UNIVERSITY OF KWAZULU-NATAL

GENETIC ENHANCEMENT OF SORGHUM FOR YIELD-RELATED TRAITS AND DROUGHT TOLERANCE THROUGH INDUCED MUTAGENESIS

MALIATA ATHON WANGA

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Genetic Enhancement of Sorghum for Yield-related Traits and Drought Tolerance through Induced Mutagenesis

By
Maliata Athon Wanga
Master of Agricultural Science (Kindai University, Nara City, Japan)
Bachelor of Technology in Agricultural Management (Polytechnic of Namibia, Windhoek, Namibia)

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College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Republic of South Africa

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Thesis Abstract

Sorghum (*Sorghum bicolor* [L.] Moench) is the foundation crop in the world's dry regions, for food, feed, and bioenergy feedstock. There has not been a systematic breeding program and farmers-preferred varieties of the crop in Namibia due to several constraints. There is a need to develop high-yielding and farmer-preferred sorghum varieties with drought-adaptive traits to boost sorghum productivity in the country. The overall goal of this study was to contribute to the national sorghum breeding program aimed at improving sorghum production and productivity through the development and deployment of climate-smart cultivars preferred by farmers and markets in Namibia via induced mutagenesis. The specific objectives of the study were: (1) to assess the present state of sorghum production in northern Namibia and document farmers' perceived production constraints and trait preferences in new varieties to guide drought-tolerance breeding; (2) to determine the optimum doses of a single and combined use of gamma radiation and ethyl methanesulfonate (EMS) for effective mutation breeding in sorghum; (3) to determine the genetic profile of elite sorghum lines developed via gamma radiation using diagnostic simple sequence repeat (SSR) markers and phenotypic traits for selection; and (4) to determine the Genotype by environment interaction (GEI) of newly-developed mutant and traditional sorghum lines for grain yield and yield related traits for drought-prone areas of Namibia.

In the first study, a survey was conducted using a participatory rural appraisal in the following six selected sorghum-growing constituencies: Kapako and Mpungu (Kavango West Region), Eenhana and Endola (Ohangwena Region), and Katima Mulilo Rural and Kongola (Zambezi Region). Data were collected using a structured questionnaire involving 198 farmers in 14 sampled villages across the regions. An equal proportion of male and female respondent farmers cultivate sorghum, suggesting the value of the crop to both genders in Namibia. Most respondent farmers (63.6%) were in productive age groups of <40 years old. In the study areas, low-yielding landrace varieties, namely Ekoko, Okambete, Makonga, Kamburo, Nkutji, Katoma, Fuba, Dommy, Kawumbe, and Okatombo, were widely cultivated, and most of the farmers did not use chemical fertilizers to cultivate sorghum. Farmers' perceived sorghum production constraints in the study areas included recurrent drought, declining soil fertility, insect pest damage, high cost of production inputs, unavailability of improved seed, lack of alternative improved varieties with farmers' preferred traits, lack of organic manure, limited access to market and limited extension service. The key farmers' preferred traits in a new sorghum variety included high grain yield, early maturity, and tolerance to drought and storage pests. The study recommends genetic improvement and new variety deployment of sorghum with the described farmers-preferred traits to increase the sustainable production of the crop in Namibia.

In the second study, two concurrent experiments were conducted as follows: in experiment I, the seeds of four sorghum genotypes (Parbhani Moti, Parbhani Shakti, ICSV 15013, and Macia) were treated using five gamma radiation doses (0, 300, 400, 500 and 600 Gray [Gy]), and three EMS doses (0, 0.5 and 1.0%), and gamma radiation followed by EMS (0 and 300 Gy and 0.1% EMS; 400 Gy and 0.05% EMS). In experiment II, the seeds of two sorghum genotypes (Macia and Red sorghum) were treated with only seven doses of gamma radiation (0, 100, 200, 300, 400, 500 and 600 Gy). The combined applied doses of gamma radiation and EMS are not recommended due to poor seedling emergence and seedling survival rate below LD_{50}. The best dosage of gamma radiation for genotypes Red sorghum, Parbhani Moti, Macia, ICSV 15013 and Parbhani Shakti ranged between 392 and 419 Gy, 311 and 354 Gy, 256 and 355 Gy, 273 and 304 Gy, and 266 and 297 Gy, respectively. The optimum dosage ranges of EMS for genotypes Parbhani Shakti, ICSV 15013, Parbhani Moti and Macia were between 0.41% and 0.60%, 0.48% and 0.58%, 0.46% and 0.51%, and 0.36% and 0.45%, respectively. The above dose rates are useful for induced mutagenesis and creating genetic variation in the tested sorghum genotypes for breeding programs.

In the third study, 20 mutant lines (which were at mutation generation 7 [M_7]) were developed using gamma-irradiation at 350 Gy from the seed of the variety Macia (SDS 3220). Also, five check varieties were used for the comparative study. DNA extraction was carried out on young and fresh leaves samples per test line 20 days after sowing. Seventeen SSR markers amplified a total of 50 alleles, which varied from 2 to 5 (mean = 2.94). The number of effective alleles per locus varied from 1.08 to 2.53, with a mean of 1.96. The observed heterozygosity ranged from 0.00 to 0.21 (mean = 0.09). The mean expected heterozygosity value was 0.45 indicating moderate genetic differentiation of the tested lines for selection and hybridization. Cluster analysis classified the genotypes into three main groups. Moderate to high genetic distance (≥ 0.50) was displayed between drought-tolerant and high-yielding genotypes that aided in selecting mutant lines such as ‘ML2, ML3, ML4, ML7 and ML14’ compared with the check varieties ‘Macia, Kotovara, ICSR 137, and ICSV 17004’. The selected lines are a useful source of genetic variation for breeding high-yielding and drought-tolerant varieties suited for the drought-prone environments of Namibia.

In the fourth study, 50 sorghum genotypes, including 10 newly-developed mutant lines (M_9), 33 landraces, two sorghum varieties widely grown in Namibia, and five standard check varieties were evaluated under field conditions using a 10 x 5 alpha lattice design with three replications. The experiments were carried out in four environments with two growing seasons in Namibia. Data were collected on grain yield and related traits and subjected to the Additive Main Effects and Multiplicative Interaction (AMMI) model. The AMMI model showed that 93.9% of the total genetic variation was attributed to days to 50% flowering (DF), while 94.04% of the variation was due to plant height (PH), 86.52% to panicle weight (PW), 70.67% to thousand-grain weight (TGW), and 90.68% to grain yield (GY). The larger variations attributed to genotypic effects for PL (36.3%), TGW (33.2%) and PH (20.7%) are useful for genotype selection for yield-related traits. Based on a multi-trait biplot and Best Linear Unbiased Prediction (BLUPs) analyses of the GEI data across all drought-prone testing environments, the medium maturity mutant line designated as L7P9-13 was selected as the best yielding (2 tons/ha) and recommended for the drought-prone areas of Namibia.

In summary, the study identified sorghum production systems, key farmers' perceived production constraints and trait preferences in new varieties in Namibia. Also, the best dosage of gamma radiation and EMS were determined for increasing the genetic diversity in sorghum for genetic enhancement. Newly developed mutant lines ML2, ML3, ML4, ML7 and ML14 displayed moderate to high genetic distance useful for breeding high-yielding and drought-tolerant varieties suited for the drought-prone environments of Namibia. The medium maturity and drought-tolerant mutant line designated as L7P9-13 was the best yielding (2 tons/ha) and recommended for large-scale production in the country.

Declaration

I, Maliata Athon Wanga, declare the following:

1. The research reported in this thesis, except where otherwise indicated, and is my original research.
2. This thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a) Their words have been re-written but the general information attributed to them has been referenced.
    b) Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed:

[redacted]

Maliata Athon Wanga

As the candidate's supervisor, I agree to the submission of this dissertation

[redacted]

Prof. Hussein Shimelis (Supervisor)

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Dedication

This thesis is dedicated to my family and all the families who have no resources to send their children to school but have faith and hope that somewhere, there is someone who cares, and they will get help to achieve their study goals.

Abbreviations

%E	Seedling emergence percentage
%G	Germination percentage
%SS	Seedling survival percentage
%SV	Seed viability
ADC	Agricultural Development Centres
AFLP	Amplified fragment length polymorphism
AMMI	Additive Main Effects and Multiplicative Interaction
ASV	AMMI stability values
BATAN	National Nuclear Energy Agency/Indonesia
BLUEs	Best linear unbiased estimates
BLUPs	Best linear unbiased predictors
CP	% crude protein
DAPEES	Directorate of Agricultural Production, Extension and Engineering Services/Namibia
DARD	Directorate of Agricultural Research and Development/Namibia
DF	days to 50% flowering
DNA	Deoxyribonucleic acid
DTF	Days to 50% flowering
EMS	Ethyl methanesulfonate
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database of the United Nations
F_{ST}	Fixation index
GEI	Genotype by environment interaction
GY	Grain yield
Gy	Gray, the unit of mutation dose for X-rays and gamma rays
H_e	Expected heterozygosity
H_o	Observed heterozygosity
IAEA	International Atomic Energy Agency
IAR	Institute of Agronomic Research/Nigeria
IBPGR	International Board for Plant Genetic Resources
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
LD_{50}	mean lethal dose of 50%
M_1	First mutation generation
M_2	Second mutation generation

M_3	Third mutation generation
M_5	Fifth mutation generation
M_6	Sixth mutation generation
M_7	Seventh mutation generation
MAS	Marker-assisted selection
MAWLR	Ministry of Agriculture, Water and Land Reform/Namibia
MET	Multi-environment trials
MVD	Mutant variety database
N_a	Total number of alleles per locus
NBRI	National Botanical Research Institute/Namibia
N_e	Number of effective alleles per locus;
NIRS	Near-infrared spectroscopy
NMU	N-methyl-N-nitrosourea
NP	Number of panicles per m^2
NPGS	National Plant Germplasm System/USA
NPP	Number of productive panicles per m^2
NSA	Namibia Statistics Agency
PH	Plant height
PHT	Plant height
PIC	Polymorphic information content
PLT	Panicle length
PRA	Participatory rural appraisal
PW	Panicle weight
QTL	Quantitative trait locus
RAPD	Rapid amplified polymorphic DNA
RCBD	Randomized complete block design
RFLP	Restriction fragment length polymorphism
SE	Standard error
SLT	Shoot length
SNP	Single nucleotide polymorphism
SPS	Single plant selection
SSA	Sub-Sahara African
SSD	Single seed descent
SSR	Simple sequence repeat
TE	Transpiration efficiency
TGW	Thousand-grain weight
XRF	X-ray fluorescence

Publications Pertaining to this Thesis

Chapter 1

Wanga, M. A., Kumar, A. A., Kangueehi, G. N., Shimelis, H., Horn, L. N., Sarsu, F., and Andowa, J. F. N. (2018). Breeding sorghum using induced mutations: Future prospect for Namibia. *American Journal of Plant Sciences*, *09*(13), 2696–2707. https://doi.org/10.4236/ajps.2018.913196

Chapter 2

Wanga, M. A., Shimelis, H., and Mengistu, G. (2022). Sorghum production in northern Namibia: Farmers' perceived constraints and trait preferences. *Sustainability*, *14*(16), 10266. https://doi.org/10.3390/SU141610266

Chapter 3

Wanga, M. A., Shimelis, H., Horn, L. N., and Sarsu, F. (2020). The effect of single and combined use of gamma radiation and ethylmethane sulfonate on early growth parameters in sorghum. *Plants*, *9*(7), 827. https://doi.org/10.3390/plants9070827

Chapter 5

Wanga, M.A., Shimelis, H., and Mashilo, J. (2022). Genotype by environment interaction of newly developed sorghum lines in Namibia. *Euphytica,* 218, 147. https://doi.org/10.1007/s10681-022-03099-5

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Introduction to Thesis

Background

Sorghum (*Sorghum bicolor* [L.] Moench, $2n=2x=20$) is a powerhouse crop in dry regions of the world, serving for food, feed, and bioenergy production (Chisi and Peterson, 2019; Taylor, 2019; FAOSTAT, 2022). In semi-arid Asia and Africa sorghum is the major food security crop (Derese et al., 2017; Rajendra et al., 2021; Chadalavada et al., 2022). Sorghum grain is processed in several food products and consumed as boiled or steamed whole grain, porridge, bread, pop sorghum, snacks, biscuit and grain flakes (Awika, 2017; Raigond et al., 2019; Castro-Campos et al., 2021). Sorghum grain is a nutrient-rich source of macro-nutrients such as starch (72%), crude protein (11%), fibre (7%), fat (3%) and vitamins, and micro-nutrients (e.g., iron and zinc) essential for human health (Kulamarva et al., 2010; Abdelhalim et al., 2019). It is also popular in the brewing industry, and various brands of industrially processed sorghum beer are manufactured in Africa (Kutyauripo et al., 2009; Takoudjou Miafo et al., 2022).

Sorghum grain production stands at 59 million tons per annum globally. This figure makes sorghum the 5th most-produced cereal after maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.) (FAOSTAT, 2022). Globally sorghum production increased from 57 million tons in 1990 to 59 million tons in 2020, whereas the land area for sorghum production decreased from 42 million in 1990 to 40 million in 2020 (FAOSTAT, 2022). Contrarily, the global yield output for sorghum increased from 1.4 to 1.5 tons/ha in three decades (from 1990 to 2020). The yield growth was mainly attributable to improved crop management practices, such as improved varieties and associated crop production technologies (Kukal and Irmak, 2018; Schlegel and Havlin, 2021; Parra et al., 2022). In Africa, sorghum production increased from an estimated 12 million tons in 1990 to 27 million tons in 2020, whereas production lands increased from 16 to 27 million ha (FAOSTAT, 2022). The yield outputs for Africa rose from 0.73 to 1.03 tons/ha in three decades, revealing the availability of improved cultivars and better crop management practices (Sultan et al., 2013; Sultan et al., 2014; Smale et al., 2018; Andiku et al., 2022).

Sorghum yields in Sub-Sahara African (SSA) countries, including Namibia, are low (< 1 ton/ha). The low yields are due to various biotic and abiotic stresses and the low input agriculture systems. In the region, sorghum is widely grown under rain-fed conditions with limited agro-chemical inputs. The notable abiotic stresses are recurrent drought, extreme temperatures, and declining soil fertility (Borrell et al., 2014; Verma et al., 2018; Ndlovu et al., 2021; Rajendra et al., 2021; Yahaya et al., 2022). The major biotic stresses for sorghum production are bird damage, parasitic weeds and pre-harvest insect pests (e.g., fall armyworm, aphid, and armoured cricket) and diseases (e.g., anthracnose, downy mildew and head smut) (Mofokeng and Shargie, 2016; Mengistu et al., 2019; Samuel et al., 2020). Therefore, development and deployment of high yielding and drought-tolerant sorghum varieties with resistant to insect pests and diseases is the best strategy to improve sorghum production and productivity in the dry regions.

In Namibia, sorghum is produced on an estimated area of 17,842 ha, making it the third most cultivated cereal crop after pearl millet (*Pennisetum glaucum* [L.]), and maize in 2020 (FAOSTAT, 2022). The crop is the second most cultivated cereal after pearl millet under the rainfed agricultural system in northern regions including Zambezi, Kavango East, Kavango West, Otjozondjupa, Oshikoto, Oshana, Ohangwena, Omusati, and Kunene regions (Hillyer et al., 2006; Mendelsohn et al., 2006; Newsham and Thomas, 2011). Namibia's national yield output is estimated at 0.2 tons/ha in 2020, which is five times lower than the yield output for continental Africa (1 ton/ha), and >14 times lower compared to the yield reported by the lead producer countries, including China (4.9 tons/ha), USA (4.6 tons/ha), Mexico (3.2 tons/ha) and Ethiopia (2.8 tons/ha) (FAOSTAT, 2022). The potential yield for the crop is up to 9.3 tons/ha under irrigation systems (Assefa and Staggenborg, 2010). Sorghum production decreased from 7,000 tons in 1990 to 3,280 tons in 2020 in Namibia, whereas the land area devoted to sorghum production dropped from 34,113 ha in 1990 to 17,842 in 2020 (FAOSTAT, 2022). The declining trends of sorghum production and productivity indicate that the economic potential for this hardy and stress-resilient crop is not realized in the country (Hillyer et al., 2006; Newsham and Thomas, 2011; Horn et al., 2015). The main reasons for low sorghum productivity are yet to be systematically studied and documented to guide the research and development of modern varieties in Namibia.

Key strategies are recommended to transform sorghum production and productivity in SSA, including the use of irrigation, cultural practices (e.g., mulching and cover crops) and adoption of high-yielding and drought-tolerant cultivars and climate-smart agriculture, among others (Mdungela et al., 2017; Bazzana et al., 2022; Gikonyo et al., 2022). Breeding and deploying high-yielding and drought-adapted cultivars is the most economical and sustainable approach in water scare and smallholder sorghum production systems. Also, there is a need for improved seed systems to deploy modern and climate-smart varieties. Therefore, economic and farmers' preferred traits and desirable product profiles of sorghum varieties are integral to adopting modern cultivars (Shimelis et al., 2019). Therefore, demand-led sorghum breeding will ensure the release and adoption of new generation climate-smart varieties to serve the diverse needs of farmers and the marketplace. This is contingent on the availability of a genetically diverse and climate-smart pool of genetic resources and genetic tools to select genotypes with desirable traits for breeding.

In Namibia, the sorghum improvement program was initiated in the 1990s by the Government in collaboration with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Bantilan et al., 2004). ICRISAT-bred and introduced lines and landraces were evaluated in multi-environment trials (MET), which led to the selection and release of variety ‘Macia’ and ‘Red sorghum’, a local line developed through mass selection, for large-scale production in 1999 and 2004, respectively (Kolberg, 1999; Saadan et al., 2000). Macia is an obsolete variety cultivated for its white grain colour and medium maturity (120 days) for drought escape. However, Macia is sensitive to moisture stress occurring between the sowing and seedling stages, leading to poor crop establishment and low yield (Madanzi et al., 2007; Hadebe et al., 2017; Wondimu et al., 2018). There are no modern sorghum varieties developed and released recently in Namibia. Therefore, cultivation of the only two obsolete varieties and low-yield traditional and unimproved varieties necessitated breeding for modern and climate-smart varieties with high yielding capacity and drought tolerance preferred by farmers and markets.

Drought is a major constraint on crop production in arid and semi-arid regions, including Namibia (Awala et al., 2019; Kapuka and Hlásny, 2020). Drought stress occurring at the reproductive growth stage has a devastating effect on sorghum yield than in the early vegetative growth stages (Tari et al., 2013; Abou-Elwafa and Shehzad, 2018; Prasad et al., 2019; Silambarasan et al., 2022). Drought stress at vegetative and reproductive stages can reduce yield by up to 60% and 80%, respectively (Upadhyaya et al., 2017; Badigannavar et al., 2018). Thus, this necessitates developing high-yielding sorghum genotypes with drought adaptive traits for production in water-scarce environments. However, drought tolerance is a quantitative trait governed by polygenes, and the expression of the trait is influenced by genotype performance and genotype by environment interaction (Katsenios et al., 2021; Enyew et al., 2021; Gerrano et al., 2022). Breeding for drought tolerance requires extensive genotype selection among a large set of breeding populations and in target environments to integrate desirable traits from contrasting parents and recommend new progenies for variety development and release.

Human selection over many years and the predominantly autogamous flower system of sorghum led to narrow genetic diversity to select high-yielding and drought tolerant genotypes

(Kimber et al., 2013; Xin et al., 2021; Enyew et al., 2022). Also, continuous changes in abiotic and biotic stresses have attributed to a shift in crop adaptation requiring ongoing enhancement in genetic resources for selecting complementary parental genotypes with novel traits. This will allow developing modern and climate-smart sorghum varieties to serve the diverse value chain demands. Induced mutagenesis is a powerful tool to broaden crops' genetic diversity for the selection of genotypes to develop climate-smart varieties with desirable agronomic traits, exploratory root and smart canopy architecture systems (Shu et al., 2012; Spencer-Lopes et al., 2018; Sarsu et al., 2021).

Success in induced mutation breeding programs depends on the lethal dose of the chosen mutagenic agents on target genotypes and the extent of the genetic changes to aid selection for novel traits (Singh et al., 2021; Kalpande et al., 2022). Mutagenic agents (physical and chemical) increase the frequency of genetic diversity by up to 10^{-5} to 10^{-8} per loci in a larger genome size species compared to a relatively smaller proportion (2×10^{-6} to 40×10^{-6}) occurred due spontaneous mutations (Rutger, 1983; Parry et al., 2009; Jiang and Ramachandran, 2010; Mba et al., 2010; Tadele, 2016; Monroe et al., 2022). In most breeding programs, mutant plants are identified through phenotypic changes. This may result in a limited selection response of novel mutant plants with low morphological expression (Waugh et al., 2006; Deng et al., 2022). Therefore, integrated selection methods such as marker-assisted selection (MAS), genomics and high-throughput phenotyping can aid in rapid selection for novel mutant genotypes to develop breeding populations.

The mutant variety database (MVD, 2022) of the International Atomic Energy Agency (IAEA) reveals that 3,388 varieties were registered in 180 plant species globally. Gamma-ray and X-ray were the most widely used physical mutagenic agents, whereas ethyl methanesulfonate (EMS) and N-methyl-N-nitrosourea (NMU) were the most widely used chemical mutagenic agents (MVD, 2022). For sorghum, the mutant variety database shows that 17 varieties were developed and released for economic traits, including high grain yield, early maturity, improved quality, and tolerance to abiotic and biotic stresses.

Breeding for drought-tolerant sorghum varieties with high grain yield and adaptive drought traits has been limited in Namibia due to the low genetic diversity of the crop in the country. Therefore, a collaborative research project was initiated between the Government of Namibia and the IAEA in 2009. The main goal was to develop novel mutant genotypes to enhance genetic diversity for developing high-yielding sorghum varieties with drought tolerance. Gamma irradiation was selected due to its high effectiveness in creating mutation and reproducibility of results and having no negative impact on the environment (Du et al., 2022;

Riviello-Flores et al., 2022). The seed for the widely cultivated sorghum variety Macia and Red sorghum were mutagenized at the Joint Food and Agriculture Organization (FAO)/IAEA laboratories in Seibersdorf, Austria, for gamma irradiation. Subsequently, the pedigree selection method was used from the M_3 to M_5 generations using phenotypic traits including plant height, maturity, panicle weight and grain yield to select the best performing populations developed from irradiated seed. The elite and genetically stable lines (M_6 generation) require rigorous testing for drought-adaption and yield potential to identify candidate varieties for recommendation and large-scale production in Namibia.

The rationale for pre-breeding sorghum using gamma irradiation

Sorghum is an important crop for food and feed with opportunity for biofuel production in drier regions, including Namibia. Sorghum varieties with drought- and heat-adaptive traits are imperative to boost the production and productivity of the crop. Systematic improvement for sorghum is integral strategy to improve yield of the crop in dryland cropping systems limited by lack of varieties for water-scarce and heat-stressed agro-ecologies. Drought and heat tolerant varieties are important to improve food security and income generation for low input farming systems, especially under rainfall agriculture for smallholder farmers. Therefore, systematic studies and documentation of sorghum production systems, farmers' perceived production constraints and trait preferences in new varieties are required to guide the development of varieties to serve diverse needs of the crop value chain. Further, developing climate-smart sorghum varieties for cultivation under the ever-changing abiotic and biotic constraints requires ongoing genetic diversity enhancements to select novel genotypes with desirable traits. Thus, breeding novel sorghum varieties is contingent on the availability of genetic diverse resources to select genotypes with desirable traits for improvement. The use of modern breeding tools such as gamma irradiation enhances genetic variation to develop mutant sorghum varieties with desirable and multiple traits (Kenga et al., 2006 Human et al., 2011; MVD, 2022). Success in inducing genetic variation through gamma radiation is dependent on the optimum dose of the mutagen and the recovery of functional mutants (Suthakar et al., 2014; Deshmukh et al., 2018). Therefore, the application of induced mutation presents an opportunity to broaden the genetic diversity for selecting mutant elite lines to recommend for large-scale production and further breeding.

Aim of research

The overall goal of this study was to contribute to the national sorghum breeding program aimed at improving production through the development and deployment of climate-smart cultivars preferred by farmers and markets in Namibia. Induced mutation breeding using gamma radiation was applied to create genetic variation for selecting mutant lines with high yield and drought adaptive traits to recommend for release for large-scale production in Namibia.

Specific objectives

The specific objectives of the study included:

1. To assess the present state of sorghum production in northern Namibia and document farmers' perceived production constraints and trait preferences in new varieties to guide drought-tolerance breeding.
2. To determine the optimum doses of a single and combined use of gamma radiation and ethyl methanesulfonate (EMS) for effective mutation breeding in sorghum.
3. To determine the genetic profile of elite sorghum lines developed via gamma radiation using diagnostic simple sequence repeat (SSR) markers and phenotypic traits for breeding.
4. To determine genotype-by-environment interaction of newly-developed mutant and traditional sorghum lines for grain yield and yield-related traits for drought-prone areas of Namibia and select desirable mutants.

Research hypothesis

This study was conducted to test the following hypotheses:

1. Participatory rural appraisal facilitates establishing the present state of sorghum production in northern Namibia and to document farmers' perceived production constraints and trait preferences in new varieties to guide drought-tolerance breeding.
2. A single or combined use of gamma radiation and ethyl methanesulfonate (EMS) will be determined for effective mutation breeding in sorghum for large-scale mutagenesis.
3. There is a wide genetic and phenotypic variation of elite sorghum lines developed via gamma radiation for selection and breeding.
4. Newly-developed sorghum mutant lines perform differently for yield and yield-related traits under multiple environments for selection.

Thesis outline

This thesis consists of six chapters outlined according to research objectives (Table 0.1). The chapters are written as discrete research papers intended for publication containing all the necessary information. Due to their interdependence, there are some overlaps and unavoidable repetition of references and introductory information between chapters. This is the dominant thesis format adopted by the University of KwaZulu-Natal. Chapter 1 presents a review of the literature on the progress, and future prospects of mutation breeding in sorghum and part of it was published in the American Journal of Plant Sciences (doi: https://doi.org/10.4236/ajps.2018.913196). Chapter 2 focuses on sorghum production in northern Namibia: farmers' perceived constraints and trait preferences and was published in Sustainability (doi: https://doi.org/10.3390/su141610266). Chapter 3 discusses the effect of single and combined use of gamma radiation and EMS on early growth parameters in sorghum and was published in Plants (doi: https://doi.org/10.3390/plants9070827). The genetic profile of newly developed sorghum lines revealed through simple sequence repeat markers and phenotypic traits is highlighted in Chapter 4. Chapter 5 evaluated genotype-by-environment interaction of newly developed sorghum lines for grain yield and yield components in Namibia and was published in Euphytica (doi: https://doi.org/10.1007/s10681-022-03099-5). The core findings and recommendations from the study are presented in Chapter 6.

Table 0.1: Outline of thesis with chapters and title

Chapter	**Title**
	Introduction to thesis
1	Induced mutation breeding to developing drought tolerant sorghum varieties: A review
2	Sorghum production in northern Namibia: Farmers' perceived constraints and trait preferences
3	The effect of single and combined use of gamma radiation and ethyl methanesulfonate on early growth parameters in sorghum
4	Genetic profile of newly developed sorghum lines revealed through simple sequence repeat markers and phenotypic traits
5	Genotype by environment interaction of newly developed sorghum lines in Namibia
6	An overview of research findings and implications for sorghum breeding

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Chapter 1. Review of the Literature

Induced mutation breeding for drought tolerant sorghum varieties: A review

Abstract

Sorghum (*Sorghum bicolor* [L.] Moench, 2n=2x=20) stands out as a high potential crop for food and feed systems in the dry regions of the world due to the increasing human population and climate change. Induced mutation breeding is a powerful tool to enhance genetic diversity required to develop climate-smart sorghum varieties for cultivation in dry regions, including Namibia. Drought tolerance is a quantitative trait governed by polygenes that affect the cumulative responses to morphological, physiological, and biochemical mechanisms under depleted moisture conditions. Therefore, the development of climate-smart sorghum varieties that are high yielding with farmers' and market-preferred traits depends on the availability of genetically diverse genotypes as a resource for recombination and select high-performing progenies. Therefore, the present review highlights progress in induced mutation breeding in sorghum and potential integration with amenable selection methods to deliver new varieties rapidly. In the first section, opportunities for induced mutation breeding techniques are discussed. In the second section, the review highlight genes associated with morphological, physiological and biochemical traits for drought adaptation mechanisms to guide selection in induced mutation breeding. Gamma-ray and X-ray were the widely used physical mutagenic agents, whereas ethyl methanesulfonate (EMS) and N-methyl-N-nitrosourea (NMU) were the most used chemical mutagenic agents in sorghum breeding programs. The traits recommended in drought tolerant sorghum varieties are agronomic traits, root systems and smart canopy architectures. Exploratory root system traits include longer and branched roots with a higher number of seminal roots, and wider root angle. Smart canopy traits include stay green, reduced number of tillers, leaf rolling, selective senescence of older lower leaves, narrow leaf angles, thick waxy cuticle and epicuticular layers. Thus, induced mutation and selection for novel mutant plants possessing essential traits using marker-assisted selection integrated with genomics, speed breeding, high-throughput phenotyping, and digital tools can rapidly deliver climate-smart varieties for cultivation in drought-prone regions.

Keywords: breeding; climate-smart varieties; drought stress; induced mutation; sorghum

1.1 Introduction

Sorghum [*Sorghum bicolor* (L.) Moench, 2n = 20] is the fifth-highest produced cereal crop after maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.) in the world (FAOSTAT, 2022). However, the impacts of climate change exacerbated the effects of biotic and abiotic constraints on sorghum, escalating the unprecedented pressure to develop and deploy modern climate-smart sorghum varieties. Therefore, modern sorghum varieties are a key strategy to improve production and productivity to meet the demands for food and crop-based products for the ever-growing human population, especially in arid and semi-arid conditions. Intense and recurrent drought stress is a major constraint for sorghum production and productivity under rainfall agriculture systems in dry regions of the world (Mrema et al., 2017; Mengistu et al., 2019; Yahaya et al., 2022; Okot et al., 2022). Therefore, an enhanced genetic pool is required to develop high-yielding sorghum genotypes with drought-tolerant traits. Induced mutation breeding is a genetic improvement procedure and involves treating plant materials (e.g., seed, stem, tuber and pollen grains) with mutagenic to create genetic changes in the deoxyribonucleic acid (DNA). New progenies will be selected in the ensuing generations based on the enhancement of genetic diversity and reshuffling of genes for breeding (Spencer-Lopes et al., 2018).

Plant breeding is the art, science, and business of developing new cultivars with desirable plant characteristics. It is assumed that the first mass selection of improved sorghum plants was carried out in Africa, given the origin of the crop in the region (de Wet and Huckabay, 1967; Winchell et al., 2017). The first selected plants had novel genes that resulted from spontaneous mutations, the only source of a selection of plants suitable for domestication (Shu et al., 2012; Oladosu et al., 2016). Since sorghum has up to 15% outcrossing, the cultivated species could have continued crossing with wild species, allowing considerable gene flow from wild species to cultivated species (House, 1985; Winchell et al., 2017). As the cultivated area expanded, the gene flow from wild species could have been stopped resulting in polymorphic populations (House, 1985). Farmers likely continued with the selection and maintenance of the best plants, creating the current five sorghum races, viz., bicolor, caudatum, durra, guinea and kafir (House, 1985).

After the discovery of the principles of heredity and the concept of genes in the 1800s and 1900s, plant genetics became essential in crop breeding programs (Acquaah, 2012; Lasky et al., 2015). This advancement in breeding tools has increased the efficiency of breeding, including induced mutation, which involves exposing plant materials to mutagens (Shu et al., 2012; Morris et al., 2013; Acquaah, 2015). Sorghum breeders' interest in utilizing induced mutation is to create genetic variability in plant population to meet the need for yield, quality, resistance and environmental adaptation (Zhang et al., 2018). However, utilizing induced mutation depends mainly on a well-defined objective for improvement (Morris et al., 2013). According to Zhang et al. (2018) induced mutation is commonly utilised to: (1) improve specific traits in a preferred cultivar, (2) induce a morphological marker in a promising line for variety registration and (3) induce male sterility or fertility restoration for hybrid variety development. Thus, the first step for sorghum mutation breeding in national breeding programs is to identify specific limitations such as susceptibility to drought, insect pests, diseases, seed shattering, and lodging in the farmers' preferred cultivars in which mutation is to be induced.

Trait-based breeding and selection of fewer superior genotypes to develop pure line varieties and inbreed lines for hybrid varieties to replace landraces, and the predominantly autogamous flower system of sorghum continue to narrow genetic diversity for the crop. This is a critical bottleneck in developing modern sorghum varieties with novel traits, including high yielding, drought-tolerant and favourable nutritional qualities such as higher digestibility and processing. Breeding programs involving the crossing of elite germplasm followed by selection and evaluation of best performance progenies across the target environment require integration of ongoing enhancement in the genetic resources. Mutation breeding enables the development of novel traits needed in modern and climate-smart sorghum varieties.

Drought tolerant cultivars are classified into three based on drought adaptive mechanisms, namely, escape, avoidance and tolerance (Fang and Xiong, 2014; Ndlovu et al., 2021; Rajendra et al., 2021). Drought escape involves early maturity by sorghum cultivars. These cultivars develop rapidly to complete their full life-cycle before drought stress. Very early maturity sorghum varieties take < 56 days to 50% flowering (DTF) from emergence. In contrast, early maturity takes between 56 and 65 DTF, compared to medium maturity, taking between 66 and 75 DTF, late maturity takes between 76 and 85 days, and > 85 days for very late maturity (Reddy et al., 2008). Drought avoidance sorghum varieties have morphological and physiological adjustments which reduce water loss and allow accessing moisture at deeper soil layers in conditions where water is depleted of the topsoil. Traits associated with drought avoidance include osmotic adjustment (Girma and Krieg, 1992), stomatal regulation (Dourado et al., 2022), exploratory root system architectures (longer and branched roots, a higher number of seminal roots, and wider root angle) (Parra-Londono et al., 2018), and smart canopy (thick waxy cuticles and epicuticular layers on leaves, and leaf rolling) (Mantilla-Perez et al., 2020; Natukunda et al., 2022). Drought-tolerant sorghum varieties can maintain a certain level of physiological activities to reduce the negative effects of limited moisture. Traits related to drought tolerance in sorghum include stay green, high membrane stability, and antioxidant capacity under limited soil moisture conditions (Borrell et al., 2014; Rajendra et al., 2021; Ndlovu et al., 2021). Thus, high-yielding sorghum genotypes with these traits are important for the development of climate-smart and drought-tolerant sorghum varieties to cultivate in drought-prone agro-ecologies.

The objectives of the present review were to outline progress made in induced mutation breeding in sorghum and genes associated with morphological, physiological and biochemical responsive mechanisms to target in induced mutation breeding programs. In the first section, the chapter discusses induced mutation breeding techniques to enhance genetic variation and select useful genotypes to recommend for large-scale production and further breeding. In the second section, the review highlight genes associated with drought mechanisms to guide induced mutation breeding. The current review highlights the potential advantages of induced mutation breeding for the successful development and release of modern climate-smart sorghum varieties to serve farmers and value-chain in drought-prone environments.

1.2 Opportunities of induced mutagenesis in breeding

1.2.1 Mutagen and dose rates

Mutation in the genome of plants raised from propagation materials exposed to mutagens occurs at random (Holme et al., 2019). Therefore, the usefulness of the mutation in the DNA is dependent on breeding objectives, the sensitivity (lethal doses) of genotypes to mutagenic agents, and genetic changes generated in the ensuing generations (M_2 and M6) after mutagenesis to aid selection for novel traits (Singh et al., 2021). In most breeding programs, mutant plants are identified through phenotypic markers which results in limited selection of novel mutant plants with low morphological expression in M_2 generations (Waugh et al., 2006; Deng et al., 2022). In sorghum, the effect of a single treatment and combination of physical and chemical mutagens on germination, survival, seedling growth, seedling fertility variation and chlorophyll mutation frequency in M_1 and M_2 has been utilized to determine the dose treatment (Xin et al., 2009; Human et al., 2012; Spencer-Lopes et al., 2018). The doses that cause between 30% and 50% reduction in germination, survival and seedling growth are selected for breeding programs (Mani, 1989; Deshmukh et al., 2018). Mutagenic effectiveness has been found to be greater at doses between 300 and 500 Gy (gray, the unit of mutation dose) for X-rays and gamma rays, whereas doses between 0.1% and 0.3% were widely applied for EMS (Bretaudeau, 1997; Human et al., 2012; Prashant, 2016). A combination of 100 Gy for gamma ray and 0.1% for EMS were found to be the most effective treatments for growth and yield components (Sree-Ramulu, 1971; Xin et al., 2009).

Mutagenic agents increase the frequency of genetic change (10^{-5} to 10^{-8} per loci in a larger genome) within the genome constitution compared to a smaller proportion (2 x 10^{-6} to 40 x 10^{-6} mutations per gamete per gene) of spontaneous mutations (Rutger, 1983; Parry et al., 2009; Jiang and Ramachandran, 2010; Mba et al., 2010; Tadele, 2016; Monroe et al., 2022). Genetic diversity resulting from induced mutation is vital for the selection of economic traits important for direct cultivation and introgression in widely adapted varieties. The International Atomic Energy Agency (IAEA)/Austria mutant variety database (MVD, 2022) reveals that from 1950 to 2022 a total of 3,388 varieties were registered in 180 plant species in different countries of the world. Asia recorded the highest number (2,087) of mutant varieties indicating the high adoption and application of induced mutation in the continent, compared to Europe (960), North America (211), Africa (82), Latin America (53), and Australia and Pacific (9) (MVD, 2022). The highest number of mutant varieties were recorded for China (817) followed by Japan (500), India (345), Russian Federation (216), and Netherland (176), suggesting that a relatively higher number of countries using induced mutation breeding approach. The highest mutant varieties recorded among food crops were for rice (873), barley (307), wheat 265, soybean (182) and maize (89) (Table 1.1). Gamma-ray and X-ray were the widely used physical mutagenic agents, whereas ethyl methanesulfonate (EMS) and N-methyl-N-nitrosourea (NMU) were the most used chemical mutagenic agents. The varieties were developed and released for economic traits including high grain yield, early maturity, improved qualities, and tolerance to abiotic and biotic stresses. Sorghum was among the crops with the least record for mutant varieties (17) indicating limited application of induced mutation breeding techniques to enhance the crop production and productivity.

Table 1.1: Desirable induced traits, mutation methods and the number of varieties for major crop varieties in the world.

