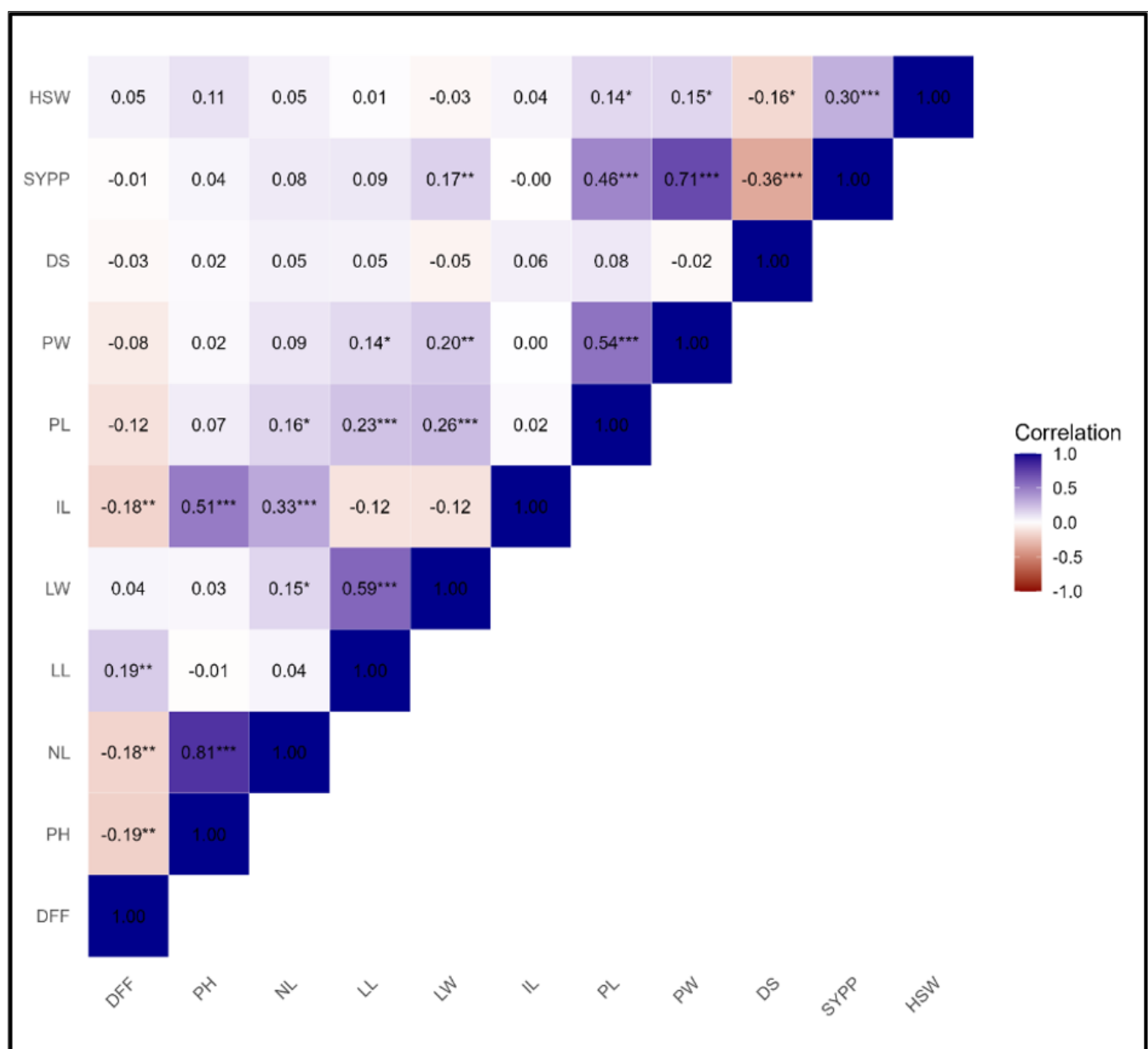


Fig. 4. Pearson's correlation matrix of agro-morphological and yield-related traits in the M₃ population of sorghum genotype ICSV 18002. Significance levels are indicated with * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. DFF: days to 50% flowering; PH: plant height; NL: number of leaves; LL: leaf length; LW: leaf width; IL: internode length; PL: panicle length; PW: panicle width; DS: disease score; SYPP: seed yield per plant; HSW: hundred seed weight.

