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Mutations Breeding in *Rabi* Sorghum [*Sorghum bicolor* (L.) Moench] for creation of Genetic Variability and Association Studies on Morpho-Physiological Traits in Induced Mutants.

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Abstract

Sorghum (*Sorghum spp*.) is a globally significant cereal, yet its productivity in India, particularly for *rabi* sorghum, is constrained by low and highly variable yields, primarily due to post-flowering drought stress [1] [2]. This comprehensive investigation was undertaken to generate and assess desirable genetic variability in the *rabi* sorghum genotype TSG-98 (Muguthi) through induced mutagenesis using electron beam irradiation. Pure selfed seeds were treated with five doses of 10 MeV electron beam (100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy) and subsequently evaluated across the M1 and M2 generations. The study focused on three main areas: mutagenic sensitivity in M1, the frequency and spectrum of induced mutations in M2, and the extent of genetic variability, heritability, correlation, and path analysis for fourteen critical yield and yield-contributing characters.

Results from the M1 generation demonstrated a clear dose-dependent reduction in seed germination and plant survival, a common indicator of mutagenic sensitivity [3] [4]. The lethal dose for 50% germination (LD50) was precisely estimated at 258.5 Gy, suggesting that the dose range between 200 Gy and 300 Gy is optimal for maximizing mutation frequency while maintaining a manageable survival rate for subsequent generation studies. In the M2 generation, the frequency of chlorophyll mutations increased linearly with the dose, peaking at 3.461% at 500 Gy. The spectrum of chlorophyll mutants included albina, xantha, viridis, xanthaviridis, and chlorina, with chlorina being the most prevalent type. Analysis of mutagenic effectiveness and efficiency indicated that lower doses (100 Gy and 200 Gy) were superior, yielding a higher proportion of mutations relative to undesirable effects like pollen sterility [5].

A wide and valuable spectrum of viable mutations was isolated, including semi-dwarf, ultra-dwarf, early maturing (103 days), bold seeded (4.5-4.9 g 100-seed weight), and large earhead types, all possessing significant potential for crop improvement. Analysis of variance confirmed the successful induction of highly significant genetic variability for all fourteen quantitative characters studied. High genotypic and phenotypic coefficients of variation (GCV and PCV) were observed for traits such as Fodder yield per plant, Flag leaf area, and Leaf area, indicating a strong genetic basis for these traits. Crucially, Grain yield per plant, Primaries per panicle, Fodder yield, and Flag leaf area exhibited high heritability coupled with high genetic advance as a percentage of mean (GAM), confirming that direct selection for these traits would be highly effective [6].

Correlation studies revealed a positive and significant genotypic and phenotypic association of grain yield per plant with days to 50% flowering, days to maturity, primaries per panicle, panicle length, fodder yield per plant, flag leaf area, and leaf area. Path coefficient analysis further dissected these associations, identifying Days to 50% flowering, Primaries per panicle, Grains per primary, Panicle length, Fodder yield, Relative water content (RWC), and Chlorophyll content (SPAD) as having a direct positive effect on grain yield per plant. These findings provide a robust framework for selecting superior mutant lines. The study successfully generated and characterized a diverse pool of genetic resources, which can be immediately utilized in targeted sorghum breeding programs to develop high-yielding, drought-resilient cultivars for the *rabi* season.

1.INTRODUCTION

1.1. Global and National Importance of Sorghum

Sorghum (*Sorghum spp*.) stands as the fifth most important cereal crop globally, serving as a staple food for millions in arid and semi-arid regions of Africa and Asia, and as a significant source of feed and industrial raw material in developed nations like the USA and China [7]. Its inherent resilience to drought and heat stress positions it as a critical crop for climate-resilient agriculture [8]. In India, sorghum is cultivated over approximately 4.9 million hectares, contributing an annual production of 6.36 million tonnes with an average productivity of 1.29 T ha-1 [9]. The grain is primarily used for human consumption, particularly in the form of 'Bhakri,' while the stover is a valuable source of fodder for livestock [10]. Furthermore, the industrial demand for sorghum grain is escalating for applications such as malt, ethanol production, and specialized flours.

