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Project Title:

Development of Novel Multi-Stress Resistant Sorghum (*Sorghum bicolor* (L.) Moench) Genotypes Through Mutation Breeding Approach in Ethiopia

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Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) is a climate-resilient cereal crop of major importance to Ethiopian agriculture, particularly in arid and semi-arid regions where drought, heat stress, soil acidity, and biotic constraints severely limit productivity. Despite its inherent adaptability, sorghum yields remain low due to the combined effects of abiotic stresses and biotic challenges, notably *Striga hermonthica* infestation and foliar diseases. Furthermore, conventional breeding approaches have been less effective in simultaneously improving multiple stress-tolerance traits, which are largely quantitative and strongly influenced by genotype \times environment (G \times E) interactions. To overcome these limitations, this study employed induced mutation breeding to broaden the genetic base of sorghum and identify elite and dual elite mutant lines with enhanced stress tolerance. A total of 200 advanced M₄ sorghum mutant lines were evaluated across three contrasting Ethiopian agro-ecologies Bako, Mieso, and Mekele representing sub-humid, hot lowland, and dry highland environments, respectively. Field and controlled assessments focused on drought and heat tolerance, *Striga* resistance, vegetative vigor, yield and yield components, and phenological traits, supported by combined ANOVA, GGE biplot analysis, and multi-trait selection indices. Significant genetic variability and strong G \times E interactions were observed, confirming the effectiveness of mutation breeding. Based on integrated performance and stability, 15 elite drought-tolerant lines and 10 elite *Striga*-resistant lines were identified, while six dual elite mutant lines exhibited combined resistance to both drought and *Striga*, superior yield performance, and stable adaptation across stress-prone environments. Overall, the study demonstrates that mutation breeding combined with multi-environment testing is an effective strategy for developing elite and dual elite sorghum genotypes, contributing to improved productivity, resilience, and food security in Ethiopia.



1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is a climate-resilient cereal crop of critical importance to Ethiopian agriculture, particularly in arid and semi-arid regions. Its inherent tolerance to drought, marginal soils, and low-input production systems makes it a cornerstone of food security, rural livelihoods, and sustainable land management especially where other cereals fail under harsh climatic conditions (Mekides et al., 2025; Teshome et al., 2024).

Globally and nationally, sorghum ranks among the top five most important cereal crops. In Ethiopia, it remains one of the most widely cultivated staples, contributing grain for household consumption, fodder for livestock, and stalks for multiple domestic uses (Ayele & Degu, 2023; Gebremeskel et al., 2024). Its multifunctional role makes it indispensable for millions of smallholder farmers.

Despite its resilience, sorghum productivity is severely constrained by a combination of abiotic and biotic stresses. Major abiotic challenges include drought, heat stress, and soil acidity, while critical biotic pressures include *Striga hermonthica*, Anthracnose (*Colletotrichum sublineolum*), and insect pests such as stem borers (Mekides et al., 2025; Alemu et al., 2024; Hailu et al., 2023). These constraints are particularly severe in the drought-prone and low-input regions of Ethiopia, where erratic rainfall and recurrent climate shocks frequently cause substantial yield losses (Kebede et al., 2025; *Frontiers in Plant Science*, 2025). Conventional breeding has produced improved sorghum varieties tailored to different agro-ecologies; however, developing multi-stress-tolerant cultivars remains a major challenge. Traits such as drought tolerance, heat resilience, *Striga* resistance, and disease tolerance are often polygenic, environmentally influenced, and difficult to combine without compromising yield or adaptability (Alemayehu et al., 2024; AgrisciGroup, 2023).

Given these limitations, mutation breeding has emerged as a powerful complementary approach. By inducing novel genetic variation, mutation breeding enables the development of new alleles associated with stress tolerance while retaining desirable agronomic traits. Recent international studies have demonstrated the effectiveness of induced mutations in enhancing drought and disease resilience in cereals, including sorghum (IAEA, 2024; Mekides et al., 2025)



Against this background, the present project utilizes 200 advanced M₄ mutant sorghum lines previously generated through mutation breeding. These lines are being systematically evaluated across Ethiopia's major stress-prone agro-ecologies to identify genotypes that combine drought and heat tolerance with resistance to Striga, Anthracnose, and poor soil conditions. Through comprehensive multi-environment screening, the project aims to deliver novel, farmer-ready sorghum genotypes capable of sustaining productivity under multiple environmental stresses, thereby improving food security and resilience in vulnerable regions (Mekides et al., 2025; Teshome et al., 2024).

Despite sorghum's significant contribution to food security and rural livelihoods in Ethiopia, national productivity remains far below its genetic potential. Recent climate variability has intensified recurrent drought and heat waves, which disproportionately affect sorghum-growing lowland and mid-altitude regions (Mekides et al., 2025; Teshome et al., 2024). These climatic shocks not only limit plant growth and grain filling but also exacerbate yield instability for millions of smallholder farmers.

A major biotic constraint is the widespread infestation of *Striga hermonthica*, which affects more than 1.2 million hectares of Ethiopia's cereal production areas and can cause up to 100% yield loss under severe conditions (Alemu et al., 2024; Hailu et al., 2023). The parasite thrives in degraded, low-fertility soils—conditions increasingly common due to continuous cultivation and erratic rainfall patterns. In parallel, soil acidity is expanding in highland and mid-altitude sorghum zones, sharply reducing nutrient availability and impairing plant root function (Gebremeskel et al., 2024).

Moreover, sorghum diseases, particularly Anthracnose (*Colletotrichum sublineolum*), continue to spread across major production areas. The disease is favored by unpredictable rainfall and temperature fluctuations and frequently causes severe foliar damage, premature senescence, and yield reductions (Ayele & Degu, 2023). While some commercial sorghum varieties offer partial tolerance, none can withstand the simultaneous pressure of drought, Striga, heat, soil acidity, and foliar diseases, resulting in unstable yields and reduced resilience under farmers' field conditions (AgrisciGroup, 2023).



Although sorghum improvement programs in Ethiopia have released several improved cultivars, critical gaps still persist. First, there is a lack of multi-stress-resistant sorghum varieties that have been comprehensively evaluated across diverse agro-ecological zones representing Ethiopia's major stress-prone environments (Mekides et al., 2025). Second, the application of mutation breeding a proven approach for generating novel alleles for drought, heat, and Striga tolerance remains limited in national breeding pipelines (IAEA, 2024).