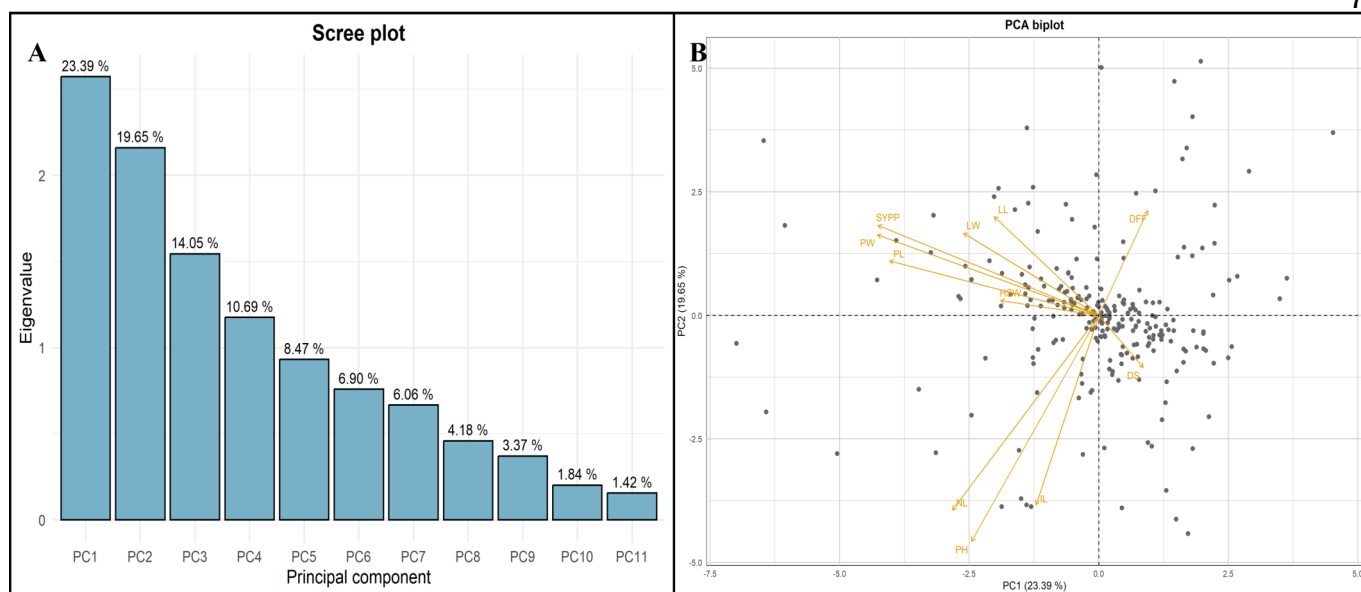


Fig. 5. Principal component analysis of agro-morphological and yield traits in the M_3 population of sorghum genotype ICSV 18002. A: scree plot showing percentage variance explained by each principal component; B: PCA biplot based on PC1 and PC2.