Crops	Reported countries	Methods used	Number of varieties released	Induced traits
Rice (*Oryza sativa* L.)	Bangladesh, Brazil, Burkina Faso, China, Costa Rica, Cote D'Ivoire, Cuba, France, Guyana, Hungary, India, Indonesia, Iran, Iraq, Japan, Korea, Malaysia, Mali, Myanmar, Nigeria, Pakistan, Philippines, Portugal, Romania, Russian Federation, Senegal, Sri Lanka, Taiwan, Thailand, USA and Viet Nam.	Gamma-ray, X-ray and EMS	873	High grain yield, improved seed quality, moderate maturity, compact plant type, big spike, higher grain number, higher grain weigh, high grain quality, more resistance to blast and bacterial leaf disease, resistance to white-backed plant hopper and resistance to lodging.
Barley (*Hordeum vulgare* L.)	Australia, Austria, Belgium, Bulgaria, Canada, Chile, China, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, India, Iraq, Japan, Korea, Netherlands, Norway, Peru, Poland, Russian Federation, Slovakia, Sweden, Syrian, Turkey, Ukraine, UK and USA	Gamma-ray, X-ray and EMS	312	High yield malting quality for brewery, resistance to lodging, feed quality, herbicide tolerance, early maturity, resistance to lodging, tolerance to powdery mildew and high 1000-kernel weight, resistance to low temperatures, short culm, resistance to nematodes, stiff straw.
Soybean (*Glycine max* L.)	Algeria, Australia, Bulgaria, China, Congo DR, Cuba, Germany, Hungary, India, Indonesia, Iraq, Japan, Korea, Mexico, Moldova, Russian Federation, Slovakia, Thailand, Turkey and Viet Nam.	Gamma-ray, X-ray and EMS	182	High yield and high protein content, early maturity, tolerant to low temperatures, resistance to drought, short plant type, yellow seeds, hypernodulation, nitrogen carry-over and nitrate tolerant nodul, stem resistance to lodging, high number of branches, dark green leaves, long pods and good quality, resistance to bacterial and virus diseases, large seed size and resistance to alkaline soil.
Maize (*Zea mays* L.)	Bulgaria, China, Congo DR, Hungary, Russian Federation, Slovakia and Viet Nam	Gamma-ray, X-ray, EMS and NMU	96	High yield, early maturity, stalk quality and stalk diseases resistance, plasticity, short stalk, stalk quality, resistance to virus and fungal diseases, high protein content, drought tolerance, resistance to leaf spot, suitable for close planting and good quality.
Sorghum	China, India, Indonesia and Mali	Gamma-ray	17	High yielding grain, fodder type sweet stem, tolerant to major insects and pathogens, white colour of the grains, longer panicle length, resistance to lodging, increased number of grains per panicle, short straw and suitable for close planting, grain quality (protein, tannin, starch).

Note: EMS, Ethyl methanesulfonate; NMU, N-Nitroso-N-methylurea; Source: MVD (2022)

1.2.2 Induced mutation for drought stress tolerance in sorghum

Drought triggers the expression of several trans-acting factors for genes governing adaptive mechanisms to cope and survive the adverse conditions of limited soil moisture combined with high atmospheric temperatures. Therefore, induced mutation breeding to create alternative alleles is important for the regulation of the expression of target quantitative trait locus (QTLs). Alternative gene creation, and introgression in adapted and widely cultivated genotypes are vital strategies in breeding programs to offset the negative effects of reduced biosynthesis of metabolites and photosynthesis (Verma et al., 2018; Ndlovu et al., 2021; Rajendra et al., 2021).

After germination and seedling emergence, a combination of drought and heat stress occurring at reproductive growth stages (i.e., pre- and post-flowering and grain filling) has a much more devastating effect on sorghum yield output compared to vegetative stages (Tari et al., 2013; Abou-Elwafa and Shehzad, 2018; Prasad et al., 2019; Silambarasan et al., 2022). Thus, inducing genotypic variations for drought and heat adaptive can be enhanced via trait-based selection targeting mechanisms, namely, escape, avoidance, tolerance and recovery (Fang and Xiong, 2014); Ndlovu et al., 2021; Rajendra et al., 2021). The following sections present morphological, physiological, biochemical and QTLs associated with drought and heat stress adaptive mechanisms to select in induced mutation breeding programs for developing modern and climate-smart sorghum varieties.

1.2.2.1 Drought stress signalling

Induced mutation is vital for creating alternative and marked genes and alleles that regulate proteins, enzymes and hormones, signalling physiological, phenological and morphological responses of plants under drought stress (Eapen et al., 2017; Hossain et al., 2022; Shelake et al., 2022; Chen et al., 2022). Drought response mechanisms are associated with trans-acting factors molecules that signal stress, including mitogen-activated protein kinase (MAPK/MPKs) (Kong et al., 2021), Ca-dependent protein kinases (CDPKs) (Geng et al., 2011), sugar as a signalling molecule (ZHANG et al., 2019), nitric oxide (NO), and phytohormone (Mukarram et al., 2022; Singh et al., 2022). The signalling molecules trigger the activation of various stress-responsive genes governing the transcription of proteins, enzymes and hormones to maintain cellular homeostasis under adverse conditions of limited moisture combined with higher temperature (Ciarmiello et al., 2011). Therefore, QTLs identified for regulation of proteins and enzymes for signalling drought and heat stress includes *PUB43* (Song et al., 2017), heat shock transcription factors (*HSFs*) and heat shock proteins (*HSPs*) (Nagaraju et al., 2015; Sudhakar et al., 2016), drought-hypersensitive mutant1 (*DSM1*) (Ning et al., 2010), Rho-type GTPase-activating protein 1 (*RGA1*), aldehyde oxidase 3 (*SbAO3*), aspartic protease G 1 (*SbASPG1*), CBL-interacting protein kinase 15 (*SbCIPK15*), cytokinin dehydrogenase 4 (*SbCKX4*), glutathione S-transferase (*SbGST*), G-type lectin Sreceptor-like serine/threonine-protein kinase (*SbGsSRK*), SbMAPKKK7, SbMAPK10 (Wang et al., 2016), pentatricopeptide repeat-containing protein (S*bPPR3*), S-type anion channel (*SbSLAH2*), thaumatin-like protein 1b (*SbTLP1b*), U-box domain-containing protein 43 (*SbPUB43*), WRKY transcription factor 46 (*SbWRKY46*) (He et al., 2016), zinc finger protein (*SbZFP*) (Abou-Elwafa and Shehzad, 2018), *CKX4* (Pospíšilová et al., 2016), *SbER1*, *SbER2-1* and *SbDREB2* (Li et al., 2019).

Plant hormones associated with response to drought and heat stress to enhance or lower expression using mutation include ethylene (ET), auxin, abscisic acid (ABA), brassinosteroids (BRs), cytokinin (CK), salicylic acid (SA) and jasmonate (JA) (Li et al., 2020). Genes associated with the regulations of the hormones include *Arabidopsis thaliana* hardy (*AtHRD*) (Shanker et al., 2012), APETELA2/ethylene responsive factor (AP2/ERF), AP2/EREB bZIP, bHLH and GRAS gene family (Abdel-Ghany et al., 2020), DNA binding one finger (*SbDof*) (Gupta et al., 2016), GRETCHEN HAGEN3 (*SbGH3*) and LATERAL ORGAN BOUNDARIES (*SbLBD*) (Wang et al., 2010; Gupta et al., 2016), SbPIN/5/8/9/11, SbPIN/3/6/7/10, SbPIN4/5/8/9/11 (Shen et al., 2010), auxin response factors (ARFs) SbARF genes (Aglawe et al., 2012; Chen et al., 2019), ABREs and CGTCA-motifs (Anami et al., 2015), DEGs (Fracasso et al., 2016b; Zhang et al., 2019). Therefore, understanding the responses of these QTLs can enhance selection through marker-assisted selection (MAS).

1.2.2.2 Osmotic adjustment and stomatal regulation

Under drought conditions, several adjustments are carried out in plants to avoid losing water under drought stress. Mechanisms such as decreasing carbon assimilation, stomatal conductance, and cell turgor triggers plants to reduced leaf area, shoot development, canopy architecture, selective senescence of older lower leaves and leaf surface properties (Earl and Davis, 2003; Assefa et al., 2010). Sorghum avoids water loss through stomatal closure when leaf osmotic potential is between −1.4 MPa to −1.5 MPa (Dourado et al., 2022). Stomatal regulation has been associated to abscisic acid level, CBL-Interacting Protein Kinase and S-type anion channel (Zhang et al., 2016; Wang et al., 2016). Therefore, selection for genotypes with higher accumulated cellular net solute to lower tissue osmotic potential allow moisture conservation within plants under drought stress conditions (Basnayake et al., 1994; Basnayake et al., 1996). Membrane thermostability is associated with tolerance to drought and heat (air temperature of 40–45 °C) stress and linked to plastidic and extra-plastidic glycerolipids, reduced photorespiration, carbon assimilation rate, energetic efficiency of photosynthesis, and photosynthetic quotient, decreased ROS production and increased antioxidant levels and antioxidant enzyme activity (Djanaguiraman et al., 2014; Djanaguiraman et al., 2018; Djanaguiraman et al., 2020).

Moisture loss is associated with cell wall stiffening, plant cell and tissue development under drought stress which are related to basic helix-loop-helix (*bHLH* proteins), TFs and phytochrome interacting factor 4 (*PIF4*) (Proveniers and Van Zanten, 2013). Genes associated with stomatal regulation include *SLAC1, SLAH2, SLAH3, KAT1, KAT2,* and *TaCIPK2* (Geiger et al., 2011; Zhang et al., 2016; Wang et al., 2016). Other genes associated with reduced water potential in sorghum include *SORBI_3006G135500, SORBI_3001G514200, SORBI_3001G313200,* and *SbMPK14* (Goche et al., 2020; Zhou et al., 2022). Under drought and heat stress NAC transcription factors families (*SbSNAC1, SbNAC2*), *HSF*, and ethylene-responsive transcription factor (*ERF*) are associated with selective senescence of older lower leaves which reduces water lose from the plant (Lu et al., 2013; Wang et al., 2016; Baillo et al., 2019; Li et al., 2019; Jin et al., 2021). Markers associated with this QTLs can be used in sorghum induced mutation breeding programs to develop drought tolerant genotypes.

1.2.2.3 Seedling vigour

Unpredictable drought stress at an early vegetative stage between germination and after seedling emergence can cause seedling death leading to poor crop establishment and yield (Queiroz et al., 2019; Abreha et al., 2021; Ndlovu et al., 2021). Genotypes aiding higher seed germination along with rapid root growth, higher root biomass, seedling vigour index, and higher root-to-shoot ratio can be selected for cultivation targeting drought-prone environments (Tolk and Howell, 2001; Singh et al., 2010; Queiroz et al., 2019). Genes associated with early seedling vigour include *dw1, dw2, dw3, KS3, GA2ox5, SbDof12, SbDof19, SbDof24, b465/140, tK12/115, bDll/65, tM5/75, tC13/150, bC18/820* (Tuinstra et al., 1996; Yamaguchi et al., 2016; Zhao et al., 2016; Gupta et al., 2016; Hao et al., 2021). Systematic studies and induced mutation to create alternative alleles to regulate the expression of rapid root growth to access soil moisture at deep soil layers when soil moisture on the topsoil layer is depleted can lead to breeding of varieties that can escape drought and heat stress at early vegetative stage.

1.2.2.4 Early flowering

Drought escape mechanism involves the cultivation of early-maturity varieties that grow rapidly and complete development stages, including the reproductive prior to adverse impact of drought and heat stress. Early maturity sorghum varieties take between < 56 and 65 DTF from emergence (Reddy et al., 2008). Genes associated with flowering time regulatory include early flowering3 *ELF3* (Guitton et al., 2018), Ma_1 (*PRR37*), Ma_2 (*PRR37*), Ma_3 (*PHYB*), Ma_5 (*PHYC*), Ma_6 (*GHD7*) and *Pfr G* (Childs et al., 1997; Sanchez et al., 2002; Murphy et al., 2011). Further, several genes were associated with post-flowering drought stress such as NAC regulating transcription, include SbNAC (*SbNAC014, SbNAC034, SbNAC035, SbNAC037, and SbNAC041*) (Sanjari et al., 2019). Induced mutation to create alternative alleles for these QTLs can be targeted to develop genotypes for early maturity varieties for cultivation in agro-ecologies prone to late season drought stress. Therefore, integrated selection approaches for early flowering sorghum genotypes require combined evaluation with responses to photoperiod, thermal duration and homeostasis of days to flowering (Craufurd and Qi, 2001; Clerget et al., 2012).

1.2.2.5 Seed set

Drought and higher atmospheric temperature lead to reduced seed set resulting from poor floret fertility due to decreased gametes (pollen and stigma) viability and embryo abortion (Prasad et al., 2011; Prasad et al., 2015; Singh et al., 2015; Djanaguiraman et al., 2018). Floret fertility under drought and heat stress conditions have been associated with increased tapetum mitochondrial number and a higher rate of respiration. The changes led to higher accumulation of ROS, damaged membrane lipids, membrane permeability and denaturation of functional proteins, misfolding of newly synthesized proteins, disruption of microtubules and cytoskeleton during pollen tube, sucrose hydrolysis and limited necessary carbohydrate and nutrients, and scavenging enzymes (Jain et al., 2007; Jain et al., 2010; Maduraimuthu et al., 2014; Sage et al., 2015). Selecting mutants with early morning and late evening flowering traits can escape heat stress to increase floret fertility. This is typical under limited moisture combined with higher atmospheric temperature during the day (Jagadish, 2020). Therefore, studies to map genes associated with higher floret fertility attributing traits are required to enhance the application of point-induced mutation and targeting induced local lesions in genomes (TILLING) for developing suitable alleles to produce a higher number of filled grains per panicle.

1.2.2.6 Root system architecture

Crop development and adaptation to drought stress is associated with the root system architecture (RSA), which depends on genotype (Khan et al., 2016; Griffiths et al., 2022). The main distinctions of RSA include length, number, spread, branching density, and angle of root components to access water in drought and heat-prone ago-ecologies (Bengough et al., 2004; Singh et al., 2012; Lynch and Brown, 2012; Yusefi-Tanha et al., 2022). RSA has been categorized into three types, namely, (1) small root systems that are shallow and narrow with few and short lateral roots, (2) compact and bushy rooting types that have relatively short lateral roots, and (3) exploratory root system which are deep and have a wide root network with long lateral roots (Parra-Londono et al., 2018). QTLs associated with RSA traits include transcription factor coding genes such *NAC, HSF, WRKY, ERF, HD-ZIP, MYB*, and *bHLH* (Parra-Londono et al., 2018; Baillo et al., 2019). For example, genes to target under mutation breeding program for improved RSA traits include *WRKY* family transcription factors (*SbWRKY30, SbWRKY74, SbWRKY75, SbWRKY19, SbWRKY5, SbWRKY45, SbWRKY79, SbWRKY25, SbWRKY 83, and SbWRKY72 SbWRKY79, SbWRKY83, and SbWRKY16*) (Abou-Elwafa and Shehzad, 2018; Yang et al., 2020; Ding et al., 2015), *SbRD1*9 (Baillo et al., 2020), and Mannitol-1-phosphate dehydrogenase (*mtlD*) (Maheswari et al., 2010; Hema et al., 2014). Induced mutation breeding combined with marker assisted selection (MAS) could lead to the development of a genotype possessing an exploratory root system with longer and branched roots combined with a higher number of seminal roots and wider root angle to explore soil moisture at both top and deeper layers (Table 1.2).

1.2.2.7 Canopy system architecture

Biochemical and physiological traits influence the canopy architecture traits and drought stress tolerance. Due to reduced biochemical and physiological performance, yield-related attributes such as plant height, stem diameter, the number of productive tillers, leaf size, panicle length, panicle weight and grain per panicle are reduced (Mantilla-Perez and Salas, 2017; Mantilla-Perez et al., 2020; Kong et al., 2020). Selection for modern sorghum genotypes requires smart canopies which consist of upright oriented upper leaves, reduced number of tillers, reduced number of leaves per culm, enhanced size of lower leaves, and decreased size of upper leaves. These attributes allow more light to reach the lower leaves and are vital for moisture conservation in plants under drought and heat stress conditions (Gitz et al., 2015; Mantilla-Perez and Salas, 2017; Mantilla-Perez et al., 2020). Alternative mutant alleles for identified QTLs associated with smart canopy traits and biosynthetic for signalling pathways for higher responsive to stress can lead to the development of smart canopies to increased water conservation during the grain filling stage to increased grain yield (Borrell, et al., 2014; Borrell, et al., 2014). Thus, selection for modern sorghum genotypes with high yield requires integrated selection with smart canopy aiding drought adaptive traits such as stay green, reduced number of tillers, leaf rolling, and selective senescence of older lower leaves, narrow leaf angles, long narrow leaves, thick waxy cuticle and epicuticular layers, and reduced number of stomata (Table 1.2).

Sorghum varieties with a smart canopy and stay green trait allows plants to retain chlorophyll molecules during post-flowering drought stress, enabling the grain filling (Mahalakshmi and Bidinger, 2002; Jordan et al., 2012; Borrell et al., 2000). Sorghum varieties with a stay green trait and higher lignin content are resistant to lodging under post-flowering drought conditions (Adeyanju et al., 2016; Kamal et al., 2018; Kamal et al., 2019). QTLs associated with stay green include *Stg1, Stg2, Stg3*, and *Stg*4 (Sabadin et al., 2012; Borrell et al., 2014; Rama et al., 2014; Li et al., 2019; Usha et al., 2020). Stay green genotypes possessing accelerated age-related senescence of lower leaves lead to a smaller canopy associated with transpiration efficiency (TE) during pre- and post-flowering drought (George-Jaeggli et al., 2017). Target QTLs associated with delayed senescence include AP2/ERF (*Sobic.010G202700*), NBS-LRR protein (*Sobic.010G205600*), ankyrin-repeat protein (*Sobic.010G205800*), senescence-associated protein (*Sobic.010G270300*), WD40 (*Sobic.010G205900*), CPK1 adapter protein (*Sobic.010G264400*), LEA2 protein (*Sobic.010G259200*), expressed protein (*Sobic.010G201100*), delta1-pyrroline-5-carboxylate synthase 2 (*P5CS2*) (Johnson et al., 2015; Usha et al., 2020). Identified QTLs associated with number of tillers under drought condition include *SBI-01, SBI-02 and SBI-06-I and SBI-10* (Alam et al., 2014), and dormancy associated protein 1 (*DRM1*) (Govindarajulu et al., 2021). Canopy temperature is highly associated with stomatal conductance, number of stomata, thick waxy cuticle, epicuticular layers, WUE and grain yield under drought and heat stress condition (Mutava et al., 2011; Rebetzke et al., 2012; Carvalho et al., 2020). Identified QTLs associated with thick waxy cuticle, and epicuticle wax on leaf and stalk to reduce evapotranspiration include mutants of the bloomless2 (*Bm2*) (Punnuri et al., 2017), *SbWINL1, FATB, and CER1*, (Sanjari et al., 2021).

Table 1.2: Phenotypic traits for climate-smart sorghum varieties for drought tolerance.

Desired phenotypic traits	Advantages	References
Early growth seedling stage vigour		
Longer root	High crop establishment, drought escape	Singh et al. (2010)
Higher root biomass	Improved photosynthesis	Singh et al. (2010)
Higher root-to-shoot ratio	Increased water availability	Singh et al. (2010)
Days to flowering and floret fertility		
Early flowering	Drought escape	Guitton et al. (2018)
Early morning flowering	Escape heat stress	Jagadish (2020)
High pollen viability	Improved floret fertility, grain yield	Jagadish (2020)
Root system architecture		
Longer roots	Access soil moisture at deeper soil layer	Singh et al. (2012)
Higher number of seminal roots	Improved water uptake	Rostamza et al. (2013)
Longer lateral roots	Improved water uptake	Yang et al. (2020)
Wider root angle	Improved water uptake	Liang et al. (2016)
Higher root biomass	Improved water uptake	Liang et al. (2016)
Canopy system architecture		
Short to medium plant height	Lodging resistance	Adeyanju et al. (2016)
Higher aboveground biomass	Enhanced photosynthesis	Borrell et al. (2000)
Thicker stem diameter	Dehydration avoidance	Perrier et al. (2017)
Reduced number of tiller	Water conservation	Kim et al. (2010)
Stay green	Grain filling under drought stress	Borrell, Mullet, et al. (2014)
Increased chlorophyll fluorescence	Enhanced photosynthesis	Abou-Elwafa and Shehzad (2018)
Leaf rolling	Dehydration avoidance	Rauf et al. (2016)
Selective senescence of older lower leaves	Reduce water demand	Harris et al. (2007)
Erect leaves (narrow angles)	Light penetration to lower leaves	Mantilla-Perez and Fernandez (2017)
Long narrow leaves	Moisture conservation	Mantilla-Perez et al. (2020)
Thick waxy cuticle and epicuticular layers	Lower canopy temperature	Punnuri et al. (2017)
Longer panicles	Increased grain yield	Abreha et al. (2021)
Heavy panicles	Increased grain yield	Naoura et al. (2019)
Higher grain number per panicle	Increased grain yield	Borrell, et al. (2014)
Higher 1,000 grain weight	Increased grain yield	Naoura et al. (2019)

1.3 Integrated selection in induced mutation breeding

Advancement in mutation breeding tools selects mutant plants based on variation at gene and alleles (i.e., recessive and dominant alleles) levels (Naito et al., 2005; Till et al., 2007; Shu et al., 2012). Knowledge of pleiotropic genes regulating quantitative traits presents an opportunity for developing alternative alleles using TILLING which locates induced point mutations (Wang et al., 2006; Xin et al., 2021; Jiang et al., 2022). Due to the random nature of mutation events, there is a need to handle larger plant populations to screen and select novel mutant plants. This requires a large area of land associated with a high cost for crop management in the field. Therefore, advancements in genomics-based technologies present a new paradigm of mutagenesis applications for gene discovery and the development of novel mutant populations to save time and resources required in breeding programs. Hence, integrated selection methods that combine qualitative and quantitative phenotypic traits with molecular genetics and functional genomics aid rapid breeding of climate-smart crops with farmers' and markets' desirable traits (Jung and Till, 2021; Awan et al., 2022; Das et al., 2022).

Selection methods can be enhanced through integrated marker-assisted selection (MAS), which is an indirect selection procedure based on molecular markers or quantitative trait loci (QTLs) linked to phenotypic traits. MAS reduces the time for selecting desirable plants to use as genetic resources and identifying traits that display low heritability (Taheri et al., 2017). Several genetic-based marker systems, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), rapid amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) have been developed to amplify particular sequence within the genome for comparative analysis (Osei et al., 2019). For example, the sorghum SSR kit consisting of 48 loci (https://sorghum.cirad.fr/SSR_kit/) is suitable for identifying genetically diverse mutant plants in M_2 generation to develop populations (Shehzad et al., 2009; Billot et al., 2013). Thus, the integration of plant breeding tools to deliver varieties rapidly include application of genotyping services (MAS) with rapid generation advancement services (speed breeding), high-throughput phenotyping and digital tools (e.g., leasy scan and lysimeter for drought, and near-infrared spectroscopy [NIRS], X-ray fluorescence [XRF] and CT-imaging for nutrition and physical qualities).

1.4 Mutant population development and evaluation

Successful breeding programs have focused on breeding goals. Higher grain yield drought tolerance, enhanced nutritional quality, and resistance to biotic and other abiotic stresses are some of the breeding goals in crop improvement programs. Induced mutation breeding program adapts crop breeding procedure namely: (1) selection of desirable parental genotype, (2) create variation (mutagenesis or crossing), (3) selection, development of progenies and genetic advancement, (4) evaluation of the best progenies in target agro-ecologies, and (5) registration of the best performing and stable candidate variety for commercialization (Shimelis and Laing, 2012). Therefore, effective selection methods are used for discrimination, the development of breeding populations, and genetic advancement. Selection for sorghum mutants with novel traits integrates various selection methods, including single seed descent (SSD), single plant selection (SPS), bulk, mass, recurrent, pedigree and pure line selection methods (Table 1.3). The use of speed breeding, which reduce the amount of time, space and resources invested in the selection and genetic advancement, is recommended to deliver improved mutant varieties more rapidly (Watson et al., 2018; Wanga et al., 2021; Temesgen, 2022; Samantara et al., 2022).

Table 1.3: Advantages and limitations of various selection methods used in sorghum.

Selection method	Target traits	Advantages	Limitations	References
SSD	Low heritable traits	Require less space, time, and resources in early breeding stage.	Loss of individual plants due to natural selection, Carry inferior genetic material forward.	Fehr (1991)
SPS	Low heritable traits	Allows a smaller population to be advanced.	Require more labor and record keeping.	Hickey et al. (2017)
Pure line	Low heritable traits	Inferior materials are discarded at early stage and high degree of uniformity.	Require more time, space, and resources.	Yan et al. (2017)
Bulk	Low heritable traits	Simple and less labour required in early generation. Large number of genotypes can be maintained.	Require large space and environmental changes can result in the loss of superior genotypes.	Kanbar et al. (2011)
Pedigree	Low to high heritable traits	Eliminates inferior material at early stage. Multi-season records allow effective selection.	Require more labour, time, space and resources and record keeping.	Crossa et al. (2010)
Recurrent	Low heritable traits	Break undesirable linkage in repeated inter-crossing.	Time consuming loss of desirable linked genes. Difficult to control pollination, Inbreeding depression may occur in CF populations.	Shelton and Tracy (2015)
Mass	Highly heritable traits	Easy and quick method for higher adaptability and stable yield.	Lack of uniformity in selected genotype, Maximum potential cannot be achieved.	Yabe et al. (2018)

SSD, single seed descent; SPS, single plant selection; low heritable traits include grain yield, drought and heat stress tolerance; high heritable traits include plant height, flower colour, panicle shape, grain texture and colour.

1.5 Global sorghum germplasm

According to Reddy et al. (2008), over 40,000 sorghum germplasm are maintained at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India; about 8,000 in Ethiopian; > 42,200 at the National Plant Germplasm System (NPGS), USA; >12,800 at National Genetic Germplasm Resources Bank, China. In Namibia, about 180 germplasm were collected from farmers and maintained at the National Botanical Research Institute (NBRI) (Bantilan et al., 2004). The diversity of these genetic resources is not fully exploited in terms of characterization, evaluation and documentation for utilization (Acquaah, 2012; Lasky et al., 2015). There is low number of researchers working on sorghum breeding (Xin et al., 2017). According to the literature, only two researchers are working on the sorghum breeding program in Namibia compared with China (200), India (150), Ethiopia (50) and Sudan (21) (Bantilan et al., 2004). Although the utilization of induced mutation has been limited in sorghum, it is an important tool in breeding superior varieties of desirable traits of different crops species. Given that sorghum as the fifth highest-produced crop in the world, only 17 mutant varieties released in Mali, China, India and Indonesia reflect limited interest and documentation in induced mutation breeding in sorghum (Table 1.1).

The mutant variety database (MVD, 2022) for the Food and Agriculture Organization (FAO)/IAEA show that mutants developed in different parts of the world were for multiple traits, high grain and fodder, semi-dwarf, synchronous maturity, more sugar content, resistance to lodging, resistant to drought, resistant to diseases and insect pest, grain quality (zinc, iron, protein, tannin, and starch) and for machine harvestability (Table 1.4). In Indonesia, irradiation with gamma were utilised to generate high-yielding sorghum varieties and tolerance to stresses such as drought and soil acidity (FAO/IAEA, 2017). Three improved varieties of drought tolerant, semi-dwarf stature, early maturity and high yielding (over 4 tons/ha) were released in Indonesia. The varieties were adopted by both farmers and private seed companies, grown for grain for developing many kinds of food products, while the plant stover was used for feed. Additionally, promising mutant lines of drought tolerance have been developed for food, feed, and starch and ethanol.

According to the National Research Council (1996) of the Institute of Agronomic Research (IAR), Samaru, Zairia, and Nigeria eight mutant lines were developed through gamma irradiation. These mutants were selected based on early and synchronous maturity, reduction in plant height, higher yield and large grain size. Some mutant lines were found to be useful directly as varieties and other lines were good combiners as they had high general combining ability (GCA). Kenga et al. (2006) reveal that agronomic traits of locally adapted sorghum in Mali were modified by gamma irradiation. This includes changes in plant height, lodging tolerance, drought tolerance, panicle length and compactness, seed size and colour, seed quality and protein content, maturity, and tillering ability. A breeding program at Research Farm of the Plant Stress and Germplasm Development Research Unit, USA induced mutation through ethyl methane sulfonate (EMS). This led to nuclear male sterility (NMS) line (*ms8*) (Suthakar and Elangovan, 2014). Mutant *ms8* had a white hairy stigma, which emerged first, and only small white anthers appeared and did not produce pollen grains, making it easily recognizable at the flowering stage.

The chemical agent ethyl methanesulfonate (EMS) was used to generate 1,600 mutant lines from an inbred line BTx623 (Xin et al., 2008). Subsequently, through field selections and TILLING analysis, their study developed two mutant lines of brown midrib (*bmr*), a trait associated with low lignin content and increased digestibility (Xin et al., 2008). A breeding program by Jiao et al. (2018) confirms creating 18 sorghum mutant lines of bloomless (*bm*), a trait known to increase resistance to greenbug and sheath blight. This study confirmed the utilization of MAS to fast-track the selection process. Thus, induced mutations might be a useful tool in generating the diversity genetic, which may supplement conventional breeding methods to desired crop varieties.

Table 1.4: Sorghum mutant varieties registered in different countries of the world

Variety name	Country (Year)	Character improvement details	Mutant development type	Type of mutagen
PAHAT	Indonesia (2011)	High yielding, semi dwarfness, early maturity, grain quality (protein, tannin, starch).	Direct use of an induced mutant	Gamma rays
Yuantian No.1	China (2002)	More sugar content (20%), excellent source both as a feed and as a bio-energy crop, or a bio-ethanol producer, grown on approximately 1,000 ha	Direct use of an induced mutant	Gamma rays
Yuanza 502	China (2002)	High yield (20%)	Crossing with one mutant	Space breeding
Fambe	Mali (1998)	Resistance to lodging and high grain yield (increased number of grains per panicle)	Direct use of an induced mutant	Gamma rays (300 Gy)
Sofin	Mali (1998)	Early maturity, dwarfness and resistance to lodging	Direct use of an induced mutant	Gamma rays (250 Gy)
Tiedjan	Mali (1998)	Longer panicle size, larger grain size and late maturity	Direct use of an induced mutant	Gamma rays (100 Gy)
Djeman	Mali (1998)	White colour of the grains, high grain yield and late maturity	Direct use of an induced mutant	Gamma rays (100 Gy)
Djemanin	Mali (1998)	White grain colour, high grain yield and longer panicle length	Direct use of an induced mutant	Gamma rays (100 Gy)
Gnoumanin	Mali (1998)	Yellow colour of the grains, high grain yield and longer panicle length	Direct use of an induced mutant	Gamma rays (100 Gy)
Gnome	Mali (1998)	Resistance to lodging and high grain yield	Direct use of an induced mutant	Gamma rays (300 Gy)
Sadje	Mali (1998)	Early maturity and shortness	Direct use of an induced mutant	Gamma rays (300 Gy)
Longfuliang1	China (1978)	Early maturity, short straw and suitable for close planting	Direct use of an induced mutant	Gamma rays (200 Gy)
Co 21	India (1977)	Tall and high yielding grain and fodder type, sweet stem, tolerant to major insects and pathogens	Direct use of an induced mutant	Gamma rays (400 Gy)
Jinza 1	China (1970	Improved quality, higher yield and wide adaptability	Crossing with one mutant variety	Gamma rays
Jinfu 1	China (1970	Better quality for machine harvest	Direct use of an induced mutant	Gamma rays (240 Gy)

Source: MVD (2022)

1.6 Conclusions and future perspectives

Induced mutation breeding is a powerful genetic improvement approach for creating alternative alleles to develop drought tolerance sorghum varieties. The present review provides progress made in induced mutation breeding in sorghum and genes associated with morphological, physiological and biochemical responsive mechanisms to target in induced mutation breeding programs. Identified genes associated with drought and heat signalling, osmotic adjustment and stomatal regulation, seedling stage vigour, early flowering and floret fertility, root system architecture and canopy system architecture are discussed. Selection methods are highlighted for discrimination, development of breeding population and genetic advancement in sorghum to integrate with marker-assisted selection. The review presents morphological, physiological and biochemical traits useful in breeding climate-smart sorghum varieties. Desired phenotypic traits to integrate with marker-assisted selection to develop smart mutant sorghum varieties include unique root and canopy architecture systems. Smart root architecture systems for cultivation in drought and heat stress include exploratory root systems with longer and branched roots combined with a higher number of seminal roots and wider root angle to explore soil moisture at both top and deeper layers. Smart canopy aiding drought adaptive traits include stay green, reduced number of tillers, leaf rolling, selective senescence of older lower leaves, narrow leaf angles, long narrow leaves, thick waxy cuticle and epicuticular layers. Plant breeding tools, including genotyping, speed breeding, high-throughput phenotyping and digital tools will deliver new generation sorghum varieties.

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Chapter 2. Sorghum Production in Northern Namibia: Farmers' Perceived Constraints and Trait Preferences

Abstract

Sorghum (*Sorghum bicolor* [L.] Moench) is a valuable crop in the dry regions of the world, including Namibia. Due to the intensity and recurrence of drought and heat stress in the traditional sorghum growing areas, there is a need to breed and deploy new generation farmer-preferred and climate-smart cultivars to serve the diverse value chains. Therefore, the objectives of this study were to assess the present state of sorghum production in northern Namibia and document farmers' perceived production constraints and trait preferences in new varieties to guide drought-tolerance breeding. A survey was conducted using a participatory rural appraisal in the following six selected sorghum-growing constituencies in Namibia: Kapako and Mpungu (Kavango West Region), Eenhana and Endola (Ohangwena Region), and Katima Mulilo Rural and Kongola (Zambezi Region). Data were collected using a structured questionnaire involving 198 farmers in 14 sampled villages across the regions. Results revealed variable trends in sorghum production among respondent farmers when disaggregated by gender, age, number of households, education level, cropping systems, types of varieties grown, and perceived production constraints. An equal proportion of male and female respondent farmers cultivate sorghum, suggesting the value of the crop to both genders in Namibia. Most respondent farmers (63.6%) were in productive age groups of <40 years old. In the study areas, low-yielding landrace varieties, namely Ekoko, Okambete, Makonga, Kamburo, Nkutji, Katoma, Fuba, Dommy, Kawumbe, and Okatombo, were widely cultivated, and most of the farmers did not use chemical fertilizers to cultivate sorghum. Farmers' perceived sorghum production constraints in the study areas included recurrent drought, declining soil fertility, insect pest damage, high cost of production inputs, unavailability of improved seed, lack of alternative improved varieties with farmers' preferred traits, lack of organic manure, limited access to market and limited extension service. The key farmers' preferred traits in a new sorghum variety included high grain yield, early maturity, and tolerance to drought, in the field and storage insect pests. The study recommends genetic improvement and new variety deployment of sorghum with the described farmers-preferred traits to increase the sustainable production of the crop in Namibia.

Keywords: farmer-preferred traits; Namibia; participatory rural appraisal; sorghum breeding; production challenges

2.1 Introduction

Sorghum (*Sorghum bicolor* [L.] Moench) is a valuable crop in the arid and semi-arid regions of the world. Sorghum is the 5th most-produced cereal crop, following maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and barley (*Hordeum vulgare* L.) globally (FAOSTAT, 2022). It is a staple food crop in Africa and Asia and a vital source of industrial raw material to manufacture feed, bio-ethanol, and syrup (Orr et al., 2020; Hossain et al., 2022). Sorghum grain is rich in macro and micro-nutrients and antioxidants (Adebo, 2020; Drub et al., 2021; Dabija et al., 2021; Khalid et al., 2022). Sorghum grain is processed to prepare various food products (e.g., porridge, bread, snacks, biscuit, and grain flakes) (Sharanagat et al., 2019; Alavi et al., 2019; Taylor and Kruger, 2019). The grain is consumed as boiled, steamed, popped, or roasted (Waniska et al., 2016). Furthermore, malting sorghum grain is used for brewing traditional household drinks and in the African beer industry (Tokpohozin et al., 2019; Coulibaly et al., 2020; Gómez Pamies et al., 2021). Globally, sorghum is produced on estimated agricultural land of 44.6 million ha producing 68.3 million tons of grain per year (FAOSTAT, 2022). The lead sorghum producer countries in Sub-Sahara Africa are Nigeria, Ethiopia, Sudan, Niger, and Burkina Faso, producing 6.4, 5.1, 2.1, 1.8, and 1.8 million tons/year, respectively (FAOSTAT, 2022).

In Namibia, sorghum is the second most cultivated cereal crop after pearl millet (*Pennisetum glaucum* [L.]), and both are produced under rainfed agricultural system (FAOSTAT, 2022). It is widely cultivated in the northern regions of the country, such as in Zambezi, Kavango East, Kavango West, Otjozondjupa, Oshikoto, Oshana, Ohangwena, Omusati, and Kunene regions (Hillyer et al., 2006; Mendelsohn et al., 2006; Newsham and Thomas, 2011). In the country, sorghum is mainly cultivated by smallholder farmers for food and cash income (Newsham and Thomas, 2011; Misihairabgwi and Cheikhyoussef, 2017). An estimated agricultural land of 17,800 ha is devoted to sorghum production providing grain outputs of 3300 tons/year in 2020 (FAOSTAT, 2022). The mean national yield of sorghum in Namibia is below 0.3 ton/ha, which is lower than the mean yield of 1 ton/ha for Africa, 4 tons/ha for South Africa (FAOSTAT, 2022), and the mean yield potential of the crop of 9.3 tons/ha (Assefa and Staggenborg, 2010).

The economic value of sorghum is not realized in Namibia (Orr et al., 2020). The main reasons for low sorghum productivity in the country are yet to be systematically studied and documented to guide research and development of the crop. Elsewhere, the major biotic stresses for sorghum production are bird damage, parasitic weeds and pre-harvest insect pests (e.g., fall armyworm, aphid, and armoured cricket), and diseases (e.g., anthracnose, downy mildew, and head smut) (Mofokeng and Shargie, 2016; Mengistu et al., 2019; Samuel et al., 2020). The major abiotic stresses of the crop are drought, extreme temperatures, and poor soil fertility (Amelework et al., 2016; Yahaya et al., 2022). In Namibia, the low sorghum productivity is partly attributable to the continued use of traditional cultivars that are low yielding and susceptible to drought and heat stress conditions. Only two sorghum varieties (Macia and Red Sorghum) introduced in 1999 are widely grown in Namibia.

Namibia is a water scarce and arid to semi-arid country. The intensity and recurrence of drought and heat stress are the major constraints to potential sorghum production in the drier and traditional growing regions of the world, including Namibia (Hillyer et al., 2006; Newsham and Thomas, 2011). Therefore, there is a need for a new variety design and deployment with farmer-preferred traits and tolerance to pre- and post-flowering drought stresses to offset the yield gap resulting from abiotic and biotic stresses (Borrell et al., 2014; Hammer et al., 2014; Mwamahonje et al., 2021). Sorghum research and development should be guided by the needs and preferences of the farmers and value chains which is to be ascertained through market research.

The participatory rural appraisal (PRA) is an effective multidisciplinary tool and one form of market research. It helps to assess and document sorghum production constraints and farmers' perceived trait preferences in new varieties for demand-led breeding and product development. Incorporation of farmers' desirable traits is essential for the wide adoption of climate-smart and drought-tolerant cultivars to serve the diverse value chains of sorghum. PRA tools have been applied by various workers and made several recommendations to define breeding goals (Mrema et al., 2017; Mengistu et al., 2019; Yahaya et al., 2022; Okot et al., 2022). Sorghum research and development is in its infancy in Namibia. No recent PRA studies have documented the major production constraints and farmer-trait preferences as a guide for demand-led sorghum breeding programs. Therefore, the objectives of this study were to assess the present state of sorghum production in northern Namibia and document farmers' perceived production constraints and trait preferences in new varieties for drought-tolerance breeding.

2.2 Materials and methods

2.2.1 Description of the study areas

This study was conducted in six selected constituencies sampled from three regions in northern Namibia, namely Kapako and Mpungu (Kavango West Region), Eenhana and Endola (Ohangwena Region), and Katima Mulilo Rural and Kongola (Zambezi Region) (Figure 2.1).

In the study areas, sorghum is popularly intercropped with pearl millet, groundnut (*Arachis hypogaea* L.), Bambara groundnut (*Vigna subterranea* [L.] Verdc), roselle (*Hibiscus sabdariffa* L.), watermelon (*Citrullus lanatus* L.), and various cucurbit species (Hillyer et al., 2006; Horn et al., 2015). The study areas have one cropping season from October to April following the main rain. The total annual rainfall ranges from 300 mm to 700 mm, with minimum and maximum temperatures of 17 °C and 35 °C, respectively (Awala et al., 2019). Mixed-farming systems of crop production and livestock husbandry are the dominant form of agriculture. Agriculture is the primary economic sector and source of income and livelihood in the study areas (Mendelsohn et al., 2006).