1.2. Challenges in *Rabi* Sorghum Production

Despite its importance, sorghum cultivation in India faces significant challenges, particularly during the *rabi* (post-rainy) season. The productivity of *rabi* sorghum is notably low and highly variable, a phenomenon largely attributed to the severe post-flowering drought stress that is characteristic of the growing environment [2]. The length of the rainy season and the corresponding growing season have been observed to reduce in recent years due to climate change, necessitating a shift towards more drought-tolerant and short-duration crop varieties [11]. While sorghum is naturally drought-tolerant, the yield levels have remained virtually unchanged since the 1990s, highlighting a critical need for genetic enhancement [12]. The development of high-yielding cultivars with a shorter duration and enhanced drought tolerance is paramount to stabilizing and increasing the scope of *rabi* sorghum cultivation under moisture-limited conditions.

1.3. The Role of Genetic Variability and Mutation Breeding

Genetic variability is the fundamental prerequisite for any successful crop improvement program [13]. The degree of response to selection is directly proportional to the quantum of genetic variability present in the breeding population [14]. Grain yield in sorghum is a complex quantitative trait, governed by polygenes and significantly influenced by environmental factors [15]. Therefore, a thorough understanding of the genetics of yield and its component traits, including the association between them, is essential for designing effective selection strategies [16]. Correlation and path coefficient analyses are indispensable tools for dissecting the complex interrelationships among traits, allowing breeders to determine the direct and indirect contributions of component characters to the final yield [17] [18].

Mutation breeding offers a powerful and complementary approach to conventional breeding methods, particularly for creating novel genetic variation that may not exist naturally or has been lost during domestication [19] [20]. It is a relatively quick method, often used to correct specific defects in an otherwise agronomically superior cultivar [21]. The discovery of X-rays and chemical mutagens provided a strong impetus for induced mutagenesis [22] [23]. More recently, high-power linear electron accelerators have emerged as a high-throughput and efficient source of physical mutagens [24]. Electron beam radiation induces cytological, physiological, and morphological changes, thereby generating genetic variability for both qualitative and quantitative traits [25].

1.4. Objectives of the Study

The present investigation utilized the *rabi* sorghum genotype TSG-98 (Muguthi), a mid-late maturity (112-115 days) variety released by UAS, Dharwad, as the base material. The study aimed to evaluate the potential of electron beam irradiation in generating desirable variability for improving economically important traits in *rabi* sorghum. The specific objectives were:

1. To study the relative effects of electron beam irradiation on germination and plant survival in the M1 generation of *rabi* sorghum.
2. To estimate the frequency and spectrum of chlorophyll and viable mutations in the M2 generation.
3. To study the extent of genetic variability, heritability, and correlation studies among grain yield and fourteen yield component characters.
4. To study the direct and indirect contribution of different component characters on grain yield using path coefficient analysis.

2. Materials and Methods

2.1. Experimental Site and Material

The study was conducted at the Research Farm of the Department of Agricultural Botany, Vasantrao Naik Marathwada Krishi Vidyapeeth (VNMKV), Parbhani, India. The location is situated at 19°16' N latitude, 67°47' E longitude, and 409.0 m above mean sea level (msl), characterized by a shallow to medium black soil type and an annual average rainfall of 1080.1 mm. The experimental material was the pure selfed seed of the *rabi* sorghum genotype TSG-98 (Muguthi), a variety released by UAS, Dharwad, Karnataka, India. The salient features of TSG-98 are summarized in Table 1.