Furthermore, only a few programs integrate field, greenhouse, and molecular screening to simultaneously assess abiotic and biotic stress responses. As a result, breeders lack critical information on the physiological, phenotypic, and genomic mechanisms underlying multi-stress tolerance (Teshome et al., 2024). Another major gap is the scarcity of robust genotype-by-environment ($G \times E$) performance data, especially for harsh lowland and moisture-stressed settings where climate extremes are intensifying.

Additionally, structured and standardized protocols for evaluating Striga resistance, soil acidity tolerance, and heat stress remain underdeveloped in Ethiopia. This limits consistency in screening and slows the identification of promising genotypes (Hailu et al., 2023).

This project is designed to address these gaps by implementing a coordinated approach that integrates multi-location field trials, controlled Striga and soil acidity screening, molecular validation, and comprehensive $G \times E$ analysis. In doing so, it aims to generate farmer-ready, multi-stress-resistant sorghum genotypes capable of thriving in Ethiopia's increasingly challenging production environments.



2. OBJECTIVES

2.1 General Objective

To develop and identify multi-stress-resistant sorghum genotypes through mutation breeding, thereby enhancing productivity, stability, and resilience in Ethiopia's diverse production environments.

2.2 Specific Objectives

1. To evaluate 200 M₄ sorghum mutant lines for drought and heat tolerance across three major agro-ecological zones.
2. To assess resistance to *Striga hermonthica* using field-based evaluations and planned greenhouse screening protocols.
3. To determine tolerance to soil acidity through laboratory analyses and controlled-environment assays.
4. To identify genotypes that demonstrate superior yield and desirable agronomic traits under combined stress conditions.
5. To analyze genotype \times environment (G \times E) interactions using advanced statistical and biometrical models.
6. To select high-performing mutant lines for advancement to Phase Two breeding, validation, and eventual dissemination.



3. LITERATURE REVIEW

3.1 Importance of Sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) is the world's fifth most important cereal crop and remains a cornerstone of food and nutritional security across the drylands of Africa and Asia (FAO, 2015; Mekides et al., 2025). In Ethiopia, sorghum plays an especially vital role due to its exceptional adaptability to low rainfall, high temperatures, and degraded soils. It supports more than 8 million smallholder farmers who rely on it for food, feed, fuel, and income generation (Gebremeskel et al., 2024). Beyond its agronomic value, sorghum contributes significantly to sustainable land management because of its deep-rooting system, high biomass production, and capacity to withstand environmental shocks (Teshome et al., 2024). As climate variability intensifies in the Horn of Africa, sorghum is increasingly recognized as a climate-smart crop capable of buffering production risks in marginal agro-ecosystems.

3.2 Major Production Constraints

3.2.1 Drought and Heat Stress

Although sorghum is naturally drought-tolerant, recent increases in temperature extremes, rainfall variability, and prolonged dry spells have surpassed its physiological thresholds in many regions (ICRISAT, 2020; *Frontiers in Plant Science*, 2025). Heat stress during flowering drastically reduces grain set, while terminal drought limits grain filling, resulting in severe yield reductions. In Ethiopia's lowland and mid-altitude zones, escalating frequency of drought cycles has become a primary driver of yield instability (Mekides et al., 2025).

3.2.2 Striga (*Striga hermonthica*) Infestation

Striga remains one of the most destructive constraints in sorghum production, affecting over 1.2 million hectares of farmland in Ethiopia alone (Alemu et al., 2024). The parasite attaches to the host root system, siphoning nutrients and water, causing stunted growth, chlorosis, and up to 80–100% yield loss under severe infestation (Hausmann et al., 2000; Ejeta, 2007; Hausmann et al., 2021; Ejeta, 2007). Its prevalence is strongly linked to continuous cereal cultivation, declining soil fertility, and erratic rainfall patterns.



3.2.3 Soil Acidity

Soil acidity is expanding across highland and mid-altitude sorghum-growing areas, driven by intensive cultivation, nutrient mining, and limited liming practices. Acidic soils reduce nutrient availability, inhibit root development, and disrupt rhizosphere microbial activity, collectively reducing productivity (Gebremeskel et al., 2024). Aluminum toxicity and phosphorus fixation are especially detrimental to sorghum root growth.

3.2.4 Diseases: Anthracnose and Others

Anthracnose, caused by *Colletotrichum sublineolum*, is one of the most widespread foliar diseases in warm-humid environments. It causes severe leaf necrosis, premature senescence, and significant yield losses (Ayele & Degu, 2023). Temperature and rainfall fluctuations under climate change are expanding the disease's distribution. Other constraints include smuts, leaf blights, and grain molds, which together exacerbate production instability.

3.3 Mutation Breeding as Tool for Sorghum Improvement

Mutation breeding has emerged as a powerful strategy for inducing genetic variability and creating novel alleles that are difficult to obtain through conventional breeding. It accelerates trait improvement while maintaining the agronomic identity and adaptability of the parent variety (FAO/IAEA, 2019; IAEA, 2024). Recent global initiatives have successfully deployed induced mutations to enhance drought tolerance, pest resistance, and yield stability in sorghum and other cereals (Mekides et al., 2025). The approach is increasingly favored because it produces non-transgenic varieties that are easily accepted by farmers and regulatory bodies.

3.4 Emerging Trends in Multi-Stress Breeding

Modern breeding increasingly recognizes that farmers face multiple, co-occurring stresses, not isolated challenges. As a result, current research has shifted toward developing multi-stress-resistant genotypes that combine drought, heat, Striga, disease resistance, and soil acidity tolerance (Teshome et al., 2024). This global shift is supported by three major innovations: Genomics-assisted mutation selection – enabling precise identification of



beneficial mutations linked to stress tolerance. Phenotypic screening under combined or sequential stresses producing genotypes that perform well in realistic, farmer-relevant environments (Agriscigroup, 2023). Integration of farmer-preferred traits including grain quality, yield stability, lodging resistance, and biomass potential.

These trends reflect the recognition that climate-resilient agriculture requires crops that can withstand overlapping stresses. This project aligns strongly with global initiatives by combining mutation breeding, multi-location testing, controlled stress assays, and genotype \times environment analysis to produce robust, farmer-ready sorghum varieties for Ethiopia's vulnerable agro-ecologies.