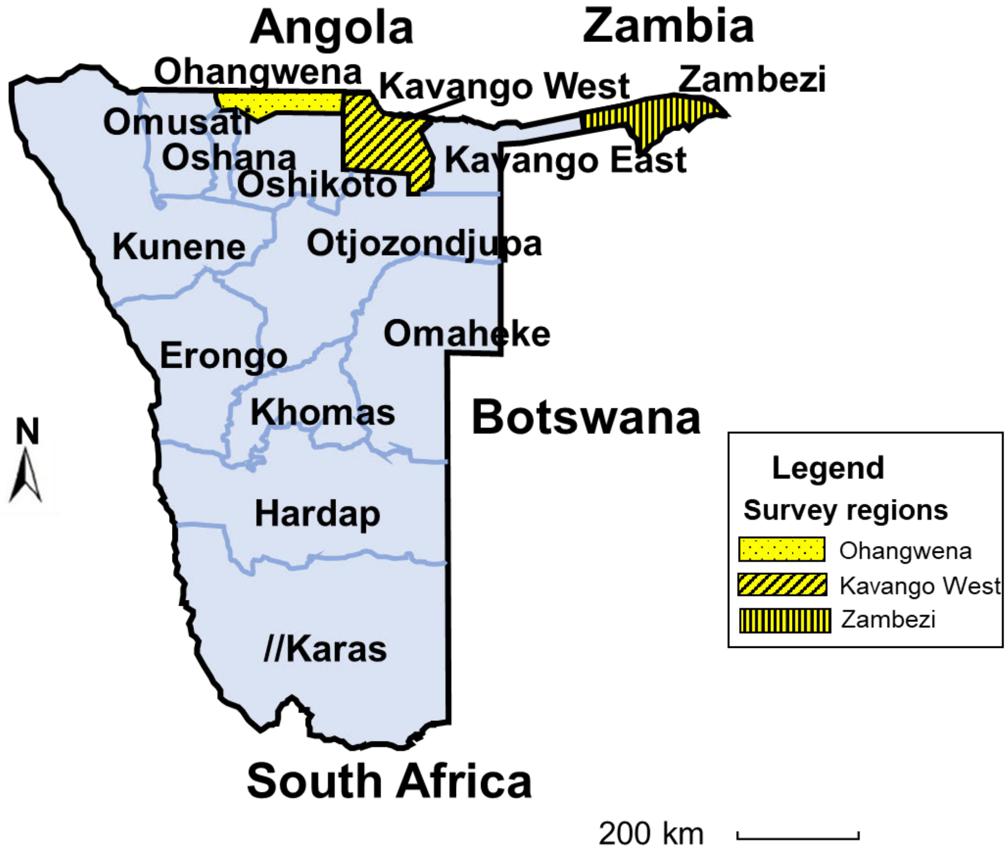

Figure 2.1: Map of Namibia showing the study regions highlighted in yellow.

2.2.2 Sampling procedures

Multistage purposive sampling was used to collect data in the selected constituencies during the 2020/21 cropping season (Table 2.1). In Namibia, a constituency is the smallest administrative unit consisting of 3600 to 60,000 people. Rural constituencies consist of villages with 600 to 2000 inhabitants with six to ten people per household (NSA, 2015). A total of 198 sorghum farming households were randomly sampled in 14 villages across six constituencies in three regions. The extension officials in the sampled constituencies assisted with the selection of villages. The officials are employees of the Ministry of Agriculture, Water and Land Reform (MAWLR) stationed at the Agricultural Development Centres (ADC) of the Directorate of Agricultural Production, Extension and Engineering Services (DAPEES). The main criterion for village selection was based on sorghum area coverage and production. The extension officials from DAPEES and the Directorate of Agricultural Research and Development (DARD) facilitated group discussions with farmers and seed growers to collect the data on the households' general socio-economic status, sorghum production constraints, and varietal preferences. Household heads or close relatives were respondent farmers in the interview.

Table 2.1: Description of the three regions and six constituencies in northern Namibia selected for the study.

Region	Constituency	Village	Number of Farmers Sampled	Gender		Ecological Region	Latitude	Longitude	Altitude (masl)
				Male	Female				
Kavango West	Kapako	Sinzogoro	10	2	8	Zambezian Baikiaea woodlands and Kalahari acacia–baikiaea woodlands	17°53′09.8″ S	19°29′42.5″ E	1120
		Mukundu	10	4	6		17°56′57.7″ S	19°32′59.7″ E	
	Mpungu	Mpungu	10	1	9		17°40′26.9″ S	18°14′38.1″ E	
		Silikunga	10	4	6		17°41′00.6″ S	18°18′54.8″ E	
Ohangwena	Eenhana	Eenhana	20	13	7	Angolan Mopane woodlands and Zambezian Baikiaea woodlands	17°29′58.9″ S	16°19′11.0″ E	1100
		Elundu	15	10	5		17°28′58.2″ S	16°25′05.3″ E	
		Ohaihana	15	7	8		17°27′20.6″ S	16°22′44.5″ E	
	Endola	Onepandaulo	15	8	7		17°39′16.7″ S	15°40′40.4″ E	
		Endola	20	9	11		17°35′30.2″ S	15°42′48.1″ E	
		Oshapwa	15	6	9		17°38′28.0″ S	15°39′38.6″ E	
Zambezi	Katima	Mubiza	18	10	8	Zambezian and mopane woodlands, and Zambezian Baikiaea woodlands	17°30′40.9″ S	24°19′20.9″ E	950
		Kwena	10	7	3		17°48′25.0″ S	24°23′04.8″ E	
	Kongola	Kongola	15	9	6		17°45′00.9″ S	23°25′52.6″ E	
		Sachona	15	9	6		17°46′45.3″ S	23°25′02.6″ E	
	Total		198	99	99				

masl = metres above sea level.

2.2.3 Data collection and analysis

Data were collected using a semi-structured questionnaire, transect walk in sorghum fields, and group discussion. Data collection through structured interviews included socio-demographic description (age, gender, number of households, and education level), sorghum production, cropping systems, and constraints. Transect walk in sorghum fields and group discussion were used to identify field insect pests and diseases, current varieties grown, and key suggested traits preferred by farmers in a new sorghum variety. Farmers' grown varieties were recognized by indigenous names, traits, and different uses such as food, local beverages, animal feed, and construction. Quantitative and qualitative data collected through questionnaires were coded and analysed through cross-tabulations to determine significant differences and compute chi-square values to make inferences. Major crops grown and sorghum production constraints were subjected to pairwise rankings based on respondent farmers' importance weights. Data were analysed using Statistical Package for Social Sciences (SPSS) for Windows, Version 27.0 (IBM Corp: Armonk, NY, USA) (IBM Corp, 2020).

2.3 Results

2.3.1 Socio-demographic description of sorghum growing farmers

Basic socio-demographics disaggregated by gender, age, number of households, and education level status among respondent farmers across constituencies are summarized in Table 2.2. Gender of the respondent farmers was significantly different (χ^2 = 13.225; *p* = 0.021) across constituencies. There was an equal gender representation of the respondent farmers across constituencies. The proportion of female respondent farmers were at 75% for Mpungu and 70% for Kapako, which were higher values than male respondent farmers for Katima Mulilo Rural (60.7%), Eenhana (60%) and Kongola (60%). Age group was significantly different (χ^2 = 43.806; *p* = 0.000) with majority of respondent farmers being 30 to 39 years (39.4%) compared to 25.3% for 40 to 49 years, 24.2% for 18 to 29 years, and 11.1% for ≥ 50 years. The highest proportion (46%) of younger respondent farmers (18 to 29 years) were in Eenhana compared to Kapako (5%), Mpungu (10%), Katima Mulilo Rural (10%), and Endola (38%). Katima Mulilo Rural had the highest percentage (16.7%) of elderly respondent farmers (>50 years); Eenhana had (8%), Endola (8%), Kapako (10%), Kongola (14.3%), and Mpungu (15%). Education level was significantly different (χ^2 = 72.954; *p* = 0.000) across constituencies. The majority of respondent farmers (37.4%) attained secondary education compared to 23.7% for no formal education, 19.7% for primary, 11.6% for a university degree, and 7.6% for a diploma from colleges or vocational training institutions. The highest respondent farmers with no formal education were in Kapako (65%) followed by Mpungu (50%), Eenhana (22%), Endola (20%), and Katima Mulilo Rural (10%). The highest percentage level of respondent farmers who attained degree level were in Katima Mulilo Rural (26.7%), Endola (16%), and Eenhana (10%). The number of family members per household was significantly different (χ^2 = 59.263; p = 0.000) across constituencies. The majority of farming households (38.4%) consisted of 4 to 6 people per household followed by 7 to 9 (30.8%), 1 to 3 (21.7%), and ≥10 (9.1%). The highest proportion of farming households with the least number of people per household (1 to 3) was scored in Eenhana (34%), followed by 26% for Endola, 23.3% for Katima Mulilo Rural, and 14.3% for Kongola. The highest percentage of farming households with ≥10 persons per household was scored in Mpungu (30%) and Kapako (25%) compared to 3.6% for Kongola, 4% for Endola, and 13.3% for Katima Mulilo Rural.

Table 2.2: The proportion of age, gender, number of households, and education level among respondent farmers in six selected constituencies of northern Namibia.

Variable	Kapako	Mpungu	Eenhana	Endola	Katima	Kongola	Mean
Gender							
Male	30.0	25.0	60.0	46.0	60.7	60.0	50.0
Female	70.0	75.0	40.0	54.0	39.3	40.0	50.0
Chi-Square test	DF = 5		χ^2 = 13.22		p-value = 0.02		
Age of farmers							
18–29	5.0	10.0	46.0	38.0	10.0	0.0	24.2
30–39	35.0	55.0	30.0	38.0	43.3	46.4	39.4
40–49	50.0	20.0	16.0	16.0	30.0	39.3	25.3
>50	10.0	15.0	8.0	8.0	16.7	14.3	11.1
Chi-Square test	DF = 15		χ^2 = 43.80		p-value = 0.00		
Level of Education							
None	65.0	50.0	22.0	20.0	10.0	0.0	23.7
Primary	15.0	35.0	18.0	20.0	13.3	21.4	19.7
Secondary	20.0	5.0	46.0	38.0	26.7	67.9	37.4
Diploma	0.0	5.0	4.0	6.0	23.3	7.1	7.6
Degree	0.0	5.0	10.0	16.0	26.7	3.6	11.6
Chi-Square test	DF = 20		χ^2 = 73.00		p-value = 0.00		
Household size							
1–3	5.0	5.0	34.0	26.0	23.3	14.3	21.7
4–6	20.0	10.0	52.0	48.0	20.0	50.0	38.4
7–9	50.0	55.0	14.0	22.0	43.3	32.1	30.8
≥10	25.0	30.0	0.0	4.0	13.3	3.6	9.1
Chi-Square test	DF = 15		χ^2 = 59.30		p-value = 0.00		

DF = degrees of freedom, χ^2 = Chi-square value.

2.3.2 Sorghum cropping systems

Sorghum production and the cropping systems across constituencies are summarized in Table 2.3. Sorghum farm size varied significantly (χ^2 = 49.77; p = 0.000) across constituencies. The majority of the respondent farmers (29.3%) had a farm size of 4 to 5 ha, while 25.8% had 6 to 7 ha, 22.5% had 2 to 3 ha, 18.2% with ≥8 ha, and ≤1 ha (4.5%). The highest proportion of smaller farm size (≤1 ha) were scored in Kapako (15%), Mpungu (10%), Katima Mulilo Rural (10%), and Kongola (3.6%), whereas the highest percentages of larger farm size (≥8 ha) were recorded in Kapako (35%), Katima Mulilo Rural (23.3%), Mpungu (20%), and Eenhana (16%).

Cropping systems among respondent farmers were significantly different ($\chi^2 = 29.541$; $p = 0.001$) across sorghum production regions. The majority of farmers (73.2%) intercropped sorghum with pearl millet, maize, cowpea, and groundnut to optimize output from small landholdings. A relatively low proportion of the respondent farmers practice crop rotation with cowpea (17.2%) as a strategy to utilise legumes for biological nitrogen fixation to maintain soil fertility levels. The small proportion of respondent farmers practicing sorghum mono-cropping (9.6%) across constituencies was attributable to its flood tolerance to grow in portions of fields prone to waterlogging after heavy rainfall. Kapako scored the highest proportion (100%) of intercropping with pearl millet, maize, and cowpea, followed by Mpungu (85%), Katima Mulilo Rural (73.3%), and Endola (72%) (Figure 2.2). Katima Mulilo Rural had the highest (20%) proportion of mono-cropping compared to 17.9% for Kongola and 16% for Eenhana. Endola had the highest rate (28%) of farmers practising crop rotation of sorghum with cowpea compared to Kongola (25%), Eenhana (16%), and Mpungu (15%).

The perception of respondent farmers on soil fertility status of their fields varied significantly ($\chi^2 = 21.155$; $p = 0.020$) across constituencies. The majority of respondent farmers perceived that the fertility status of their soils was medium fertile (69.7%) followed by fertile (24.7%) and infertile (5.6%) across the study areas. Kapako recorded the highest number of respondent farmers who reported poor soil fertility (20%) followed by 10% for Mpungu and 6.7% for Katima Mulilo Rural. Respondent farmers in Kongola (42.9%), Katima Mulilo Rural (30%) and Mpungu (30%) had the highest proportion of reported fertile soils.

Fertilizer use among constituencies was significantly different ($\chi^2 = 33.529$; $p = 0.000$). The majority of respondent farmers (70.2%) do not use fertilizers, compared to 29.8% who use them. Kraal manure was the most used organic fertilizer by respondent farmers, whereas NPK and urea acquired through the Government Dry Land Crop Production Program (DCPP) were widely used inorganic fertilizers (data not shown). Mpungu had the most (100%) respondent farmers without fertilizer use followed by Kapako (95%), Kongola (85.7%), and Katima Mulilo Rural (76.7%). Respondent farmers use different land preparation methods. The majority of them (86.9%) prepare land using mouldboard or disc plough pulled by a tractor or draft animal power. Land preparation using hand hoeing (6.6%) and conservation agriculture (CA) using a ripper (6.6%) were the least practised across constituencies. Conservation agriculture which involves ridge tillage and planting between raised beds to provide better drainage, soil health, and reduce erosion and runoff, is a newly introduced land preparation method in sorghum production constituencies (Figure 2.2C). Katima Mulilo Rural scored the highest proportion of land preparation by hand hoeing (20%) compared to Kapako (15%) and Mpungu (15%). Kongola had the highest proportion of land preparation with a ripper (28.6%) followed by Katima Mulilo Rural (13.3%) and Mpungu (5%).

The perception of respondent farmers on soil fertility status of their fields varied significantly ($\chi^2 = 21.155$; $p = 0.020$) across constituencies. The majority of respondent farmers perceived that the fertility status of their soils was medium fertile (69.7%) followed by fertile (24.7%) and infertile (5.6%) across the study areas. Kapako recorded the highest number of respondent farmers who reported poor soil fertility (20%) followed by 10% for Mpungu and 6.7% for Katima Mulilo Rural. Respondent farmers in Kongola (42.9%), Katima Mulilo Rural (30%) and Mpungu (30%) had the highest proportion of reported fertile soils.

Table 2.3: Sorghum production and cropping systems in six selected constituencies of northern Namibia

Variables	Kapako	Mpungu	Eenhana	Endola	Katima	Kongola	Mean
Land size (ha)							
≤1	15.0	10.0	0.0	0.0	10.0	3.6	4.5
2–3	20.0	35.0	8.0	16.0	43.3	28.6	22.2
4–5	25.0	15.0	44.0	34.0	13.3	25.0	29.3
6–7	5.0	20.0	32.0	38.0	10.0	28.6	25.8
≥8	35.0	20.0	16.0	12.0	23.3	14.3	18.2
Chi-Square test	DF = 20		$\chi^2 = 49.77$		*p*-value = 0.00		
Cropping system							
Mono-cropping	0.00	0.0	16.0	0.0	20.0	17.9	9.6
Intercropping with pearl millet, maize, cowpea and groundnut	100.0	85.0	68.0	72.0	73.3	57.1	73.2
Crop rotation with cowpea	0.00	15.0	16.0	28.0	6.7	25.0	17.2
Chi-Square test	DF = 10		$\chi^2 = 29.54$		*p*-value = 0.00		
Perception of respondent farmers on soil status of their crop lands							
Poor	20.0	10.0	2.0	4.0	6.7	0.0	5.6
Medium	55.0	60.0	80.0	80.0	63.3	57.1	69.7
Fertile	25.0	30.0	18.0	16.0	30.0	42.9	24.7
Chi-Square test	DF = 10		$\chi^2 = 21.16$		*p*-value = 0.02		
Fertilizer use							
Yes	5.0	0.0	52.0	42.0	23.3	14.3	29.8
No	95.0	100.0	48.0	58.0	76.7	85.7	70.2
Chi-Square test	DF = 5		$\chi^2 = 33.53$		*p*-value = 0.00		
Land preparation method							
Hand hoeing	15.0	15.0	0.0	0.00	20.0	3.6	6.6
Plough	85.0	80.0	100.0	100.0	66.7	67.9	86.9
Conservation agriculture	0.0	5.0	0.0	0.0	13.3	28.6	6.6
Chi-Square test	DF = 10		$\chi^2 = 54.90$		*p*-value = 0.00		

DF = degrees of freedom, χ^2 = Chi-square value. Note: conservation agriculture involves ridge tillage and planting between raised beds.

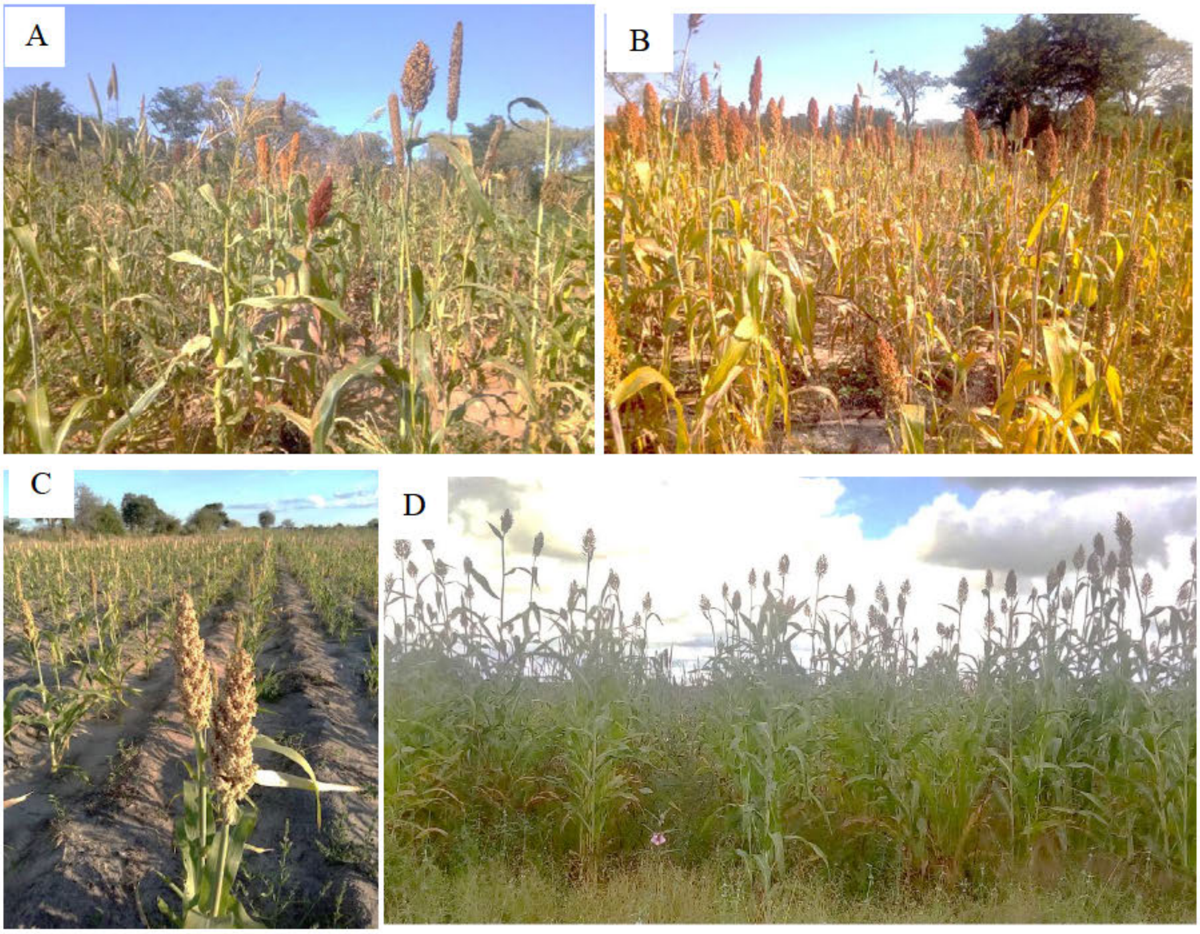

Figure 2.2: Sorghum cultivation in northern Namibia; note intercropping with pearl millet and maize (A), mono-cropping with hand broadcasting method (B), the introduced variety Macia planted in between ridges prepared using a ripper (C), and the tall traditional variety (Nakare) planted in rows in flat beds (D). (Photos: Maliata Athon Wanga. Pictures were taken from respondent farmers' fields during the transect walk in Kavango West Region).

2.3.3 Major crops grown in northern communal areas of Namibia

Pairwise ranking of the major crops grown in studied constituencies is presented in Table 2.4. In terms of total production area sorghum was ranked 4th after pearl millet, maize, and cowpea across the study area. Pearl millet was considered most important in Mpungu, Eenhana, and Endola, whereas maize was the most crucial crop in Kapako, Katima Mulilo Rural, and Kongola. Sorghum was ranked as less important in Kapako, Mpungu, and Endola than pearl miller, maize, cowpea, and groundnut. In addition, farmers listed other important food or horticultural crops such as Bambara groundnut, roselle, and cucurbits (e.g., watermelon, pumpkins and squashes) (data not shown).

Table 2.4: Pairwise ranking of major crops grown in six selected constituencies of northern communal areas of Namibia.

Crop	Kapako (N = 20)		Mpungu (N = 20)		Eenhana (N = 50)		Endola (N = 50)		Katima (N = 30)		Kongola (N = 28)		Total (N = 198)	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
Millet	1.6	2	1.3	1	1.0	1	1.0	1	2.4	2	2.1	2	1.5	1
Maize	1.4	1	1.9	2	3.2	3	2.6	2	1.1	1	1.0	1	2.1	2
Cowpea	3.3	3	3.7	3	2.7	2	3.2	3	3.9	4	4.0	4	3.4	3
Sorghum	4.5	5	4.2	5	3.9	4	4.2	5	2.8	3	2.9	3	3.7	4
Groundnut	4.3	4	4.0	4	4.2	5	3.9	4	4.8	5	5.0	5	4.3	5

2.3.4 Sorghum varieties grown in the study areas and uses

Sorghum production and use among respondent farmers in the studied constituencies is presented in Table 2.5. The type of varieties grown across the study areas were significantly different (χ^2 = 21.425; *p* = 0.000). The majority of the respondent farmers (89.9%) grew landraces such as Ekoko, Okambete, Makonga, Kamburo, Nkutji, Katoma, Fuba, Dommy, Kawumbe, and Okatombo compared to introduced varieties (10.1%) such as Macia and Red sorghum. Kongola had the highest proportion of respondent farmers growing introduced varieties (28.6%) compared to Katima Mulilo Rural (20%), Kapako (10%), and Mpungu (10%). Endola had the highest proportion of farmers growing landraces (100%) followed by Eenhana (96%), Kapako (90%), and Mpungu (90%).

Sorghum uses across constituencies varied significantly (χ^2 = 86.169; *p* = 0.000). The crop was popularly produced for multi-purposes as food for households and markets (39.9%) followed by solely for food for households (38.4%), and household food and feed (7.1%) across the constituencies. Kapako had the highest proportion of respondent farmers (85%) for growing sorghum for food compared to 70% for Mpungu, 60% for Katima Mulilo Rural, and 46.4% for Kongola. Sorghum production for food and market was higher in Endola (62%), followed by Eenhana (42%), Kongola (35.7%), and Katima Mulilo Rural (30%). Across constituencies, sorghum was least used for animal feed (2%); feed and market (2.5%); market (4%); and for food, feed, and market (6.1%). Eenhana was the only constituency that recorded the production of sorghum solely for feed (8%). Respondent farmers in Endola (62%) had the highest score for producing sorghum for feed and market, followed by Eenhana (42%), Kongola (35.7%), and Katima Mulilo Rural (30%). Eenhana had the highest number of respondent sorghum farmers growing the crop solely for the marketplace (6%) compared to 5% for Mpungu, 4% for Endola, and 3.6% for Kongola. The use of sorghum for food, feed, and market was the highest in Endola (14%), then 10.7% for Kongola and 4% for Eenhana. Household requirements of sorghum grain varied significantly (χ^2 = 48.666; *p* = 0.000) across constituencies. Majority of households (40.9%) across constituencies required 200 to 299 kg/year of sorghum grain followed by 100 to 199 kg/year (39.4%), 300 to 399 kg/year (8.6%), ≥400 kg/year (7.6%), and <99 kg/year (3.5%). Kongola had the highest proportion of households with the lowest (7.1%) sorghum grain requirement of <99 kg/year compared to Mpungu (5%), Eenhana (4%), and Katima Mulilo Rural (3.3%). The highest household sorghum grain requirement (≥400 kg) was 23.3% for Katima Mulilo Rural, 17.9% for Kongola, 5% for Kapako, and 4% for Eenhana.

Table 2.5: Sorghum varieties and uses in six selected constituencies of northern communal areas of Namibia.

Variable	Kapako	Mpungu	Eenhana	Endola	Katima	Kongola	Mean
Varieties grown							
Introduced	10.0	10.0	4.0	0.0	20.0	28.6	10.1
Landraces	90.0	90.0	96.0	100.0	80.0	71.4	89.9
Chi-Square test	DF = 5		$\chi^2 = 21.43$		p-value = 0.00		
Use type							
Food	85.0	70.0	18.0	10.0	60.0	46.4	38.4
Feed	0.0	0.0	8.0	0.0	0.0	0.0	2.0
Market	0.0	5.0	6.0	4.0	3.3	3.6	4.0
Food and feed	0.0	0.0	16.0	6.0	6.7	3.6	7.1
Food and Market	15.0	25.0	42.0	62.0	30.0	35.7	39.9
Feed and market	0.0	0.0	6.0	4.0	0.0	0.0	2.5
Food, feed and market	0.0	0.0	4.0	14.0	0.0	10.7	6.1
Chi-Square test	DF = 30		$\chi^2 = 86.17$		p-value = 0.00		
Household requirements of sorghum grain (kg)							
<99	0.0	5.0	4.0	2.0	3.3	7.1	3.5
100–199	55.0	60.0	30.0	36.0	30.0	46.4	39.4
200–299	40.0	35.0	50.0	58.0	23.3	17.9	40.9
300–399	0.0	0.0	12.0	4.0	20.0	10.7	8.6
≥400	5.0	0.0	4.0	0.0	23.3	17.9	7.6
Chi-Square test	DF = 20		$\chi^2 = 48.67$		p-value = 0.00		

DF = degrees of freedom, χ^2 = Chi-square value.

2.3.5 Constraints to sorghum production

The major constraints to sorghum production in the study constituencies is presented in Table 2.6. Respondent farmers across the study areas ranked drought and heat stress as major constraints followed by declining soil fertility, insect pest damage, high cost of production inputs, unavailability of improved seed, lack of alternative improved varieties with farmers' preferred traits, lack of organic manure, limited access to market, and limited extension service. Declining soil fertility is caused by farmers' traditional farming practices of continued cultivation without fertilizer application to replenish soil nutrients. This is due to majority of farmers not applying organic or inorganic fertilizers. Major crop pests observed during the transect walk included birds (quelea and dove birds), fall armyworms, midges, aphids, head caterpillars, stink bugs, and armoured bush crickets. Diseases observed included grain mold, anthracnose, loose smut, rust and ergot, and the parasitic red flowered *Striga* (*Striga asiatica* L.). High costs of production inputs were reported in the areas attributed to ploughing services and crop management such as weed, pest and disease control, harvesting, and post-harvest handling of the grain. Farmer-saved seed of low-yielding landraces were widely used due to the unavailability of improved seed and lack of alternative improved varieties with farmers' preferred traits. Farmers sell sorghum grain mainly in the informal markets such as open markets and trading with neighbours, and there were no formal markets and incentives to promote the production of the crop. Due to limited extension service, farmers lacked information on improved agronomic management for sorghum production and its value chain.

Table 2.6: The major constraints to sorghum production ranked by respondent farmers in six selected constituencies of northern communal areas of Namibia.

Constraints	Kapako (N = 20)		Mpungu (N = 20)		Eenhana (N = 50)		Endola (N = 50)		Katima (N = 30)		Kongola (N = 28)		Total (N = 198)	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
Drought and heat stress	1.9	2	2.2	2	1.9	3	1.5	1	1.6	2	2.0	4	1.8	1
Poor soil fertility	2.4	6	1.8	1	1.7	2	2.0	6	2.1	4	1.7	3	1.9	2
Pests (aphid, fall armyworms, birds)	3.1	8	3.0	9	2.3	7	1.5	2	1.5	1	1.5	2	2.0	3
High cost of production inputs	2.7	7	2.9	8	1.5	1	1.6	4	2.8	7	2.1	6	2.1	4
Unavailability of improved seed	1.8	1	2.6	5	1.9	4	1.5	3	3.0	8	2.4	8	2.1	5
Lack of varieties with farmers' preferred traits	2.3	4	2.2	3	2.6	9	2.4	8	2.0	3	1.4	1	2.2	6
Lack of organic manure	3.2	9	2.7	7	2.1	5	1.8	5	2.3	5	2.2	7	2.2	7
Limited access to market	2.3	5	2.7	6	2.2	6	2.0	7	3.2	9	3.3	9	2.5	8
Limited extension service	2.2	3	2.4	4	2.3	8	3.0	9	2.4	6	2.0	5	2.5	9

2.3.6 Varieties grown by farmers and preferred traits

A summary of the results of the group discussions on sorghum varieties and preferred traits across the study areas is presented in Table 2.7. The variety Macia was widely cultivated for white grain colour, short plant height, and early maturity, and the variety Red sorghum was preferred for high grain yield, medium plant height, and medium to late maturity. The seed for these varieties was mainly sourced from the ADCs of the MAWLR. Farmer-saved seed was the major source of planting material derived from landraces and the informal markets. Distinguished sorghum landraces grown for the white grain colour included Ekoko, Okambete, Saye-saye, Makonga, Mombe, Kamburo, Nkutji, and Katoma. The white grains were mainly used to prepare porridge locally referred to as pap, isima, inkoko, and oshifima; traditional pancake (oshikwiila and mungome); and to brew non-alcoholic beverages (sikundu, chilubu, oshikundu, and maheu). Traditional non-alcoholic beverages are used for feeding the sick and lactating women. Red grain colour sorghum is widely grown to prepare local beverages such as non-alcoholic and alcoholic (marovhu) drinks. Prominent landraces with red grain colour were Fuba, Dommy, Murwa, Nehutu, Kawumbe, and Mutjuma gongombe. Other landraces widely grown for high stem sugar content for chewing are Nyova, Okalya, Nswe, Nakafo, and Kamburo. Farmers identify sorghum varieties for high stem sugar by the dull green leaf midrib colour. Landrace varieties with gooseneck type panicle are generally called Kotovava, Kakumbama, Kankota, and Omusamane iteka ondaku and were grown for grain production. Landrace Esha/Eha was preferred for fresh grain roasting quality as a snack. Varieties identified for tall plant height were Oshilyalyaka, Tjwatama, Tou, Nakare, and Makonga. Tall varieties were important for animal feed and the construction of a fence around the homestead. Landrace Shorty, Tumbi, Kawumbe, and Okatombo are important for short plant height and early maturity for late planting and drought escape. Landraces such as Nakafo are known for drought tolerance, Fuba for flooding tolerance, and Siboni zuba, which have longer glumes, are important for bird damage resistance.

Table 2.7: Names of landraces and varieties grown by farmers and preferred traits in northern Namibia.

Variety	Preferred Traits	Drawbacks
Macia	Early- to medium-maturity, short plant height, white grain colour	Sensitive to moisture stress at germination, susceptible to bird
Red sorghum	Medium- to late-maturity, medium plant height, red grain colour	Susceptible to stalk borer and weevil
Ekoko, Okambete, Saye-saye, Makonga, Tumbi, Tou, Mombe, Nakare, Kamburo, Nkutji and Katoma	White grain colour for flour to prepare porridge and non-alcoholic beverages	Late-maturity, susceptible to aphid and ergot
Okatombo, Dommy, Murwa, Nehutu, Kawumbe and Mutjuma gongombe	Red grain colour to prepare local beverages non-alcoholic and alcoholic	Late-maturity, susceptible to weevil
Nyova, Okalya, Nswe and Kamburo	High stem sugar	Poor grain yield
Esha/Eha	Fresh grain roasted and eaten as a snack	Poor grain yield
Oshilyalyaka, Tjwatama, Tou, and Makonga.	Tall plant height for animal feed and construction of a fence around the homestead	Poor grain yield, lodges and late maturity
Tumbi, Kawumbe, and Okatombo	Short plant height and early maturity	Susceptible to bird
Nakafo	Drought tolerance and stem sugar	Poor grain yield
Fuba	Flooding tolerance	Late-maturity and poor grain yield
Siboni zuba	Non preferred by birds	Poor grain yield and difficult to thresh

2.4 Discussion

Incorporating farmer-preferred traits is key to the adoption of new generation varieties in the traditional sorghum growing areas. This study focused on an assessment of the present state of sorghum production to understand farmers' perceived production constraints and trait preferences in new varieties as a guide for current and future breeding. This is the first study for documenting farmer' perceptions on sorghum production and trait preferences in northern communal areas of Namibia using a PRA.

Results revealed clear trends in sorghum production amongst the sampled respondent farmers disaggregated by gender, age, number of households, education level, cropping systems, types of varieties grown, and perceived production constraints. This suggest that the adoption of improved sorghum varieties in the study area depends on new varieties that possess farmers' trait preferences. The data assembled in this study are useful to select market-preferred traits to incorporate in breeding drought-tolerant varieties for the dry conditions and serve the diverse value chains in Namibia.

2.4.1 Socio-economic status

The socio-economic status of smallholder farmers strongly influences the adoption of new varieties (Martey et al., 2020; Mugumaarhahama et al., 2021; Attipoe et al., 2021). In the present study, the sampled respondent farmers were male (50%) and female (50%) across constituencies, indicating equal economic value of sorghum production to men and women in northern communal areas of Namibia (Table 2.2). The higher proportion of female respondent farmers (75%) in Mpungu constituency suggested the important role women had in sorghum production. Hence, a high percentage of men were involved in none crop production activities in urban areas, mining, and fishing industries. This proportion was higher than 53.5% of females reported in the Agriculture Communal Sector Census in the Kavango West region (NSA, 2015). A higher proportion of male respondent farmers (>60%) in Katima Mulilo Rural, Kongola and Eenhana areas could be attributed to the land tenure system and tradition in Namibia where men are the majority of the landowner and household leaders. The male proportion in the present study corroborates with the Namibia Census of Agriculture 2013/2014 for Ohangwena (54.8%) and Zambezi (51.9%) (NSA, 2015). Therefore, gender variations in the present study indicates the necessity of awareness of traits preference by both genders to integrate into new sorghum varieties.

Younger farmers can be targeted for being flexible and ready in risk-taking to introduce improved varieties for adoption (Adenegan et al., 2013). In the present study, the proportion of respondent farmers in productive age groups of <40 years old (63.6%) is higher than 50.9% reported in the previous study (NSA, 2015), indicating it is relatively easy to introduce improved sorghum varieties in the northern communal area of Namibia (Table 2.2). Formal education is essential for farmers to search for information on new varieties compared to non-formal or uneducated farmers (Gebretsadik et al., 2014; Mrema et al., 2017; (Mengistu et al., 2019). In the present study, the majority of the respondent farmers (56.6%) had attained secondary education. This indicates that farmers in the study area can understand the need to cultivate improved varieties. The proportion of farmers who attained secondary education in the present study is higher than 33% reported in northern Namibia (NSA, 2015). The household size is essential for labour and farm activity distribution in smallholder agriculture (Bryceson, 2019; Pierotti et al., 2022; Tsuda, 2022). In the present study, a higher proportion of respondent farmers scored 4–6 (38.4%) and 7–9 (30.8%) persons per household, indicating limited availability of labour for crop production and other agricultural activities. In the study areas, smallholder farmers practiced mixed farming involving animal husbandry and crop production, which requires labour distribution (Mendelsohn et al., 2006). Therefore, sorghum varieties that can be easily handled using mechanization can be recommended to reduce labour requirements in sorghum production.

2.4.2 Sorghum production and cropping systems

The majority of sorghum producers in northern communal areas of Namibia are smallholder farmers with a farm size of <6 ha (Hillyer et al., 2006; Newsham and Thomas, 2011; Horn et al., 2015). In the present study, farmers with farm sizes of ≤5 ha (56%) agree with the ideal land size that smallholder farmers can manage to produce food and cash income for the households. Newsham and Thomas (2011), and Iijima et al. (2018) reported that sorghum was relatively tolerant to waterlogging stress, which farmers planted near seasonal wetland (ndombe) prone to water-logging after heavy rainfall. In the present study, the majority of respondent farmers (73.2%) intercropped sorghum with other crops such as pearl millet, maize, cowpea, and groundnut, agreeing with Hillyer et al. (2006) and Horn et al. (2015). Thus, new varieties for intercropping compatibility require novel traits to compete with the companion crops. Intercropping is vital for the optimization of output from small landholdings, maintaining soil fertility through legume crops' biological nitrogen fixation and providing food diversity and nutrition security at the household level. Further, legume crops such as cowpea, groundnut, and Bambara groundnut are essential companion crops for biological nitrogen fixation (Ghosh et al., 2009; Traoré et al., 2022). In the present study, a small proportion of respondent farmers with the perception that their soil fertility was medium fertile (69.7%) and fertile (24.7%) could be the reason for the majority of farmers (70.2%) not applying fertilizers. The present study agrees with spatial fertilizer use advocated for developing countries (Bouwman et al., 2017) due to availability, high cost, lack of awareness, and incentives on production input for smallholder farmers in sub-Saharan Africa (Holden, 2018; Cedrez et al., 2020). The current status of respondent farmers applying fertilizer reveals the need for varieties that can grow in low fertile soils to improve productivity. Further, the land preparation method is vital for sustainable land use and management of the crop. The most used conventional land preparation methods were mouldboard plough and disc harrow, and mono-cropping revealed the need to develop varieties with high root biomass for soil carbon sequestration, higher yield, and drought tolerant (Datta et al., 2018).

Sorghum is a relatively drought tolerant and low water requirement crop (450–650 mm) (Hadebe et al., 2017) compared to 500–800 mm for maize (Hadebe et al., 2016; Parra et al., 2022). In the present study, sorghum was ranked the fourth most important crop in production area after pearl millet, maize and cowpea. Hence, the crop's economic potential was not realized in drought-prone areas of Namibia. Thus, farmers risked crop failure by growing maize in drought-prone areas due to its well-established market system in the country. Therefore, policies and regulations to establish the market are required to increase sorghum production. In the present study, 89.9% of farmers cultivated low-yielding landrace varieties, indicating the need to develop farmers' preferred varieties combining high grain yield with abiotic and biotic tolerant traits (Reddy et al., 2004; Shimelis et al., 2019). The use of landraces was aggravated by limited seed availability due to the country's lack of seed and variety regulation system. Therefore, there is a need for the implementation of the Seeds and Seeds Varieties, and Plant Breeders' Rights Acts to promote seed dealers and new variety registration in Namibia. Sorghum grain was mainly produced for household consumption and the surplus (78.3%) was sold for cash income. Moreover, the need of sorghum grain per household <300 kg/year (83.8%) suggested that the crop is consumed in a small quantity. This revealed the need to promote sorghum beyond household consumption such as exploiting value addition for various food products such as bread, biscuit, and grain flakes (Alavi et al., 2019; Taylor and Kruger, 2019); popped sorghum as snacks (Waniska et al., 2016; Sharanagat et al., 2019) and animal feed and the brewing industry.