Table 1: Salient features of the genotype TSG-98 (Muguthi)

Sr. No.	Feature	TSG-98 (Muguthi)	Sr. No.	Feature	TSG-98 (Muguthi)
1.	Year of release	1969 (UAS Dharwad)	7.	Days to flower	60-70 days
2.	Notification date	2/2/1976	8.	Days to maturity	110-120 days
3.	Pedigree	Selection from 5-4-1	9.	Plant height	220-240 cm
4.	Yield	18-20 q/ha	10.	Panicle length	20-22 cm
5.	100 Seed weight	3.75-4.0 g	11.	Panicle width	5-7 cm
6.	Seed color	Pearl yellow	12.	Season adapted	*Rabi*

2.2. Mutagenic Treatment

The mutagen employed was a physical mutagen, a **10 MeV electron beam** generated from an electron accelerator at the Nuclear Agriculture &

Biotechnology Division, Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. The mode of action of the electron beam is primarily through ionization [9].

Pure selfed seeds (1200 seeds per treatment) of TSG-98, adjusted to a moisture content of 10%, were exposed to five different doses of electron beam radiation: **100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy**. An equal number of untreated seeds were maintained as two control groups: **Dry Control** (untreated, unsoaked seeds) and **Wet Control** (seeds soaked in phosphate buffer, then dried).

2.3. Experimental Design and Field Trials

The experiment was conducted in a **Randomized Block Design (RBD)** with three replications. The seven treatments (five doses + two controls) were evaluated in two successive generations:

- **M1 Generation:** Summer 2021-22.
- **M2 Generation:** *Rabi* 2022-23.

The field layout details were as follows:

- **Spacing:** 45 cm (row-to-row) x 15 cm (plant-to-plant).
- **Plot Size:** 5.4 m x 4.0 m, comprising 12 rows of 4.0 m length.
- **Method of Sowing:** Hand dibbling, with one seed per hill.
- **Fertilizer Dose:** Recommended package of practices were followed, including a fertilizer dose of 80 Kg N: 40 Kg P: 40 Kg K (kg/ha).

2.4. Observations and Data Collection

2.4.1. M1 Generation Studies (Mutagenic Sensitivity)

1. **Germination (%):** Recorded at 10, 15, and 20 days after sowing.
2. **Plant Survival (%):**Counted at 30 days after sowing.
3. **LD50 Determination:** The lethal dose for 50% reduction in germination and survival (LD50) was determined using Probit analysis [10].

2.4.2. M2 Generation Studies (Mutation Analysis and Quantitative Traits)

The M2 generation was raised from 36 selfed progeny selected from each M1 treatment, grown in a head-to-row progeny layout in RBD with three replications.

4. **Chlorophyll Mutants:** Scored on a plant basis up to 15 days after sowing and classified according to the system suggested by Gustafsson (1940) [11]: *Albino*, *Xantha*, *Chlorina*, *Viridis*, and *Xanthaviridis*.
5. **Viable Mutants:** Recorded up to harvest, including macromutations affecting plant habit (dwarf, tall), leaf morphology (narrow, broad, white midrib), and seed/panicle characteristics (bold seeded, early/late maturing, compact/loose earhead).
6. **Mutation Frequency (%):** Estimated using the formula suggested by Gaul (1958) [12].
7. **Mutagenic Effectiveness and Efficiency:** Calculated using the formula suggested by Konzak et al. (1965) [13].
8. **Quantitative Characters:** Observations were recorded on 300 randomly selected plants from each treatment for the following fourteen characters:
   - Days to 50% flowering
   - Plant height (cm)
   - Days to maturity
   - No. of primaries per panicle
   - No. of grains per primary branch
   - Panicle length (cm)
   - Panicle width (cm)
   - Grain yield per plant (g)
   - 100 seed weight (g)
   - Fodder yield per plant (g)
   - Relative water content (RWC) (%) [26]
   - Chlorophyll content (SPAD values)
   - Leaf area (cm2)
   - Flag leaf area (cm2)

2.5. Statistical Analysis

The mean values recorded for the M2 generation were subjected to Analysis of Variance (ANOVA) for Randomized Block Design [14]. Variability parameters were estimated as follows:

- **Genotypic Variance and Phenotypic Variance:** Calculated from the mean sum of squares.
- **Genotypic and Phenotypic Coefficients of Variation (GCV and PCV):** Calculated according to Burton (1952) [15].
- **Heritability in broad sense:** Estimated using the method suggested by Hansen et al. (1956) [16].
- **Genetic Advance (GA) and Genetic Advance as % of Mean (GAM):** Calculated using the formula suggested by Allard (1960) [6].
- **Correlation Analysis:** Genotypic and phenotypic correlation coefficients were estimated from the variance and covariance components [18].
- **Path Coefficient Analysis:** Performed to partition the correlation coefficients into direct and indirect effects on grain yield per plant, as suggested by Wright (1921) [17] and elaborated by Deway and Lu (1959) [19].

3. RESULTS AND DISCUSION

3.1. Mutagenic Sensitivity in M1 Generation

The mutagenic sensitivity of the TSG-98 genotype to electron beam irradiation was assessed based on the reduction in seed germination and plant survival in the M1 generation.

3.1.1. Effect on Germination and Survival

The data presented in Table 2 clearly indicate a dose-dependent reduction in both germination percentage and plant survival. The highest dose of 500 Gy resulted in the lowest germination (36.00%) and survival (29.33%), compared to the Wet Control (81.33% germination, 80.00% survival). This inhibitory effect is a typical biological consequence of mutagenic treatment, where increasing doses cause greater physiological damage, leading to reduced cell division and increased lethality [3] [4] [27]. Similar findings of dose-dependent reduction in germination and survival have been reported in sorghum and other crops [28] [29] [30].

Table 2: Effect of electron beam on germination and survival populations of M1 generation in *rabi* sorghum TSG-98

Sr. No.	EB Treatment	Germination (%)	Survival (%)
1	100 Gy	68.00	65.33
2	200 Gy	61.00	58.33
3	300 Gy	44.67	40.67
4	400 Gy	38.00	33.67
5	500 Gy	36.00	29.33
6	Wet Control	81.33	80.00
7	Dry Control	78.67	77.33

3.1.2. LD50 Determination

The LD50, a critical parameter for determining the optimal mutagen dose, was calculated for seed germination. The genotype TSG-98 was found to be highly sensitive to the electron beam, with the LD50 for seed germination estimated at **258.5 Gy** (Table 3). This result suggests that the dose range between 200 Gy and 300 Gy is ideal for inducing a high frequency of mutations while ensuring a sufficient number of surviving plants for M2 generation studies [31].

Table 3: LD50 for germination of TSG-98 *rabi* sorghum population

Sr. No.	Population	LD50 Germination (Gy)
1	TSG-98	258.5

3.2. Chlorophyll Mutations in M2 Generation

Chlorophyll mutations are widely used as a reliable index for assessing the genetic effects of mutagens, as they are easily detectable and represent changes at the gene level [32].

3.2.1. Frequency of Chlorophyll Mutations

The frequency of chlorophyll mutations, expressed on an M2 seedling basis, showed a linear increase with the increasing dose of electron beam (Table 4). The maximum mutation frequency of **3.461%** was recorded at the highest dose of 500 Gy. This positive correlation between mutagen dose and mutation frequency is consistent with the general principles of induced mutagenesis [33] [34].

Table 4: Frequency of chlorophyll mutations in M2 generation of TSG-98 *rabi* sorghum genotype

Treatment	No. of seedlings scored	No. of chlorophyll mutants	Frequency of chlorophyll mutations (%)
100 Gy	684	7	1.023
200 Gy	657	10	1.522
300 Gy	630	13	2.063
400 Gy	576	16	2.778
500 Gy	549	19	3.461
Wet Control	742	-	-
Dry Control	718	-	-
Total	**4556**	**65**	**10.848**

3.2.2. Spectrum of Chlorophyll Mutations

The spectrum of chlorophyll mutations observed in the M2 generation included five distinct types: *albina*, *xantha*, *chlorina*, *viridis*, and *xanthaviridis* (Table 5). The most frequently observed mutant type was **chlorina** (17 mutants), followed by **xantha** (14) and **albina** (12). The presence of *albina* and *xantha* mutants, which are lethal, indicates the induction of severe genetic damage, while the presence of *chlorina*, *viridis*, and *xanthaviridis* mutants, which are often viable, suggests the induction of less drastic, potentially useful mutations [35]. The predominance of *chlorina* types is a common observation in many mutagenic studies [36].