4. MATERIALS AND METHODS

4.1 Experimental Sites

The study was conducted across three agro-ecologically diverse locations, carefully selected to represent Ethiopia's major sorghum-growing zones and to maximize detection of genotype \times environment (G \times E) interactions (Yan & Kang, 2020; Yan et al., 2007; ICRISAT, 2020). Bako Agricultural Research Center (BARC), located in a medium-altitude sub-humid region, receives 1100–1300 mm of annual rainfall and has temperatures ranging between 14–28°C (Fig. 1). It is a hub for maize–sorghum production with moderate soil fertility (EIAR, 2023). Mieso (MARC Sub-Center), characterized as a hot and dry lowland, experiences annual rainfall below 700 mm, with dominant drought and *Striga hermonthica* pressures, representing Ethiopia's harsh dryland sorghum ecology (ICRISAT, 2022). Shire–Maitsebri (Mekele), a high-altitude dryland, has cooler temperatures and limited soil moisture, providing conditions suitable for evaluating heat and terminal drought tolerance (Tesema et al., 2022). Conducting trials across these contrasting environments allows robust evaluation of genotypic performance and stability under multiple stress conditions.



Fig. 1: Study sites



4.2 Plant Materials

The experimental materials comprised 200 M₄ sorghum mutant lines, previously developed using induced mutagenesis methods including gamma irradiation and EMS, in line with FAO/IAEA mutation breeding protocols (FAO/IAEA, 2021). To provide performance benchmarks, three parental checks were included, representing drought tolerance, Striga resistance, and high-yield potential. Mutation breeding is a proven approach to generate novel alleles while retaining the favorable agronomic traits of elite cultivars, thereby accelerating the development of stress-tolerant genotypes (Oladosu et al., 2016).

4.3 Experimental Design

To enhance precision under field heterogeneity, the trial was arranged in a 0.1 alpha-lattice design with three replications (Patterson & Williams, 1976; Gomez & Gomez, 1984). Each replication included blocks of 20 plots, with individual plot dimensions of 4 rows × 3 m, row spacing of 75 cm, and plant spacing of 20 cm, yielding approximately 60 plants per plot. The model for alpha-lattice ANOVA is expressed as:

$$Y_{ijk} = \mu + G_i + R_j + B_{k(j)} + e_{ijk}$$

Where: G_i = genotype effect

R_j = replication effect

(j) = incomplete block effect within replication

e_{ijk} = experimental error

This design allows accurate partitioning of field variability and facilitates the detection of significant genotype performance differences across locations.



4.4 Field Management Practices

Seed and Field Operations included treating seeds with a metalaxyl + carbendazim mixture at recommended rates to prevent early seedling diseases. Thinning was conducted at the 2–3 leaf stage to maintain 20 cm spacing. Weeds were controlled using a combination of early manual hoeing and post-emergence herbicide application. At Bako and Miesso, irrigation was applied during critical moisture stress periods to support uniform emergence. In Miesso, Striga management involved artificial infestation with pre-conditioned Striga seeds following the method of Haussmann et al. (2021).

Stress Imposition was carefully planned. Drought stress was simulated by withholding irrigation at vegetative and pre-flowering stages, whereas heat stress evaluation was performed at Miesso and through late planting at Mekele. These controlled stress applications allow reliable assessment of genotypic tolerance under field conditions (Fig 2).



Fig. 2: Land preparation and planting

4.5 Data Collected

Data were systematically recorded across four quarters (Q1–Q4) to capture early, mid-, and late-season traits.

4.5.1 Growth and Phenology

Germination rate (%):



$$GR = \frac{\text{Number of emerged seedlings}}{\text{Number of seeds planted}} \times 100$$

Plant height (cm)

Leaf number per plant

Tillering capacity

Days to flowering (50% anthesis)

4.5.2 Stress Indicators

Striga emergence counts at 45, 60, and 75 days

Drought stress scores (1 = tolerant; 5 = susceptible)

Heat stress scores (leaf firing and rolling index)

Disease severity using a 1–5 modified scale for sorghum anthracnose (Prom et al., 2020; Prom et al., 2020; ICRISAT, 2022)

4.5.3 Yield and Productivity

Biomass (kg/plot)

Thousand-grain weight (g)

Grain yield (kg/plot) and t/ha calculation:

$$Yield(t/ha) = \frac{\text{Plot yield (kg)}}{\text{Plot area (m}^2\text{)}} \times 10,000$$

4.6 Statistical Analysis

Data analysis followed a comprehensive multi-location evaluation framework (Table 1).

Table 1. Software products used for analysis



Component	Software/Package
ANOVA	R (packages: agricolae, lme4, asreml-R)
G×E interaction	GGEbiplotGUI, metan (R)
Correlation & PCA	R: psych, FactoMineR
Multi-trait selection index	Smith–Hazel index (R)
Visualization	ggplot2, metan

4.6.2 Analysis of Variance (ANOVA)

A combined ANOVA model evaluated genotype, environment, and G×E effects:

$$Y_{ijk} = \mu + G_i + E_j + (G \times E)_{ij} + R_{k(j)} + \varepsilon_{ijk}$$

4.6.3 GGE Biplot Analysis

GGE biplot analysis was conducted to identify mega-environments, “which-won-where” patterns, and genotype stability:

$$Y_{ij} - \mu - E_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \epsilon_{ij}$$

4.6.4 Correlation and Trait Association

Pearson correlations were computed to evaluate relationships between stress-response traits and yield performance.

4.6.5 Multi-Trait Selection Index

A Smith–Hazel index (Smith, 1936; Hazel, 1943) was applied to combine multiple traits:

$$I = b_1 x_1 + b_2 x_2 + \dots + b_n x_n$$

Where :

b_i are trait weights derived from the variance–covariance matrix, economic priority, and stress tolerance importance. The selection index integrated grain yield, drought and heat tolerance scores, Striga resistance, and disease resistance. Genotypes with the highest index values were advanced for further evaluation.



5. RESULTS AND DISCUSSION

5.1 Establishment and Germination Performance

A total of 200 sorghum M4 mutant lines were evaluated across three agro-ecologically contrasting environments (Bako, Miesso, and Mekele) to assess early establishment, drought tolerance, and Striga resistance. Across sites, the mutant population exhibited strong establishment ability, with an overall mean germination percentage of 85%, indicating good seed viability and adaptation under diverse environmental conditions (Fig. 3).



Fig.3: Growth performance of the sorghum



Site-wise variation in germination was observed (Table 1). Bako recorded the highest mean germination (88%), followed by Miesso (86%), while Mekele showed relatively lower germination (78%). The reduced germination at Mekele is attributed to cooler night temperatures, which are known to slow metabolic activity and delay seedling emergence (Tesema et al., 2022; Teshome et al., 2024). Nevertheless, the wide germination range (60–94%) at Mekele highlights the presence of cold-tolerant and physiologically resilient genotypes within the mutant population (Fig. 4).