2.4.3 Constraints to sorghum production

There is a high yield gap of sorghum in Namibia and elsewhere in Africa. This is mainly attributed to abiotic and biotic stresses in the country. Farmers' perceived sorghum production constraints identified elsewhere in Africa included drought, lack of seed for improved varieties, storage pests and field pests, poor soil fertility, lack of market, heavy rainfall, *Striga* infestation, bird damage, high cost of production inputs (e.g., fertilizers, insecticides, fungicides and herbicides), anthracnose and smut diseases, and poor agronomic knowledge (Mrema et al., 2017; Mengistu et al., 2019; Yahaya et al., 2022; Okot et al., 2022). In agreement with these reports, respondent farmers' perceived sorghum production constraints in the present study included recurrent drought, declining soil fertility, insect pest damage, high cost of production inputs, unavailability of improved seed, lack of alternative improved varieties with farmers' preferred traits, lack of organic manure, limited access to market, and limited extension service. Drought is rampant in Namibia, attributed to the country's arid to semi-arid climate conditions and low and erratic rainfall (Mendelsohn et al., 2006; Awala et al., 2019). Declining soil fertility was mainly due to a low input farming system practised in small-scale and inaccessibility to organic manure and chemical fertilizers due to the high cost (Hillyer et al., 2006; Newsham and Thomas, 2011; Horn et al., 2015). The unavailability of improved seed, lack of alternative improved varieties with farmers' preferred traits limited access to market, and limited extension service were attributed to lack of established value chains for the crop in the country. Thus, there is a need for further studies to guide the production and breeding of sorghum for the market in the country. Therefore, a demand-led breeding and product development approach is recommended to assist with establishing sorghum value chains in Namibia.

2.4.4 Varieties grown by farmers and suggested traits

Landraces are well-adapted to low-input farming systems and possess essential quality traits for food and beverages (Misihairabgwi and Cheikhyoussef, 2017; Orr et al., 2020). In the present study, most of the landraces were described as late maturity and low grain yielders. Grain yield was the main trait of interest, and grain colour was of secondary importance depending on the household consumption and market. The majority of farmers cultivated both red and white grain colour sorghum in the same field. The landrace varieties were not evaluated for cultivation and further breeding. Therefore, there is a need to evaluate and characterize landraces to select useful breeding populations for different trait-based breeding programs, including grain yield, fodder, and dual-purpose cultivars. Due to the intensity and recurrence of drought and heat stress, drought tolerance was identified as the main trait to incorporate in new sorghum varieties for the northern agro-ecologies of Namibia. The respondent farmers expressed willingness to adopt new drought tolerant sorghum variety with high grain yield and early maturity, whereas field and storage insect pests are of secondary importance. Sorghum variety with early-maturity and short plant height are important for drought escape and lodging resistance (Abreha et al., 2021; Gano et al., 2021; Raymundo et al., 2021). Therefore, this study reveals the need for breeding and deploying new sorghum varieties with a high grain yield, early maturity, and tolerance to drought, field, and storage insect pests to increase the production and productivity of the crop in Namibia.

2.5 Conclusions

Developing and deploying sorghum varieties to serve the diverse needs of farmers and value chains is vital for adopting new climate-smart cultivars. Participatory Rural Appraisal (PRA) was carried out in six constituencies to assess the present state of sorghum production in northern Namibia, and document farmers' perceived production constraints and trait preferences in new varieties to guide drought-tolerance breeding in Namibia. Results revealed variable trends in sorghum production changes among respondent farmers. An equal proportion of male and female respondent farmers cultivate sorghum, suggesting the crop's value to both genders in northern Namibia. Sorghum was mainly intercropped with pearl millet, maize, cowpea, and groundnut to optimize output from small landholdings and maintaining soil fertility. The majority of the respondent farmers grew landrace varieties, namely Ekoko, Okambete, Makonga, Kamburo, Nkutji, Katoma, Fuba, Dommy, Kawumbe, and Okatombo without fertilizers. Recurrent drought was identified as a major production constraint, followed by declining soil fertility, insect pest damage, high cost of production inputs, unavailability of improved seed, lack of alternative improved varieties with farmers' preferred traits, lack of organic manure, limited access to market, and limited extension service. The study recommends breeding and deployment of improved seed of sorghum varieties with farmers' preferred traits, including the high grain yield; early maturity; and tolerance to drought, field, and storage insect pests to increase the production and productivity of the crop in Namibia.

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Chapter 3. The Effect of Single and Combined Use of Gamma Radiation and Ethyl Methanesulfonate on Early Growth Parameters in Sorghum

Abstract

Success in inducing genetic variation through mutagenic agents is dependent on the source and dose of application. The objective of this study was to determine the optimum doses of a single and combined use of gamma radiation and ethyl methanesulfonate (EMS) for effective mutation breeding in sorghum. The study involved two concurrent experiments as follows: in experiment I, the seeds of four sorghum genotypes (Parbhani Moti, Parbhani Shakti, ICSV 15013, and Macia) were treated using gamma radiation (0, 300, 400, 500 and 600 Gy), EMS (0, 0.5 and 1.0%), and gamma radiation followed by EMS (0 and 300 Gy and 0.1% EMS; 400 Gy and 0.05% EMS). In experiment II, the seeds of two genotypes (Macia and Red sorghum) were treated with seven doses of gamma radiation only (0, 100, 200, 300, 400, 500 and 600 Gy). Overall, the combined applied doses of gamma radiation and EMS are not recommended due to poor seedling emergence and seedling survival rate below LD_{50}. The best dosage of gamma radiation for genotypes Red sorghum, Parbhani Moti, Macia, ICSV 15013 and Parbhani Shakti ranged between 392 and 419 Gy, 311 and 354 Gy, 256 and 355 Gy, 273 and 304 Gy, and 266 and 297 Gy, respectively. The EMS optimum dosage ranges for genotypes Parbhani Shakti, ICSV 15013, Parbhani Moti and Macia were between 0.41% and 0.60%, 0.48% and 0.58%, 0.46% and 0.51%, and 0.36% and 0.45%, respectively. The above dose rates are useful to induce genetic variation in the tested sorghum genotypes for greater mutation events in sorghum breeding programs.

Keywords: ethyl methanesulfonate; gamma radiation; sorghum

3.1 Introduction

Use of climate-smart crop cultivars is a key mitigation strategy against the unpredictable impacts of climate change associated with threats such as drought and heat stress, flooding, soil erosion, and salinization (Lobell, 2014). Sorghum (*Sorghum bicolor* [L.] Moench) is one of the key crops adapted to grow under arid and semiarid and harsh conditions where other crops fail to survive under similar environmental conditions. This makes sorghum a crop of choice in sub-Saharan Africa (SSA), Asia and similar agro-ecologies. However, yields in sorghum are low in SSA, with a mean grain yield level of less than 1 t/ha far below the potential grain yield of the crop, which reaches up to more than 4 t/ha (FAOSTAT, 2022). Therefore, plant breeders need to broaden the genetic variation of sorghum to develop high-performing cultivars with enhanced grain and biomass yields in SSA.

Natural genetic variation is created via spontaneous mutation. However, the frequency of natural mutation is very low, varying from 10^{-5} to 10^{-8} per loci in higher plants (Jiang and Ramachandran, 2010). Induced genetic variation using physical and chemical mutagens is an alternative method of creating the new genetic variation needed for the breeding of climate-resilient crop cultivars. Different mutagenic agents have a variable effect on mutations. For example, physical mutagens such as gamma irradiation result in deletion, translocation and aberrations of chromosomes (Shu et al., 2012). Meanwhile, chemical mutagens such as ethyl methanesulfonate (EMS) result in modification of certain nucleotides (Kim et al., 2006; Sikder et al., 2013). The use of artificial mutagenesis emulates the natural process of spontaneous mutation by maintaining the genetic status quo and without introducing alien genes, unlike in genetically modified organisms (Gruère and Rosegrant, 2008; Gruère and Sengupta, 2009; Davison and Ammann, 2017). Induced mutation increases the proportion of genetic variation multi-fold (1000 to a million times) (Parry et al., 2009; Mba et al., 2010; Wolabu and Tadege, 2016). Artificial mutagenesis coupled with innovative breeding approaches such as speed breeding techniques and use of single seed descent selection method can deliver desired cultivars with a relatively shorter breeding cycle (Jankowicz-Cieslak et al., 2016; Chiurugwi et al., 2018; Watson et al., 2018).

Success in inducing genetic variation using mutagenic agents is dependent on the source and dose of application (Mani, 1989; Suthakar et al., 2014; Deshmukh et al., 2018). Gamma radiation and EMS are widely used mutagenic agents to create genetic variation in plant breeding programs (Kodym and Afza, 2003; Acquaah, 2012; Oladosu et al., 2016). The use of gamma radiation and EMS in sorghum breeding programs has been reported by several researchers. For example, in Nigeria, eight sorghum mutant lines were developed by irradiating seed at a dose rate of 300 Gy (Kenga et al., 2006a). In Mali, genetic variation was achieved for agronomic traits including plant height, drought tolerance, grain quality and maturity period when sorghum seeds were irradiated with a range of 200 and 300 Gy (Bretaudeau, 1997). In Japan, sorghum seed irradiated at 400 Gy produced brown midrib (*bmr*) lines, a phenotype associated with low lignin content and increased digestibility (Mizuno et al., 2013). In the USA, nuclear male sterility, a trait important for hybridization, was generated when seeds were treated with EMS doses ranging between 0.1 and 0.3% (Xin et al., 2017). Bloomless (*bm*) mutant lines, linked with resistance to the greenbug pest and sheath blight disease in sorghum, were produced when the seed was treated with EMS doses ranging between 0.1 and 0.6% (Xin et al., 2008; Jiao et al., 2018). From the foregoing, it can be concluded that variable ranges of gamma radiation and EMS doses were used in different crop genotypes. Hence, there is a need to select optimum doses for each mutagenic source for specific sorghum genotypes to achieve a higher proportion of mutation events and genetic variation.

The mutant variety database (MVD) holds records of 3,320 varieties officially released across 73 countries and 228 plant species by different nations of the world (MVD, 2022). Single treatments of plant materials with gamma radiation and EMS are the most studied mutagenic agents. Gamma radiation is the most preferred mutagenic agent because of its relatively higher degree of plant tissue penetration, reproducibility and greater mutation frequency (Gruère and Rosegrant, 2008; MVD, 2022). It was reported that gamma radiation accounted for 1,666 of mutant plant varieties on the MVD (2022). In Indonesia, Human et al. (2011) developed three genetically stable high-yielding varieties through gamma irradiation. EMS is the most preferred chemical mutagenic agent and requires relatively simple equipment and facilities (Kodym and Afza, 2003; Acquaah, 2012). EMS was used to generate 107 mutant plant varieties in the MVD. This demonstrates the significance of mutation breeding as an important tool to enhance the genetic diversity of plant species.

The combined use of physical and chemical mutagens has been used to develop 37 of the mutant plant varieties in the MVD. However, there is a limited record of sorghum mutant varieties developed by combined treatment of gamma radiation and EMS in the MVD. Reports reveal that combined use of gamma radiation and EMS might yield higher mutation frequency than using a single mutagenic agent (Sree-Ramulu, 1971a; Reddy and Smith, 1981). Thus, the optimum dose of single or combined use of gamma radiation and EMS needs to be assessed and established for large-scale mutagenesis (Sree-Ramulu, 1971b; Mani, 1989). Higher doses of gamma radiation and EMS lead to undesirably low seed germination; poor seedling emergence, survival, growth and development; and reduced flowering, seed set, and seed viability (Omar et al., 2008; Deshmukh et al., 2018; MVD, 2022). A dose of a mutagenic treatment which results in a mean lethal dose of 50% (LD_{50}) and a mean growth reduction of 50% (GR_{50}) is suggested to provide a higher chance of producing effective mutations and mutagenic events for targeted selection (Gruère and Rosegrant, 2008; Mudibu et al., 2012). Therefore, the objective of this study was to determine the effect of a single or combined use of gamma radiation and ethyl methanesulfonate (EMS) for effective mutagenesis and breeding in sorghum.

3.2 Materials and methods

3.2.1 Study site and plant material

The study involved two concurrent experiments carried out under field tests at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, (17°30′32.9″ N 78°16′50.8″ E) and Mannheim Crop Research Station, Tsumeb, Namibia (19°10′06.7″ S 17°45′54.1″ E). The study used seeds of five sorghum genotypes, viz., Parbhani Moti (SPV 1411), Parbhani Shakti (ICSR 14001), ICSV 15013, Macia (SDS 3220) and Red sorghum. The genotypes were selected for their unique traits; e.g., Macia is one of the popular cultivars in the Southern African region. It is a high-yield semi-dwarf and early-maturity cultivar (ICRISAT, 2000). Meanwhile, the genotype Red sorghum is a late-maturity cultivar widely cultivated in Namibia (Wanga et al., 2018), and the genotypes Parbhani Shakti, Parbhani Moti and ICSV15013 have unique traits including high contents of grain Fe and Zn and are currently being tested for wider adoption and drought tolerance.

3.2.2 Seed treatment, planting and experimental design

3.2.2.1 Experiment I

This study examined the effect of a single or combined use of gamma radiation and EMS. Four sorghum genotypes (Parbhani Moti, Parbhani Shakti, ICSV 15013, and Macia) were subjected to mutagenesis using five doses of gamma radiation (0, 300, 400, 500 and 600 Gy), three EMS doses (0, 0.5 and 1.0%) and subsequent use of gamma radiation followed by EMS (0 and 300 Gy and 0.1% EMS; 400 Gy and 0.05% EMS). Batches of 1000 dry seeds per treatment were irradiated at Bhabha Atomic Research Centre, Nuclear Agriculture and Biotechnology, Mumbai, India. The minimum gamma radiation dose (300 Gy) was selected following favorable responses as previously reported by Bretaudeau (1997), Human et al. (2012) and Mizuno et al. (2013). EMS dose rates were selected following the germination rate and seedling responses reported by Mani (1989), Xin et al. (2008) and Jiao et al. (2016). Prior to EMS treatment, 100 seeds per treatment were placed in mesh bags replicated five times for pre-soaking under running tap water for six hours at room temperatures, according to the procedure described by Mba et al. (2007) and Ndou et al. (2012). Seeds were removed to dry off excess water and placed in respective prepared EMS solutions for five hours. The seed was rinsed under running tap water for three hours. Treated seeds were sown in 10-m-long single-row plots using a randomized complete block design (RCBD), with five replications in the experimental field of ICRISAT, Patancheru, India. Inter-row spacing of 75 cm and intra-row spacing of 10 cm was used for planting. To ensure adequate soil moisture, supplemental irrigation was provided. Compound synthetic fertilizer as a source of nitrogen (N), phosphorus (P) and potassium (K) was used at the rate of 30 kg N ha^{-1}, 45 kg P_2O_5 ha^{-1} and 30 kg K_2O ha^{-1}, in that order. The fertilizer was homogeneously broadcasted to the plots before planting.

3.2.2.2 Experiment II

This experiment examined the effect of gamma radiation from germination to maturity. The experiment involved two genotypes ('Macia' and 'Red sorghum'), which were treated with seven doses of gamma radiation (0, 100, 200, 300, 400, 500 and 600 Gy). The doses of 100 and 200 Gy were included following the result of Experiment I. Batches of 1000 dry seeds per treatment were irradiated at the Joint FAO/IAEA laboratories in Seibersdorf, Austria. Gamma-irradiated seeds were sown in 10-m-long single-row plots laid in an RCBD with three replications in the field at Mannheim Crop Research Station, Namibia. All standard agronomic practices were followed as described above. Urea (46% N) was applied at the rate of 30 kg N ha^{-1} after a 50% emergence.

3.2.3 Data collection

The following traits were assessed following the methods described by Mba et al. (2010), Horn and Shimelis (2013) and Spencer-Lopes et al. (2018). Germination percentage was recorded by counting sprouted seeds per treatment after 3 days using the moist paper towel germination method under room temperature as described by Sako et al. (2001). Emergence percentage was recorded by counting the number of seedlings raised above ground from sowing to seven days after sowing. Seedling survival percentage was recorded by counting the number of surviving seedlings at 21 days after 50% emergence. The shoot length was recorded from measuring five randomly selected plants per plot from the base to the tip of the top leaf, and the average was expressed in cm 21 days after 50% emergence. The total number of panicles and productive panicles per plot was counted at harvest and expressed in the number of panicles m^{-2}. Panicle length was recorded from five randomly selected panicles per plot by measuring from the base to the tip of panicles and expressed in cm. The plant height was measured from five randomly selected plants from the base of the plant to the tip of the panicle and expressed in cm. Seed viability was recorded using a germination test as described above.

3.2.4 Data analysis

Data on germination percentage, emergence percentage, seedling survival percentage, shoot length, number of panicles m^{-2}, number of productive panicles m^{-2}, panicle length, plant height and seed viability were subjected to analysis of variance (ANOVA) using Genstat (18th edition) statistical software (VSN International, 2015). Mean comparisons were conducted using Fisher's least significant difference procedure when significant differences were detected in the ANOVA. The mean lethal dose (LD_{50}) and mean growth reduction (GR_{50}) were estimated through the simple linear regression model by fitting the straight-line equation $y = mx + c$; where y is the response variable, x is the independent variable, m and c represent the slope and constant, respectively.

3.3 Results

3.3.1 Experiment I

3.3.1.1 Effect of single doses of gamma radiation

Highly significant interaction effects ($p < 0.01$) were recorded between genotype and gamma radiation dosage on seedling emergence percentage and survival percentage (Table 3.1). This suggests that the optimum dose of gamma radiation in sorghum mutation breeding is significantly influenced by the genotype. Table 3.2 summarizes the mean of seedling emergence percentage, survival rate and shoot length. Results showed a significant reduction in assessed traits with increased doses of gamma radiation when compared with their respective controls (Table 3.2). Genotype Macia showed the lowest seedling emergence value of 2.4% at 600 Gy, showing non-significant differences when compared with genotypes ICSV 15013, Parbhani Shakti and Parbhani Moti with values of 5.2%, 4.6% and 4.4%, respectively. Genotype Macia could not survive doses ≥ 500 Gy, whereas genotypes Parbhani Moti, ICSV 15013 and Parbhani Shakti had relatively better survival rates at 600 Gy, 5.8%, 5.3% and 2.2%, respectively. The shoot length was significantly decreased by doses of ≥400 Gy in all genotypes when compared to controls (Table 3.2). The optimum radiation doses aiming at

LD_{50} for seedling emergence for genotypes Parbhani Moti, ICSV 15013, Parbhani Shakti and Macia were 311, 304, 297, and 278 Gy, respectively (Figure 3.1). The LD_{50} for seedling survival rate for genotypes Parbhani Moti, ICSV15013, Parbhani Shakti and Macia were 354, 273, 266 and 256 Gy, respectively. The optimum radiation dose aiming for GR_{50} for shoot length for genotypes ICSV15013, Parbhani Shakti, Macia and Parbhani Moti were 521, 510, 479 and 436 Gy, respectively (Figure 3.1).

Table 3.1. Mean-square values and significance tests for emergence percentage (%E), seedling survival percentage (%SS) and shoot length (SLT) of four sorghum genotypes evaluated with variable doses of gamma radiation, EMS concentrations and combined treatments in Experiment I.

Mutagenic Treatments	Source of Variation	df	%E	%SS	SLT (cm)
Gamma radiation	Replication	4	94.6	57.7	30.0
	Genotype (G)	3	126.7***	268.2***	110.5***
	Dose (D)	4	9415.7***	6512.8***	1460.6***
	Genotype x dose	12	202.7***	326.9***	40.8
	Error	76	16.4	22.8	35.3
EMS	Replication	4	62.9	62.4	38.1
	Genotype (G)	3	339***	275.7***	173.2***
	Dose (D)	2	11,831.3***	9367.7***	1391.4***
	Genotype x dose	6	148.8***	460.2***	26.3
	Error	44	22.8	23.5	35.5
Gamma radiation followed by EMS	Replication	4	49.7	53.5	45.7
	Genotype (G)	3	185.4***	431.7***	58.2
	Dose (D)	2	15,731.7***	11,334.6***	1805.3***
	Genotype x dose	6	168.9***	379.4***	30.2
	Error	44	11.2	19.3	25.0

*** denotes significant differences at $p \leq 0.001$; df = degree of freedom.

Table 3.2. Means of emergence percentage (%E), seedling survival percentage (%SS) and shoot length (SLT) among four sorghum genotypes tested using four gamma radiation, two EMS and two subsequent treatments of gamma and EMS in experiment I.

Mutagen	Dose	Variety	%E		%SS		SLT (cm)	
Gamma radiation	300 Gy	Parbhani Moti	27.40	±3.63	22.20	±3.76	30.70	±2.26
		Parbhani Shakti	31.00	±1.76	24.20	±1.69	27.42	±3.18
		ICSV 15013	23.00	±1.79	14.00	±2.17	27.16	±5.31
		Macia	40.80	±1.66	32.20	±1.83	23.06	±2.48
	400 Gy	Parbhani Moti	16.80	±5.24	14.20	±4.65	26.26	±3.79
		Parbhani Shakti	19.20	±0.58	10.60	±1.60	20.92	±2.88
		ICSV 15013	13.60	±1.47	8.60	±1.44	19.34	±2.99
		Macia	3.80	±1.02	0.80	±0.49	16.08	±1.60
	500 Gy	Parbhani Moti	9.80	±2.35	2.00	±0.32	13.94	±1.92
		Parbhani Shakti	6.00	±0.95	1.60	±0.40	12.93	±1.69
		ICSV 15013	7.60	±1.33	1.80	±0.86	16.50	±2.64
		Macia	2.60	±1.12	0.00	±0.00	-	
	600 Gy	Parbhani Moti	4.40	±0.81	1.60	±0.24	5.50	±1.35
		Parbhani Shakti	4.60	±1.94	1.20	±0.49	10.53	±1.53
		ICSV 15013	5.20	±0.58	1.80	±0.58	11.00	±1.22
		Macia	2.40	±0.81	0.00	±0.00	-	
EMS	0.50%	Parbhani Moti	15.60	±3.01	11.20	±2.03	21.96	±1.33
		Parbhani Shakti	25.80	±2.42	7.80	±2.18	17.26	±2.94
		ICSV 15013	21.00	±0.71	10.60	±1.17	22.82	±4.51
		Macia	16.00	±0.71	4.20	±0.49	15.60	±1.88
	1%	Parbhani Moti	4.60	±0.87	3.00	±0.32	18.46	±3.86
		Parbhani Shakti	17.60	±4.62	6.40	±2.18	15.67	±1.05
		ICSV 15013	11.00	±0.63	4.80	±1.07	20.40	±3.15
		Macia	8.00	±3.35	2.40	±1.69	9.80	±0.60
Gamma radiation followed by EMS	300 Gy + 0.1% EMS	Parbhani Moti	8.80	±1.36	4.40	±0.68	20.00	±1.74
		Parbhani Shakti	17.00	±2.00	9.00	±2.49	19.64	±2.04
		ICSV 15013	6.60	±2.82	1.20	±0.73	17.90	±4.67
		Macia	12.80	±0.86	2.20	±0.37	13.56	±1.88
	400 Gy + 0.05% EMS	Parbhani Moti	8.40	±1.72	1.40	±0.51	10.28	±2.64
		Parbhani Shakti	3.80	±0.66	0.60	±0.40	12.43	±1.97
		ICSV 15013	6.00	±1.82	1.00	±0.77	12.83	±3.61
		Macia	1.00	±0.45	0.00	±0.00	-	
Control	0	Parbhani Moti	52.40	±1.12	27.60	±4.80	37.68	±1.54
		Parbhani Shakti	61.60	±1.36	55.60	±2.94	30.40	±3.15
		ICSV 15013	46.20	±2.58	34.00	±2.76	31.22	±1.85
		Macia	65.00	±1.82	57.20	±2.35	30.20	±2.63
Grand Mean			17.4		10.6		19.4	
LSD (5%)			5.3		4.9		2.7	
CV (%)			24.3		37.3		29.9	
R^2 (%)			83.6		77.5		53.8	

± denotes standard error.

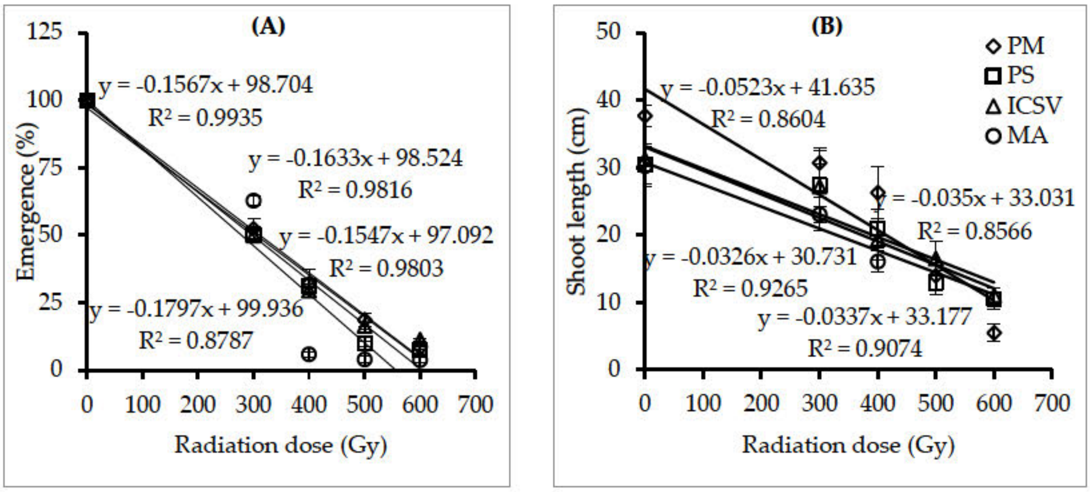

Figure 3.1: Emergence percentage (A) and shoot length (B) and fitted straight lines of four sorghum genotypes treated with five gamma radiation doses in experiment I (bars represent standard error). Note: PM = Parbhani Moti, PS = Parbhani Shakti, ICSV = ICSV 15013 and MA = Macia.

3.3.1.2 Effect of single doses of EMS

Significant interactions ($p < 0.01$) were observed between genotype and EMS concentrations on seedling emergence percentage and survival rate (Table 3.1). This suggests that the optimum EMS dose in sorghum mutation breeding is significantly influenced by the genotype. The means of seedling emergence percentage, survival percentage and shoot length showed a significant reduction with increased EMS concentration (Table 3.2). The lowest seedling emergence value, 4.6%, was recorded in genotype Parbhani Moti at 1% EMS, showing significant differences when compared with the 17.6% and 11.0% recorded in genotypes Parbhani Shakti and ICSV 15013, respectively. The lowest seedling survival rate value, 2.4%, was recorded in genotype Macia at 1% EMS, showing non-significant differences when compared with genotypes Parbhani Shakti, ICSV 15013 and Parbhani Moti with values of 6.4%, 4.8% and 3.0%, respectively. The LD_{50}s for seedling emergence for genotypes Parbhani Shakti, ICSV15013, Parbhani Moti and Macia were 0.60%, 0.58%, 0.46% and 0.45%, respectively (Figure 3.2). The LD_{50}s for seedling survival rate for genotypes Parbhani Moti, ICSV 15013, Parbhani Shakti, and Macia were 0.51%, 0.48%, 0.41% and 0.36% EMS, respectively. The GR_{50}s for shoot length for genotype ICSV 15013, Parbhani Shakti, Parbhani Moti, and Macia were 1.35%, 0.90%, 0.87% and 0.67% EMS, respectively (Figure 3.2).

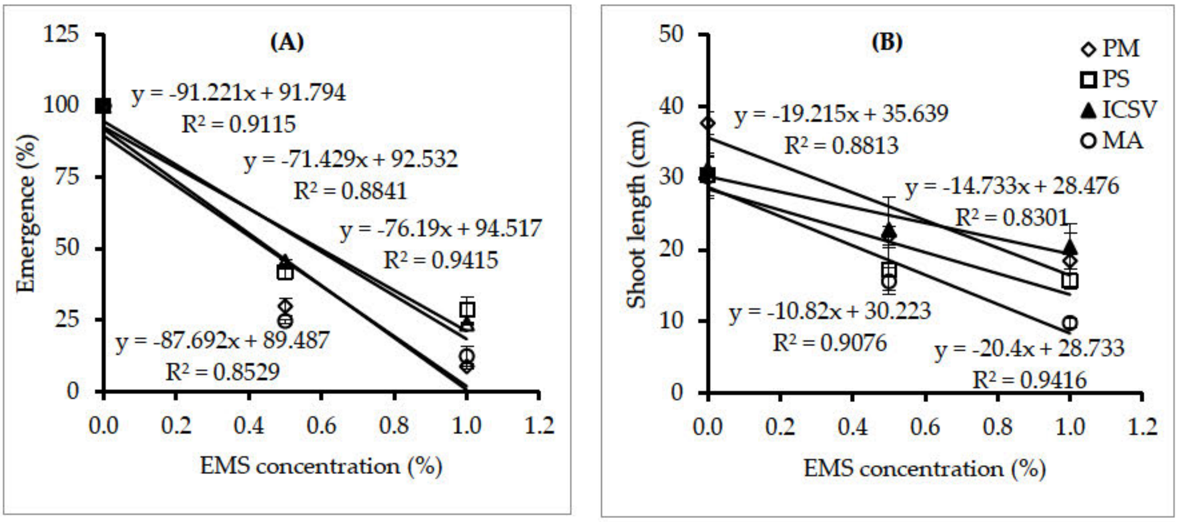

Figure 3.2: Emergence percentage (A) and shoot length (B) and fitted straight lines of four sorghum genotypes treated with three EMS concentrations in experiment I (bars represent standard error). Note: PM = Parbhani Moti, PS = Parbhani Shakti, ICSV = ICSV 15013 and MA = Macia

3.3.1.3 Effect of combined doses of gamma radiation and EMS

Highly significant interactions ($p < 0.01$) were observed between genotype and combined use of gamma radiation doses and EMS concentrations on seedling emergence percentage and survival percentage (Table 3.1). The mean of seedling emergence percentage, survival percentage and shoot length showed a significant reduction with increased combined dosage (Table 3.2). Seedling emergence percentages of genotypes Parbhani Moti, Macia, Parbhani Shakti and ICSV 15013 at dose rates of 300 Gy and 0.1% EMS were 27.6%, 19.7%, 16.8% and 14.3%, respectively (Figure 3.3). The lowest seedling emergence value, 1%, was recorded in genotype Macia at dose rates of 400 Gy and 0.05% EMS (Table 3.2). Genotype Macia could not survive dose rates of 400 Gy and 0.05% EMS, compared with Parbhani Moti, ICSV 15013 and Parbhani Shakti, which survived with 1.4%, 1.0% and 0.6%, respectively. Genotype Parbhani Moti showed the lowest mean shoot length value of 10.3 cm, recorded at dose rates of 400 Gy and 0.05% EMS, showing non-significant differences when compared with genotypes ICSV 15013 and Parbhani Shakti, with 12.8 and 12.4 cm, respectively. The growth reduction of shoot length for genotypes Parbhani Shakti, ICSV 15013, Parbhani Moti and Macia at the dose rate of 300 Gy and 0.1% EMS were 64.6%, 57.3%, 53.1% and 44.9% of control, respectively (Figure 3.3).

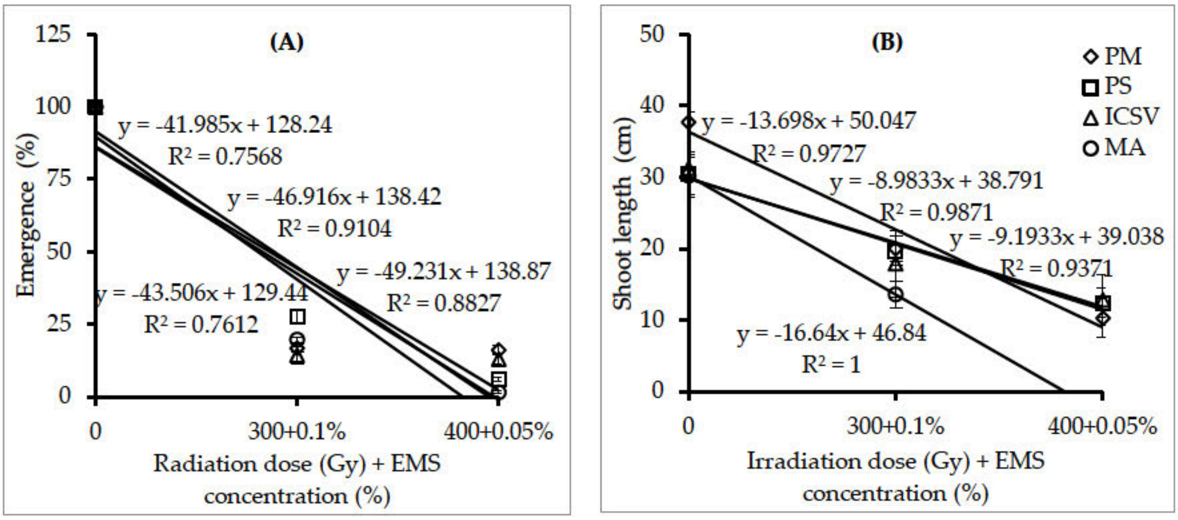

Figure 3.3: Emergence percentage (A) and shoot length (B) and fitted straight lines of four sorghum genotypes treated with three sequential combined treatments of gamma followed by EMS in experiment I (bars represent standard error). Note: PM = Parbhani Moti, PS = Parbhani Shakti, ICSV = ICSV 15013 and MA = Macia.

3.3.2 Experiment II

Significant interactions ($p < 0.01$) were observed between genotype and gamma radiation dosage on germination percentage, seedling emergence percentage, seedling survival percentage, number of panicles per m^2, number of productive panicles per m^2, panicle length, plant height and seed viability (Table 3.3). Table 3.4 summarizes the means of traits studied. The germination percentage for genotype Macia was significantly reduced by doses ≥300 Gy while there was a non-significant reduction in the genotype Red sorghum (Table 3.4). The lowest seedling emergence was recorded in genotype Macia (0.7%) at a dose of 600 Gy showing significant difference compared to genotype Red sorghum (13.9%). The seedling survival rate and shoot length demonstrated a similar trend as emergence percentage. Genotype Macia could not survive at 600 Gy as compared with Red sorghum, which survived the dose with 9.2% (Table 3.4). The mean of shoot length was significantly decreased by doses of ≥400 Gy in both genotypes. The LD_{50}s for germination percentage for genotypes Red sorghum and Macia were 6158 and 1092 Gy, respectively (Figure 3.4). The LD_{50}s for seedling emergence for the genotypes Red sorghum and Macia were 419 and 355 Gy, respectively (Figure 3.4). The LD_{50}s for seedling survival rate for genotypes Red sorghum and Macia were 392 and 288 Gy, respectively. The GR_{50}s for shoot length for genotypes Red sorghum and Macia were 686 and 658 Gy, respectively (Figure 3.4).

The number of panicles m^{-2} was significantly reduced by doses ≥400 and 600 Gy in Macia and Red sorghum, respectively (Table 3.4). The number of panicles m^{-2} in Red sorghum was significantly increased at 100 Gy and decreased at 600 Gy, revealing different responses of genotypes to gamma radiation doses. Plant height of the genotype Red sorghum was significantly reduced by doses ≥100 Gy whereas genotype Macia was reduced by doses ≥400 Gy. Seed viability was significantly decreased by doses ≥200 and 500 Gy for genotype Macia and Red sorghum, respectively. In general, the result showed that genotype Red sorghum demonstrated stronger tolerance to the tested gamma radiation doses, suggesting that high doses are needed to induce mutagenesis.

Table 3.3. Mean-square values and significance tests for germination percentage (%G), seedling emergence percentage (%E), seedling survival percentage (%SS), shoot length (SLT), number of panicles per m^2 (NP), number of productive panicles per m^2 (NPP), panicle length (PLT), plant height (PHT) and seed viability (%SV) of two sorghum genotypes evaluated with variable doses of gamma radiation in experiment II.

Source of Variation	df	%G	%E	%SS	SLT (cm)	NP
Replication	2	13.0	3.3	4.5	66.1	1.2
Genotype (G)	1	3498.7***	92.7***	398.7***	7238.2***	151.2***
Dose (D)	6	205.8***	1515.2***	1174.9***	1494***	56.1***
G x D	6	109.2***	132.3***	53.0***	18.6	12.3***
Error	26	13.49	3.3	3.8	36	0.9
Source of variation	**df**	**NPP**	**PLT (cm)**	**PHT (cm)**	**%SV**	
Replication	2	0.6	6.8	77.7	25.9	
Genotype (G)	1	105.4***	3.3	25834.6***	5208***	
Dose (D)	6	50.4***	10.5***	1252.1***	1187.3***	
G x D	6	4.6***	13***	78.1	456***	
Error	26	0.8	2.4	42.0	76.3	

*** denotes significant differences at $p \leq 0.001$; df = degree of freedom.

Table 3.4. Mean germination percentage (%G), emergence percentage (%E), seedling survival percentage (%SS), shoot length (SLT), plant height (PHT), number of panicles per m^2 (NP), number of productive panicles per m^2 (NPP), panicle length (PLT) and seed viability (%SV) of sorghum involving six gamma irradiations using three replications in experiment II.

Genotype	Dose (Gy)	%G	%E	%SS	SLT (cm)	PHT (cm)	NP	NPP	PLT (cm)	%SV
Macia	0	91.1±2.9	46.0±4.4	41.5±5.2	63.2±3.8	137.8±3.2	8.6±4.4	5.2±2.6	17.6±1.2	80.0±2.9
	100	91.1±1.1	43.5±2.0	38.9±5.2	61.6±2.3	133.4±4.1	8.5±4.4	7.1±2.1	16.7±0.1	71.7±1.7
	200	88.9±1.1	41.6±4.4	27.7±4.4	62.2±3.4	132.1±3.3	8.2±2.4	6.8±3.3	17.2±2.2	70.0±2.9
	300	74.4±2.9	40.4±7.4	16.1±7.3	59.4±2.5	129.4±3.8	7.6±5.5	5.3±4.9	17.7±1.7	58.3±6.0
	400	72.2±2.2	16.7±4.9	12.3±2.8	44.4±5.3	112.5±4.4	3.3±1.7	1.2±0.3	20.1±0.5	51.7±7.3
	500	72.2±4.0	4.7±3.8	2.2±1.5	35.1±3.2	96.2±4.8	1.0±1.7	0.2±0.9	23.3±0.5	14.0±8.3
	600	68.9±4.0	0.7±0.9	0.0±0.0	-	-	-	-	-	-
Red sorghum	0	98.9±1.1	47.3±4.1	42.3±8.5	90.3±1.7	197.7±2.8	8.5±6.0	7.0±14.8	20.2±0.1	88.3±4.4
	100	100.0±0.0	41.7±6.5	35.8±7.9	92.0±0.8	184.2±2.7	14.8±4.3	12.1±31.0	16.8±0.6	88.3±3.3
	200	100.0±0.0	40.6±5.1	31.9±5.6	86.3±6.0	176.6±1.7	10.0±3.2	8.4±19.7	17.6±0.8	85.0±2.9
	300	98.9±1.1	27.7±8.4	24.0±7.2	85.0±2.4	176.4±3.5	8.5±5.8	6.5±16.8	19.4±0.4	80.0±2.9
	400	98.9±1.1	25.1±3.3	22.1±3.3	78.2±6.0	169.6±6.2	8.6±6.1	6.3±16.6	16.7±0.7	76.7±1.7
	500	95.6±1.1	18.1±6.1	16.6±7.4	64.2±2.6	158.4±5.1	8.1±1.0	4.8±11.9	18.4±0.8	71.7±8.8
	600	94.4±1.1	13.9±6.8	9.2±5.3	36.8±1.3	153.7±2.0	5.2±4.3	2.8±3.0	19.1±0.1	70.0±2.9
Grand Mean		89.0	29.1	22.9	66.1	150.6	7.8	5.7	18.5	69.7
LSD (5%)		6.2	3.0	3.3	4.0	4.3	0.6	0.6	1.0	5.8
CV (%)		4.1	6.2	8.5	9.1	4.3	12.5	16.1	8.4	12.5
R^2 (%)		87.8	88.9	96.3	77.6	93.7	62.9	62.5	48.4	83.0

± denotes standard error.