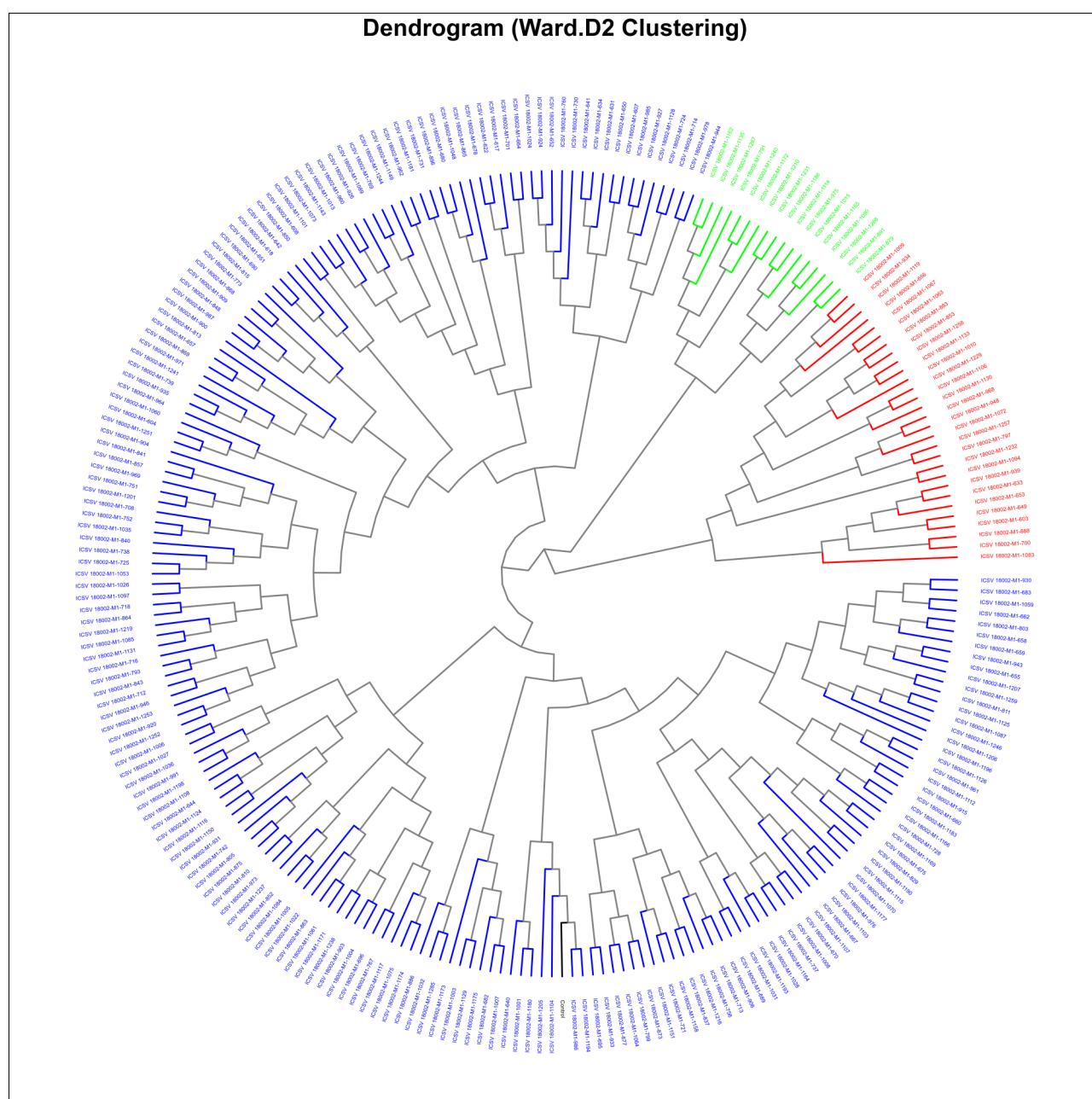


Fig. 6. Circular dendrogram of the M_3 population of sorghum genotype ICSV 18002 constructed using Ward's minimum variance method (Ward.D2) based on standardised agro-morphological and yield-related traits.

Table 2. Identification of superior mutants in the ICSV 18002 M₃ population using standardised deviation (Z-score) analysis

Mutant ID	DFF	PH	NL	LL	LW	IL	PL	PW	DS	SYPP	HSW
Control	79.33	360.56	15.00	66.89	6.23	18.72	17.58	4.19	4.08	16.06	2.21
ICSV 18002-M1-1232	78	323.1*	13.5	68.085	6.524	18.411	25***	7.5***	2*	63.9***	2.55**
ICSV 18002-M1-888	78	371.667	15.333	66.333	6.933**	20	25***	6**	3	42.9**	2.75***
ICSV 18002-M1-1094	78	335	13.333	71.333	6.8*	20.667*	25***	6.5***	2*	55.5***	2.19
ICSV 18002-M1-700	74***	361.667	14.667	65	6.767*	20.667*	25***	6**	3	24.4	3.26***
ICSV 18002-M1-797	80	338.333	14.333	68.667	6.667	19.333	20	6**	4	48.5***	2.66***
ICSV 18002-M1-1072	78	329	13.4	66.905	6.533	19.041	28***	5.3	3	40.8**	2.85***
ICSV 18002-M1-1173	80	305**	12.333*	73	6.867**	19	19	4.5	2*	31.4	3.43***
ICSV 18002-M1-1165	80	286.667***	11.667**	66	6.533	19.667	19.5	4.35	3	18.35	2.68***
ICSV 18002-M1-617	74***	338.333	13.667	68.667	7.167***	20	17.833	4.833	3.333	24.53	2.52**
ICSV 18002-M1-879	78	291.667***	11.333***	71.667	6.8*	17.333	16.667	4.833	3.667	18.07	2.45*
ICSV 18002-M1-891	78	288.333***	10.667***	68	6.967**	17.333	17.5	4.5	4	21.85	2.38
ICSV 18002-M1-1007	79	329.1	13.4	67.495	6.524	19.199	22.5*	4.25	2*	28.3	2.74***
ICSV 18002-M1-1285	80	328.333	13.667	74	6.933**	20.333	19	4.7	2.5	26.3	3.26***
ICSV 18002-M1-1003	80	330	13.5	69.118	6.521	19.252	18	4.5	2*	32.1	2.73***
ICSV 18002-M1-1063	78	332.4	13.4	68.676	6.503	19.357	22.333*	5.167	4.333	18.6	3.49***
ICSV 18002-M1-933	80	329	13.5	68.971	6.519	19.146	19.333	4.333	5	11.07	3.29***
ICSV 18002-M1-1015	79	278.333***	10.667***	63.667	6.133	18.333	18.25	4	4.5	14.65	1.66***
ICSV 18002-M1-1114	79	288.3***	11.667**	67	6.267	19	17	5.15	3	22.5	2.03
ICSV 18002-M1-649	74***	373.333	14.667	68.333	6.333	19	21.333	4	3.333	11.8	2.52**
ICSV 18002-M1-607	74***	320*	14	59.667	6.233	20	17	3.5	3.333	12.3	2.36