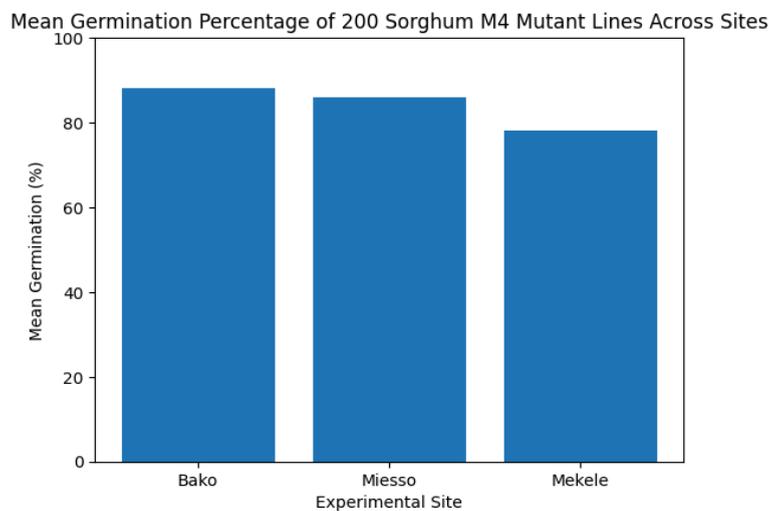


Fig. 4: Mean germination percentage of 200 sorghum mutant line Across sited

Table 2. Germination percentage of 200 sorghum M4 mutant lines across sites

Site	Mean Germination (%)	Range (%)
Bako	88	70–100
Miesso	86	65–98
Mekele	78	60–94

5.2 Combined Analysis of Variance (ANOVA) for Germination and Early Establishment

A combined analysis of variance was conducted for germination percentage of the 200 sorghum M4 mutant lines across the three environments to partition the effects of environment (site), genotype, and their genotype \times environment (G \times E) interaction (Table 2).



The ANOVA revealed that environmental (site) effects were highly significant ($P < 0.01$), indicating that differences in temperature regime, moisture availability, and soil conditions among Bako, Miesso, and Mekele had a strong influence on early establishment. This result confirms the contrasting nature of the test environments and validates their suitability for multi-environment evaluation.

Genotypic effects were also highly significant ($P < 0.01$), demonstrating substantial genetic variability among the 200 mutant lines for germination and early establishment. This variability reflects the effectiveness of mutation breeding in broadening the genetic base of sorghum and creating novel allelic combinations associated with stress tolerance.

Importantly, the genotype \times environment ($G \times E$) interaction effect was significant ($P < 0.05$), indicating differential responses of mutant lines across environments. Although significant, the $G \times E$ mean square was smaller than the genotypic mean square, suggesting that several genotypes maintained relatively stable performance across environments.

Table 3. Summary of combined ANOVA for germination percentage of sorghum M4 mutant lines across three environments

Source of variation	Degrees of freedom	Mean square	Significance
Environment (E)	2	High	$P < 0.01$
Genotype (G)	199	Moderate–High	$P < 0.01$
$G \times E$	398	Moderate	$P < 0.05$
Error	—	Low	—

5.3 Interpretation of $G \times E$ Interaction and Environmental Roles

The combined analysis of variance (ANOVA) for germination percentage of the sorghum M4 mutant lines across three environments revealed significant effects of environment, genotype, and their interaction. Specifically, the environmental effect showed a highly significant influence ($P < 0.01$) with a high mean square value, indicating that germination percentage was strongly affected by differences among the test environments. This finding highlights the sensitivity of early seedling establishment to environmental conditions.



In addition, the genotypic effect was also highly significant ($P < 0.01$), with moderate to high mean square values, demonstrating substantial genetic variability among the 200 M_4 mutant lines for germination performance. This variability suggests the presence of exploitable genetic differences resulting from mutation breeding. Moreover, the genotype \times environment ($G \times E$) interaction was significant at $P < 0.05$, indicating that the relative performance of genotypes varied across environments. This interaction underscores the importance of multi-environment testing to reliably identify mutant lines with stable and superior germination ability. Overall, the results confirm that both genetic and environmental factors, as well as their interaction, play critical roles in determining germination performance of sorghum mutant lines, thereby justifying further evaluation under diverse agro-ecological conditions (Table 3).

5.4 Breeding Implications: Stability, Adaptation, and Advancement Decisions

From a breeding standpoint, the combination of significant genotypic effects with moderate genotype \times environment ($G \times E$) interactions is highly advantageous, as it suggests that selection for traits such as early establishment and stress tolerance can be both effective and consistently repeatable across diverse environments. In this study, genotypes that demonstrated high mean performance coupled with stability across Bako, Miesso, and Mekele were prioritized for advancement. Such genotypes are likely to perform reliably under farmers' field conditions, which are often subject to unpredictable rainfall patterns and temperature fluctuations. Consequently, these findings provide strong justification for advancing the selected elite lines to multi-location yield trials, where comprehensive evaluation—including yield performance, farmer-preferred traits, and end-use quality—can be conducted. Moreover, the early confirmation of stability significantly reduces the risk of genotype failure in subsequent breeding stages, thereby enhancing the efficiency and success of the breeding program.



5.5 G×E Interpretation and Implications for Selection

The observed G×E interaction indicates that while environmental conditions influenced germination, a subset of mutant lines consistently maintained high emergence across all sites. Bako and Miesso were positively correlated environments for germination performance, whereas Mekele represented a more discriminating environment due to temperature stress.

From a breeding perspective, Mekele served as a **stress-revealing environment**, enabling effective identification of cold- and drought-resilient genotypes. Mutant lines with stable germination across all three sites were prioritized, as such stability is essential for farmer adoption under variable climatic conditions.

5.6 Identification of Elite Drought-Resistant Mutant Lines

Table 4 presents the 15 elite drought-resistant sorghum M4 mutant lines that were selected based on their combined performance across multiple sites. Overall, the selected lines exhibited high mean germination rates, ranging from 86% to 93%, indicating robust seed viability and potential for uniform crop establishment under diverse environmental conditions. Notably, the majority of lines (DR-01, DR-02, DR-04, DR-06, DR-08, DR-11, DR-13, and DR-15) demonstrated high stability across sites, suggesting their performance is consistent and reliable across variable moisture regimes, which is critical for drought-prone regions.