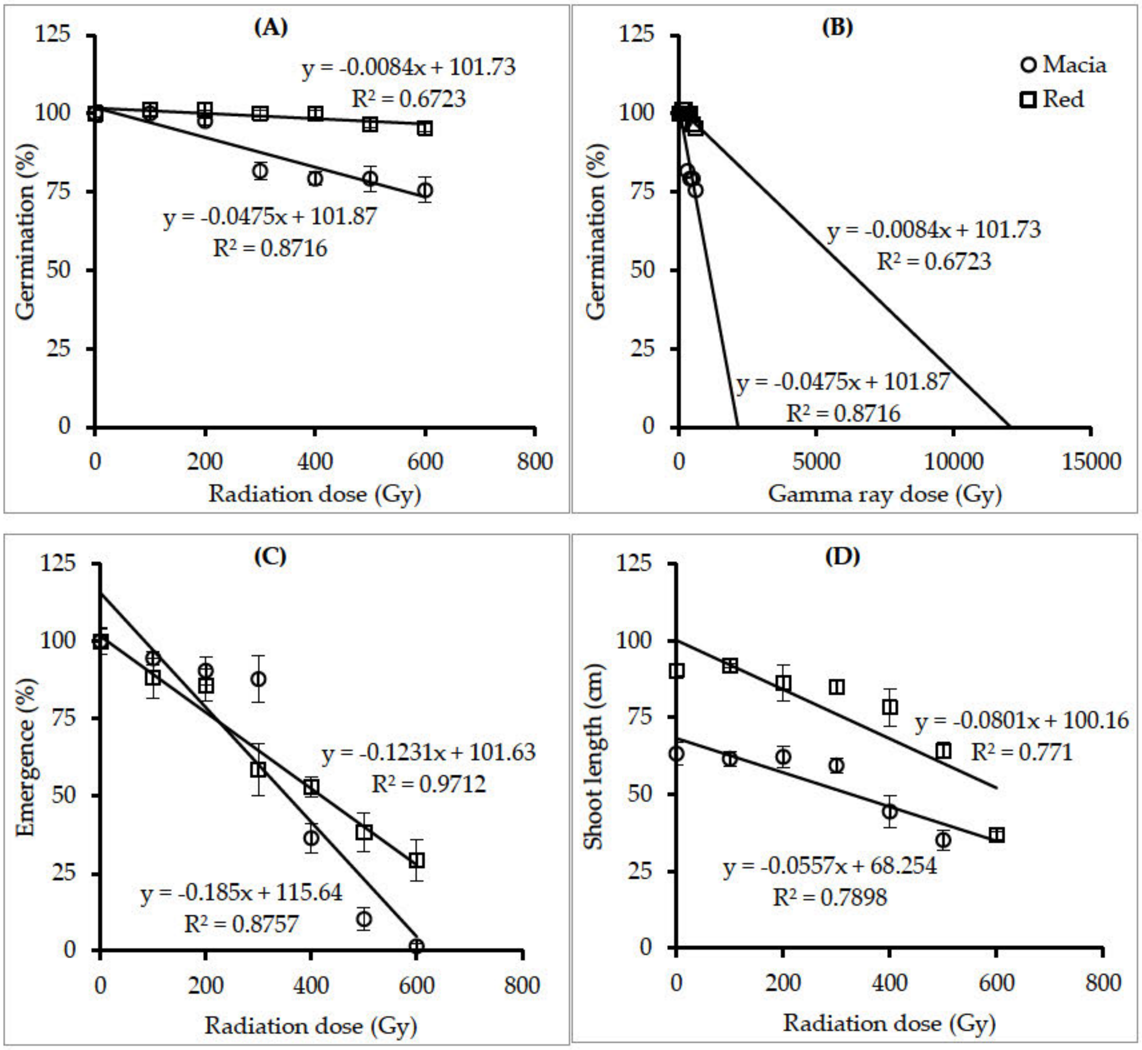

Figure 3.4: Actual (A) and extrapolated (B) germination percentage, seedling emergence percentage (C), and shoot length (D), fitted with straight lines to estimate the LD_{50}s of two sorghum genotypes treated with seven gamma radiation doses in experiment II (bars represent standard error).

3.4 Discussion

3.4.1 Effect of single doses of gamma radiation

The present study found highly significant interactions between genotypes and gamma radiation doses on assessed traits, viz., germination percentage, seedling emergence percentage, seedling survival percentage, number of panicles per m^2, number of productive panicles per m^2, panicle length and seed viability (Table 3.1 and Table 3.3). The optimum dose rates calculated on the basis of seedling emergence and survival rates for genotypes Red sorghum, Parbhani Moti, Macia, ICSV 15013 and Parbhani Shakti ranged between 392 and 419 Gy, 311 and 354 Gy, 256 and 355 Gy, 273 and 304 Gy, and 266 and 297 Gy, respectively (Figure 3.1 and Figure 3.4). Moreover, results showed that GR50 for shoot length ranged between 436 and 686 Gy, respectively (Figure 3.1 and Figure 3.4). However, these dose rates will mean lower seedling emergence, poor seedling survival rate and weak plants. High dose rates produce low plant population and poor plant growth in the M_2 and M_3 generations, in turn affecting the identification and selection of useful mutants (Table 3.2 and Table 3.4). Golubinova and Gecheff (2011) applied gamma radiation dose rates ranging between 100 to 400 Gy on Sudan grass and found that the LD_{50} values estimated on the basis of seedling survival rate were more suitable for large scale mutagenesis than using data on germination and sterility. The authors stressed the importance of using the optimum dose rate which obtains good and healthy plant populations to be grown to maturity. Mudibu et al. (2012) suggested that a high mutagen dose rate leads to an increased proportion of changes such as chromosomal aberrations, lethality, injury, and sterility. The present study found that low gamma radiation dose rates (≤200 Gy) showed no significant effect on plant growth except for the germination rate and the number of panicles per m^2 (Table 3.4). Meanwhile, a high dose reduced seedling emergence and survival rates, the number of panicles and the number of productive panicles per m^2, plant height and seed viability below the target of 50% (Table 3.4). Similar findings were reported in chili pepper (Dhamayanthi and Reddy, 2000) and African nightshade (Ojiewo et al., 2005). The differences observed in biological traits suggest that the identified optimum doses of single gamma radiation may produce useful mutants, as reported by Tadele (2016) and Manova and Gruszka (2015).

3.4.2 Effect of Single Doses of EMS

Highly significant interactions were found between genotypes and EMS concentration on seedling emergence and survival rates (Table 3.1). The LD_{50}s calculated on the basis of seedling emergence and survival rate for genotypes Parbhani Shakti, ICSV 15013, Parbhani Moti and Macia was between 0.41% and 0.60%, 0.48% and 0.58%, 0.46% and 0.51%, and 0.36% and 0.45%, respectively (Figure 3.2). The GR_{50}s for shoot length for genotypes ICSV 15013, Parbhani Shakti, Parbhani Moti, and Macia were 1.35%, 0.90%, 0.87% and 0.67% EMS, respectively (Figure 3.2). GR_{50} may be associated with undesirable effects on growth promoters and various chromosomal aberrations which affect emergence, seedling survival rate and seed set (Rajib and

Jagatpati, 2011). Therefore, estimated GR_{50}s in the present study were not suitable for use in mass mutagenesis breeding program. Mani (1989) applied an EMS concentration of 0.3% for 8 h in three sorghum varieties and found germination rates between 63% and 80% and a survival rate between 66% and 82%. This result suggest the need to increase the concentrations to obtain LD_{50}s for each genotype. In the present study, the mean seedling emergence, survival rates and seedling height had significant reductions with increased EMS concentrations (Table 3.2). This result corroborates the findings in rice (Talebi et al., 2012), soybean (Mudibu et al., 2012) and wheat (Olaolorun et al., 2019) which reported that seedling emergence, survival and growth were influenced by genetic variations and EMS concentrations.

3.4.3 Effect of combined doses of gamma radiation and EMS

Significant interactions were found between genotypes and dose rates for the combined use of gamma radiation followed by EMS concentrations on seedling emergence and survival rates (Table 3.1). However, results were unable to determine the optimum dose rates for combined applications of gamma radiation and EMS for large scale mutagenesis on the test genotypes. The test dose rates were high and produced undesirable effects, including reduction of seedling emergence and survival rates. The mean of all traits assessed was drastically decreased with the test doses (Table 3.2). Results showed that, with a combined treatment of 300 Gy followed by 0.1% EMS concentration, seedling emergence percentages of genotypes Parbhani Moti, Macia, Parbhani Shakti and ICSV 15013 were 27.6%, 19.7%, 16.8% and 14.3%, respectively (Figure 3.3). At the same dose rate, the seedling survival rates of genotypes Parbhani Shakti, Parbhani Moti, Macia, and ICSV 15013 were 16.2%, 15.9%, 3.8% and 3.5%, respectively. These results can be explained by the optimum dose rates obtained in a single use of gamma radiation and EMS on the test genotypes shown above. This result suggests the need to apply lower gamma radiation doses and EMS concentrations to obtain desired level of seedling emergence and survival rates and in turn to promote healthy plant growth for effective selection.

3.5 Conclusions

This study determined doses of gamma radiation and EMS singly or in combination in sorghum based on early growth parameters. Combined doses of gamma radiation and EMS produced poor seedling emergence and seedling survival rate below the target of LD_{50}. Therefore, combined doses were not recommended for large scale mutation induction in sorghum. The optimum gamma radiation dose rates aiming at LD_{50} for seedling emergence and survival rate for genotypes Red sorghum, Parbhani Moti, Macia, ICSV 15013 and Parbhani Shakti ranged between 392 and 419 Gy, 311 and 354 Gy, 256 and 355 Gy, 273 and 304 Gy, and 266 and 297 Gy, respectively. The optimum EMS dose rates aiming at LD50 for seedling emergence and survival rate for genotypes Parbhani Shakti, ICSV 15013, Parbhani Moti and Macia were between 0.41% and 0.60%, 0.48% and 0.58%, 0.46% and 0.51%, and 0.36% and 0.45%, respectively. These dose rates may induce genetic variation in the tested sorghum genotypes for genetic enhancement in sorghum breeding programs.

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Chapter 4. Genetic Profile of Newly Developed Sorghum Lines Revealed through Simple Sequence Repeat Markers and Phenotypic Traits

Abstract

The autogamous mating system of sorghum (*Sorghum bicolor* [L.] Moench) limits the inherent genetic variation for the selection of economic traits, including grain yield and drought tolerance. Induced mutagenesis generates marked genetic variation useful for sorghum selection and breeding programs. The objective of this study was to determine the genetic profile of elite sorghum lines developed via gamma radiation using diagnostic simple sequence repeat (SSR) markers and phenotypic traits for breeding. Seventeen SSR markers amplified a total of 50 alleles, which varied from 2 to 5 (mean = 2.94). The number of effective alleles per locus varied from 1.08 to 2.53, with a mean of 1.96. The observed heterozygosity ranged from 0.00 to 0.21 (mean = 0.09). The mean expected heterozygosity value was 0.45 indicating moderate genetic differentiation of the tested lines for selection and hybridization. Cluster analysis classified the genotypes into three main groups. Moderate to high genetic distance (≥ 0.50) was displayed between drought-tolerance and high-yield performance and aided in selecting mutant lines such as ‘ML2, ML3, ML4, ML7 and ML14’ compared with the check varieties Macia, Kotovara, ICSR 137, and ICSV 17004’. The selected lines are a useful source of genetic variation for breeding high-yielding and drought-tolerant varieties suited for the drought-prone environments of Namibia.

Keywords: Genetic analysis, mutation breeding, simple sequence repeat markers, sorghum

4.1 Introduction

Sorghum [*Sorghum bicolor* (L.) Moench, 2n=2x=20] is one of the important and widely grown grain crops worldwide (FAOSTAT, 2022). The annual production of the crop is approximately 57.9 million tons making it the 5th most-produced cereal globally (FAOSTAT, 2022). Sorghum is a major food and feed crop in the semi-arid tropics of Africa, Australia and America. Sorghum grains are gluten-free and rich in essential nutrients including protein (15%), minerals (e.g., Calcium [114 mg/kg], Iron [52 mg/kg] and Zinc [26 mg/kg]), vitamins (e.g., B_1 [Thiamine], B_2 [Riboflavin], B_6 [Pyridoxine]), fibre (8%) and antioxidants (e.g., flavonoids, phenolic acids and tannin) (Adebo, 2020; Drub et al., 2021; Dabija et al., 2021).

In sub-Saharan Africa (SSA), the average sorghum yield is low (0.97 ton/ha) compared to Northern America (4.24 tons/ha), Europe (3.67 tons/ha), South America (3.33 tons/ha), and Asia (1.18 tons/ha) (FAOSTAT, 2022). High sorghum yields attained are attributed to the use of modern production technologies, including high yielding varieties, precision agriculture and input management systems (e.g., irrigation, fertiliser, pest, and disease control), farm mechanisation, market linkages, and well-established value chains (Fischer and Connor, 2018). Low sorghum productivity in SSA is confounded with an array of challenges including biotic stresses (i.e., insect pests, diseases, and parasitic weeds) and abiotic factors (e.g., drought and heat stress), limited market access and opportunity, and high prices of production inputs (Mundia et al., 2019). A lack of improved sorghum varieties that possess desirable traits such as higher grain yield is the grand constraint to the sustainable and profitable crop production in SSA (Assefa et al., 2010; Ogbaga et al., 2014; Gano et al., 2021).

Namibia is one of the driest countries in SSA, with a mean annual rainfall below 400 mm in the major sorghum production regions (Awala et al., 2019). As a result, the country is the least sorghum producer in the region with an annual grain production of approximately 3,280 tons from a total land area of about 17,842 ha (FAOSTAT, 2022). The average sorghum yield in the country is 0.22 ton/ha, compared to the relatively higher potential yields such as 7.5 tons/ha attained in Malawi (Sato et al., 2020), and national mean yields of 2.68 tons/ha in South Africa, 2.38 tons/ha in Ethiopia, 1.21 tons/ha in Nigeria, and 0.63 ton/ha in Sudan (FAOSTAT, 2022). Farmers in Namibia use farm-saved seeds of traditional and unimproved varieties that are low-yielding, because of their adaptability to the local conditions (Wanga et al., 2018). Currently, Macia (SDS 3220) and Red sorghum varieties are the only commercially available and widely cultivated sorghum cultivars in Namibia (NSA, 2015; Wanga et al., 2018). The country requires new sorghum varieties tolerant to drought and heat stress and preferred by farmers and markets. This is dependent on the availability of a dedicated sorghum breeding program and the presence of adequate genetic variation.

Sorghum is predominantly an autogamous crop limiting its inherent genetic variation for the selection of economic traits, including grain yield and drought tolerance. Induced mutagenesis is a powerful tool to create genetic variation to select desirable traits (Shu et al., 2012; Spencer-Lopes et al., 2018; Sarsu et al., 2021). For example, Human et al. (2011), Human et al. (2020), and Kurniadi et al. (2021) used gamma-radiation to develop sorghum mutants with high-yield and enhanced contents of protein (12.8%), fat (2.8%), and fibre (2.2%), and low tannin composition (0.03%). Nikièma et al. (2020) used ethyl-methane sulfonate (EMS) mutagenesis to induce random mutations, which aided in developing lines with a reduced date to flowering and tolerant to water deficit. Further, whole-genome sequencing of EMS mutagenised lines revealed higher mutagenic events (>95%) in sorghum (Jiao et al., 2016). Thus, induced mutagenesis can increase the genetic diversity required to select and develop promising lines with high-yield potential and good adaptability.

To improve sorghum yields in Namibia, the Ministry of Agriculture, Water and Land Reform, in collaboration with the joint Food and Agriculture Organization (FAO) of the United Nations and the International Atomic Energy Agency (IAEA) developed 148 sorghum mutant populations using the gamma irradiation protocol. This was achieved using the seed of the locally adapted sorghum variety Macia. From this population, a set of promising mutants with potential high grain yield and other suitable yield attributes were selected based on morpho-physiological traits under water-limited conditions (Wanga et al., 2020). The genetic profile of the selected elite lines should be adequately established for practical breeding. This will identify distantly related lines to maximise genetic recombination of alleles with high-yield potential and desirable yield components. Molecular markers are powerful tools for genotyping, genetic grouping and selection, among others. Simple sequence repeat (SSR) markers are popularly used for genetic diversity evaluations due to their high polymorphism and abundance in the complete genome sequences for genome analysis and plant breeding (Hong et al., 2011). Billot et al., (2013) revealed high genetic differentiation in a global sorghum collection using the SSR markers. Adugna (2014) found wide genetic diversity among landraces of sorghum cultivated in Ethiopia. South African sorghum genotypes were genetically distinctive based on SSR marker genotyping (Mofokeng et al., 2017). A collection of Southern African (i.e., Botswana, Namibia, Swaziland, Zambia and Zimbabwe) sorghum germplasm was reportedly genetically unrelated when assayed using the SSR markers (Motlhaodi et al., 2016). Therefore, the objective of this study was to determine the genetic profile of elite sorghum lines developed via gamma radiation using diagnostic simple sequence repeat (SSR) markers and phenotypic traits for breeding.

4.2 Materials and methods

4.2.1 Plant material

The study used 25 sorghum genotypes of which 20 were newly developed elite mutant lines of M_7 generations derived from variety Macia (SDS 3220), while five were check varieties (Table 4.1). Namibia cultivated check varieties Macia and Red sorghum (*Sorghum bicolour* [L.] Moench) were used as comparative controls. Further, variety Kotovara was included in the study. Kotovara was selected from locally grown landraces for its desirable gooseneck type panicle and for being widely grown by smallholder farmers in Namibia. Genotypes ICSR 137 and ICSV 17004 were sourced from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT)/India and served as checks. The elite mutant lines were selected after gamma irradiation of the seed of the progenitor variety Macia at a dose rate of 350 Gy. The pedigree selection method was used from the M_3 to M_5 generations using phenotypic traits such as desirable plant height, early-to-medium maturity, and higher panicle and grain yield.

Table 4.1: Name, pedigree and traits of sorghum genotypes used in the study.

Sr. No	Genotype name/designation	Pedigree	Traits
1	ML1 (L7P9-2)	Macia	Medium maturity, white seed and short plant height
2	ML2 (L7P9-4-1)	Macia	Medium maturity, white seed and medium plant height
3	ML3 (L7P7-3C)	Macia	Medium maturity, white seed and medium plant height
4	ML4	Macia	Medium maturity, white seed and medium plant height
5	ML5 (L7P9-4)	Macia	Medium maturity, white seed and short plant height
6	ML6 (L7P9-13)	Macia	Medium maturity, red seed and short plant height
7	ML7	Macia	Medium maturity, red seed and medium plant height
8	ML8	Macia	Early maturity, white seed and short plant height
9	ML9 (L7P9-14-1)	Macia	Medium maturity, red seed and short plant height
10	ML10 (L7P9-4-2)	Macia	Medium maturity, white seed and short plant height
11	ML11	Macia	Late maturity, red seed and medium plant height
12	ML12	Macia	Medium maturity, red seed and short plant height
13	ML13	Macia	Medium maturity, white seed and medium plant height
14	ML14	Macia	Medium maturity, white seed and medium plant height
15	ML15	Macia	Late maturity, white seed and short plant height
16	ML16 (L3P15-9)	Macia	Early maturity, white seed and short plant height
17	ML17	Macia	Medium maturity, white seed and short plant height
18	ML18	Macia	Late maturity, white seed and short plant height
19	ML19	Macia	Late maturity, white seed and short plant height
20	ML20	Macia	Medium maturity, white seed and medium plant height
21	Macia (SDS 3220)	M91057 (pedigree [GPR 148 x E35-1] x CS 3541)	Early-to-medium maturity, high yield and short plant height, white seed
22	Red sorghum	Landrace	Late maturity and medium plant height and red seed
23	Kotovara	Landrace	Medium maturity and medium plant height and reed seed
24	ICSR 137	[MR 836 × (CK 60B × IS 84)]-6-4-3	Early maturity, heat tolerance and medium plant height
25	ICSV 17004	IS 26962-2-1-1-2-2	Early-to-medium maturity, high yield and Grain Zn

Sr. No, serial number; entries 1 to 20 are M_6 mutant lines derived from variety Macia using gamma radiation with a dose of 350Gy

4.2.2 Genomic DNA extraction

DNA extraction was carried out on young and fresh leaves samples from 14 seedling plants per test line 20 days after sowing. The lines were established by sowing 28 seeds per line under glasshouse condition at the University of KwaZulu-Natal (latitude 29°37’51.75″ S; longitude 30°23’59.10″ E), South Africa. The samples were bulked and transported to the SciCorp Laboratories (Pty) Ltd, Pietermaritzburg, 3201, South Africa for SSR analysis. Sampled leaves were homogenised using liquid nitrogen, and in-house ionic detergent cetyltrim ethylammonium bromide (CTAB) method was used for DNA extraction (Aboul-Maaty & Oraby, 2019). The DNA concentration was quantified using a Nano-Drop 1000 spectrophotometer (ND-1000 V3.5, NanoDrop Technologies, Inc.).

4.2.3 DNA amplification and polymerase chain reaction

Twenty SSR markers were selected based on distribution across 10 sorghum chromosomes and for being highly polymorphic (https://sorghum.cirad.fr/SSR_kit/data/msat_char.html) (Table 4.2). The SSR marker mSbCIR276 produced poor amplification after numerous optimisations attempts and was not included in the final analysis. The PCR settings for denaturation were adjusted to 94ºC for 30 seconds. The primer annealing temperature was set based on the expected amplicon size and dye (Table 4.2). The PCR products were fluorescently labelled and separated by capillary electrophoresis on an ABI 3130xl automatic sequencer (Applied Biosystems, Johannesburg, South Africa). Electropherograms were analysed using Gene Mapper 4.0, and data on fragment sizes was presented in an Excel spreadsheet.

Table 4.2: Names of SSR markers, primer sequences, and annealing temperatures (Tm) used for genetic analysis of sorghum lines.

S no.	Marker	Forward primer	Reverse primer	Tm (ºC)	Allele size
1	gpsb067	TAGTCCATACACCTTTCA	TCTCTCACACACATTCTTC	49	170-180
2	gpsb123	ATAGATGTTGACGAAGCA	GTGGTATGGGACTGGA	50	288-296
3	mSbCIR223	CGTTCCAATGACTTTTCTTC	GCCAATGTGGTGTGATAAAT	55	108-118
4	mSbCIR240	GTTCTTGGCCCTACTGAAT	TCACCTGTAACCCTGTCTTC	55	108-112
5	mSbCIR246	TTTTGTTGCACTTTTGAGC	GATGATAGCGACCACAAATC	55	98-100
6	mSbCIR248	GTTGGTCAGTGGTGGATAAA	ACTCCCATGTGCTGAATCT	56	89-101
7	mSbCIR262	GCACCAAAATCAGCGTCT	CCATTTACCCGTGGATTAGT	57	208-220
8	mSbCIR276	CCCCAATCTAACTATTTGGT	GAGGCTGAGATGCTCTGT	53	230-234
9	mSbCIR283	TCCCTTCTGAGCTTGTAAAT	CAAGTCACTACCAAATGCAC	54	113-139
10	mSbCIR286	GCTTCTATACTCCCCTCCAC	TTTATGGTAGGATGCTCTGC	55	112-134
11	mSbCIR300	TTGAGAGCGGCGAGGTAA	AAAAGCCCAAGTCTCAGTGCTA	61	104-110
12	mSbCIR306	ATACTCTCGTACTCGGCTCA	GCCACTCTTTACTTTTCTTCTG	56	120-124
13	mSbCIR329	GCAGAACATCACTCAAAGAA	TACCTAAGGCAGGGATTG	55	109-117
14	Xcup02	GACGCAGCTTTGCTCCTATC	GTCCAACCAACCCACGTATC	54	192-204
15	Xgap206	ATTCATCATCCTCATCCTCGTAGAA	AAAAACCAACCCGACCCACTC	55	106-146
16	Xgap72	TGCCACCACTCTGGAAAAGGCTA	CTGAGGACTGCCCCAAATGTAGG	55	183-203
17	Xtxp012	AGATCTGGCGGCAACG	AGTCACCCATCGATCATC	55	161-205
18	Xtxp15	CAC AAA CAC TAG TGC CTT ATC	CAT AGA CAC CTA GGC CAT C	55	199-223
19	Xisep0327	CTGTTTGTGCTTGCAACTCC	TCATCGATGCAGAACTCACC	63	200-210
20	Xiabtp515	TGCCACATCGATCTTGTCAC	AGGCAGTCACCCACACTACC	63	214-218

4.2.4 Estimation of genetic parameters

The following monomorphic markers: mSbCIR248 and Xcup02 were excluded, resulting in the 17 SSR markers in the final analysis. The following genetic diversity parameters were computed based on 17 SSR markers, including the total number of polymorphic alleles per locus (N_a), the number of effective alleles per locus (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{ST}) and the polymorphic information content (PIC). GenAlex 6.5 was employed for data analysis (Peakall and Smouse, 2012). The PIC values were computed using Gene Calc (https://www.gene-calc.pl/pic). Genetic relationships, distance, and cluster analysis were carried out using a neighbour-joining (NJ) algorithm using the unweighted pair group method (UWPGM) with PowerMarker version 3.25 statistical software (Liu and Muse, 2005).

4.3 Results

4.3.1 Characterisation of the SSR markers

The estimated genetic parameters by the SSR markers are presented in Table 4.3. The 17 polymorphic SSR markers detected a total of 50 alleles. The total number of alleles per locus (N_a) ranging from 2 to 5 with a mean of 2.94. The number of effective alleles per locus (N_e) ranged from 1.08 to 2.53, with a mean of 1.96. The SSR markers mSbCIR223, gpsb067, Xisep0327, and Xtxp15 detected the lowest (≤1.57) N_e, whereas markers mSbCIR286, Xgap72, gpsb123, mSbCIR283, and Xiabtp515 detected the highest N_e (≥2.32). The observed heterozygosity (H_o) ranged from 0.00 for markers Xisep0327, Xtxp15, mSbCIR223, and gpsb067 to 0.21 for markers gpsb123 and mSbCIR329. The expected heterozygosity (H_e) values ranged from 0.08 to 0.61 for mSbCIR223 and mSbCIR286, respectively. The mean H_e value of 0.45 was recorded across the SSR markers. The fixation index (F_{ST}) values ranged from 0.61 for marker mSbCIR329 to 1.0 for markers gpsb067, mSbCIR223, Xtxp15, and Xisep0327 with a mean value of 0.88. The polymorphic information content (PIC) ranged between 0.08 for loci mSbCIR223 and 0.61 for loci mSbCIR286, with a mean value of 0.61.

Table 4.3: Genetic parameters generated by 17 simple sequence repeat (SSR) markers among 25 sorghum test lines.

Marker	Genetic parameters					
	Na	Ne	Ho	He	F_{ST}	PIC
gpsb067	3	1.18	0.00	0.15	1.00	0.15
gpsb123	5	2.43	0.20	0.59	0.66	0.52
mSbCIR223	2	1.08	0.00	0.08	1.00	0.07
mSbCIR240	2	2.00	0.12	0.50	0.76	0.38
mSbCIR246	2	1.92	0.16	0.48	0.67	0.36
mSbCIR262	2	1.97	0.04	0.49	0.92	0.36
mSbCIR283	4	2.33	0.12	0.57	0.79	0.48
mSbCIR286	4	2.53	0.04	0.61	0.93	0.53
mSbCIR300	2	1.92	0.13	0.48	0.74	0.36
mSbCIR306	2	2.00	0.04	0.50	0.92	0.38
mSbCIR329	3	2.15	0.21	0.54	0.61	0.43
Xgap206	3	1.99	0.08	0.50	0.83	0.41
Xgap72	4	2.49	0.12	0.60	0.80	0.52
Xtxp012	4	2.20	0.12	0.54	0.78	0.46
Xtxp15	3	1.57	0.00	0.36	1.00	0.33
Xisep0327	2	1.20	0.00	0.17	1.00	0.15
Xiabtp515	3	2.32	0.17	0.57	0.71	0.48
Mean	2.94	1.96	0.09	0.45	0.83	0.37
SE	0.23	0.11	0.02	0.04	0.03	0.03

N_a, total number of alleles per locus; N_e, number of effective alleles per locus; H_o, observed heterozygosity; H_e, expected heterozygosity; F_{ST}, fixation index; PIC, polymorphic information content; SE, standard error.

4.3.2 Genetic distance

The genetic distance matrix of 25 sorghum lines based on 17 polymorphic SSR markers is presented in Table 4.4. The genetic distance between test lines varied from 0.03 to 0.91. Lowest genetic distances (≤0.09) were detected between mutant lines ML2 and ML5, ML3 and ML5, ML4 and ML14, ML5 and ML10, and ML13 and ML14. High genetic distance (> 0.60) was detected among the following mutant lines: ML2 and ML11, ML2 and ML12, ML2 and ML15, ML2 and ML19, ML3 and ML19, ML4 and ML8, ML4 and ML19, ML8 and ML9, ML8 and ML13, ML8 and ML14, ML8 and ML17, ML9 and ML19, ML10 and ML19, ML11 and ML17, ML19 and ML16, ML13 and ML116, ML17 and ML119. Moderate to high genetic distance (~ 0.50) was displayed between the mutant lines ML1, ML4, ML6, ML9, ML11, ML12, ML13, ML14, ML15, ML17, and ML18 with their parental genotype Macia. Very high genetic distance (>0.80) was recorded among the following test lines: ML15 and ICSR 137, Macia and Red sorghum, ICSR 137 and ML12, ML4 and ICSR 137, Kotovara and ML9, ML13 and ICSR 137, Kotovara and ICSV 17004, Red sorghum and ICSV 17004, and ICSR 137 and ML14.

Table 4.4: Genetic distance matrix of 20 sorghum lines derived via gamma radiation, and five check varieties based on 17 simple sequence repeat (SSR) markers.

Genotype	ML1	ML2	ML3	ML4	ML5	ML6	ML7	ML8	ML9	ML10	ML11	ML12	ML13	ML14	ML15	ML16	ML17	ML18	ML19	ML20	Macia	Red sorgh	Kotovara	ICSR 137	ICSV 17004
ML1	0.00																								
ML2	0.29	0.00																							
ML3	0.24	0.12	0.00																						
ML4	0.47	0.47	0.41	0.00																					
ML5	0.24	0.06	0.06	0.47	0.00																				
ML6	0.41	0.59	0.53	0.41	0.59	0.00																			
ML7	0.50	0.44	0.38	0.38	0.44	0.41	0.00																		
ML8	0.41	0.41	0.47	**0.65**	0.41	0.35	0.38	0.00																	
ML9	0.59	0.56	0.47	0.35	0.53	0.53	0.38	**0.65**	0.00																
ML10	0.24	0.12	0.00	0.41	0.06	0.53	0.38	0.47	0.47	0.00															
ML11	0.47	**0.65**	0.59	0.53	0.59	0.35	0.50	0.41	0.59	0.59	0.00														
ML12	0.50	**0.62**	0.56	0.56	0.56	0.50	0.41	0.56	0.50	0.56	0.26	0.00													
ML13	0.50	0.53	0.47	0.12	0.53	0.41	0.38	**0.65**	0.29	0.47	0.47	0.44	0.00												
ML14	0.44	0.50	0.44	0.03	0.50	0.38	0.35	**0.62**	0.38	0.44	0.50	0.53	0.09	0.00											
ML15	0.50	**0.62**	0.59	0.29	0.59	0.47	0.50	0.53	0.53	0.59	0.44	0.53	0.41	0.32	0.00										
ML16	0.35	0.35	0.35	0.53	0.29	0.41	0.50	0.29	0.59	0.35	0.41	**0.62**	**0.65**	0.56	0.53	0.00									
ML17	0.56	0.53	0.44	0.32	0.50	0.56	0.41	**0.68**	0.15	0.44	**0.62**	0.41	0.26	0.35	0.56	0.56	0.00								
ML18	0.26	0.41	0.35	0.41	0.35	0.44	0.38	0.41	0.41	0.35	0.41	0.38	0.47	0.44	0.35	0.35	0.44	0.00							
ML19	0.59	0.56	**0.62**	**0.62**	0.56	0.53	0.59	0.26	**0.65**	**0.62**	0.38	0.59	0.50	0.59	0.56	0.44	**0.65**	0.56	0.00						
ML20	0.44	0.41	0.38	0.44	0.38	0.53	0.41	0.44	0.50	0.38	0.50	0.47	0.38	0.41	0.47	0.44	0.47	0.44	0.35	0.00					
Macia	0.50	0.47	0.41	**0.53**	0.47	**0.56**	0.44	0.41	**0.53**	0.41	**0.59**	**0.68**	**0.56**	**0.53**	**0.59**	0.41	**0.50**	**0.50**	0.38	0.35	0.00				
Red sorghum	0.47	0.47	0.47	0.41	0.41	0.47	0.56	0.53	0.53	0.47	0.41	0.38	0.41	0.44	0.47	0.47	0.44	0.41	**0.62**	**0.62**	**0.88**	0.00			
Kotovara	0.59	**0.71**	**0.65**	**0.76**	**0.65**	**0.71**	**0.79**	**0.79**	**0.82**	**0.65**	**0.62**	**0.68**	**0.74**	**0.76**	**0.71**	**0.65**	**0.74**	**0.68**	**0.74**	**0.68**	**0.71**	**0.71**	0.00		
ICSR 137	**0.68**	0.53	0.59	**0.82**	0.53	**0.76**	**0.68**	0.56	**0.79**	0.59	**0.76**	**0.85**	**0.82**	**0.82**	**0.91**	0.47	**0.71**	**0.76**	**0.65**	**0.65**	**0.65**	**0.71**	**0.71**	0.00	
ICSV 17004	0.59	0.59	**0.65**	**0.71**	0.59	**0.68**	0.50	0.41	**0.71**	**0.65**	**0.59**	**0.65**	**0.74**	**0.68**	**0.62**	0.41	**0.71**	0.56	0.47	0.50	0.47	**0.82**	**0.82**	0.47	0.00

Note: Genotypes ML1 to ML20 represent the mutant lines (M_7) derived from variety Macia using gamma radiation with a dose of 350Gy.

4.3.3 Cluster analysis

A dendrogram based on genetic distances showing relationships between newly developed sorghum mutant lines and check varieties is presented in Figure 4.1. The tested sorghum lines were grouped into three distinct genetic clusters (I, II, and III), consisting of a variable number of entries. Fifteen sorghum lines, including 11 mutant lines and 4 standard varieties, were grouped in Cluster I. Cluster II comprised of 7 mutant lines, whereas 2 mutants and 1 check variety were allocated in Cluster III.

Morphologically, the mutant lines and varieties showed varied differences for the following phenotypic traits: root system architecture traits, panicle length, panicle weight, and seed colour (Figures 4.2, 4.3, and 4.4). For example, mutants ML2, ML3, ML4, and ML7 had white and red seeds, derivatives of the white seed sorghum variety Macia (Figure 4.2). Mutant lines ML2, ML8, ML9, and ML14 were early flowering compared to late flowering mutant lines ML11, ML15, ML18, and ML19 (Table 4.1). Longer panicles were observed for mutant lines ML3, ML4, ML11 and ML16 compared to mutant lines ML1, ML12, ML14, and ML20 (Figure 4.3). Mutant lines ML4, ML3, ML9 and ML14 displayed higher panicle weight than mutant lines ML8, ML15, ML16 and ML19 (Data not shown). For root system architecture, mutants ML14, ML18 and ML20 had longer roots, high root biomass and root-to-shoot ratio, whereas mutants ML1 and ML7, had shorter root length and low root volume (Figure 4.4).

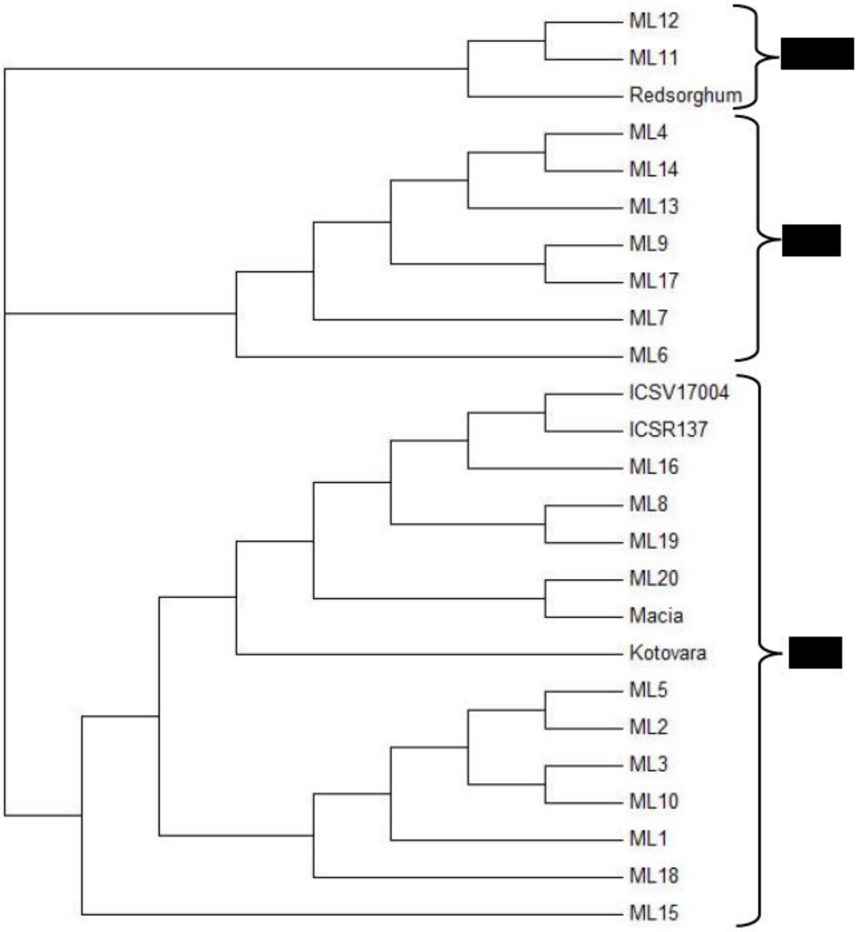

Figure 4.1: Dendrogram based on neighbour-joining algorithm with the unweighted pair group method (UPGMA) showing genetic relationships among 25 sorghum lines using 17 SSR markers. M1 to M20 are mutant lines derived using gamma radiation from the variety Macia.