Table 5: Spectrum of chlorophyll mutations in M2 generation of TSG-98 *rabi* sorghum genotype

Genotype	Treatment	Albina	Xantha	Chlorina	Viridis	Xanthaviridis	Total
TSG-98	100 Gy	1	2	3	-	1	7
	200 Gy	2	3	2	1	2	10
	300 Gy	2	2	4	3	2	13
	400 Gy	3	3	3	4	3	16
	500 Gy	4	4	5	2	4	19
	Wet Control	-	-	-	-	-	-
	Dry Control	-	-	-	-	-	-
Total		**12**	**14**	**17**	**10**	**12**	**65**

3.2.3. Mutagenic Effectiveness and Efficiency

Mutagenic effectiveness, a measure of the frequency of mutations induced per unit dose, and mutagenic efficiency, the proportion of mutations relative to undesirable effects (pollen sterility), are presented in Table 6.

Table 6: Mutagenic effectiveness and efficiency of mutagen in inducing chlorophyll mutations in TSG-98 *rabi* sorghum genotype

Treatment	Mutagenic effectiveness (%)	Mutagenic efficiency (%)
100 Gy	10.23	8.53
200 Gy	7.61	9.51
300 Gy	6.88	9.38
400 Gy	6.94	8.96
500 Gy	6.92	8.87

The **100 Gy** treatment exhibited the highest mutagenic effectiveness (10.23%), while the **200 Gy** treatment showed the highest mutagenic efficiency (9.51%). In general, both effectiveness and efficiency decreased with increasing dose. This observation is consistent with the principle that lower doses tend to be more effective and efficient, as they induce a higher frequency of gene mutations relative to the frequency of chromosomal aberrations and physiological damage (e.g., lethality and sterility) caused by higher doses [5] [37]. The optimal dose for practical mutation breeding is often the one that maximizes efficiency, which in this study is the 200 Gy dose.

3.3. Viable Mutations in M2 Generation

Viable mutations, or macromutations, are of direct practical importance as they represent changes in morphological and agronomic traits that can be selected for crop improvement [38].

3.3.1. Frequency and Spectrum of Viable Mutations

The frequency of viable mutations also increased with the dose, reaching a maximum of **2.732%** at 500 Gy (Table 7). A wide spectrum of viable mutations was observed, affecting plant habit, leaf morphology, and seed/panicle characteristics (Table 8).

Table 7: Frequency of viable mutations in M2 generation of TSG-98 *rabi* sorghum genotype

Treatment	No. of seedlings scored	No. of viable mutants	Frequency of viable mutations (%)
100 Gy	684	7	1.023
200 Gy	657	8	1.218
300 Gy	630	10	1.587
400 Gy	576	11	1.910
500 Gy	549	15	2.732
Wet Control	742	-	-
Dry Control	718	-	-
Total	**4556**	**51**	**8.470**

Table 8: Spectrum of viable mutations in M2 generation of TSG-98 *rabi* sorghum genotype (Summary of key types)

Mutant Type	Key Characteristic	Dose Range (Gy)	Practical Utility
Stature			
Semi dwarf	120-140 cm height	100, 400, 500	Lodging resistance, high harvest index
Ultra dwarf	35 cm height	400	Extreme dwarfism, potential for high-density planting
Maturity			
Early maturing	103 days to maturity	500	Escape from terminal drought
Late maturing	125-130 days to maturity	200, 400	Increased biomass/fodder yield
Seed/Panicle			
Bold seeded	4.5-4.9 g 100-seed weight	100, 200, 300, 500	Direct yield enhancement
Large earhead	Increased panicle size	100, 500	Direct yield enhancement
Compact earhead	Tightly packed grains	300, 500	Potential for mechanized harvesting
Leaf/Stem			
White midrib	Leaf morphology change	400, 500	Potential physiological marker for drought tolerance
Thick stem	Increased stem girth	100, 400	Increased fodder yield/strength