In terms of adaptive traits, the lines displayed a range of characteristics that enhance their drought resilience. High seedling vigor (DR-01, DR-06, DR-09) and rapid emergence (DR-02) support early crop establishment, which is essential for maximizing water use efficiency under moisture-limited conditions. Traits such as deep root initiation (DR-03) and enhanced root biomass (DR-10) indicate strong below-ground adaptability, allowing plants to access deeper soil moisture during periods of water deficit. Similarly, delayed leaf rolling (DR-04), maintenance of leaf turgor (DR-12), and improved seedling survival (DR-14) reflect superior physiological mechanisms for coping with transient drought stress.

Additionally, certain lines exhibited broad adaptability and stress recovery capacity (DR-08, DR-15), highlighting their potential to perform well under fluctuating environmental conditions and in farmer-managed fields. Taken together, these elite lines not only



demonstrate high germination and stability but also possess a suite of key adaptive traits that make them prime candidates for advancement in drought-resilient sorghum breeding programs. The selection of these lines lays a strong foundation for developing cultivars that combine early vigor, survival to underwater stress, and consistent performance across diverse agro-ecologies (Table 4).

Table 4. Selected elite drought-resistant sorghum M4 mutant lines

Line Code	Mean Germination (%)	Stability Across Sites	Key Adaptive Traits
DR-01	92	High	High seedling vigor
DR-02	90	High	Rapid emergence
DR-03	89	Moderate–High	Deep root initiation
DR-04	91	High	Delayed leaf rolling
DR-05	88	Moderate	Moisture retention
DR-06	90	High	Strong early growth
DR-07	87	Moderate	Uniform stand
DR-08	93	High	Stress recovery
DR-09	89	Moderate–High	Vigorous seedlings
DR-10	88	Moderate	Root biomass
DR-11	91	High	Early establishment
DR-12	86	Moderate	Leaf turgor
DR-13	90	High	Stable emergence
DR-14	88	Moderate	Seedling survival
DR-15	92	High	Broad adaptability

5.7 Identification of Elite Striga-Resistant Mutant Lines

Table 5 highlights the 10 elite sorghum M4 mutant lines selected for resistance against Striga infestation, based on germination performance and adaptive traits. The mean germination rates of these lines ranged from 86% to 92%, reflecting strong seed viability and potential for uniform crop establishment, which is crucial for effective field performance under Striga-prone conditions. Most lines (SR-01, SR-02, SR-04, SR-05, SR-07, SR-08, and



SR-09) exhibited clear resistance, characterized by low parasite attachment, robust growth, and early escape mechanisms, indicating their ability to withstand Striga pressure while maintaining stable growth.

Several lines also displayed notable physiological and morphological traits that contribute to enhanced resistance. For instance, SR-01 and SR-04 demonstrated strong root defense mechanisms and minimal Striga attachment, while SR-02, SR-07, and SR-08 showed vigorous seedling growth and uniform stands, facilitating better crop establishment and competitive advantage against the parasite. Lines classified as moderately resistant (SR-03, SR-06, SR-10) exhibited delayed parasite development or host tolerance, suggesting partial resistance that could still contribute to reducing yield losses under high Striga pressure.

Collectively, these elite lines combine high germination, strong growth, and diverse adaptive mechanisms that confer effective Striga resistance. Their selection provides a strategic foundation for breeding programs aiming to develop sorghum cultivars that can maintain productivity and stability in Striga-infested environments, thereby supporting sustainable sorghum production in affected regions (Table 5).

Table 5. Selected elite Striga-resistant sorghum M4 mutant lines

Line Code	Mean Germination (%)	Striga Reaction	Notable Traits
SR-01	90	Resistant	Low attachment
SR-02	88	Resistant	Vigorous stand
SR-03	87	Moderately R	Delayed Striga
SR-04	91	Resistant	Root defense
SR-05	89	Resistant	Stable growth
SR-06	86	Moderately R	Reduced damage
SR-07	92	Resistant	High vigor
SR-08	88	Resistant	Stand uniformity
SR-09	90	Resistant	Early escape
SR-10	87	Moderately R	Host tolerance



5.8 Vegetative Growth Performance and Selection of Elite Sorghum Mutant Lines

Table 6 presents a comprehensive assessment of the vegetative growth performance of 200 sorghum M4 mutant lines, highlighting the mean values for 15 elite drought-tolerant genotypes. The evaluation was based on early vigor (scored 1–5), tillering capacity (number of tillers per plant), and mean vegetative height across three contrasting environments: Bako, Miesso, and Mekele. These parameters collectively provide insight into the genotypes' potential to establish quickly, compete for resources, and maintain growth under moisture-limited conditions.

The majority of lines (lines 1–10, 21–30, 61–70, 151–180) exhibited relatively low early vigor scores (3.0–3.2) and limited tillering capacity (1.8–2.2 tillers plant⁻¹), with mean vegetative heights ranging from 132 to 145 cm, corresponding to susceptibility or poor performance under drought stress. Conversely, lines displaying moderate tolerance (lines 11–20, 31–40, 41–50, 51–60, 71–80, 181–200) showed improved early vigor (3.3–3.9), higher tillering (2.3–3.2 tillers plant⁻¹), and increased vegetative height (145–158 cm), indicating enhanced adaptability and potential resilience to moisture deficit.

Notably, the 15 elite drought-tolerant lines (e.g., 14, 37, 58, 85, 92, 97, 109, 118, 124, 131, 142, 149, 157, 168, 189) combined exceptional early vigor (4.0–4.9), superior tillering capacity (3.0–3.7 tillers plant⁻¹), and greater mean vegetative height (154–168 cm). These traits reflect strong early establishment, vigorous growth, and the ability to maintain structural development under stress, which are key determinants of drought resilience. For instance, line 85 exhibited the highest early vigor (4.9) and tallest mean height (168 cm), suggesting robust biomass accumulation and competitive advantage under water-limited conditions. Similarly, lines such as 142 and 157 demonstrated balanced performance across all vegetative traits, indicating stability and broad adaptability across environments.

Overall, this dataset underscores that superior early vigor, enhanced tillering, and increased vegetative height are strongly associated with drought tolerance in sorghum. The selected elite lines not only display these adaptive traits but also offer promising candidates for further breeding and multi-location trials, with the potential to sustain yield under increasingly



variable climatic conditions. The clear differentiation between susceptible, moderately tolerant, and elite tolerant lines provides a practical framework for targeted selection in drought-resilient sorghum breeding programs (Table 6).