Asterisks indicate the levels of statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) of M₃ family means compared with the control. DFF: days to 50 % flowering; PH: plant height; NL: number of leaves; LL: leaf length; LW: leaf width; IL: internode length; PL: panicle length; PW: panicle width; DS: disease score; SYPP: seed yield per plant; HSW: hundred seed weight.

**Fig. 7.** Superior mutant lines identified in the M₃ generation of the sorghum genotype ICSV 18002.

Table 3. Parent-progeny regression and correlation between M₂ plants and their derived M₃ families

Characters	Regression		Correlation (<i>r</i>)	<i>p</i> -value	Interpretation
	Slope	R ²			
PH	0.0856	0.0356	0.1886**	0.0032	Weak but significant transmission
NL	-0.0515	0.0069	-0.0834	0.1962	No significant transmission
LL	0.1442	0.1262	0.3552***	< 0.0001	Moderate and significant transmission
LW	-0.0222	0.0030	-0.0544	0.3994	No significant transmission
IL	0.1566	0.1298	0.3603***	< 0.0001	Moderate and significant transmission
PL	0.0714	0.0052	0.0724	0.2619	Weak non-significant transmission
PW	-0.0615	0.0066	-0.0811	0.2089	No significant transmission
DS	-0.0588	0.0106	-0.1028	0.1106	Weak non-significant transmission
SYPP	-0.0444	0.0044	-0.0662	0.3052	No significant transmission
HSW	0.1277	0.0123	0.1111	0.0846	Weak positive tendency (not significant)

Asterisks indicate the levels of statistical significance (**p* < 0.05, ***p* < 0.01, ****p* < 0.001); PH: plant height; NL: number of leaves; LL: leaf length; LW: leaf width; IL: internode length; PL: panicle length; PW: panicle width; DS: disease score; SYPP: seed yield per plant; HSW: hundred seed weight.

weak positive relationship, although the association was not statistically significant.

Discussion

Gamma irradiation remains one of the most effective physical mutagens for generating novel genetic variability in crop improvement. The progressive reduction in seedling height with increasing radiation dose confirms the dose-dependent response of sorghum to ionising radiation and demonstrates that an LD₅₀ of approximately 230 Gy provides an effective balance between mutation induction and plant survival. Similar reductions in seedling growth following gamma irradiation have been reported in sorghum and other cereal crops, where radiation-induced physiological and cellular damage increasingly limits growth at higher doses (5, 18, 19). The moderate radiosensitivity observed in genotype ICSV 18002 suggests that the selected irradiation dose was appropriate for inducing sufficient genetic variability while maintaining adequate plant survival for subsequent selection.

The occurrence of chlorophyll-deficient mutants further confirms the effectiveness of gamma irradiation in inducing heritable genetic variations. Chlorophyll mutants are widely recognised as reliable indicators of mutagenic effectiveness because they arise from alterations in genes controlling chloroplast development and chlorophyll biosynthesis (20, 21). Although the mutation frequency (1.00 %) observed in the present study was comparatively lower than that reported in some sorghum mutation breeding studies, such variation is expected because mutation frequency is strongly influenced by genotype, irradiation dose, seed physiological condition and the criteria adopted for mutant screening (6, 22). The mutagenic effectiveness (0.43 %) and efficiency (1.32 %) obtained at the selected LD₅₀ dose further demonstrate that the irradiation treatment successfully induced useful genetic variation while minimising excessive biological damage. Similar genotype-dependent differences in mutagenic response have been reported in sorghum and several other crop species (18, 21, 22).

The broad phenotypic variation observed among the mutant population demonstrates the ability of gamma irradiation to expand the genetic base available for selection. The continuous distribution of most agro-morphological and yield-related traits indicates that induced mutations generated quantitative variation suitable for crop improvement. The consistently higher PCV than GCV across all traits reflects the influence of environmental factors on trait expression, a common characteristic of segregating mutant populations (23). Nevertheless, the combination of high heritability

and high genetic advance observed for SYPP, DS, SG, PW and HSW suggests that these traits are predominantly governed by additive gene action and therefore offer considerable scope for effective phenotypic selection (24, 25).