The isolation of mutants with reduced plant height (semi-dwarf and ultra-dwarf) is highly desirable for improving lodging resistance and harvest index [39]. The early maturing mutant (103 days) is particularly valuable for *rabi* sorghum, as it allows the crop to escape the severe terminal drought stress [40]. Furthermore, the bold seeded and large earhead mutants directly contribute to increased grain yield, representing a significant achievement in the breeding program [41]. These findings confirm the efficacy of electron beam irradiation in generating a broad spectrum of agronomically useful mutations in sorghum [42].

3.4. Genetic Variability and Heritability

The successful induction of genetic variability was confirmed by the Analysis of Variance (ANOVA), which showed highly significant differences among the treatments for all fourteen quantitative characters studied.

3.4.1. Coefficients of Variation

The magnitude of Phenotypic Coefficient of Variation (PCV) was consistently greater than the Genotypic Coefficient of Variation (GCV) for all characters, indicating that the expression of these traits is influenced by environmental factors (Table 9). However, the relatively small difference between PCV and GCV for most traits suggests a high degree of genetic control, making selection effective [15].

High GCV and PCV values were observed for **Fodder yield per plant**, **Flag leaf area**, and **Leaf area**, suggesting a wide range of genetic variation for these traits, which is favorable for selection. Conversely, traits like Days to 50% flowering, Days to maturity, and 100 seed weight showed lower GCV and PCV, indicating less induced variability for these characters.

Table 9: Variability parameters for key quantitative characters in M2 generation of TSG-98

Character	GCV (%)	PCV (%)	Heritability (h^2 B.S.) (%)	Genetic Advance as % of Mean (GAM)
Days to 50% flowering	Medium	Medium	High	Medium
Plant height	High	High	High	High
Days to maturity	Medium	Medium	High	Medium
Primaries panicle-1	High	High	High	High
Grains primary-1	Medium	Medium	High	Medium
Panicle length	Medium	Medium	High	Medium
Panicle width	Medium	Medium	High	Medium
Grain yield plant-1	**High**	**High**	**High**	**High**
100 seed weight	Medium	Medium	High	Medium
Fodder yield plant-1	**High**	**High**	**High**	**High**
Relative water content	High	High	High	High
Chlorophyll content	Medium	Medium	High	Medium
Leaf area	**High**	**High**	**High**	**High**
Flag leaf area	**High**	**High**	**High**	**High**

3.4.2. Heritability and Genetic Advance

High heritability (broad sense) estimates were observed for all fourteen characters, indicating that a large proportion of the total phenotypic variation is attributable to genetic factors. However, heritability alone is not sufficient to predict the gain from selection. The combination of high heritability with high Genetic Advance as a Percentage of Mean (GAM) is a more reliable indicator of the effectiveness of selection [6].

Crucially, **Grain yield per plant**, **Primaries per panicle**, **Fodder yield per plant**, **Leaf area**, and **Flag leaf area** showed high heritability coupled with high GAM. This suggests that these traits are primarily governed by additive gene action, and therefore, direct selection based on the phenotypic performance of these traits would lead to substantial genetic improvement in the subsequent generations [43] [44]. Traits with high heritability but medium GAM (e.g., Days to 50% flowering, 100 seed weight) are likely controlled by both additive and non-additive gene effects, suggesting that selection may be slightly less effective.

3.5. Correlation and Path Analysis

Understanding the association between yield and its component traits is crucial for indirect selection, where selection for an easily measurable trait leads to a simultaneous improvement in the target trait (grain yield) [18].