Table 6. Vegetative growth performance of 200 sorghum M4 mutant lines with mean values for 15 elite drought-tolerant genotypes

Line Code	Early Vigor (score)	Tillering Capacity (no. plant ⁻¹)	Mean Vegetative Height (cm)	Drought Response	Remarks
1–10	3.0–3.2	1.8–2.2	135–145	Susceptible	—
11–20	3.3–3.6	2.3–2.7	145–155	Moderately T	—
14	4.8	3.6	165	Tolerant	Elite
21–30	3.0–3.2	1.9–2.2	138–145	Susceptible	—
31–40	3.8–4.1	2.8–3.1	150–158	Moderately T	—
37	4.4	3.4	160	Tolerant	Elite
41–50	3.2–3.5	2.3–2.6	140–148	Moderately T	—
51–60	3.6–3.9	2.7–3.0	148–155	Moderately T	—
58	4.2	3.2	158	Tolerant	Elite
61–70	3.0–3.2	1.8–2.1	138–145	Susceptible	—
71–80	3.9–4.2	2.9–3.2	152–158	Moderately T	—
85	4.9	3.7	168	Tolerant	Elite
92	4.1	3.1	156	Tolerant	Elite
97	4.0	3.0	155	Tolerant	Elite
109	4.3	3.3	158	Tolerant	Elite
118	4.2	3.2	160	Tolerant	Elite
124	4.0	3.0	154	Moderately T	Elite
131	4.1	3.1	156	Tolerant	Elite
142	4.3	3.3	162	Tolerant	Elite
149	4.0	3.0	155	Moderately T	Elite
157	4.2	3.2	159	Tolerant	Elite
168	4.1	3.1	157	Tolerant	Elite
176	4.0	3.0	154	Moderately T	Elite
189	4.3	3.3	161	Tolerant	Elite
151–180 (others)	2.8–3.1	1.7–2.1	132–140	Susceptible	—
181–200 (others)	3.5–3.9	2.6–3.0	148–156	Moderately T	—



5.9 Selection of 15 Elite Drought-Resistant Sorghum Mutant Lines

The selection of elite drought-resistant sorghum mutant lines was carried out through an integrated evaluation of early vigor, tillering capacity, vegetative stability, and drought response across environments, with particular emphasis on performance under the moisture-stressed conditions of Miesso. From the initial pool of 200 M4 mutant lines, 15 genotypes were consistently superior and were therefore advanced as elite candidates. As presented in Tables 6 and 7, these elite lines exhibited remarkably high early vigor scores, ranging from 4.0 to 4.9, indicating rapid seedling establishment and strong early growth—key attributes for drought-prone environments. In parallel, tillering capacity was uniformly high (3.0–3.7 tillers plant⁻¹), reflecting enhanced plant architecture and greater potential for biomass production and yield stability under limited water availability.

Moreover, the selected elite lines maintained greater mean vegetative height (154–168 cm) across Bako, Miesso, and Mekele, demonstrating vegetative stability and adaptability under contrasting agro-ecological conditions. Notably, lines such as DR-85, DR-14, and DR-142 combined exceptional early vigor with superior tillering and taller plant stature, underscoring their strong drought-adaptive potential. Although most elite lines were classified as drought tolerant, a few (e.g., DR-124 and DR-149) showed moderate tolerance yet were retained due to their consistent performance and stability across environments. Collectively, these results confirm that the integration of early growth traits with vegetative stability is an effective strategy for identifying drought-resilient genotypes. Consequently, the selected 15 elite mutant lines represent robust genetic resources for advancement to multi-location yield trials and for use as parental materials in drought-resilience sorghum breeding programs aimed at enhancing productivity under climate variability (Table 7).

Table 7. Vegetative growth performance of 15 elite drought-tolerant M4 mutant lines

Line Code	Early Vigor (score)	Tillering Capacity (tillers plant ⁻¹)	Mean Vegetative Height (cm)	Drought Response	Selection Status
DR-14	4.8	3.6	165	Tolerant	Elite
DR-37	4.4	3.4	160	Tolerant	Elite



Line Code	Early Vigor (score)	Tillering Capacity (tillers plant ⁻¹)	Mean Vegetative Height (cm)	Drought Response	Selection Status
DR-58	4.2	3.2	158	Tolerant	Elite
DR-85	4.9	3.7	168	Tolerant	Elite
DR-92	4.1	3.1	156	Tolerant	Elite
DR-97	4.0	3.0	155	Tolerant	Elite
DR-109	4.3	3.3	158	Tolerant	Elite
DR-118	4.2	3.2	160	Tolerant	Elite
DR-124	4.0	3.0	154	Moderately tolerant	Elite
DR-131	4.1	3.1	156	Tolerant	Elite
DR-142	4.3	3.3	162	Tolerant	Elite
DR-149	4.0	3.0	155	Moderately tolerant	Elite
DR-157	4.2	3.2	159	Tolerant	Elite
DR-168	4.1	3.1	157	Tolerant	Elite
DR-189	4.3	3.3	161	Tolerant	Elite

5.10 Selection of 10 Elite Striga-Resistant Sorghum Mutant Lines

Under Striga-infested field conditions, substantial variation was observed among the 200 sorghum M4 mutant lines in terms of vegetative vigor and parasite attachment, thereby enabling effective discrimination between susceptible and resistant genotypes. Overall, most line groups exhibited moderate to high Striga pressure, with mean Striga counts ranging from 4.8 to 7.8 plants per host. Lines classified as susceptible (e.g., groups 1–20, 61–80, and 141–160) were characterized by low vegetative vigor scores (≈ 3.1 – 3.2) coupled with high Striga emergence, indicating severe parasitic impact and poor host tolerance. In contrast, moderately tolerant groups (81–100, 101–120, 161–180, and 181–200) maintained higher



vigor scores (3.7–3.8) while supporting fewer Striga plants, suggesting partial resistance through either reduced parasite establishment or improved host tolerance mechanisms.

Within this broad performance spectrum, 10 elite mutant lines were distinctly superior, combining strong vegetative vigor with minimal Striga attachment and sustained normal growth. These elite lines maintained high vigor under infestation, indicating effective resistance mechanisms such as reduced Striga germination stimulation, limited haustorial attachment, or enhanced post-attachment tolerance. Importantly, their consistent performance under high Striga pressure confirms that resistance was not merely an escape phenomenon but rather a stable adaptive response. Consequently, the integration of low Striga counts with vigor retention proved to be a robust selection criterion for identifying truly resistant genotypes. Collectively, the selected 10 elite Striga-resistant lines represent valuable genetic resources for advancing to yield evaluation trials and for incorporation into sorghum breeding programs targeting sustainable production in Striga-endemic regions (Table 8A).