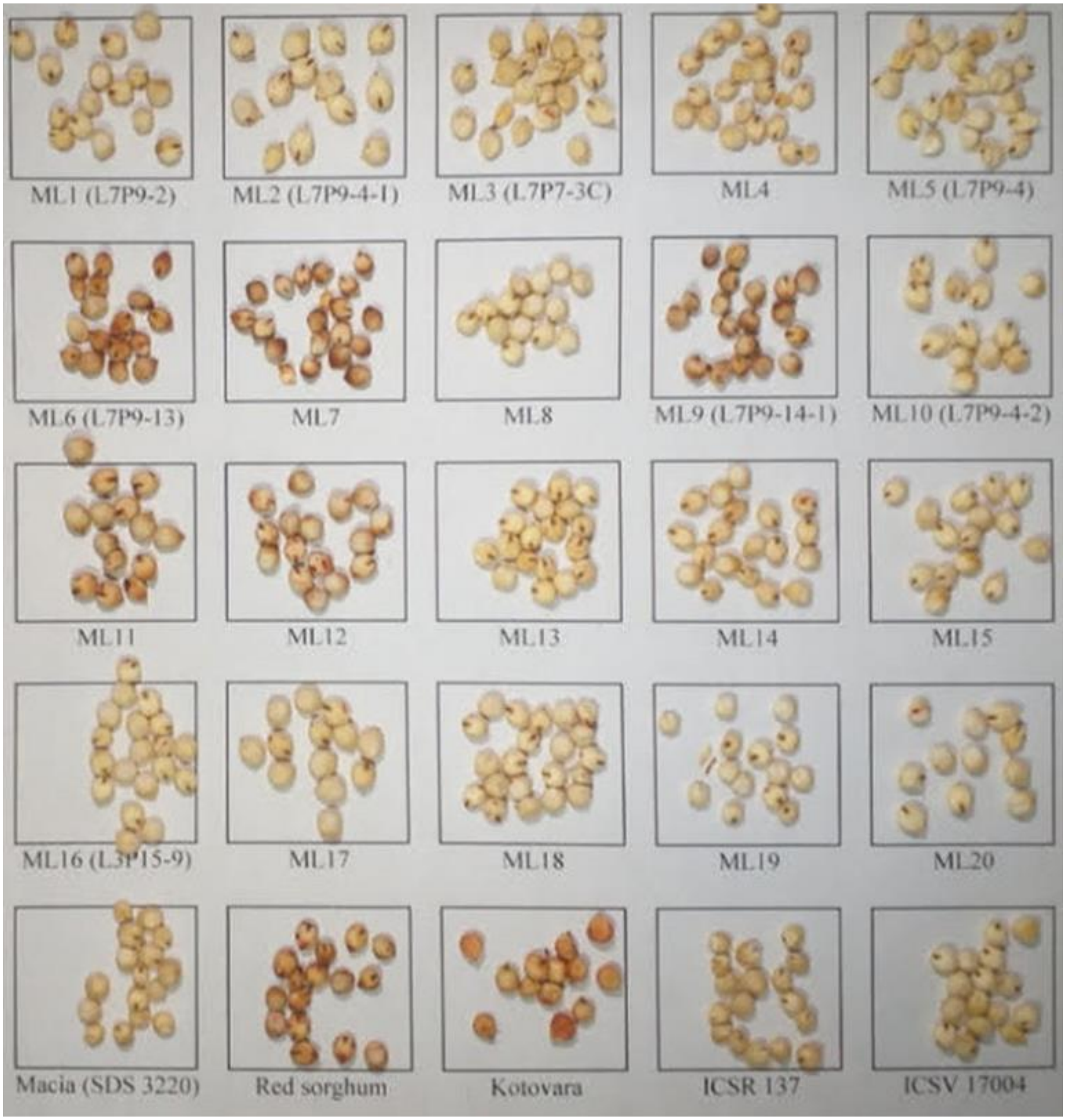

Figure 4.2: Comparison of seed colour among sorghum mutant lines (ML1 to ML20) derived using gamma radiation from the parental genotype Macia (SDS 3220) and check varieties Red sorghum, Kotovara, ICSR 137 and ICSV 17004.

Figure 4.3: Panicle shape, seed colour and glume colours of sorghum mutant lines derived using gamma radiation from the variety Macia. Note: mutant lines ML3 (A), ML4 (B) and ML2 (C), at the M_7 selected based on morphological traits including panicle shape, seed colour and glume colours in M_2 generation and check varieties Macia (D), Kovara (E) and ICSV 17004 (F).

Figure 4.4: Variation in root length and biomass among mutant lines derived using gamma radiation from the parental variety Macia. A, ML 18, B; ML 4, C, ML1; D, LM 7; E, ML 20; F, ML14; G, ML19; H, ML 15.

4.4 Discussion

A mutation breeding program was initiated by Namibia's Ministry of Agriculture, Water and Land Reform. The aim was to develop and release high-yielding sorghum mutant varieties that are drought tolerant and possess excellent nutritional qualities for cultivation in Namibia. The widely cultivated and locally adapted sorghum variety Macia was used for mutagenesis using gamma-radiation (350 Gy), resulting in selecting 20 superior candidate mutant lines. The newly-developed mutant lines require genetic analysis to distinguish widely distant lines for breeding, further genetic advancement, and subsequent release for adoption in Namibia. As a result, 17 highly polymorphic SSR markers were used for genotyping of the selected mutant lines and check varieties in the present study to complement the phenotypic selection.

The expected heterozygosity revealed moderate diversity in mutant lines and check varieties (i.e., Macia, Red sorghum, Kotovara, ICSR 137, and ICSV 17004). The diversity indicated the success of gamma-radiation in inducing genetic variability in the parental variety Macia (Table 4.3). The mutant lines ML1, ML4, ML6, ML9, ML11, ML12, ML13, ML14, ML15, ML17, and ML18 had wide divergence compared with the parental genotype Macia (Table 4.4). Also, mutant lines ML4, ML12, ML13, ML14 and ML15 were distantly related from the check variety ICSR 137 (Table 4.4). Further, ML15 was genetically different compared to the check varieties ICSR 137, Macia, and Red sorghum, whereas ML12 was divergent from ICSR 137. These revealed the possibility of creating new recombinants through crosses involving the mutant lines and checking varieties to breed a new generation of sorghum adapted to Namibia conditions.

Seed coat color is a crucial trait for grading and influencing sorghum grains' nutritive quality (Ng'uni et al., 2012; Ratnavathi, 2019; Zhou et al., 2020). For example, white sorghum grain has no glume coloured pigments and is vital to developing baked products, noodles, extruded snacks, and brewing beer (Taylor et al., 2006). Further, coloured sorghum grains are rich in polyphenolic compounds, which affect the end-use quality (Taylor et al., 2014; Niaba et al., 2017). The newly-developed mutants showed seed coat colour variation, from red and white (Figure 4.2). Mutant lines ML 6, ML 7, and ML 9 had red-white seed coat colour compared to white seed coats of their parental variety Macia. Also, these mutant lines showed varied seed coat colour from ICSR 137 and ICSV 17004 (Figure 4.2). These indicated that the induced mutations via gamma radiation could have altered the expression of genes influencing seed coat colour in the sorghum genome.

Sorghum panicle architecture is a crucial trait contributing to grain yield. The newly developed mutants showed varied panicle architectures allowing selection to improve grain yield potential. Longer panicles observed for mutants ML3, ML4 and ML2 may allow for more grains per panicle and high grain yield potential (Figure 4.3). Also, these mutants exhibited a high number of branches per panicle associated with a high number of grains for targeted selection. These traits may increase the number of spikelets and grains per panicle to improve yield potential (Hmon et al., 2013; Burow et al., 2014). Therefore, these mutant lines will be evaluated for grain yield potential under water-limited environments of Namibia. The variation in panicle morphology of the newly-derived mutants indicated the genetic influence of gamma radiation on genes associated with panicle structure.

Root-system architecture is helpful for maximising water absorption under dry environments (Nippert et al., 2012; Correa et al., 2019; Parra-Londono et al., 2018). The newly developed mutants showed variation in root-system architecture traits for selection and breeding for enhanced drought tolerance under water-limited environments of Namibia (Figure 4.4). The mutant lines ML 14, ML 18, and ML 20 with longer roots and high root volume are ideal genetic resources for higher drought tolerance or cultivation in drought-prone environments.

4.5 Conclusions

A mutation breeding program was initiated to develop high-yielding sorghum mutant varieties that are drought tolerant and possess excellent nutritional qualities for cultivation in Namibia. Induced mutagenesis on the widely cultivated and locally adapted sorghum variety Macia using gamma-radiation (350 Gy), resulted in selection of 20 superior candidate mutant lines based on phenotypic traits. The present study on the genetic profile of the elite mutant lines and five check varieties using 17 polymorphic diagnostic simple sequence repeat (SSR) markers amplified a total of 50 alleles to select genetically distinct lines for further breeding or genetic advancement. The mean number of alleles, effective alleles per locus, observed heterozygosity and expected heterozygosity were 2.94, 1.96, 0.09 and 0.45, respectively, indicating moderate genetic differentiation of the tested lines for selection and hybridisation. Moderate to high genetic distance (≥ 0.50) was displayed between drought-tolerance and high-yield performance and aided in selecting mutant lines such as ‘ML2, ML3, ML4, ML7 and ML14’ compared with the check varieties Macia, Kotovara, ICSR 137, and ICSV 17004’. The selected lines are a useful source of genetic variation for breeding high-yielding and drought-tolerant varieties suited for the drought-prone environments of Namibia.

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Chapter 5. Genotype by Environment Interaction of Newly Developed Sorghum Lines in Namibia

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Abstract

The magnitude of genotype by environment interaction (GEI) is crucial for selecting high performing and adapted genotypes for targeted breeding. The aim of the study was to determine GEI of newly-developed mutant and traditional sorghum lines for grain yield and yield related traits for drought-prone areas of Namibia. Fifty sorghum genotypes were evaluated under field conditions using a 10 x 5 alpha lattice design with three replications. The experiments were carried out in four environments with two growing seasons in Namibia. Data were collected on grain yield and related traits and subjected to the Additive Main Effects and Multiplicative Interaction (AMMI) model. The AMMI model showed that 93.9% of the total genetic variation was attributed to days to 50% flowering (DF), while 94.04% of the variation was due to plant height (PH), 86.52% to panicle weight (PW), 70.67% to thousand-grain weight (TGW), and 90.68% to grain yield (GY). The larger variations attributed to genotypic effects for PL (36.3%), TGW (33.2%) and PH (20.7%) are useful for genotype selection for yield related traits. Based on a multi-trait biplot and Best Linear Unbiased Prediction (BLUPs) analyses of the GEI data across all drought-prone testing environments, the medium maturity mutant line designated as L7P9-13 was selected as the best yielding (2 tons/ha) and recommended for drought-prone area of Namibia.

Keywords: AMMI; BLUEs; BLUPs; drought tolerance, mutation breeding; sorghum

5.1 Introduction

Sorghum (*Sorghum bicolor* [L.] Moench) is a multi-purpose crop grown for food and fodder, notably in tropical and subtropical agro-ecologies of Africa and Asia (Taylor, 2019). The grains of improved varieties are rich in protein and essential nutrients (carbohydrates, fat, fibre, calcium, iron, and zinc) (Adebo, 2020; Drub et al., 2021; Dabija et al., 2021). In addition the crop is a vital source of vitamins (vitamin B1 and B6) and phytochemical compounds, including phenolic and flavonoids (Khalid et al., 2022). Sorghum grain is popular for preparation of several food products and brewing industry in Africa (Kutyauripo et al., 2009; Awika, 2017; Raigond et al., 2019; Miafo et al., 2022). Currently, the estimated world production of sorghum is 68.3 million tons per annum from 44.6 million ha of land (FAOSTAT, 2022). Africa accounts for 44.5% (30.4 million tons) of the world's sorghum production from an estimated cultivated area of 30 million hectares.

The mean yield of sorghum in Namibia is 0.2 tons/ha compared to the relatively high average yields recorded in other Southern African Development Community (SADC) countries, including South Africa (3.7 tons/ha), Botswana (1.8 tons/ha), Malawi (1.2 tons/ha) and Tanzania (1.1 tons/ha) (FAOSTAT, 2022). The cultivation of unimproved and low-yielding varieties are the main cause of the yield gap in Namibia and other SADC countries (Wanga et al., 2022). Variety Macia (SDS 3220) introduced in Namibia from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in the 1990 and farmers' varieties such as Red sorghum, Ekoko, Okambete, Makonga, Kamburo, Nkutji, Katoma, Fuba, Dommy, Kawumbe, and Okatombo are widely grown in diverse agro-ecologies (Wanga et al., 2022). However, the low yield levels of the obsolete and other traditional and unimproved varieties is not profitable to farmers and markets (Wanga et al., 2018). No other modern varieties are locally bred and released with high-yields and local adaptation for production in Namibia. These necessitated the initiation of a collaborative mutation breeding program by the Ministry of Agriculture and Land Reform (MAWLR)/Namibia and the Food and Agriculture Organization (FAO) of the United Nations, and the International Atomic Energy Agency (IAEA) to develop and release high yielding varieties with traits desired by farmers and the marketplace (FAO/IAEA, 2018).

Induced mutagenesis using gamma irradiation has been recommended as a suitable breeding approach to creating genetic diversity for developing improved cultivars with economic traits (Mba et al., 2010). Sorghum lines developed via gamma irradiation are catalogued in the Mutant Variety Database of the IAEA (MVD, 2022). Reportedly, gamma irradiation created marked genetic variation for yield and yield related traits, including variable maturity, short plant height, long panicle, white grain colour and large grain size (Kenga et al., 2006; Human et al., 2011).

Multi-environment trials (MET) are an integral part of crop breeding programs aiming to release and recommend well-suited genotypes for adoption and large-scale production for the target environments as per the farmers' trait preferences. However, genotypes tested across MET tend to vary in performance for client-preferred traits (Katsenios et al., 2021; Enyew et al., 2021; Gerrano et al., 2022). This requires an understanding of genotype by environment interaction (GEI) to aid selection for best-performing genotypes to recommend for cultivation in different production environments (Baker, 1988; Yan and Kang, 2002). GEI has been reported as the main cause of sorghum genetic variation for grain yield and related traits (Hamidou, et al. 2019; Ndiaye et al., 2019; Mumtaz et al., 2019). The GEI and stability responses for yield and yield related traits were used for selection and recommendation of newly bred sorghum genotypes for release and commercialization (Delgado et al., 2019; Belay et al., 2020; Admas and Tesfaye, 2017). To analyse GEI, various statistic models such as the Additive Main Effects and Multiplicative Interaction (AMMI) has been widely used (Yan and Kang, 2002; Gauch, 2006; Gauch et al., 2008). AMMI model is useful for selecting the best-performing genotypes with specific and wide adaptation (Andiku et al., 2020; Seyoum et al., 2020; Enyew et al., 2021; Worede et al., 2021).

Systematic research on the effect of genotype, environment, and their interactions on newly bred sorghum genotypes for cultivation in drought-prone agro-ecologies is limited in Namibia. There is a need to undertake multi-location evaluation in targeted production environments to determine yield performance and stability to identify and recommend suitable sorghum genotypes for official registration, release, commercialization and large-scale production. Therefore, the objective of this study was to determine the GEI of newly-developed elite sorghum mutants for grain yield and yield related traits for drought-prone areas in Namibia.

5.2 Materials and methods

5.2.1 Plant material

Fifty sorghum genotypes, including 10 newly-developed mutant lines (M_9 generations), 33 landraces, two sorghum varieties widely grown in Namibia, and five standard check varieties are presented in Table 5.1. The seed of the variety Macia widely cultivated in Namibia was gamma-irradiated at 350 Gy at the IAEA Laboratories, Seibersdorf, Austria, which created a variation for yield and yield components (Wanga et al., 2020). This resulted in individual plant selection of mutants in the M_2 generation. The selected plants were advanced and subjected to the pedigree selection procedure from the M_3 to M_5 generations allowing the development of genetically stable mutant populations. The mutant lines were selected based on superior agronomic performance, including short-to-medium plant height, early-to-medium maturity, and high grain yield and drought tolerance. The selected elite mutant pure lines were at the M_9 generation and are a useful genetic resource for variety release and local breeding programs. The landraces were selected from 150 landraces accessions sourced from the National Botanical Research Institute (NBRI), Namibia. The two improved varieties were introduced from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in 1999. Genotypes ICSR 55 and ICSR 59 were sourced from the ICRISAT, India, whereas varieties Pahat, Samurai I and Samurai II were sourced from the National Nuclear Energy Agency (BATAN), Indonesia, and were used as comparative controls.

Table 5.1: The list of sorghum genotypes with their origin, seed colour and crude protein content (%).

Code	Genotype	Origin	Seed colour	CP (%)	Code	Genotype	Origin	Seed colour	CP (%)
G01	Macia	ICRISAT	White	9.33	G26	NAM 1195/2	Omusati	Red	9.20
G02	Red Sorghum	MAWLR	Red	7.93	G27	NAM 3798	Omusati	Tan	9.32
G03	L3P15-13	MAWLR	Buff	11.60	G28	NAM 3821	Omusati	White	7.78
G04	L3P15-16	MAWLR	Tan	7.55	G29	NAM 3857	Omusati	Red	7.32
G05	L3P15-40	MAWLR	Buff	8.39	G30	NAM 3872	Omusati	Red	11.00
G06	L3P15-9	MAWLR	White	8.42	G31	NAM 707/2	Omusati	White	9.80
G07	L7P7-3	MAWLR	Tan	9.20	G32	NAM 802/2	Omusati	Red	9.59
G08	L7P9-13	MAWLR	Red	6.71	G33	NAM 858/2	Omusati	White	9.16
G09	L7P9-14	MAWLR	White	7.24	G34	NAM 858/2/1	Omusati	White	8.77
G10	L7P9-2	MAWLR	Tan	6.85	G35	NAM 859/2	Omusati	White	9.49
G11	L7P9-4	MAWLR	Tan	9.31	G36	NAM 868/2	Omusati	White	10.90
G12	L7P9-9	MAWLR	Tan	9.05	G37	NAM 972/2	Omusati	White	11.20
G13	NAM 1676	Kavango East	Red	8.76	G38	NAM 972/2/1	Omusati	Red	9.75
G14	NAM 405/2	Kavango West	Red	6.40	G39	NAM 984/2	Omusati	Tan	11.40
G15	NAM 454/2	Kavango West	Red	8.85	G40	NAM 980/2	Oshana	White	9.18
G16	NAM 476/2	Kavango West	White	8.69	G41	NAM 100/2	Zambezi	White	10.20
G17	NAM 543/2	Kavango West	White	10.10	G42	NAM 1116/2	Zambezi	Red	8.45
G18	NAM 640/2	Kavango West	Red	9.09	G43	NAM 52/2	Zambezi	White	8.27
G19	NAM 1220/2	Kunene	Tan	9.94	G44	NAM 57/3	Zambezi	White	10.90
G20	NAM 1221/2	Kunene	Red	7.82	G45	NAM 60/2	Zambezi	White	9.88
G21	NAM 1831/3	Kunene	White	9.75	G46	ICSR 55	ICRISAT	White	8.93
G22	NAM 1856/3	Kunene	Buff	9.29	G47	ICSR 59	ICRISAT	White	7.65
G23	NAM 1057	Ohangwena	White	9.05	G48	Pahat	BATAN	White	11.70
G24	NAM 1058	Ohangwena	Red	11.00	G49	Samurai I	BATAN	White	9.01
G25	NAM 1058/2	Ohangwena	Red	10.20	G50	Samurai II	BATAN	White	7.21

CP = % crude protein; ICRISAT = International Crop Research Institute for the Semi-Arid Tropics, India; MAWRL = Ministry of Agriculture, Water and Land Reform, Namibia; BATAN = National Nuclear Energy Agency, Indonesia. Landraces and their regions of in Namibia.

5.2.2 *Description of study*

5.2.3 *Study sites*

Field experiments were conducted at two sites of the Crop Research Stations of the Ministry of Agriculture, Water and Land Reform/Namibia, namely, Mannheim in Oshikoto Region and Bagani in Kavango East Region of Namibia across two seasons (2019/2020 and 2020/2021) (Table 5.2). The two sites and two seasons provided four environments in the present study and denoted as: E1 - 2019/20 rainy season at Mannheim, E2 - 2020/21 rainy season at Mannheim, E3 -2019/20 rainy season at Bagani and ‘E4 2020/21 rainy seasons at Bagani. Planting in environments E1, E2, E3 and E4 were conducted on the 16 January 2020, 23 November 2020, 17 January 2020 and 15 January 2021, respectively. The average rainfall across all environments was below 500 mm per annum, representing the drought-prone production environments of Namibia.

Table 5.2: Description of the study area during 2019/20 and 2020/21.

Month	Temperature [°C]				Humidity [%]		Rainfall (mm)		Site information		
	Minimum		Maximum								
	Mannheim										
	E1	E2	E1	E2	E1	E2	E1	E2	Latitude	Longitude	Altitude
October	9.6	12.4	39.4	40.4	29.0	40.9	0.0	0.0	19°10' S	17°45' E	1222 masl
November	11.2	12.0	39.4	40.5	39.7	48.1	11.1	51.1			
December	15.5	14.7	38.6	39.3	59.6	51.2	131.9	88.0			
January	15.3	18.8	36.7	33.4	62.4	62.9	70.0	199.1			
February	15.3	15.6	35.5	34.5	67.1	57.5	215.0	151.5			
March	13.2	11.1	33.9	37.2	67.6	58.2	55.0	85.75			
April	10.2	9.1	32.7	32.3	65.8	48.3	42.0	0.0			
May	3.9	0.5	31.1	31.6	68.1	47.1	0.0	0.0			
Month	Bagani										
	E3	E4	E3	E4	E3	E4	E3	E4	Latitude	Longitude	Altitude
October	11.5	18.2	41.2	39.6	22.0	17.8	0.0	3.5	18°05' S	21°33 E	1008 masl
November	16.3	13.6	39.2	39.7	46.2	50.6	14.0	131.0			
December	15.0	19.2	38.7	35.4	56.2	65.5	50.9	59.0			
January	17.3	17.8	37.2	32.2	68.0	82.1	101.1	220.0			
February	17.4	16.6	35.7	32.7	68.9	52.4	137.0	114.0			
March	17.9	12.7	33.2	35.4	73.0	58.5	132.0	85.0			
April	11.1	7.5	34.8	33.2	58.4	57.6	24.0	0.0			
May	7.0	2.0	33.2	31.1	44.1	54.3	0.0	0.0			

E = environment, masl = metres above sea levelE1 - 2019/20 rainy season at Mannheim, E2 - 2020/21 rainy season at Mannheim , E3 -2019/20 rainy season at Bagani and E4 2020/21 rainy seasons at Bagani.

5.2.4 Experimental design and field management

Field trials were conducted using a 10 × 5 alpha lattice design with three replicates. The size of the experimental units were 9 m^2 (3 m × 3 m), and seeds were sown in four rows of 3m long. The spacing was 75 cm between rows and 20 cm between plants, providing a plant density of 66,667 plants/ha. Three to five seeds were sown per hill and thinned to a single plant per hill at two weeks after emergence. Compound synthetic fertilizers consisting of Nitrogen [N], Phosphorus [P] and Potassium [K] with a ratio of 2:3:2 was basally applied at the rate of 200 kg/ha. Urea (46-0-0) was applied at the rate of 100 kg/ha at 35 days after sowing. Standard agronomic management were adopted, including hand weeding and chemical control of fall armyworm (*Spodoptera frugiperda*), aphid (*Melanaphis sacchari* and *Rhopalosiphum maydis*) and armoured cricket (*Acanthoplus discoidalis*). A two-week rotation program of Belt® 480 SC (480 g/L flubendiamide) at 100 mL/ha, Steward® 150 EC (150 g/L Indoxacarb) at 250 mL/ha, and Warlock® 19.2 EC (emamectin benzoate) at 625 ml/ha was adapted for controlling fall armyworm. APEX 500 WDG (pirimicarb [carbamate]) at 17g/10l water was applied for aphid and Bulldock® 25 EC (25 g/L Beta-cyfluthrin) at 60 mL/ha was adapted for armoured cricket.

5.2.5 Data collection

Data on grain yield (GY) and yield related traits were recorded according to methods described by the International Board for Plant Genetic Resources (IBPGR) and ICRISAT (1993). The data collected included days to 50% flowering (DF), plant height (PH), panicle length (PL), panicle weight (PW) and thousand-grain weight (TGW). DF was recorded as a number of days from sowing to the date when 50% flowering of plants per plot was observed. Five randomly selected and tagged plants were used to measure PH, PL and PW. PH was measured in cm from the ground to the tip of the panicle at physiological maturity. PL was measured from the lower panicle branch to the tip of the panicle and expressed in cm. PW in grams was computed, before threshing. GY in kilograms was measured by weighing grains from the middle two rows recorded after threshing and converted to tons/ha. TGW in grams was measured by weighing 1000 randomly sampled grains at 12% moisture content.

5.2.6 Data analysis

5.2.6.1 AMMI analysis

Data collected on grain yield and yield related traits were subjected to AMMI analysis (Gauch, 2006; Gauch et al., 2008; Gauch and Gauch, 2013) using Genstat 18^{h} edition (Payne et al., 2015).

The following AMMI analysis using a linear model (Gauch and Zobel, 1996) was used to determine the effects of genotype, environment and GEI:

$$\bar{\Upsilon}_{ij} = \mu - G_i + \mathrm{E}_j + \sum_{k=1}^{m} \lambda_k \, \alpha_{ik} \, \gamma_{jk} + \rho_{ij}$$

where: $\bar{\Upsilon}_{ij}$ = the observed trait value of the i^{th} genotype in the j^{th} environment, μ = the grand mean, G_i = the mean of the i^{th} genotype minus the grand mean, E_j = the mean of the j^{th} environment minus the grand mean, λ_k = the square root of the eigen value of the k^{th} IPCA axis, α_{ik} and γ_{jk}= the i^{th} genotype and j^{th} environment principal component scores for IPCA axis k, and ρ_{ij}= the deviation from the model.

5.2.6.2 AMMI biplots and stability value

AMMI biplots were constructed in Genstat 18th edition to visualize the relationship between genotypes and the environments for the assessed traits. AMMI stability values (ASV) were calculated using Genstat 18th edition using the Purchase (1997) procedure as follows:

$$AMMI\ Stability\ Value\ (ASV) = \sqrt{\left[\left(\frac{SSIPCA1}{SSIPCA2}(IPCA1)\right)^{2} + [IPCA2]^{2}\right]}$$

where: SSIPCA1 and SSIPCA2 are the sum of squares for interaction principal component axis 1 (IPCA1) and interaction principal component axis 2 (IPCA2). The ASV is the distance from zero in a two-dimensional scatter plot of IPCA1 scores against IPCA2 scores. Low ASV indicates high genotype stability, whereas high ASV indicates low stability.

5.2.6.3 Estimates of best linear unbiased predictors (BLUPs) and best linear unbiased estimates (BLUEs)

BLUPs and BLUEs for yield and yield-component traits were computed using Multi-Environment Trial Analysis with R (META-R) Version 6.0 using a random-effects model (Piepho, 1994; Alvarado et al., 2020). Genotypic main effects under a random effect model is referred to as the best linear unbiased predictors (BLUPs). Genotypic main effects under a fixed model effects are termed the best linear unbiased estimates (BLUEs) (Haslett and Puntanen, 2009). The linear model for combined analysis across environments based on the lattice procedure is given below:

$$\bar{\mathrm{Y}}_{ijk} = \mu + Env_i + \mathrm{Rep}_j\ (\mathrm{Env})_{\mathrm{I}} + \mathrm{Block}_k(Env_i\mathrm{Rep}_j) + \mathrm{Gen}_k + Env_i \times \mathrm{Gen}_k + \varepsilon_{ijk}$$

where: $\bar{\mathrm{Y}}_{ijk}$ = the effect of ith environment, jth replications and kth genotype on the trait of interest, μ = the overall mean effect, Env_i = the effects of the ith environment, Rep_j = the effect of the jth replication, $\mathrm{Rep}_j\ (\mathrm{Env})_{\mathrm{I}}$ = the effect of jth replications within ith environment, Block$_k$ (Env$_i$Rep$_j$) = the effect of the kth incomplete block within the ith environment and jth replicate, Gen_k = the effect of the kth genotype, and ε_{ijk} = the residual/error terms associated with ith environment, jth replications and kth genotype. The replications and blocks were treated as fixed factors to calculate BLUEs, whereas genotypes, environment, and interactions were treated as random factors effects to calculate BLUPs and broad-sense heritability.

5.2.6.4 Correlation and multi-trait biplot analyses

Phenotypic and genotypic correlations between yield and yield related traits across four drought-prone test environments were estimated using META-R version 6.0 (Alvarado et al., 2020). A t-test procedure was used to determine the significance level for each pair of traits in the correlation matrix. A multi-trait biplot using BLUPs estimates was used to summarise the associations for yield and yield-component traits involving 50 sorghum genotypes across four drought-prone testing environments. The biplot was constructed using FactoMineR (Lê et al., 2008) and factoextra (Kassambara and Mundt, 2016) in R version 4 (R Core Team 2020).

5.3 Results

5.3.1 AMMI analysis for yield and yield related traits

AMMI analysis revealed a highly significant ($p \leq 0.001$) effect of genotype, environment and GEI for grain yield and yield related traits (Table 5.3). Environment accounted for 42.6% variation for DF compared to 27.4% and 17.4% of the variation for genotype and GEI, respectively. The explained GEI value of 93.8% was recorded for DF, attributing to 79.1% and 14.7% for IPCA1 and IPCA2, respectively. For PH, the environment effect accounted for 64.4% of the variation, whereas genotype and GEI contributed 20.7% and 10.1%. GEI explained that 94.1% of the variation for PH, of which IPCA1 and IPCA2 accounted for 78.8% and 15.3%, respectively. Genotype accounted for 36.3% of the variation for PL, whereas 17.8% and 11.3% were attributed to GEI and environmental effects. Only IPCA1 was significant in the AMMI model for PL contributing 47.2% of the variation of the GEI effect. For PW, environment, GEI and genotypic effects accounted for 39.4%, 19.4% and 11.5% of the variations, in that order. Explained GEI effect for the total variation on PW was 86.8% attributed to 46.3% and 40.2% by IPCA1 and IPCA2. Genotypic effect accounted for 33.2% of the variation for TGW compared to 24.1% and 12.2% attributed to GEI and environmental effects. A total of 70.7% variation for GEI on TGW was contributed by IPCA1 only, IPCA2 was not significant. Environment accounted for 47.8% of the sum of squares larger than 18.1% for GEI and 7.8% for genotypic effect for GY. The AMMI model explained that 90.7% of the variation for GEI for GY and attributed to 65.3% and 25.4% of the explained variation for IPCA1 and IPCA2, respectively. GEI effect was larger for DF and TGW. Based on the number of significant IPCAs for assessed traits in the AMMI model, AMMI 1 model was the best fit for PL and TGW. AMMI 2 was the best diagnostic model for DF, PH, PW and GY.

Table 5.3: AMMI model summary showing the sum of squares (SS), mean-squares (MS) explained variance and significant tests for yield and yield-component traits for 50 sorghum genotypes evaluated across four drought-prone environments in Namibia.

Trait		**DF (days)**			**PH (cm)**			**PL (cm)**		
Source	**d.f.**	**SS**	**MS**	**TV (% SS)**	**SS**	**MS**	**TV (% SS)**	**SS**	**MS**	**TV (% SS)**
Total	599	102713.0	171.0		4593100.0	7668.0		9069.0	15.1	
Treatments	199	89620.0	450.0**		4372850.0	21974.0**		5936.0	29.8**	
Genotypes (G)	49	17832.0	364.0**	17.4	948534.0	19358.0**	20.7	3296.0	67.3**	36.3
Environments (E)	3	43717.0	14572.0**	42.6	2958425.0	986142.0**	64.4	1027.0	342.5**	11.3
Block	8	1838.0	230.0**		34838.0	4355.0**		121.0	15.1*	
GEI	147	28072.0	191.0**	27.3	465892.0	3169.0**	10.1	1612.0	11.0**	17.8
IPCA1 (GEI)	51	22239.0	436.0**	79.2	366956.0	7195.0**	78.8	761.0	14.9**	47.2
IPCA2 (GEI)	49	4121.0	84.0**	14.7	71188.0	1453.0**	15.3			
Residuals	47	1712.0	36.0		27749.0	590.0		851.0	8.9	
Error	391	11255.0	29.0		185411.0	473.0		3012.0	7.7	
Trait		**PW (g)**			**TGW (g)**			**GY (t ha^{-1})**		
Source	**d.f.**	**SS**	**MS**	**TV (% SS)**	**SS**	**MS**	**TV (% SS)**	**SS**	**MS**	**TV (% SS)**
Total	599	431415.0	720.0		15090.0	25.2		469.6	0.8	
Treatments	199	303201.0	1524.0**		10486.0	52.7**		345.9	1.7**	
Genotypes (G)	49	49442.0	1009.0**	11.5	5010.0	102.2**	33.2	36.6	0.7**	8
Environments (E)	3	170117.0	56706.0**	39.4	1841.0	613.8*	12.2	224.6	74.9**	48
Block	8	17345.0	2168.0**		1240.0	155.0**		10.5	1.3**	
GEI	147	83642.0	569.0**	19.4	3634.0	24.7**	24.1	84.8	0.6**	18
IPCA1 (GEI)	51	38727.0	759.0**	46.3	2568.0	50.4**	70.7	55.4	1.1**	65
IPCA2 (GEI)	49	33644.0	687.0**	40.2				21.5	0.4*	25
Residuals	47	11271.0	240.0		1066.0	11.1		7.8	0.2	
Error	391	110869.0	283.0		3365.0	8.6		113.2	0.3	

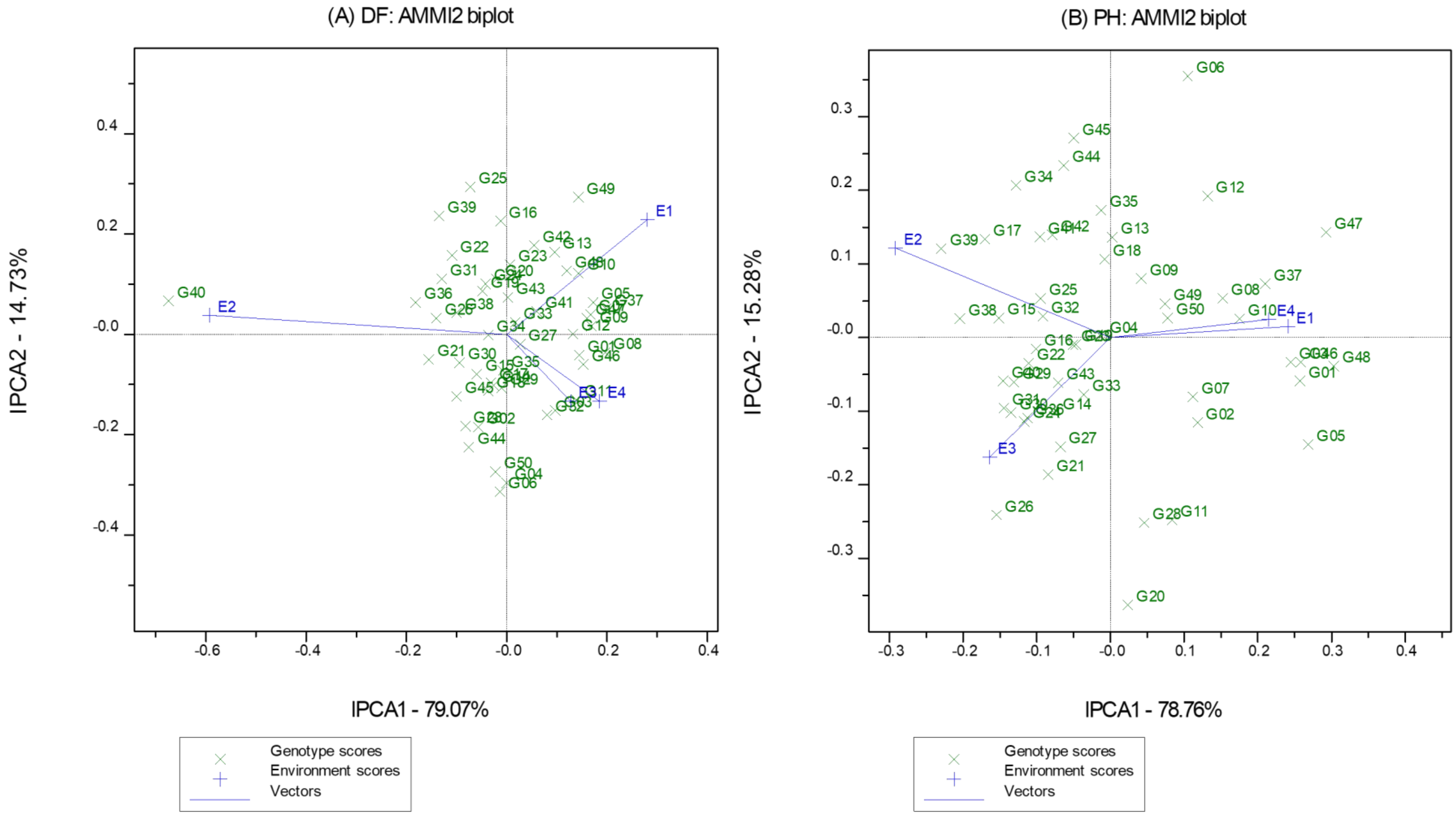
DF = days to 50% flowering, PH = plant height (cm), PL = panicle length (cm), PW = panicle weight (g/panicle), TGW = thousand grain weight (g/1000-grain), GY = grain yield (tons/ha), IPCA = interaction principal component analysis, GEI = genotype-by-environment interaction, d.f. = degrees of freedom, * and ** denote significant at 5% and 1% probability level, respectively, ns = non-significant.

5.3.2 AMMI biplots and stability values for yield and yield related traits

AMMI biplots showing explained variation due to GEI among sorghum genotypes tested across test environments are presented in Figure 5.1. AMMI model 1 biplot (IPCA1 vs Genotype means) is presented for PL and TGW due to IPCA1 being significant in the AMMI model (Table 5.3). AMMI model 2 biplots are presented for DF, PH, PW and GY based on the significant IPCA1 vs IPCA2 effect in the AMMI model summary (Table 5.3). Based on high positive or negative IPCA1 and IPCA2 scores, genotypes such as G06, G08, G25, G37, G39, G40 and G49 were unstable across the test environments for DF. These genotypes were located furthest from the biplot origin. Stable genotypes for DF included G23, G29, G33, G35 and G43, recording ASV of <0.95. These genotypes had IPCA1 and IPCA2 scores close to zero and were located near the biplot origin. Environments E2 was the most discriminatory for DF among the sorghum genotypes with the longest vector, followed by environment E1. The least discriminating environments for DF were environments E3 and E4, with the shortest vectors from the biplot origin.

According to IPCA1 and IPCA2 scores for PH, genotypes G05, G38, G39, G47 and G48 were the least stable, recording ASV of >19. Genotypes near the biplot origin such as G04, G18, and G35 were the most stable for PH and recorded ASV values of 1.24, 1.53 and 2.47, respectively. Environment E2 followed by environment E1 had the longest vectors from the biplot origin and were the most discriminatory environments for PH among the tested sorghum genotypes. Environments E3 and E4 were least discriminating for PH. AMMI1 biplot summaries the main effect of genotypes and environments against the IPCA1 score, thus showing interaction effects. For PL, genotypes such as G04, G14, G39, G42 and G43 recorded large IPCA1 scores and were the least stable, recording ASV of >1.5. ASV values of <0.4 were identified in genotypes G01, G03, G10 and G37, being the most stable for PL. These genotypes had low interaction with the environment. Contrastingly, genotypes G04, G39, G43 and G44 were located furthest from the biplot origin because of their high interaction with the environmental variance.