3.5.1. Correlation Studies

Genotypic and phenotypic correlation coefficients were estimated to determine the degree and direction of association between grain yield per plant and the other thirteen characters. The results indicated that **Grain yield per plant** exhibited a positive and significant genotypic and phenotypic correlation with:

- Days to 50% flowering
- Days to maturity
- Primaries per panicle
- Panicle length
- Fodder yield per plant
- Flag leaf area
- Leaf area

The strong positive correlation with yield components like **Primaries per panicle** and **Panicle length** is expected and confirms their importance in determining final yield [45]. The positive association with physiological traits like **Flag leaf area** and **Leaf area** highlights the role of photosynthetic capacity and biomass in yield formation [46]. The positive correlation with Days to 50% flowering and Days to maturity, while indicating that later-maturing plants tend to be higher yielding, also suggests a trade-off with the goal of developing early-maturing, drought-escaping varieties. This necessitates a careful selection strategy that balances yield potential with maturity duration.

3.5.2. Path Coefficient Analysis

Path coefficient analysis was performed to partition the total correlation into direct and indirect effects, providing a clearer picture of the causal relationships [17] [19]. The analysis revealed that several characters exerted a **direct positive effect** on grain yield per plant (Table 10).

Table 10: Summary of Direct Positive Effects on Grain Yield per Plant (Genotypic Level)

Character	Direct Effect on Grain Yield	Implication for Selection
Days to 50% flowering	Positive	Selection for moderate flowering time is beneficial.
Primaries per panicle	**Strong Positive**	**Most important direct contributor to yield.**
Grains per primary	**Positive**	**Second most important direct contributor.**
Panicle length	**Positive**	**Directly influences the number of grains.**
Fodder yield	**Positive**	**Dual-purpose selection is possible.**
Relative water content (RWC)	**Positive**	**Key physiological trait for drought tolerance.**
Chlorophyll content (SPAD)	**Positive**	**Key physiological trait for photosynthetic efficiency.**

The strong direct positive effects of **Primaries per panicle**, **Grains per primary**, and **Panicle length** confirm that these are the most reliable morphological traits for direct selection to improve grain yield. The positive direct effects of the physiological traits, **Relative water content** and **Chlorophyll content**, are particularly significant in the context of *rabi* sorghum, as they represent the plant's ability to maintain turgor and photosynthetic activity under moisture stress [47].

The residual effect was found to be low, indicating that the fourteen characters included in the path analysis adequately accounted for the total variation in grain yield. This comprehensive analysis provides a strong basis for a selection index that prioritizes these traits for maximum genetic gain.

4. CONCLUSION

The present investigation successfully demonstrated the utility of electron beam irradiation as an effective mutagenic tool for inducing a broad spectrum of genetic variability in the *rabi* sorghum genotype TSG-98 (Muguthi). The determination of the LD50 at **258.5 Gy** provides a crucial benchmark for future mutagenesis experiments in this crop.

The study successfully isolated a range of agronomically superior viable mutants, including **semi-dwarf**, **early maturing**, **bold seeded**, and **large earhead** types. These mutants represent valuable genetic stocks for targeted breeding efforts aimed at improving lodging resistance, escaping terminal drought, and enhancing grain quality and yield.

The genetic analysis confirmed the successful induction of high genetic variability, with **Grain yield per plant**, **Primaries per panicle**, **Fodder yield per plant**, and **Flag leaf area** exhibiting high heritability and high genetic advance. This indicates that direct selection for these traits will be highly effective.

The path analysis further refined the selection criteria, highlighting the strong direct positive effects of **Primaries per panicle**, **Grains per primary**, and the physiological traits **Relative water content** and **Chlorophyll content** on grain yield. A breeding strategy that focuses on selecting early-maturing mutants with high values for these key yield components and physiological traits is recommended to develop high-yielding, drought-resilient *rabi* sorghum cultivars. The isolated mutants are now available for further evaluation and integration into advanced breeding programs.

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