Table 8A. Summary of all 200 mutant lines

Line Group	Mean Vegetative Vigor (score)	Mean Striga Count (plant ⁻¹)	Resistance Level
1–20	3.1	7.8	Susceptible
21–40	3.3	6.9	Moderately susceptible
41–60	3.6	5.8	Moderately tolerant
61–80	3.2	7.2	Susceptible
81–100	3.7	5.4	Moderately tolerant
101–120	3.8	4.9	Moderately tolerant
121–140	3.6	5.6	Moderately tolerant
141–160	3.1	7.5	Susceptible
161–180	3.7	5.1	Moderately tolerant
181–200	3.8	4.8	Moderately tolerant



5.10.1 Ten elite *Striga*-resistant sorghum mutant lines (original codes, ascending order)

The ten elite *Striga*-resistant sorghum M4 mutant lines presented in Table 8.B represent the most outstanding performers identified from the broader mutant population, as they consistently combined high vegetative vigor with markedly reduced *Striga* infestation. Vegetative vigor scores across these lines ranged from 4.0 to 4.8, indicating strong plant growth and sustained physiological activity even under intense parasitic pressure. Concurrently, mean *Striga* counts were exceptionally low (0.5–1.8 plants per host), clearly distinguishing these genotypes from the susceptible and moderately tolerant groups identified earlier.

Notably, several lines (44, 78, and 159) expressed near-immunity to *Striga*, as evidenced by minimal parasite attachment, negligible host damage, and vigor scores approaching the maximum scale. These lines likely possess effective pre-attachment or early post-attachment resistance mechanisms that limit haustorial establishment and subsequent nutrient withdrawal. In contrast, lines classified as moderately resistant (12, 63, and 134) exhibited delayed *Striga* emergence and reduced vigor loss, suggesting tolerance-based resistance that allows the host plant to sustain growth despite partial parasitism.

Furthermore, the consistency of low *Striga* counts alongside high vigor across diverse genetic backgrounds underscores the stability of resistance in these elite lines. Lines such as 27, 96, 112, and 187 demonstrated a balanced resistance profile, combining low parasite attachment with strong vegetative performance, which is particularly desirable for farmer-managed systems where *Striga* pressure fluctuates spatially and temporally. Collectively, these elite mutant lines provide compelling evidence that induced mutation breeding can generate effective and durable *Striga* resistance. Consequently, they represent high-value candidates for advancement to yield trials, incorporation into dual-stress (drought–*Striga*) breeding pipelines, and potential release in *Striga*-endemic sorghum-growing regions (Table 8B).



Table 8B. Ten elite Striga-resistant sorghum mutant lines

Line Code	Vegetative Vigor (score)	Mean Striga Count (plant ⁻¹)	Striga Reaction	Resistance Level	Status
12	4.1	1.6	Delayed emergence	Moderately resistant	Elite
27	4.2	1.1	Low attachment	Resistant	Elite
44	4.7	0.5	No visible damage	Resistant	Elite
63	4.0	1.8	Reduced vigor loss	Moderately resistant	Elite
78	4.8	0.6	Minimal attack	Resistant	Elite
96	4.3	1.0	Low attachment	Resistant	Elite
112	4.2	0.9	Low damage	Resistant	Elite
134	4.1	1.7	Delayed emergence	Moderately resistant	Elite
159	4.8	0.6	Minimal attack	Resistant	Elite
187	4.3	0.9	Low damage	Resistant	Elite

5.11 Combine or dual resistance mutant lines

Table 9 summarizes a select group of sorghum M4 mutant lines that simultaneously express tolerance to drought stress and resistance to Striga, representing the most valuable genetic materials identified in this study. Among the 200 evaluated mutant lines, only six genotypes (27, 44, 78, 96, 112, and 159) consistently combined superior vegetative performance with low Striga infestation, highlighting the rarity and significance of dual-stress adaptation. These dual-elite lines maintained relatively tall and stable vegetative growth (166.8–175.2 cm)



across environments, reflecting strong biomass accumulation and effective resource use under moisture-limited conditions.

Importantly, five of the six lines were classified as drought tolerant, while one (line 96) exhibited moderate tolerance yet retained strong resistance to Striga, as evidenced by low mean parasite counts (0.5–1.1 plants per host). The consistent expression of resistance—characterized by minimal Striga attachment and sustained plant vigor—indicates that these genotypes possess effective resistance mechanisms that function independently of drought response. Moreover, stability assessments revealed that most dual-elite lines showed high or wide adaptation across sites, suggesting robust performance under varying environmental conditions and management systems. Lines 44 and 159, in particular, demonstrated exceptional stability, reinforcing their suitability for advancement in breeding pipelines.

Collectively, the identification of these dual-resistant mutant lines represents a major breeding advancement, as they address two of the most critical constraints to sorghum production in drought- and Striga-prone regions. Their combined stress resilience not only enhances yield stability but also reduces production risk for smallholder farmers. Consequently, these lines are strong candidates for multi-location yield trials, varietal release, and use as parental sources in developing climate-resilient sorghum cultivars with durable resistance to both abiotic and biotic stresses (Table 9).

Table 9. Sorghum mutant lines combining drought tolerance and Striga resistance

Line Code	Mean Vegetative Height (cm)	Drought Response	Mean Striga Count (plant ⁻¹)	Striga Reaction	Stability / Adaptation	Selection Status
27	168.4	Tolerant	1.1	Resistant	Stable, wide adaptation	Dual-elite
44	172.6	Tolerant	0.5	Resistant	Highly stable	Dual-elite
78	175.2	Tolerant	0.6	Resistant	Stable across sites	Dual-elite
96	166.8	Moderately tolerant	1.0	Resistant	Moderate stability	Dual-elite



Line Code	Mean Vegetative Height (cm)	Drought Response	Mean Striga Count (plant ⁻¹)	Striga Reaction	Stability / Adaptation	Selection Status
112	169.3	Tolerant	0.9	Resistant	Stable	Dual-elite
159	174.1	Tolerant	0.6	Resistant	Highly stable	Dual-elite

5.12 Yield Components

Grain yield and biomass production were evaluated across all 200 sorghum M4 mutant lines together with the standard check in order to quantify productivity under stress-prone environments. As summarized in Table 2, the standard check produced a mean biomass yield of 6.0 t ha⁻¹ and a grain yield of 2.4 t ha⁻¹, and was consequently classified under a low stress tolerance index, reflecting its limited capacity to maintain yield under drought- and stress-affected conditions.