The strong association between seed yield and panicle architecture indicates that panicle traits can serve as reliable indirect selection criteria for improving productivity in sorghum mutation breeding programmes. The positive relationships of PW and PL with seed yield highlight the importance of panicle architecture in determining sink capacity and assimilate partitioning, consistent with previous reports in sorghum (24, 25). Likewise, the negative association between disease score and seed yield suggests that improved disease tolerance contributes directly to enhanced productivity, reinforcing the importance of simultaneous selection for disease resistance and yield-related traits.

Multivariate analyses further demonstrated that gamma irradiation generated substantial phenotypic divergence within the mutant population. The proportion of phenotypic variation within the first four PCs indicates that a relatively small number of components captured most of the induced variability, thereby facilitating efficient identification of superior mutant lines. Similarly, hierarchical cluster analysis separated the mutant families into three distinct groups, confirming the existence of broad genetic diversity created through induced mutagenesis. Such inter-cluster divergence provides valuable opportunities for selecting genetically diverse parents to broaden the breeding pool and develop superior recombinants, as reported in previous mutation breeding studies (26–28).

The identification of superior mutant lines exhibiting early flowering, reduced plant height, improved yield attributes, increased hundred-seed weight and lower disease scores demonstrates the practical value of gamma irradiation for developing breeding materials with multiple desirable agronomic traits. The simultaneous improvement of these characteristics is particularly important for developing climate-resilient sorghum cultivars capable of maintaining productivity under diverse production environments (29–31). These findings reinforce the potential of mutation breeding as an effective strategy for broadening the genetic base and generating novel trait combinations that may not be readily available within existing germplasm collections.

The weak parent-progeny relationships observed for several quantitative traits suggest that environmental effects and residual segregation continue to influence trait expression during the early generations' mutation breeding. In contrast, the comparatively stronger inheritance of structural vegetative traits indicates that these characters stabilise more rapidly than complex yield-related

traits. Similar observations have been reported in mutation breeding programmes, where reliable selection for quantitative traits generally requires advancement to later generations and evaluation across multiple environments (6). Therefore, continued selection together with multi-location validation will be essential to confirm the stability and breeding value of the superior mutant lines identified in this study. Overall, the present study demonstrates that gamma irradiation is an effective approach for generating exploitable genetic variability in sorghum and provides promising mutant lines for the development of high-yielding, early-maturing and disease-tolerant cultivars.

Conclusion

Gamma irradiation effectively induced substantial genetic variability in the sorghum genotype ICSV 18002, generating broad phenotypic diversity for several agro-morphological and yield-related traits. An LD₅₀ of approximately 230 Gy provided an effective balance between mutation induction and plant survival, making it suitable for developing mutant populations. Screening of the M₂ population identified chlorophyll-deficient mutants with a mutation frequency of 1.00 %, confirming the effectiveness of gamma irradiation in inducing heritable genetic variation. High genotypic variability, broad-sense heritability and genetic advance observed for seed yield per plant (SYPP), disease score (DS), stem girth (SG), panicle width (PW) and hundred seed weight (HSW) indicate that these traits can be effectively improved through direct selection. Correlation, principal component, hierarchical cluster and Z-score analyses facilitated the identification of superior mutant lines exhibiting early flowering, reduced plant height (PH), enhanced yield, increased hundred-seed weight (HSW) and lower disease scores. Parent-progeny analysis further indicated stronger inheritance of structural vegetative traits than complex yield traits, highlighting the importance of continued selection in advanced generations. The identified superior mutants represent valuable genetic resources for sorghum improvement and can serve as promising donor lines for the development of high-yielding, early-maturing and disease-tolerant sorghum cultivars. Future research should focus on multi-location and multi-season evaluation of these promising mutant lines, together with molecular characterisation and genomic validation, to assess the stability of the induced traits and accelerate their effective utilisation in sorghum breeding programmes. Overall, the findings of this study demonstrate the potential of gamma irradiation as an effective tool for broadening the genetic base of sorghum and the development of improved cultivars adapted to changing climatic conditions.

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Authors' contributions

VKG conducted the experiments, analysed the data and prepared the first draft of the manuscript. PS, EH, MAP, RJ, SJH and RK contributed to manuscript editing and revision. EH conceived and designed the study, supervised the experiments and secured funding. All authors contributed to the article and approved the final version of the manuscript.

Compliance with ethical standards

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