Based on larger IPCA1 and IPCA2 scores, genotypes such as G06, G08, G17, G20, G25, G39 and G46 were the least stable for PW and recorded ASV values of >2.4. Stable genotypes with lower ASV value of <0.6 were G03, G14, G15, G23, G37 and G45 and were placed near the biplot origin. G06, G08, G12, G20, G27 and G39 had high interaction with the environment. Based on longer vectors, environments E3 and E4 were the most discriminatory for PW. The least discriminating environment for PW was environment E1, with the shortest vector from biplot origin. Genotypes such as G09, G21, G24, G24, G49 and G50 with more significant IPCA1 scores were the least stable for TGW. Stable genotypes including G01, G04, G17, G19, G37 and G42 with ASV value of <0.9 were displayed near the biplot origin. These genotypes had low interaction with the environment. Environments E1 followed by E2 was the most discriminatory for TGW. Environments E4 followed by E3 located near the biplot origin were the least discriminating environments for TGW. Based on larger IPCA1 and IPCA2 scores for GY, genotypes such as G36, G08, G20, G40, and G06 were the least stable, with high environmental interaction. These genotypes were located further away from the biplot origin and recorded ASV values of >0.8. Stable genotypes with lower ASV value of <0.2 were G10, G15, G16, G21, G23 and G43 and were displayed near the biplot origin. These genotypes show low interaction with the environment. Environment E3 had the longest vector, followed by E2 and was the most discriminatory environment for GY. Environments E1 and E4 had shorter vectors from biplot origin and were identified as the least discriminating environments for GY.



(A) DF: AMMI2 biplot

(B) PH: AMMI2 biplot

Figure 5.1: AMMI biplots showing relationships of the 50 sorghum genotypes and four testing environments for days to 50% flowering (A), plant height (B), panicle length (C), panicle weight (D), thousand grain weight (E) and grain yield (F). E1 - 2019/20 rainy season at Mannheim, E2 - 2020/21 rainy season at Mannheim, E3 -2019/20 rainy season at Bagani, and E4 2020/21 rainy seasons at Bagani, G1-G50 = genotype codes as presented in Table 5.1.

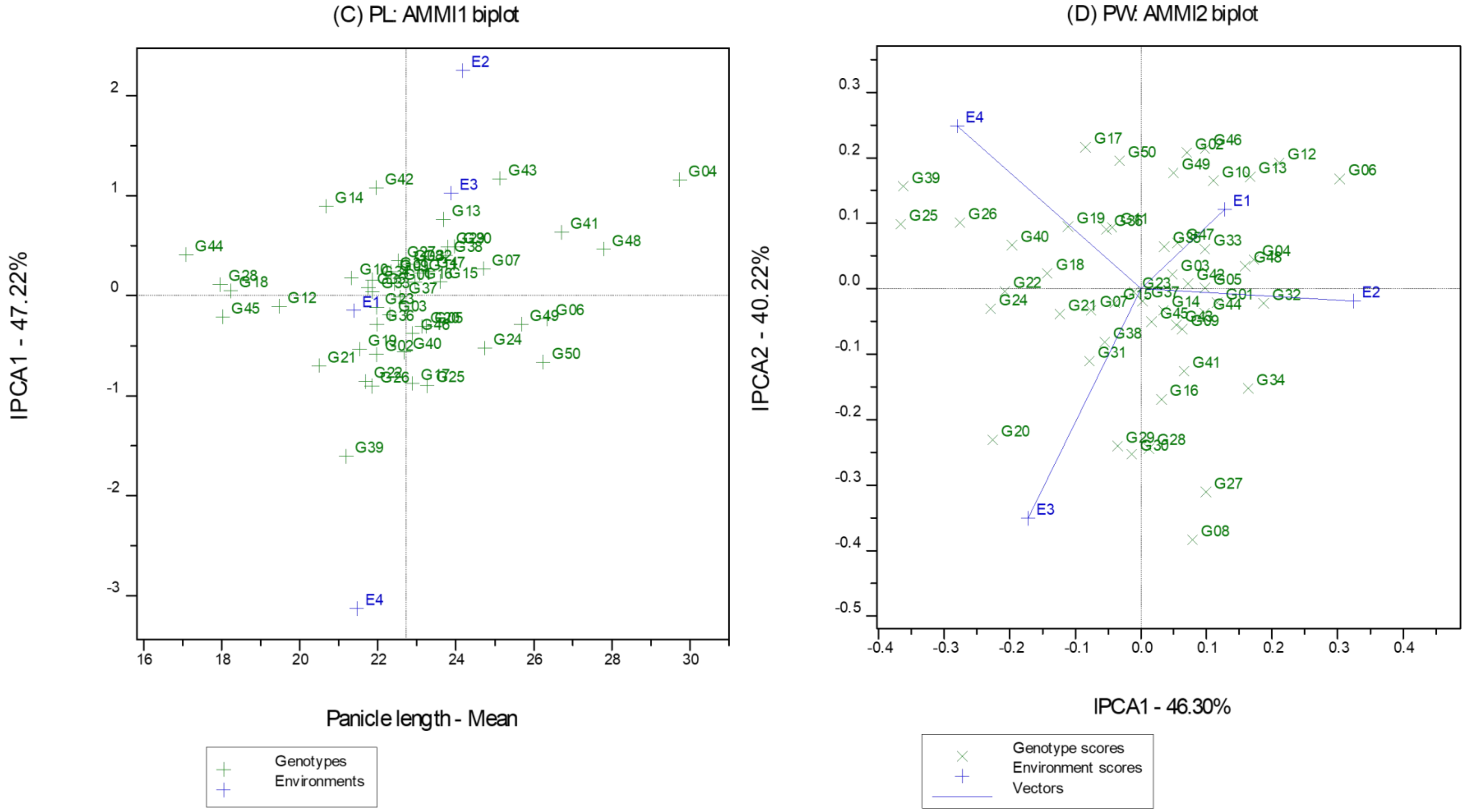


Figure 5.1: Continued

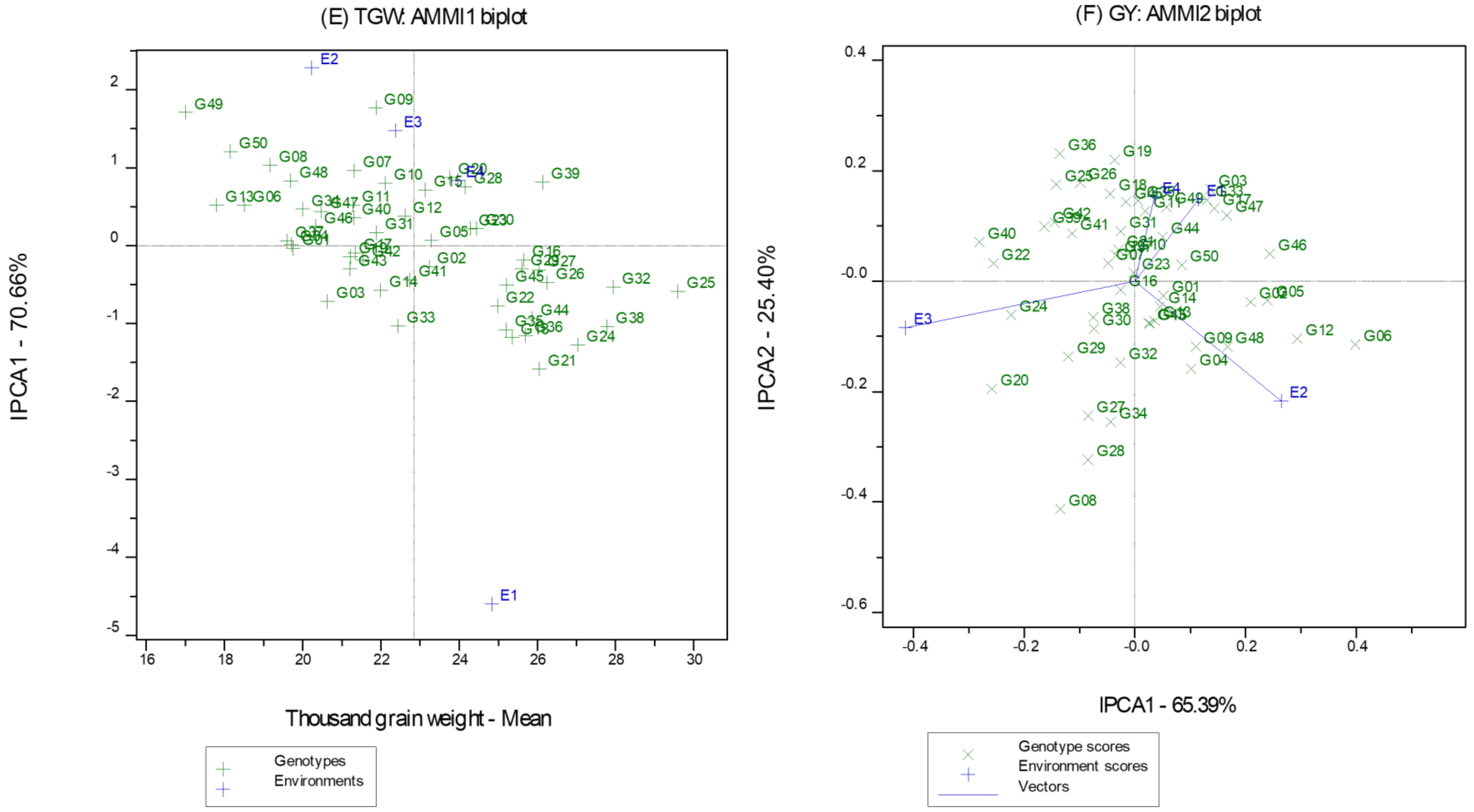


Figure 5.1: Continued

5.3.3 Identification of best-performing sorghum genotypes

The AMMI model showing the best-performing sorghum genotypes for assessed traits in four drought-prone environments of Namibia are presented in Table 5.4. AMMI model 1 identified G17 as a superior genotype for DF in environments E3 and E4, and genotypes G25 and G40 as higher performers for DF in environments E1 and E2, respectively. Top performers for DF based on AMMI model 2 family were genotype G39 in environments E1 and E2, and G50 in environments E3 and E4. Based on AMMI model 3, genotypes G49, G26, G40 and G6 were top performers for DF in environments E1, E2, E3 and E4, in that order. According to AMMI model 4 family, the best performers for DF were genotype G24 in environments E1 and E4, and genotypes G36 and G06 in environments E2 and E3, respectively. For PH, AMMI model 1 family identified genotype G31 as the top performer for PH in environments E1 and E4, and genotypes G39 and G26 in environments E2 and E3, respectively. AMMI model 2 family identified genotype G40 as the top performer for PH in environments E1 and E4, and genotypes G17 and G31 in environments E2 and E3, respectively. AMMI model 3 family identified G25 as top performer for PH in environments E1 and E4, whereas G31 and G40 were best performing genotypes in environments E2 and E3. AMMI model 4 family identified G26 as the top performer for PH in environments E1 and E4, and genotypes G40 and G30 in environments E2 and E3, respectively.

According to AMMI model 1 family, genotype G48 was the top performer for PL in environments E1 and E3, whereas genotypes G04 and G50 were identified as high performers in environments E2 and E4. AMMI model 2 family identified G04 as the top performer for PL in environments E1, E3 and E4, respectively, and genotype G41 was the highest performer for PL in environment E2. AMMI model 3 family identified genotypes G50, G43, G41 and G06 for PL in environments E1, E2, E3 and E4, in that order. According to the AMMI model 4 family, genotypes G06, G38, G50 and G49 were identified the best for PL in environments E1, E2, E3 and E4, respectively. For PW, the AMMI model 1 family identified genotypes G02, G08, G20 and G39 as best performers in environments E1, E2, E3 and E4. AMMI model 2 family identified G06 as the top performer for PW in environments E1 and E2, and genotypes G08 and G25 as the best performers in environments E3 and E4. AMMI model 3 family identified G28 as the top performer for PW in environments E2 and E3, whereas genotypes G08 and G26 were the best for PW in environments E1 and E4. AMMI model 4 family identified genotypes G39, G05, G27 and G24 as the best performers for PW in environments E1, E2, E3 and E4, in that order.

AMMI model 1 family identified G25 as the top performer for thousand-grain weight in environments E2 and E4, whereas G21 and G39 were the best performers in environments E1 and E3. AMMI model 2 family identified G32 as the top performer for thousand-grain weight in environments E2 and E4, whereas G24 and G27 were identified as the best performers in environments E1 and E3. According to AMMI model 3 family, genotypes G38 was top performer for thousand-grain weight in environments E1 and E4, whereas G16 and G25 were best performing under environments E2 and E3, respectively. AMMI model 4 family identified genotypes G25, G15, G24 and G39 as top-performing for thousand-grain weight in environments E1, E2, E3 and E4, in that order. For GY, the AMMI model 1 family identified G08 as the top performer in environments E2 and E4, whereas G02 and G25 were the best performers in environments E1 and E4. AMMI model 2 family identified G25, G06, G20 and G21 as best performing genotypes for GY in environments E1, E2, E3 and E4. AMMI model 3 family identified G40 as the top performer for GY in environments E3 and E4, whereas G21 and G28 were the best performers in environments E1 and E2, respectively. AMMI model 4 family identified G06, G02, G28 and G02 for GY in environments E1, E2, E3 and E4, respectively.

Table 5.4: AMMI model family showing winning sorghum genotypes for grain yield and yield-component traits in four drought-prone environments of Namibia.

Trait	ENV	Mean	IPCA1	IPCA2	Score	AMMI model family			
						1	2	3	4
DF	E1	98.91	-3.75	4.68	-3.75	G25	G39	G49	G24
	E2	89.87	7.94	0.77	7.94	G40	G39	G26	G36
	E3	75.30	-1.72	-2.74	-1.72	G17	G50	G40	G06
	E4	85.04	-2.48	-2.72	-2.48	G17	G50	G06	G24
PH	E1	133.80	-9.78	-0.91	-9.78	G31	G40	G25	G26
	E2	295.80	11.74	-7.33	11.74	G39	G17	G31	G40
	E3	197.60	6.50	9.82	6.50	G26	G31	G40	G30
	E4	115.90	-8.46	-1.58	-8.46	G31	G40	G25	G26
PL	E1	21.38	-0.16	1.48	-0.16	G48	G04	G50	G06
	E2	24.18	2.26	-2.31	2.26	G04	G41	G43	G38
	E3	23.89	1.02	1.95	1.02	G48	G04	G41	G50
	E4	21.45	-3.12	-1.12	-3.12	G50	G04	G06	G49
PW	E1	28.67	2.73	-2.83	2.73	G02	G06	G08	G39
	E2	66.88	7.20	-0.25	7.20	G08	G06	G28	G05
	E3	68.13	-3.16	8.42	-3.16	G20	G08	G28	G27
	E4	41.10	-6.77	-5.34	-6.77	G39	G25	G26	G24
TGW	E1	24.83	-4.61	0.19	-4.61	G21	G24	G38	G25
	E2	20.23	2.28	2.01	2.28	G25	G32	G16	G15
	E3	22.35	1.46	-3.02	1.46	G39	G27	G25	G24
	E4	23.87	0.87	0.82	0.87	G25	G32	G38	G39
GY	E1	0.83	-0.48	0.76	-0.48	G02	G25	G21	G06
	E2	1.98	-1.10	-1.12	-1.10	G08	G06	G28	G02
	E3	1.51	1.70	-0.45	1.70	G08	G20	G40	G28
	E4	0.38	-0.12	0.81	-0.12	G25	G21	G40	G02

DF = days to 50% flowering, PH = plant height, PL = panicle length, GY = grain yield, PW = panicle weight, TGW = thousand-grain weight, ENV = environment, IPCAe = interaction principal component axes for environment, E1 - 2019/20 rainy season at Mannheim, E2 - 2020/21 rainy season at Mannheim, E3 -2019/20 rainy season at Bagani, and E4 2020/21 rainy seasons at Bagani, G1-G50 = genotype codes as presented in Table 5.1. Only best-performing genotypes are shown in Table 5.4.

5.3.4 BLUPs and BLUEs estimates for grain yield and related traits among genotypes

BLUPs and BLUEs estimates for grain yield and yield-component traits for fifty sorghum genotypes across four drought-prone environments in Namibia are shown in Table 5.5. BLUPs and BLUEs estimates for each environment for assessed agronomic traits are presented in supplemental Tables 5.1, 5.2, 5.3 and 5.4. Mean BLUPs and BLUEs for DF was 87 days across test environments. BLUEs for DF varied between 63 and 159 days. Genotypes G40, G17, G39 and G24 were late flowering, recording BLUPs estimates of 97, 93, 92 and 92 days across the test environments. Genotypes G32, G11, G47, G37, G01 and G07 were early maturing, recording BLUPs of 83 and 84 for DF across environments. BLUPs for PH varied between 79.6 and 401.4 cm, whereas BLUEs estimates varied between 64.9 and 408.0 cm. Genotypes G31, G26, G40, G39 and G25 were taller, recording BLUPs estimates of 236.1, 233.6, 232.5, 229.3 and 222.5 cm across environments, in that order. Contrastingly, genotypes G01, G03 and G46 were shorter, recording BLUPs estimates of 114.6, 117.2 and 119.7 cm across environments.

BLUPs for PL varied between 21.0 and 32.1 cm, and BLUEs estimates varied between 18.7 and 37.3 cm. The mean BLUPs and BLUEs estimates for PL was 22.7 cm across test environments. Genotypes G04, G48, G41, G50 and G06 recorded longer BLUPs values of >25 cm for PL, and genotypes G44, G45 and G28 had shorter panicles of <19 cm. Based on BLUPs estimates across all test environments, genotypes G20, G39, G28, G08 and G25 had heavier panicles with BLUPs of 60.0, 59.4, 59.1, 58.9 and 56.8g, in that order. Contrarily, genotypes G49, G12, G36 and G45 recorded low PW of 42.6, 45.5, 45.5 and 46.5g, respectively.

Mean TGW was 22.8g across test environments, which varied from 13.2g to 33.9g (BLUPs) and 11.0g to 35.7g (BLUEs). BLUPs values for TGW were 27.9g for G25, 26.6g for G38, 26.6g for G32, 26.1g for G24 and 25.4g for G26. Genotypes G08, G06, G50, G13 and G49 scored low BLUPs for TGW with estimates of 18.4, 19.1, 19.3, 19.6 and 20.1g across environments, respectively. For GY, the mean value of 1.2 tons/ha was recorded for the tested sorghum genotypes across the four test environments. BLUPs varied from 0.20 and 2.7 tons/ha, whereas BLUEs estimates varied from 0.03 to 3.2 tons/ha. Genotypes G08, G28, G20, G27 and G24 were identified as high grain yielders recording BLUEs values of 1.9, 1.8, 1.7, 1.5 and 1.4 tons/ha across environments, respectively. Genotypes G35, G36, G03, G12, G33, G45 and G49 were identified as poor grain yielders recording BLUEs values of <1 tons/ha. BLUPs estimates of 1.33, 1.30, 1.28, 1.24 and 1.23 tons/ha were recorded for genotypes G08, G28, G20, G27 and G24, which were much higher than other test genotypes.

Table 5.5: Best linear unbiased predictors (BLUPs) and best linear unbiased estimates (BLUEs) for yield and yield-component traits for 50 sorghum genotypes evaluated across four drought-prone test environments in Namibia.

Genotype	DF (days)		PH (cm)		PL (cm)		PW (g)		TGW (g)		GY (tons/ha)	
	BLUPs	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**
G01	84.4	81.2	114.6	100.5	22.6	22.6	49.4	46.2	20.6	19.9	1.2	1.1
G02	86.7	86.0	169.4	166.2	22.1	22.0	54.6	58.9	23.1	23.2	1.2	1.4
G03	87.3	87.2	117.2	103.7	22.4	22.3	49.3	46.4	21.2	20.7	1.1	0.9
G04	86.9	86.4	185.5	185.5	28.8	29.9	50.4	48.7	20.6	19.8	1.2	1.3
G05	85.0	82.5	127.6	116.1	23.1	23.2	54.3	58.3	23.1	23.3	1.2	1.1
G06	88.5	89.9	172.3	169.6	25.7	26.3	50.4	49.8	19.6	18.6	1.2	1.2
G07	84.4	81.1	175.7	173.7	24.2	24.6	53.4	55.9	21.6	21.2	1.2	1.3
G08	85.1	82.9	153.9	147.7	22.6	22.6	58.9	69.6	20.1	19.3	1.3	1.9
G09	84.6	81.6	169.3	166.0	22.1	22.1	53.4	55.6	22.1	21.9	1.2	1.1
G10	85.2	82.8	143.3	134.9	21.6	21.3	46.6	39.8	22.2	22.0	1.1	1.0
G11	83.7	79.8	168.7	165.4	23.0	23.1	52.2	53.0	21.7	21.3	1.2	1.3
G12	87.4	87.6	159.2	154.0	19.9	19.4	45.5	37.7	22.6	22.5	1.1	0.9
G13	85.8	84.3	184.6	184.4	23.8	24.0	47.0	41.0	19.1	17.9	1.2	1.2
G14	86.9	86.3	206.2	210.2	21.0	20.7	48.1	43.5	22.3	22.0	1.2	1.0
G15	86.8	86.2	218.5	224.9	23.5	23.7	53.9	57.1	23.1	23.2	1.2	1.0
G16	88.5	89.9	214.8	220.5	22.8	22.8	53.4	56.9	25.0	25.7	1.2	1.3
G17	92.5	98.1	220.6	227.4	23.0	23.0	49.8	48.2	21.7	21.3	1.1	1.0
G18	86.7	86.1	178.3	176.9	19.0	18.3	52.8	55.2	24.7	25.3	1.2	1.1
G19	88.9	90.7	191.3	192.4	21.8	21.6	48.3	44.4	21.6	21.2	1.1	1.0
G20	88.1	89.1	200.6	203.5	23.1	23.1	60.0	72.0	23.5	23.7	1.3	1.7
G21	89.4	91.8	194.5	196.1	20.8	20.4	55.2	60.6	25.2	26.0	1.2	1.4
G22	90.3	93.5	194.0	195.5	21.9	21.7	48.9	45.2	24.5	25.0	1.2	1.2
G23	88.9	90.7	205.1	208.9	22.1	21.9	52.3	53.9	23.9	24.2	1.2	1.2
G24	91.6	96.2	211.4	216.3	24.4	24.7	55.5	60.8	26.1	27.1	1.2	1.4
G25	90.7	94.5	222.5	229.7	23.1	23.2	56.8	64.4	27.9	29.6	1.2	1.4
G26	91.1	95.1	233.6	243.0	21.9	21.7	51.4	52.3	25.4	26.3	1.1	1.0
G27	86.5	85.7	205.9	209.8	22.6	22.5	53.4	56.6	25.3	26.1	1.2	1.5
G28	87.5	87.7	174.4	172.1	18.8	18.1	59.1	70.0	23.8	24.1	1.3	1.8
G29	85.5	83.5	216.7	222.8	23.8	23.9	52.9	55.7	24.9	25.6	1.2	1.4
G30	86.2	85.1	216.5	222.4	23.6	23.8	51.2	51.2	24.1	24.5	1.2	1.1
G31	89.8	92.4	236.1	246.0	22.4	22.3	51.2	51.3	22.1	21.8	1.2	1.0
G32	83.2	78.8	209.4	214.0	23.0	23.0	49.9	48.1	26.6	27.8	1.2	1.3
G33	85.4	83.4	192.1	193.3	21.9	21.7	47.5	42.3	22.4	22.3	1.1	0.9
G34	86.7	86.0	200.4	203.2	22.0	21.8	51.1	50.2	20.6	19.9	1.2	1.2
G35	85.0	82.6	191.2	192.3	22.0	21.8	47.0	40.8	24.6	25.2	1.1	0.9
G36	88.3	89.5	210.7	215.6	22.1	22.0	45.5	37.5	25.1	25.7	1.1	0.9
G37	84.2	80.8	127.6	116.1	22.6	22.6	50.1	48.2	20.4	19.6	1.2	1.2
G38	89.7	92.2	210.0	214.7	23.7	23.9	55.3	60.5	26.6	27.8	1.2	1.3
G39	91.8	96.7	229.3	237.8	21.6	21.4	59.4	70.7	25.3	26.1	1.2	1.2
G40	96.7	106.7	232.5	241.6	22.6	22.6	52.8	54.3	21.7	21.4	1.2	1.4
G41	85.1	82.7	212.9	218.2	25.8	26.5	49.7	47.0	22.8	22.7	1.2	1.2
G42	86.6	85.7	203.7	207.2	22.2	22.0	47.0	40.6	21.9	21.5	1.2	1.3
G43	88.4	89.6	212.5	217.8	24.8	25.2	52.9	55.2	21.6	21.2	1.2	1.4
G44	87.1	86.8	196.2	198.3	18.2	17.3	49.3	46.5	25.1	25.9	1.1	1.0
G45	85.4	83.3	191.9	193.0	18.7	17.9	46.5	39.4	24.6	25.1	1.1	0.7
G46	84.9	82.3	119.7	106.7	22.9	22.9	51.4	51.7	20.9	20.2	1.2	1.1
G47	83.9	80.1	131.2	120.4	23.3	23.4	50.5	49.0	21.1	20.5	1.1	1.0
G48	86.3	85.3	128.4	117.1	26.8	27.6	48.6	45.3	20.5	19.8	1.1	1.0
G49	86.5	85.6	149.3	142.1	25.1	25.6	42.6	31.3	18.4	17.0	1.1	0.6
G50	89.4	91.7	165.1	161.0	25.8	26.3	49.6	47.9	19.3	18.2	1.1	1.0
V_G	14.6		1349.4		4.6		34.2		6.4		0.0	

VGE	53.7		905.0		1.0		97.1		5.3		0.1	
Mean	87.3		185.9		22.7		51.3		22.8		1.2	
LSD (5%)	7.7	11.1	42.0	45.5	2.4	2.6	12.5	19.1	3.5	4.0	0.3	0.6
CV (%)	6.0	5.9	11.6	11.6	11.8	11.8	30.8	30.8	12.6	12.6	44.1	44.2
Genotype	**	**	**	**	**	**	*	**	**	**	ns	ns
VGE	**	**	**	**	*	*	**	**	**	**	**	**

DF = days to 50% flowering, PH = plant height (cm), PL = panicle length (cm), PW = panicle weight (g/panicle), TGW = thousand grain weight (g/1000-grain), GY = grain yield (tons/ha), VG = genetic variance, VGE = genotype-by-environment interaction variance, CV, coefficient of variation; LSD, least significant difference, * and ** denote significant at 5% and 1% probability level, respectively. ns = non-significant, G1-G50 = genotype codes presented in Table 5.1.

5.3.5 Phenotypic and genotypic correlation coefficients analysis for yield and yield related traits

Phenotypic (*rp*) and genotypic (*rg*) correlation coefficients showing relationships between yield and yield related traits among 50 sorghum genotypes tested across four drought-prone environments based on BLUPs estimates is presented in Table 5.6. GY positively and significantly correlated with DF (*rg* = 0.99; $p \leq 0.001$), and PW (*rp* = 0.80 and *rg* = 0.88; $p \leq 0.001$). DF significantly and positively correlated with PH (*rp* = 0.54 and *rg* = 0.77; $p \leq 0.001$), PL (*rg* = 0.99; $p \leq 0.001$) and TGW (*rg* = 0.59; $p \leq 0.001$). PH showed positive and moderate correlations with PW (*rg* = 0.35; $p \leq 0.05$) and TGW (*rp* = 0.55 and *rg* = 0.77; $p \leq 0.001$). PL displayed negative and moderate correlations with TGW (*rp* = -0.39; $p \leq 0.05$ and *rg* = -0.49; $p \leq 0.001$). PW showed positively and moderate correlations with TGW (*rp* = 0.34; $p \leq 0.05$ and *rg* = 0.41; $p \leq 0.01$).

Table 5.6: Genotypic (above-diagonal) and phenotypic (below diagonal) correlation coefficients showing pair-wise association for yield and yield-component traits among 50 sorghum genotypes evaluated across four drought-prone testing environments of Namibia.

Traits	DF	PH	PL	PW	TGW	GY
DF	-	0.77**	0.05ns	0.99**	0.59**	0.99**
PH	0.54**	-	-0.14ns	0.35*	0.77**	0.29*
PL	-0.06ns	-0.11ns	-	-0.22ns	-0.49**	-0.08ns
PW	0.26ns	0.22ns	0.01ns	-	0.41**	0.88**
TGW	0.24ns	0.55**	-0.39**	0.34*	-	0.13ns
GY	0.12ns	0.19ns	0.09ns	0.80**	0.2ns	-

DF = days to 50% flowering, PH = plant height (cm), PL = panicle length (cm), GY = grain yield (t ha^{-1}), PW = panicle weight (g panicle^{-1}), TGW = thousand grain weight (g 1000-grain^{-1}), * and ** denote significant at 5 and 1% probability level, respectively. ns = non-significant.

5.3.6 *Multi-trait biplot for selection of sorghum genotypes with grain yield and yield-related traits*

A multi-trait PCA biplot showing relationships between yield and yield-component traits and 50 sorghum genotypes across the four drought-prone environments is presented in Figure 5.2. The biplot explained 63.2% of the total variation, of which PC1 and PC2 accounted for 38.7% and 24.5% of the variation, respectively. Based on the biplot, a strong positive relationship was displayed between DF, PH and thousand-grain weight. GY was strongly associated with PW and weakly related with DF, PH, PL and TGW. PL was negatively correlated with DF, PH and TGW. High-performing genotypes for each trait were displayed furthest from the origin of the biplot in the same quadrant of the PCA biplot. Accordingly, genotypes G18, G21, G22, G24, G25, G26, G31 and G40 were grouped being late maturing, taller plants and high TGW. Genotypes G01, G04, G05, G06, G07, G11, G13, G37 and G46 were grouped based on longer PL, whereas genotypes G08, G20 and G28 were grouped based on high PW and GY.

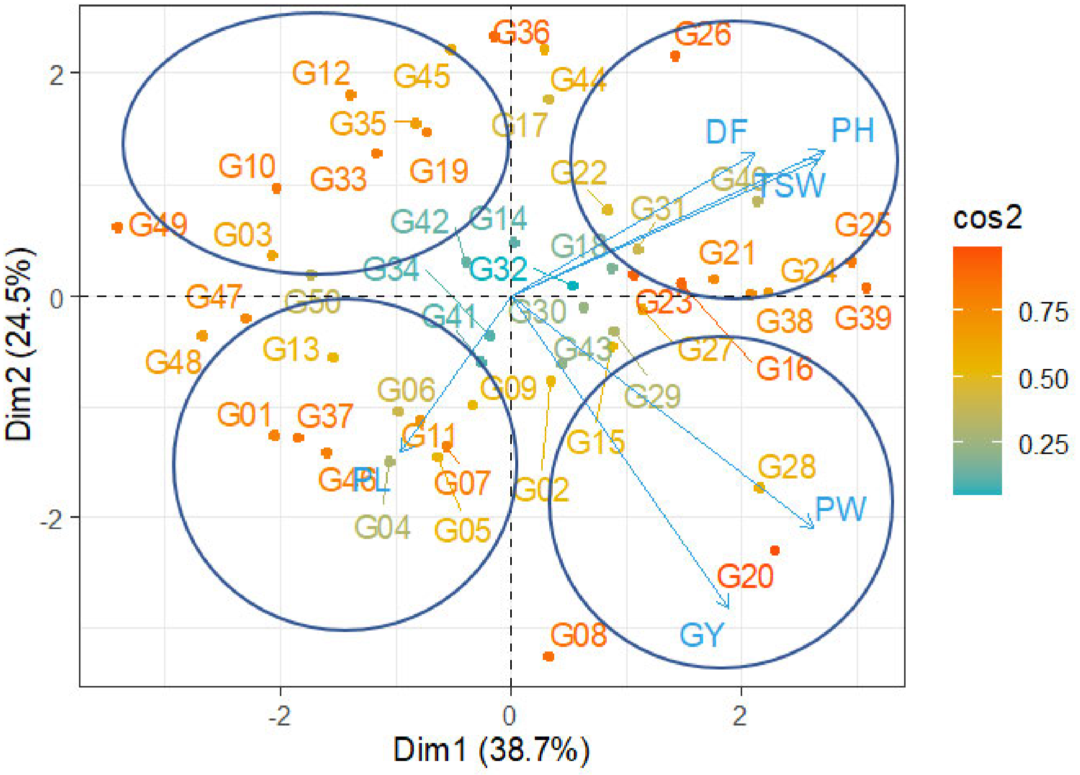


Figure 5.2: Multi-trait biplot of PC1 and PC2 displaying associations between yield and yield-component traits and 50 sorghum genotypes tested across four drought-prone environments of Namibia. DF = days to 50% flowering, PH = plant height (cm), PL = panicle length (cm), GY = grain yield (t ha^{-1}), PW = panicle weight (g panicle^{-1}), TGW = thousand grain weight (g 1000-grain^{-1}), G1-G50 = genotype codes presented in Table 5.1.

5.4 Discussion

Testing of genotypes across environments is a critical step in a breeding program to identify best-performing genotypes for release, registration and production. In the present study, genotype-by-environment interaction was carried out among 50 sorghum genotypes, including ten, newly-developed sorghum mutants derived using gamma radiation.

Genotype by environment analysis in the present study revealed significant variation attributed to genotype, environment and genotype-by-environment for grain yield and yield-component traits (Table 5.3). Large variations were attributed to genotypic effects for traits such as panicle length (36.3%), thousand-grain weight (33.2%) and plant height (20.7%). These indicated large genetic variation in the present sorghum population for genotype selection for yield-component traits. Previously, several studies reported a significant genetic variation in sorghum genetic resources based on phenotypic and genetic markers (Chapman et al., 2000; DeLacy et al., 2010), agreeing with the present study. Enyew et al. (2021) reported a large explained variation due to the genotypic effect of 54.9% for days to flowering, 61.4% for plant height, 66.3% for panicle length and 53.9% for panicle weight in a large population among Ethiopian sorghum genotypes tested in multiple environments. Genetic analysis using SSR markers revealed wide genetic variation among mutant lines derived from Macia (Data not shown), agreeing with the large genotypic variation for yield related traits. For the newly developed sorghum genotypes developed using gamma radiation, mutagenesis could have resulted in unique genetic make-up leading to genetic variation of the sorghum mutants. On the contrary, the present study revealed low variation for grain yield among the tested sorghum genotypes (Table 5.3). In agreement with the present GEI analysis, Rono et al. (2016), Admas and Tesfaye (2017), and Al-Naggar et al. (2018) reported low explained variation of 7.1%, 4.6% and 14.6% for grain yield attributed to genotypic effects in sorghum genotypes tested in multiple environments.

Phenotypic assessment of African sorghum genetic resources revealed high variations in grain yield for improvement (Mofokeng et al., 2017; Mengistu et al., 2020). In the USA and China, several studies also revealed considerable genotypic variation in selecting of high grain sorghum yielders (Abu et al., 2005; Smith et al., 2010; Li et al., 2010; Burow et al., 2012). Despite the low genotypic variation for grain yield in the present study, the newly developed and advanced mutant lines evaluated in the present study yielded much higher than the “old” varieties cultivated in Namibia. For example, the highest yield output of 1.9 tons/ha across environments was recorded for mutants L7P9-13 (G08), higher than 1.4 and 1.1 tons/ha recorded for released varieties Red sorghum and Macia (Table 5.5). The mutant lines L7P9-13 (G08), L7P7-3 (G07), L3P15-16 (G04) and L7P9-4 (G11) recorded grain yields of ≥1.2 tons/ha higher than the yield outputs of Macia (1.1 t/ha) (Table 5.5). These suggested mutation breeding using gramma radiation favoured the expression of grain-yield-promoting alleles, leading to high-yielding mutant lines. Several studies (Human et al., 2012; Brenton et al., 2016; Nikièma et al., 2020) reported the beneficial effect of gamma radiation on grain yield agreeing with the present findings. On the contrary, some mutant lines, including mutant lines L3P15-13 (G03), L7P9-2 (G10) and L7P9-9 (G12) yielded lower (≤1.0 tons/ha) compared to landrace varieties such as NAM 3821 (G28), NAM 1221/2 (G20), NAM 3798 (G27), NAM 1058 (G24) and NAM 1058/2 (G25) (Table 5.5). These suggested that the landraces collected across northern Namibia are a useful genetic resource as they might have evolved to respond to drought stresses and adapted to the farming practices in the environments.

The larger proportion of variations attributed to environmental effect for days to 50% flowering (42.6%), plant height (64.4%), panicle weight (39.4%) and grain yield (47.8%) revealed that the response of these traits were influenced by changes in the prevailing environmental conditions. Namibia is regarded as one of the driest countries in sub-Saharan Africa. The annual rainfall in the country is below 300mm, and sorghum production occurs under drought stress conditions (Wanga et al., 2022). In agreement with the present study, Seyoum et al. (2019) and Belay et al. (2020) also reported a larger proportion of >72% attributed to the environmental effect on grain yield among sorghum genotypes tested in multi-environment trials. Also, Nagesh et al. (2021) and Enyew et al. (2021) reported a large variation due to environmental effects on days to flowering, fodder and panicle weight traits agreeing with the present findings. These suggested selecting genotypes with high buffering capacity for yield and yield-component traits to avoid crop failure in dry environments.

Genotype-by-environment interaction accounted for lower variation (<20%) for plant height, panicle length, panicle weight and grain yield, which suggested some genotypes with consistent and stable performance could be selected for cultivation in drought-prone environments of Namibia (Figure 5.1). Also, lower variations due to GEI (<20%) were reported in sorghum for days to flowering, plant height, and panicle length grain yield (Nagesh et al., 2021; Enyew et al., 2021; Muluneh and Kuru, 2022). Stable genotypes for days to flowering such as NAM 1057 (G23), NAM 3857 (G29), NAM 858/2 (G33), NAM 859/2 (G35) and NAM 52/2 (G43) would be ideal genetic resources for developing drought-responsive varieties. Genotypes Macia (G01), L3P15-13 (G03), L3P15-40 (G05), ICSR 55 (G46), Pahat (G48), ICSR 59 (G47) and NAM 972/2 (G37) with short plant statue are ideal for enhanced lodging resistance.

Yield-component traits that improve yield response are useful to estimate selection response in breeding schemes. The present study determined phenotypic and genotypic correlations between grain yield and yield-component traits. Genetic correlation is the correlation of breeding values and it results mainly from pleiotropy (Neyhart et al., 2019). Phenotypic correlations is a measure of proxies for genetic correlations, whereas genotypic correlations is breeding values to enable predictions of selection response traits for simultaneous selection of yield-component traits to improve yield (Oliveira et al., 2020; Demelash et al., 2021). Strong and positive (r >0.80) phenotypic and genotypic associations between grain yield and panicle weight suggested simultaneous selection and improvement in the presently evaluated sorghum population. The higher genotypic correlation than phenotypic correlation indicates that the probability of development of higher-performing genotypes can be increased with crosses and selection of individuals with higher expressed traits (Forsman 2014). Also, the strong and positive genotypic correlation between grain yield and days to 50% flowering suggested growth duration may cause difficulties in selecting early maturity genotypes among assessed genotypes. Mengistu et al. (2020) and Nikam et al. (2021) reported a positive association between grain yield and panicle weight in sorghum.

A multi-trait biplot is helpful to identify and select the best genotypes based on desirable traits. The multi-trait biplot identified genotypes L7P9-13 (G08), NAM 1221/2 (G20), NAM 3821 (G28), Red Sorghum (G02), NAM 52/2 (G43) and NAM 3857 (G29) as high-yielding (>1.4 tons/ha) with high panicle weight (> 55g). These genotypes comprised mostly of old varieties and one mutant line. These genotypes are recommended for further breeding of high grain yield and drought-tolerant genotypes for drought-prone environments of Namibia. The newly-identified mutant L7P9-13 will add to the existing pool of genotypes for adoption and as useful germplasm for cultivar development. Induced mutation breeding is an important source of genetic variation to create novel raw material in sorghum breeding programs due to reduced genetic diversity among cultivated sorghum (Girma et al., 2019; Zhou et al., 2020). In the present study, one mutant line identified out of ten mutant lines from initially148 M_2 plants suggests the need to select a large number of M_2 plants to increase the chance of developing genetically diverse mutant varieties.