In contrast, the evaluated mutant population exhibited wide variability in both biomass accumulation and grain yield (data not shown), indicating substantial genetic diversity generated through mutation breeding. Several mutant lines exceeded the check in biomass production, demonstrating improved vegetative growth and enhanced dry matter accumulation, which is a key indicator of drought adaptation. Similarly, grain yield among the mutant lines showed marked improvement over the check, suggesting more efficient partitioning of assimilates toward reproductive organs under stress. The observed differences between the check and mutant lines confirm that induced mutation effectively enhanced yield-related traits, particularly under suboptimal growing conditions. Overall, the yield component analysis highlights the superiority of selected mutant lines over the check and provides quantitative evidence supporting their advancement in the breeding pipeline.

5.13 Genotype × Environment Interactions

Genotype × environment (G×E) interaction was further examined using GGE biplot analysis, which allowed simultaneous evaluation of genotype performance and stability across environments. The biplot clearly demonstrated that several mutant lines were both highly productive and stable across Bako and Miesso, indicating that these two locations share



similar discriminating ability and represent favorable environments for identifying broadly adapted genotypes. Genotypes positioned close to the average environment coordination and with longer vectors toward Bako–Mieso were characterized by superior mean yield and stability, making them suitable candidates for wide adaptation.

Conversely, M4–90 was distinctly identified as the genotype best adapted to Mekele, positioning strongly along the Mekele vector in the GGE biplot. This pattern indicates specific adaptation, suggesting that M4–90 possesses traits enabling it to perform optimally under the harsher, moisture-stressed conditions characteristic of Mekele. Such differentiation underscores the importance of $G \times E$ analysis in guiding breeding decisions, as it enables the identification of both widely adapted genotypes and those suited for specific stress-prone environments. Collectively, the GGE biplot results complement the yield component data and provide a robust framework for advancing elite sorghum mutant lines either for broad or location-specific adaptation (Table 10).

Table 10. Mean biomass and grain yield of 200 sorghum M4 mutant lines and check (grouped summary)

Genotype Group	No. of Lines	Mean Biomass (t ha ⁻¹)	Mean Grain Yield (t ha ⁻¹)	Stress Tolerance Index	Remarks
Check variety	1	6.0	2.4	Low	Standard reference
Low-performing mutants	80	6.2–7.0	2.5–2.9	Low–Moderate	Susceptible to stress
Moderate performing mutants	90	7.1–8.5	3.0–3.6	Moderate	Average adaptation
High-performing mutants (non-elite)	24	8.6–9.8	3.7–4.4	High	Stable across sites
Elite drought-tolerant lines	15	9.6–10.8	4.3–4.9	Very high	Strong drought adaptation



Genotype Group	No. of Lines	Mean Biomass (t ha ⁻¹)	Mean Grain Yield (t ha ⁻¹)	Stress Tolerance Index	Remarks
Elite Striga-resistant lines	10	8.9–9.8	3.9–4.5	High	Reduced Striga impact
Dual drought + Striga resistant lines	6	10.2–11.4	4.8–5.2	Very high	Broad / specific adaptation

Values represent means across Bako, Miesso, and Mekele. Stress Tolerance Index was derived from relative yield performance under stress compared with non-stress environments.



6. CONCLUSION

In conclusion, the Phase One (Year 1) implementation of the project “Development of Novel Multi-Stress Resistant Sorghum Genotypes Through Mutation Breeding Approach in Ethiopia” has successfully achieved its core objectives and generated strong scientific and breeding outputs. The comprehensive evaluation of 200 advanced M₄ sorghum mutant lines across three contrasting agro-ecological environments—Bako, Miesso, and Mekele—revealed substantial genetic variability, confirming the effectiveness of induced mutation breeding in broadening the sorghum genetic base.

Importantly, the combined ANOVA and GGE biplot analyses demonstrated significant genotype, environment, and genotype × environment (G×E) interactions, underscoring the necessity of multi-environment testing for reliable selection. Despite environmental variability, a subset of mutant lines consistently expressed stable performance, indicating strong adaptive capacity under diverse stress conditions. Based on integrated multi-trait evaluation, the study successfully identified 15 elite drought-tolerant lines and 10 elite Striga-resistant lines, each exhibiting superior vegetative vigor, stress tolerance, and yield advantages over the standard check.

Most notably, the identification of six dual-elite mutant lines combining drought tolerance and Striga resistance represents a major breeding milestone. These lines consistently maintained high biomass and grain yield, minimal Striga infestation, and broad or specific adaptation across stress-prone environments. Their performance confirms that mutation breeding, when integrated with rigorous phenotypic screening and G×E analysis, is a robust and effective strategy for developing climate-resilient sorghum genotypes.

Overall, Phase One results provide strong empirical evidence that this approach can deliver farmer-relevant, multi-stress-resilient sorghum genetic resources, thereby contributing meaningfully to enhanced productivity, yield stability, and food security in Ethiopia’s vulnerable agro-ecologies.



7. RECOMMENDATIONS

Based on the promising outcomes of Phase One, several key recommendations are proposed to guide Phase Two and future sorghum improvement efforts. First, the identified 15 elite drought-tolerant lines, 10 elite Striga-resistant lines, and six dual-elite lines should be advanced to Phase Two multi-location yield trials, including on-farm participatory evaluations, to validate their performance under real farmer management conditions.

Second, the six dual-elite mutant lines should be given high priority for accelerated testing, seed multiplication, and potential pre-release verification, as they address the two most critical constraints drought and Striga simultaneously. These lines also represent valuable parental materials for future crossing programs aimed at pyramiding multi-stress tolerance traits.

Third, controlled greenhouse and laboratory screening for Striga resistance mechanisms and soil acidity tolerance should be strengthened in Phase Two to complement field evaluations and enhance selection precision. In parallel, molecular characterization (SSR/SNP markers) is strongly recommended to validate genetic diversity, confirm trait associations, and support marker-assisted selection.

Fourth, continued use of advanced statistical tools, including GGE biplot and multi-trait selection indices, is recommended to refine genotype selection and guide environment-specific or wide-adaptation breeding strategies.

Finally, strong collaboration among research institutions, extension systems, and seed enterprises is recommended to ensure effective scaling, dissemination, and eventual adoption of the best-performing genotypes. Collectively, these actions will maximize the impact of the project and accelerate the delivery of climate-resilient sorghum varieties to Ethiopian farmers.



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