5.5 Conclusions

In the present study, the Additive Main Effects and Multiplicative Interaction (AMMI) analysis was used to select newly developed sorghum lines for grain yield and yield components to recommend for variety release and commercialization in drought-prone areas in Namibia. The

AMMI model showed total variation attributed to days to 50% flowering (93.9%), plant height (94.04%), panicle weight (86.52%), thousand-grain weight (70.67%) and grain yield (90.68%). This indicated adequate genetic variation among newly-developed sorghum mutant lines, landraces, widely adapted varieties and international check varieties to select genotypes for higher yield and yield component traits. Newly developed mutant lines designated as L7P9-13, L7P7-3, L3P15-16 and L7P9-4 recorded grain yields of ≥1.2 tons/ha higher than the yield output for their parental genotype Macia (1.1 tons/ha). Mutation breeding using gramma radiation created genetic variation to create novel raw material of grain-yield promoting alleles for sorghum breeding programs. The multi-trait biplot based on Best Linear Unbiased Prediction (BLUPs) analyses across test drought-prone environments identified a medium maturity mutant line designated as L7P9-13 as the best yielding (2 tons/ha) and recommended for variety release and commercialization in drought-prone areas in Namibia.

5.6 Reference

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5.7 Supplemental Tables

Supplemental Table 5.1: Best linear unbiased predictors (BLUPs) and best linear unbiased estimates (BLUEs) of yield and yield-component traits for 50 sorghum genotypes evaluated during the 2019/20 season in Mannheim.

	2019/20 Season in Mannheim (Environment 1)											
Genotype	DF (days)		PH (cm)		PL (cm)		PW (g)		TGW (g)		GY (tons/ha)	
	BLUPs	BLUEs	BLUPs	BLUEs	BLUPs	BLUEs	BLUPs	BLUEs	BLUPs	BLUEs	BLUPs	BLUEs
G01	97.2	96.3	110.5	103.8	21.7	21.7	29.7	31.7	22.5	22.0	0.9	1.0
G02	93.3	90.7	139.5	140.9	23.2	23.6	30.3	33.1	26.1	26.3	0.9	1.0
G03	98.5	98.0	99.0	89.1	21.4	21.4	31.5	36.3	25.8	26.0	1.0	1.1
G04	93.0	89.7	144.9	148.5	26.6	27.7	28.7	28.3	22.5	22.0	0.9	0.9
G05	100.7	102.0	106.8	99.5	20.6	20.4	31.6	37.7	24.7	24.7	0.9	1.1
G06	93.9	92.0	144.8	148.1	26.0	27.0	30.5	34.3	19.6	18.7	0.9	1.0
G07	98.9	99.7	146.3	149.4	24.2	24.8	30.6	34.5	19.6	18.7	0.9	1.1
G08	99.5	100.0	125.9	124.2	22.3	22.6	28.2	27.5	18.0	16.7	0.9	0.9
G09	99.2	99.7	123.8	121.3	20.0	19.7	29.5	31.0	17.1	15.7	0.8	0.7
G10	101.7	103.0	123.9	121.2	20.3	20.0	27.0	23.9	21.0	20.3	0.8	0.7
G11	94.8	92.7	132.4	132.4	21.9	22.0	28.6	28.2	21.6	21.0	0.9	1.0
G12	102.6	104.0	118.0	113.8	17.7	16.9	25.0	18.0	23.0	22.7	0.7	0.5
G13	102.7	103.7	137.2	138.3	23.5	24.0	27.0	23.8	18.5	17.3	0.8	0.7
G14	95.9	94.3	145.9	149.5	19.2	18.8	28.0	26.5	26.4	26.7	0.8	0.7
G15	95.1	93.3	141.7	143.3	19.7	19.3	26.6	22.1	22.5	22.0	0.7	0.5
G16	104.7	107.7	146.6	150.7	21.1	21.0	32.3	39.5	28.1	28.7	0.9	1.1
G17	103.7	105.7	140.3	142.1	20.5	20.2	28.6	29.0	23.9	23.7	0.8	0.8
G18	94.8	93.0	127.0	125.3	18.9	18.3	30.3	33.9	31.1	32.3	0.9	0.9
G19	101.6	103.0	133.9	133.9	21.0	20.9	29.7	32.5	24.1	24.0	0.9	0.9
G20	101.7	103.0	133.6	133.3	21.8	21.8	28.9	29.3	22.2	21.7	0.8	0.8
G21	97.0	96.3	133.2	132.7	19.7	19.4	31.0	35.7	33.9	35.7	0.9	1.0
G22	103.4	105.3	124.4	121.0	19.3	18.9	24.6	16.5	29.7	30.7	0.8	0.6
G23	103.9	106.3	137.3	138.0	21.9	21.9	30.4	33.9	25.0	25.0	0.9	0.9
G24	105.7	109.0	139.9	141.0	21.3	21.3	29.4	31.0	33.1	34.7	0.9	0.9
G25	107.7	112.0	136.7	137.6	20.5	20.3	28.9	29.9	32.5	34.0	0.8	0.8
G26	101.5	103.0	147.9	152.5	21.8	21.9	27.1	24.7	29.7	30.7	0.8	0.7
G27	97.9	97.3	140.0	141.0	21.9	22.0	26.4	21.8	29.2	30.0	0.7	0.6
G28	93.7	91.3	129.8	129.9	18.9	18.4	30.4	33.9	23.3	23.0	0.9	1.0
G29	93.9	91.7	148.1	153.1	22.9	23.3	27.3	25.1	28.6	29.3	0.8	0.8
G30	93.6	91.7	143.4	145.2	22.2	22.5	24.7	16.8	25.3	25.3	0.7	0.5
G31	101.2	102.7	159.9	167.3	21.7	21.8	30.1	33.1	23.3	23.0	0.9	0.9
G32	92.0	89.0	144.7	147.9	21.0	20.9	27.0	23.7	31.4	32.7	0.8	0.7
G33	96.7	96.3	133.8	132.8	22.4	22.6	29.6	31.4	28.6	29.3	0.8	0.8
G34	97.3	96.3	120.2	116.0	19.2	18.6	25.6	19.5	20.5	19.7	0.6	0.4
G35	94.8	92.0	142.4	145.6	21.5	21.4	27.5	25.1	31.1	32.3	0.8	0.8
G36	97.1	96.3	141.0	142.3	21.2	21.2	26.8	23.3	31.4	32.7	0.7	0.6
G37	100.1	100.7	107.3	99.6	20.9	20.9	29.5	30.9	21.6	21.0	0.9	0.9
G38	100.6	101.3	137.2	138.4	19.7	19.4	28.7	28.9	33.1	34.7	0.9	0.9
G39	107.1	110.3	126.8	125.5	19.1	18.6	26.9	23.6	23.6	23.3	0.7	0.5
G40	98.0	97.0	142.8	145.4	21.0	20.9	28.4	27.3	22.5	22.0	0.8	0.7
G41	98.0	98.0	153.7	159.1	23.8	24.4	30.8	34.9	26.1	26.3	1.0	1.3
G42	102.9	104.3	147.8	151.3	21.1	21.0	28.7	29.0	24.4	24.3	1.1	1.4
G43	102.5	103.3	162.8	171.4	22.0	22.0	30.9	34.9	24.7	24.7	1.0	1.2
G44	93.1	89.7	138.0	139.5	16.3	15.2	30.2	33.1	30.6	31.7	0.9	0.9
G45	91.9	88.3	132.5	132.2	16.6	15.5	26.8	23.1	28.6	29.3	0.7	0.6
G46	97.8	97.3	114.7	108.8	22.6	22.8	32.3	39.0	21.6	21.0	1.0	1.2
G47	98.1	98.0	111.5	105.3	22.0	22.2	31.1	35.4	21.0	20.3	0.9	1.0

G48	102.5	104.7	120.5	116.6	26.2	27.3	26.6	22.8	19.4	18.3	0.7	0.6
G49	106.2	110.0	114.5	109.1	22.5	22.8	25.8	20.3	13.2	11.0	0.6	0.3
G50	96.2	94.7	132.8	133.0	26.3	27.4	26.9	23.6	16.3	14.7	0.8	0.7
V_G	25.6		246.8		5.9		11.9		28.2		0.0	
Mean	98.9		133.8		21.4		28.7		24.8		0.8	
LSD (5%)	7.9	9.5	20.9	23.6	2.9	3.1	7.9	13.7	5.9	6.5	0.3	0.5
CV (%)	5.8	5.9	10.5	10.5	8.9	8.8	28.6	28.8	16.1	16.1	35.8	35.8
G significance	**		**		**		ns		**		*	

DF = days to 50% flowering, PH = plant height (cm), PL = panicle length (cm), PW = panicle weight (g/panicle), TGW = thousand grain weight (g/1000-grain), GY = grain yield (tons/ha), VG = genetic variance, CV, coefficient of variation; LSD, least significant difference, * and ** denote significant at 5% and 1% probability level, respectively, ns = non-significant, G1-G50 = genotype codes presented in Table 5.1.

Supplemental Table 5.2: Best linear unbiased predictors (BLUPs) and best linear unbiased estimates (BLUEs) of yield and yield-component traits for 50 sorghum genotypes evaluated in the 2020/21 season in Mannheim.

	2020/21 Season in Mannheim (Environment 2)											
Genotype	**DF (days)**		**PH (cm)**		**PL (cm)**		**PW (g)**		**TGW (g)**		**GY (tons/ha)**	
	BLUPs	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**
G01	74.3	73.1	157.6	148.9	24.4	24.7	66.8	66.5	18.3	17.5	2.0	2.1
G02	92.0	92.2	243.3	240.0	22.9	22.1	79.4	87.2	19.9	19.8	2.5	2.8
G03	83.1	82.7	164.7	156.5	23.3	22.7	60.9	57.5	17.6	16.5	1.8	1.7
G04	87.5	87.3	298.5	298.7	32.1	37.3	74.1	78.9	19.2	18.9	2.3	2.6
G05	74.1	72.9	161.9	153.5	24.8	25.4	75.0	79.6	20.5	20.6	2.3	2.5
G06	92.9	93.3	289.7	289.4	26.3	27.5	84.3	95.0	20.1	20.0	2.7	3.1
G07	73.8	72.5	254.9	252.3	25.1	26.0	65.7	64.9	20.4	20.5	2.0	2.0
G08	73.5	72.6	232.8	228.9	23.3	22.7	89.1	102.7	19.6	19.4	2.7	3.2
G09	73.1	71.9	275.4	274.1	23.4	23.7	74.8	79.9	21.8	22.4	2.2	2.4
G10	76.3	75.3	214.2	209.1	23.8	23.4	66.4	66.0	20.7	20.9	1.8	1.7
G11	72.5	71.2	237.7	234.1	25.3	26.3	66.6	66.6	20.4	20.5	1.9	1.9
G12	80.0	79.4	254.2	251.6	22.9	22.0	72.3	75.7	19.8	19.6	2.3	2.5
G13	81.3	80.6	305.5	306.1	25.6	26.6	67.6	68.0	18.2	17.4	2.1	2.2
G14	90.0	89.9	325.4	327.3	23.2	22.9	60.8	57.3	19.3	19.0	2.0	2.0
G15	93.2	93.4	365.5	369.9	24.6	24.8	71.9	75.0	22.5	23.4	2.0	2.0
G16	94.1	94.4	347.8	351.0	24.0	23.6	69.3	70.4	22.7	23.7	2.0	2.0
G17	101.7	102.4	382.0	387.4	23.9	23.4	58.8	53.9	19.2	18.9	1.9	1.9
G18	90.7	90.8	298.7	298.9	21.0	18.7	58.7	53.7	19.1	18.7	1.7	1.5
G19	96.7	97.2	310.9	311.9	22.3	20.7	52.4	43.0	19.1	18.7	1.5	1.2
G20	93.7	93.9	275.4	274.2	23.0	22.0	71.3	74.2	22.0	22.7	2.1	2.2
G21	104.5	105.6	307.5	308.2	21.9	20.2	66.7	66.2	21.2	21.5	2.0	2.1
G22	105.1	106.0	324.4	326.2	22.3	21.4	53.1	44.8	20.8	21.0	1.6	1.4
G23	93.7	94.0	326.3	328.2	23.6	23.0	67.5	67.5	20.1	20.0	1.9	1.9
G24	103.0	103.8	339.2	341.9	24.4	24.8	60.7	57.0	19.8	19.6	1.9	1.9
G25	102.7	103.7	359.5	363.5	24.3	24.0	60.6	56.3	23.7	25.1	1.8	1.6
G26	106.9	108.1	360.1	364.1	22.6	21.3	54.7	47.1	22.1	22.8	1.6	1.3
G27	87.8	87.8	319.8	321.3	24.0	23.8	77.8	85.0	22.1	22.8	2.3	2.6
G28	95.3	95.7	251.6	248.8	21.6	19.8	82.8	92.5	23.0	24.0	2.6	3.0
G29	87.1	87.0	352.4	355.9	24.5	24.5	68.7	69.8	23.1	24.2	2.1	2.2
G30	95.9	96.3	349.5	352.9	25.2	26.2	68.0	69.1	21.6	22.0	1.9	1.9
G31	104.6	105.5	373.9	378.7	24.1	23.9	61.3	57.8	18.9	18.4	1.8	1.6
G32	75.3	74.3	343.9	346.9	25.0	25.2	76.7	82.4	24.6	26.2	2.2	2.3
G33	85.7	85.4	303.7	304.2	24.0	24.0	62.7	60.0	19.8	19.6	1.8	1.7
G34	91.1	91.1	355.8	359.5	24.9	25.3	78.5	85.4	18.5	17.9	2.2	2.4
G35	85.1	84.8	319.5	321.0	22.7	21.8	59.1	54.8	20.8	21.0	1.7	1.5
G36	105.0	106.0	337.1	339.7	23.6	23.1	53.0	44.2	19.4	19.0	1.4	1.0

G37	70.3	69.0	192.2	185.8	24.1	23.9	63.4	60.8	16.8	15.5	1.9	1.8
G38	102.3	103.0	368.4	372.9	27.3	29.4	70.2	72.3	22.1	22.8	2.0	2.0
G39	108.9	110.2	401.4	408.0	22.1	20.6	68.1	69.2	20.1	20.1	1.7	1.5
G40	154.9	159.2	372.2	376.9	22.0	20.9	59.3	55.1	20.2	20.2	1.7	1.5
G41	81.6	80.9	358.5	362.4	27.1	29.5	63.7	61.9	17.8	16.9	1.8	1.6
G42	85.0	84.6	345.1	348.2	25.1	25.5	59.8	55.2	18.7	18.1	1.7	1.5
G43	91.7	91.9	336.4	338.9	27.5	29.7	70.1	72.7	19.0	18.6	2.2	2.3
G44	92.6	92.8	341.0	343.9	21.9	20.2	63.2	61.0	19.5	19.3	1.8	1.7
G45	91.2	91.3	335.9	338.4	21.4	19.6	57.8	52.1	19.9	19.8	1.5	1.2
G46	74.5	73.5	165.8	157.7	24.6	24.6	70.6	72.8	18.8	18.2	2.2	2.3
G47	72.8	71.4	184.4	177.4	25.1	25.8	66.7	66.4	19.1	18.8	2.0	2.0
G48	81.5	81.0	165.1	156.9	26.5	28.3	73.7	78.0	21.1	21.5	2.3	2.5
G49	81.1	80.4	243.6	240.3	25.3	26.3	54.5	47.3	19.5	19.2	1.6	1.3
G50	94.0	94.4	259.4	257.2	25.5	26.1	64.7	63.7	18.8	18.2	2.0	2.0
V_G	219		4802.1		6.0		112.6		3.8		0.2	
Mean	90.1		295.8		24.2		66.9		20.2		2	
LSD (5%)	10.6	10.9	47.2	48.7	4.4	5.6	18.4	23.3	2.9	3.4	0.7	0.9
CV (%)	7.3	7.3	10.1	10.1	13.8	14	21.4	21.5	10.2	10.2	27	27
G significance	**		**		**		**		ns		**	

DF = days to 50% flowering, PH = plant height (cm), PL = panicle length (cm), PW = panicle weight (g/panicle), TGW = thousand grain weight (g/1000-grain), GY = grain yield (tons/ha), VG = genetic variance, CV, coefficient of variation; LSD, least significant difference, ** denote significant at 1% probability level, respectively, ns = non-significant, G1-G50 = genotype codes presented in Table 5.1.

Supplemental Table 5.3: Best linear and unbiased predictions (BLUPs) and best linear and unbiased estimates (BLUEs) of yield and yield-component traits for 50 sorghum genotypes evaluated in the 2019/20 season in Bagani.

	2019/20 Season in Bagani (Environment 3)											
Genotype	**DF (days)**		**PH (cm)**		**PL (cm)**		**PW (g)**		**TGW (g)**		**GY (tons/ha)**	
	BLUPs	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**
G01	72.1	71.4	90.3	87.5	23.3	23.1	64.0	59.3	20.2	19.5	1.4	1.4
G02	77.6	78.0	177.1	176.4	21.7	20.5	61.4	55.4	21.3	21.1	1.2	1.0
G03	78.9	79.3	93.3	90.9	23.6	23.3	63.5	59.6	19.7	19.1	1.1	0.7
G04	79.2	79.8	196.6	196.6	26.9	28.3	62.6	57.4	20.0	19.4	1.5	1.6
G05	72.0	71.3	111.9	109.7	23.2	23.0	70.1	72.1	21.0	20.7	1.1	0.7
G06	81.5	82.8	125.7	124.0	24.5	25.2	47.9	33.3	17.9	16.9	0.7	0.0
G07	71.2	70.5	180.6	179.9	24.8	25.7	73.3	77.0	21.2	20.9	1.6	1.7
G08	70.8	70.1	134.6	133.0	24.9	25.8	89.8	107.7	21.5	21.4	2.2	2.8
G09	71.9	71.0	162.2	161.1	23.9	24.3	71.1	72.5	24.8	25.4	1.3	1.1
G10	71.0	70.0	124.6	122.8	23.0	22.5	52.0	38.3	22.4	22.4	1.4	1.3
G11	73.0	72.4	194.8	194.8	23.0	22.5	66.1	63.6	22.0	21.8	1.5	1.6
G12	81.7	82.8	129.1	127.6	21.2	19.5	48.7	32.9	23.7	24.1	0.9	0.3
G13	70.9	70.1	178.4	177.8	25.3	25.8	52.8	40.4	17.4	16.1	1.6	1.6
G14	75.8	75.7	240.5	241.5	24.2	24.4	65.4	62.3	20.2	19.6	1.4	1.4
G15	74.2	74.0	251.2	252.6	26.5	27.8	71.9	75.0	22.7	22.7	1.4	1.4
G16	76.4	76.9	244.3	245.6	25.1	25.9	75.3	82.5	23.6	23.9	1.6	1.6
G17	84.7	86.6	242.8	244.0	23.5	23.1	59.9	54.7	20.2	19.7	1.1	0.7
G18	74.8	74.9	177.1	176.7	21.5	20.1	74.5	80.5	22.9	23.0	1.5	1.5
G19	77.6	78.2	211.5	212.0	23.7	23.4	62.6	58.5	20.6	20.3	1.4	1.3
G20	76.8	77.2	257.4	259.2	25.7	26.4	95.4	118.3	24.8	25.4	2.3	3.1
G21	77.5	78.1	241.0	242.1	22.3	21.4	78.9	87.1	22.5	22.6	1.7	1.7
G22	73.2	72.6	226.6	227.4	23.2	22.9	68.0	67.0	23.8	24.1	2.0	2.4
G23	75.9	76.1	230.4	231.5	23.0	22.3	68.1	69.0	25.2	26.0	1.5	1.5
G24	79.2	79.9	252.3	253.2	25.5	26.6	80.1	89.3	26.2	27.0	2.1	2.6
G25	79.2	80.1	246.4	247.7	23.4	23.2	76.6	83.4	25.7	26.5	1.8	2.0

G26	83.2	84.9	299.7	302.4	21.8	21.0	66.6	67.1	25.7	26.5	1.5	1.4
G27	73.1	72.7	248.8	250.2	24.2	24.3	82.4	94.8	28.4	29.9	1.9	2.2
G28	79.7	80.9	208.3	208.7	20.4	18.5	89.0	106.9	25.0	25.7	2.1	2.7
G29	73.5	73.6	256.2	257.8	26.6	27.9	82.3	94.9	23.2	23.5	1.9	2.3
G30	73.0	72.5	260.9	262.3	24.5	25.1	78.1	86.4	23.9	24.2	1.6	1.6
G31	78.9	79.9	286.1	288.3	23.6	23.7	72.8	77.2	23.0	23.2	1.4	1.3
G32	72.0	71.4	232.0	232.9	24.7	25.0	64.2	60.9	23.6	24.0	1.7	1.8
G33	71.9	71.1	219.1	219.7	21.5	20.3	58.1	50.1	17.4	16.3	1.1	0.7
G34	70.6	69.4	208.7	209.1	22.9	22.3	69.8	70.4	20.1	19.5	1.7	1.8
G35	65.3	63.2	184.1	183.8	24.4	24.4	61.9	56.1	22.6	22.7	1.4	1.3
G36	70.7	69.8	252.7	253.9	23.1	22.6	57.7	47.8	24.0	24.3	1.6	1.6
G37	70.7	69.8	95.1	92.5	24.4	24.6	67.4	65.1	21.0	20.6	1.6	1.6
G38	74.2	74.0	244.7	245.7	23.5	23.3	80.1	89.9	26.0	26.8	1.8	2.1
G39	82.5	84.2	262.3	264.0	21.9	20.5	81.9	93.9	28.8	30.3	1.8	2.1
G40	79.6	80.1	275.8	277.5	24.9	25.4	72.4	74.2	22.8	22.9	2.2	2.7
G41	71.9	71.0	222.8	223.2	26.4	28.2	70.0	70.5	22.7	22.8	1.7	1.9
G42	71.8	70.8	212.3	212.5	24.3	24.5	61.8	55.3	21.5	21.3	1.8	2.1
G43	75.8	76.0	244.2	245.5	26.9	28.2	74.8	80.6	20.6	20.3	1.7	1.8
G44	80.4	81.2	189.3	189.1	20.8	18.9	68.9	69.1	23.9	24.2	1.3	1.2
G45	71.5	70.4	178.9	178.5	20.8	18.8	66.7	63.3	24.8	25.4	1.3	1.2
G46	74.8	74.7	96.6	94.5	22.6	21.5	57.2	49.2	19.3	18.7	1.0	0.6
G47	71.3	70.6	81.8	78.8	24.1	24.3	62.2	56.2	20.7	20.2	1.1	0.7
G48	74.1	73.8	95.5	92.7	27.9	30.6	56.9	48.2	19.5	18.8	1.1	0.6
G49	72.2	71.8	137.6	135.8	25.9	27.3	47.0	30.9	16.8	15.5	1.0	0.5
G50	84.6	86.5	160.6	159.8	25.2	25.8	58.6	52.5	21.2	21.0	1.2	1.0
V_G	22.1		3608.1		4.7		211.5		8.7		0.3	
Mean	75.3		197.5		23.9		68.2		22.4		1.5	
LSD (5%)	5.3	5.7	26.7	26.8	3.6	4.5	27.6	37.2	3.7	4.1	1.0	1.3
CV (%)	4.6	4.5	8.2	8.2	11.2	11.5	32.5	32.6	11.0	11.0	52.6	52.4
G significance	**		**		**		**		ns		**	

DF = days to 50% flowering, PH = plant height (cm), PL = panicle length (cm), PW = panicle weight (g/panicle), TGW = thousand grain weight (g/1000-grain), GY = grain yield (tons/ha), VG = genetic variance, CV, coefficient of variation; LSD, least significant difference, ** denote significant at 1% probability level, respectively, ns = non-significant, G1-G50 = genotype codes presented in Table 5.1.

Supplemental Table 5.4: Best linear and unbiased predictions (BLUPs) and best linear and unbiased estimates (BLUEs) of yield and yield-component traits for 50 sorghum genotypes evaluated in the 2020/21 season in Bagani.

2020/21 Season in Bagani (Environment 4)												
Genotype	**DF (days)**		**PH (cm)**		**PL (cm)**		**PW (g)**		**TGW (g)**		**GY (tons/ha)**	
	BLUPs	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**	**BLUPs**	**BLUEs**
G01	84.30	83.80	81.40	66.00	21.00	20.80	32.30	27.50	21.70	20.80	0.30	0.20
G02	84.00	82.90	110.60	107.90	21.70	21.70	54.70	59.30	25.00	25.40	0.60	0.80
G03	86.60	88.40	90.20	79.30	21.40	21.40	36.70	34.00	21.80	21.00	0.30	0.30
G04	86.40	87.90	105.50	100.50	24.70	26.10	36.70	34.10	20.70	19.50	0.30	0.20
G05	84.60	84.30	105.10	100.20	23.10	23.80	43.40	44.70	26.30	27.20	0.30	0.20
G06	88.20	91.70	116.70	117.60	24.20	25.30	36.40	35.10	19.90	18.40	0.40	0.40
G07	84.30	83.10	114.70	113.40	21.90	22.10	45.60	46.30	24.40	24.60	0.50	0.50
G08	86.90	89.10	107.80	105.20	20.10	19.50	39.10	38.90	20.50	19.20	0.50	0.60
G09	84.70	84.20	109.50	106.10	21.00	20.90	42.30	41.10	23.80	23.70	0.30	0.30
G10	84.20	83.00	95.00	86.10	20.10	19.50	33.70	28.90	24.10	24.20	0.40	0.30
G11	83.80	82.70	105.20	101.00	21.60	21.60	51.90	56.00	22.90	22.50	0.60	0.80
G12	84.20	83.70	118.40	119.50	20.00	19.40	29.10	24.10	23.50	23.30	0.30	0.20
G13	83.30	81.80	119.30	119.20	20.00	19.30	36.10	33.90	22.20	21.60	0.40	0.40
G14	85.00	85.10	121.20	123.30	18.10	16.60	33.50	30.20	23.50	23.30	0.30	0.20

G15	84.50	84.10	131.10	137.00	22.30	22.60	52.80	57.90	24.90	25.20	0.40	0.30
G16	83.00	81.10	126.80	132.60	21.20	21.00	33.70	32.00	25.60	26.10	0.30	0.30
G17	91.00	97.40	128.20	133.10	23.90	25.00	50.60	55.90	23.50	23.50	0.50	0.50
G18	85.00	85.40	107.10	103.50	17.60	15.90	48.80	53.40	26.70	27.70	0.40	0.50
G19	84.70	84.60	112.60	111.50	21.40	21.40	39.90	39.50	21.90	21.30	0.40	0.30
G20	83.50	82.20	136.20	144.80	22.00	22.20	59.00	67.80	25.10	25.50	0.50	0.60
G21	86.00	87.30	104.00	99.30	21.20	21.20	47.10	50.40	23.80	23.80	0.60	0.80
G22	87.70	90.30	114.10	112.30	23.00	23.70	50.60	53.40	24.70	24.90	0.40	0.40
G23	85.80	86.70	131.30	138.20	20.90	20.60	41.90	42.20	25.00	25.40	0.40	0.30
G24	88.50	92.30	127.10	130.10	24.50	25.80	60.50	68.30	26.30	27.00	0.40	0.40
G25	83.70	82.60	153.30	169.20	24.40	25.60	70.20	82.60	29.90	31.90	0.80	1.20
G26	85.00	85.10	140.00	151.10	22.50	23.00	58.80	67.30	24.60	24.90	0.40	0.50
G27	84.70	84.90	124.10	127.00	20.20	19.70	31.20	27.90	22.50	21.90	0.50	0.50
G28	84.00	82.80	105.20	101.40	17.40	15.70	43.60	44.90	23.80	23.90	0.40	0.40
G29	83.50	81.80	121.80	124.60	20.50	20.00	33.90	31.30	25.00	25.40	0.40	0.40
G30	82.70	80.50	127.50	131.10	21.30	21.30	37.30	35.50	25.80	26.30	0.40	0.30
G31	83.90	82.50	139.90	150.00	20.40	20.00	36.30	33.70	22.80	22.50	0.30	0.20
G32	82.90	80.60	123.20	126.50	21.00	20.80	28.90	23.50	27.20	28.40	0.40	0.40
G33	83.30	81.60	117.10	116.90	20.50	20.10	31.50	27.00	23.70	23.60	0.40	0.30
G34	86.00	86.90	125.50	128.70	21.20	21.10	31.20	25.40	23.10	22.80	0.30	0.20
G35	86.80	88.80	116.80	116.70	20.00	19.40	34.10	29.90	24.70	24.90	0.40	0.30
G36	85.40	85.80	126.00	129.50	21.50	21.50	34.50	31.60	26.00	26.60	0.40	0.50
G37	84.40	83.80	97.50	89.50	21.40	21.40	35.30	32.80	21.90	21.10	0.30	0.30
G38	87.40	90.10	107.00	102.00	22.50	22.90	49.70	53.10	26.40	27.30	0.40	0.30
G39	83.40	81.80	142.30	152.40	24.40	25.60	80.10	97.00	29.20	31.00	0.60	0.80
G40	87.20	89.70	151.50	165.30	22.50	22.90	57.80	63.10	21.20	20.10	0.60	0.80
G41	83.30	81.60	124.20	126.70	23.20	24.00	29.40	22.80	24.40	24.60	0.30	0.10
G42	83.80	82.70	117.00	116.40	18.30	17.00	28.70	21.90	22.90	22.50	0.30	0.10
G43	85.30	85.90	113.50	112.30	21.00	20.70	37.70	36.60	22.30	21.80	0.30	0.20
G44	83.50	82.40	119.80	120.20	16.70	14.70	29.30	23.70	27.60	28.80	0.30	0.10
G45	83.70	82.50	119.40	120.70	19.00	18.00	26.00	18.10	25.00	25.40	0.20	0.00
G46	84.30	83.70	79.60	64.90	22.30	22.70	43.40	44.80	23.20	22.90	0.30	0.20
G47	83.10	80.80	122.50	124.10	21.40	21.40	38.70	36.60	23.10	22.80	0.40	0.40
G48	83.50	82.30	107.00	102.70	23.50	24.30	35.40	33.20	21.10	20.10	0.30	0.20
G49	83.10	81.00	93.90	84.20	24.50	25.80	31.80	27.70	22.40	21.90	0.30	0.20
G50	87.60	90.60	99.10	92.10	24.70	26.10	48.40	52.80	20.40	19.20	0.40	0.40
V_G	6.33		353.18		5.38		189.87		6.40		0.03	
Mean	84.91		116.68		21.46		41.60		23.96		0.39	
LSD (5%)	5.08	7.28	29.03	34.59	3.56	4.25	21.65	25.83	3.65	4.23	0.31	0.43
CV (%)	5.14	5.14	17.65	17.73	12.22	12.22	36.78	36.74	10.60	10.61	64.83	65.77
G significance	**		**		**		**		**		**	

DF = days to 50% flowering, PH = plant height (cm), PL = panicle length (cm), PW = panicle weight (g/panicle), TGW = thousand grain weight (g/1000-grain), GY = grain yield (tons/ha), VG = genetic variance, CV, coefficient of variation; LSD, least significant difference, ** denote significant at 1% probability level, respectively, ns = non-significant, G1-G50 = genotype codes presented in Table 5.1.

Chapter 6. Overview and Implications of the Study

6.1 Introduction and objectives of the study

Sorghum (*Sorghum bicolor* [L.] Moench) is the foundation crop in the world's dry regions for food, feed, and bioenergy feedstock. Sorghum, pearl millet and maize are the major cereal crops widely cultivated in Namibia. However, in the past, there was no systematic plant breeding program to deliver new crop varieties in the country. Also, there are no current studies that document the major production systems, farmers' perceived production constraints and trait preferences in new varieties to guide breeding. Sorghum has a low genetic base in Namibia limiting its genetic diversity for selective breeding of drought-adapted and high yielding varieties. Therefore, this study adopted an induced mutation procedure to broaden the genetic diversity for breeding and variety registration and commercialisation. The overall goal of this study was to contribute to the national sorghum breeding program aimed at improving production through the development and deployment of climate-smart cultivars preferred by farmers and markets in Namibia. Gamma radiation method was adopted, and the pedigree selection method pursued to create heritable genetic variation and identify functional mutant lines with high yield and drought adaptive traits for variety development and release in Namibia.

The specific objectives of the study were:

1. To assess the present state of sorghum production in northern Namibia and document farmers' perceived production constraints and trait preferences in new varieties to guide drought-tolerance breeding.
2. To determine the optimum doses of a single and combined use of gamma radiation and ethyl methanesulfonate (EMS) for effective mutation breeding in sorghum.
3. To determine the genetic profile of elite sorghum lines developed via gamma radiation using diagnostic simple sequence repeat (SSR) markers and phenotypic traits for breeding.
4. To determine genotype-by-environment interaction of newly-developed mutant and traditional sorghum lines for grain yield and yield related traits for drought-prone areas of Namibia.

6.2 Research findings in brief

Sorghum production in northern Namibia: Farmers' perceived constraints and trait preferences

A survey was conducted using a participatory rural appraisal in six selected sorghum-growing constituencies in Namibia, including Kapako and Mpungu (Kavango West Region), Eenhana and Endola (Ohangwena Region), and Katima Mulilo Rural and Kongola (Zambezi Region).

The core findings of the study were:

- The trends of sorghum production were variable among respondent farmers when disaggregated by gender, age, number of households, education level, cropping systems, types of varieties grown, and perceived production constraints.
- An equal proportion of male and female respondent farmers cultivate sorghum, suggesting the value of the crop to both genders in Namibia.
- Most respondent farmers (63.6%) were in productive age groups of < 40 years old.
- In the study areas, low-yielding landrace varieties, namely Ekoko, Okambete, Makonga, Kamburo, Nkutji, Katoma, Fuba, Dommy, Kawumbe, and Okatombo, were widely cultivated, and most of the farmers did not use chemical fertilizers to cultivate sorghum.
- Farmers' perceived sorghum production constraints in the study areas included recurrent drought, declining soil fertility, insect pest damage, high cost of production inputs, unavailability of improved seed, lack of alternative improved varieties with farmers' preferred traits, lack of organic manure, limited access to market and limited extension service.
- The key farmers' preferred traits in a new sorghum variety included high grain yield, early maturity, and tolerance to drought, in the field and storage insect pests.
- The study recommends genetic improvement and new variety deployment of sorghum with the described farmers-preferred traits to increase the sustainable production of the crop in Namibia.

The effect of single and combined use of gamma radiation and ethyl methanesulfonate on early growth parameters in sorghum

The study involved two concurrent experiments as follows: In experiment I, the seeds of four sorghum genotypes (Parbhani Moti, Parbhani Shakti, ICSV 15013, and Macia) were treated using gamma radiation (0, 300, 400, 500 and 600 Gy), EMS (0, 0.5 and 1.0%), and gamma radiation followed by EMS (0 and 300 Gy and 0.1% EMS; 400 Gy and 0.05% EMS). In experiment II, the seeds of two genotypes (Macia and Red sorghum) were treated with seven doses of gamma radiation only (0, 100, 200, 300, 400, 500 and 600 Gy).

The major findings of the study were:

- The combined doses of gamma radiation and EMS are not recommended due to poor seedling emergence and seedling survival rate below LD_{50}.
- The best dosage of gamma radiation for genotypes Red sorghum, Parbhani Moti, Macia, ICSV 15013 and Parbhani Shakti ranged between 392 and 419 Gy, 311 and 354 Gy, 256 and 355 Gy, 273 and 304 Gy, and 266 and 297 Gy, respectively.
- The EMS optimum dosage ranges for genotypes Parbhani Shakti, ICSV 15013, Parbhani Moti and Macia were between 0.41% and 0.60%, 0.48% and 0.58%, 0.46% and 0.51%, and 0.36% and 0.45%, respectively.
- The above dose rates are recommended to induce genetic variation in the tested sorghum genotypes for higher mutation events in sorghum breeding programs.

Genetic profile of newly developed sorghum lines revealed through simple sequence repeat markers and phenotypic traits

Twenty mutant lines (M_7 generations) developed using gamma-irradiated (350 Gy) from the seed of variety Macia (SDS 3220), and five check varieties were used for the study. The DNA samples of the test genotypes were extracted using young and fresh leaves samples from 14 plants per test line 20 days after sowing. Twenty highly polymorphic SSR markers were selected based on distribution across 10 sorghum chromosomes and for being highly polymorphic.

The main findings of the study were:

- The SSR marker mSbCIR276 produced poor amplification after numerous optimisations attempts and was not included in the final analysis.
- The following monomorphic markers: mSbCIR248 and Xcup02 were excluded, resulting in the 17 SSR markers in the final analysis.
- Seventeen SSR markers amplified a total of 50 alleles, which varied from 2 to 5 (mean = 2.94).
- The number of effective alleles per locus varied from 1.08 to 2.53, with a mean of 1.96. The observed heterozygosity ranged from 0.00 to 0.21 (mean = 0.09). The mean expected heterozygosity value was 0.45 indicating moderate genetic differentiation of the tested lines for selection and hybridization.
- Cluster analysis classified the genotypes into three main groups.
- Moderate to high genetic distance (≥ 0.50) was displayed between drought-tolerance and high-yield performance and aided in selecting mutant lines such as ‘ML2, ML3, ML4, ML7

and ML14' compared with the check varieties Macia, Kotovara, ICSR 137, and ICSV 17004'.

- The selected lines are a useful source of genetic variation for breeding high-yielding and drought-tolerant varieties suited for the drought-prone environments of Namibia.

Genotype by environment interaction of newly developed sorghum lines in Namibia

Fifty sorghum genotypes, including 10 newly-developed mutant lines (M_9 generation), 33 landraces, two sorghum varieties widely grown in Namibia, and five standard check varieties were evaluated under field conditions using a 10 x 5 alpha lattice design with three replications. The experiments were carried out in four environments with two growing seasons in Namibia.

The core findings of the study were:

- The AMMI model showed that 93.9% of the total genetic variation was attributed to days to 50% flowering (DF), while 94.04% of the variation was due to plant height (PH), 86.52% to panicle weight (PW), 70.67% to thousand-grain weight (TGW), and 90.68% to grain yield (GY).
- The larger variations attributed to genotypic effects for PL (36.3%), TGW (33.2%) and PH (20.7%) are useful for genotype selection for yield related traits.
- Based on a multi-trait biplot and Best Linear Unbiased Prediction (BLUPs) analyses of the GEI data across all drought-prone testing environments, the medium maturity mutant line designated as L7P9-13 was selected as the best yielding (2 tons/ha) and recommended for drought-prone area of Namibia.

6.3 The implications of the study findings

- The study identified sorghum production systems, key farmers' perceived production constraints and trait preferences in new varieties in Namibia. These attributes are key for demand-led sorghum breeding and commercialisation programs.
- The best dosage of gamma radiation and EMS were determined for increasing the genetic diversity in sorghum for genetic enhancement.
- New mutant lines ML2, ML3, ML4, ML7 and ML14 were selected with moderate to high genetic distance useful for breeding high-yielding and drought-tolerant varieties suited for the drought-prone environments of Namibia.

- The medium maturity and drought-tolerant mutant line designated as L7P9-13 was the best yielding (2 tons/ha) and recommended for large-scale production in the country.
- Future induced mutation breeding programs is recommended to rapidly develop novel sorghum mutant populations and select genetically diverse genotypes through integrated marker-assisted selection, genomics, speed breeding, high-throughput phenotyping, and digital tools.
- Rapid genetic diversity enhancement and selection for climate-smart varieties with farmers' and markets' preferred traits are the drivers to improve sorghum production and productivity to contribute to sustainable food systems to feed the increasing human population under the ever-changing climate conditions.